INTER-POPULATION DIFFERENCES IN GROWTH AND ENERGY ALLOCATION OF NORTHWEST ATLANTIC COD (*Gadus morhua* L.) REVEALED BY COMMON ENVIRONMENT EXPERIMENTS

CENTRE FOR NEWFOUNDLAND STUDIES

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Canada

Inter-population differences in growth and energy allocation of northwest Atlantic cod (*Gadus morhua* L.) revealed by common environment experiments

by

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©

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ABSTRACT

Geographic variation in life history traits is frequently observed among fishes. Although much of this variation has been shown to be based on environmental variability, genetic differences among populations have been found. Atlantic cod (*Gadus morhua* L.) occur throughout much of the north Atlantic Ocean. Factors such as growth rates vary tremendously among stocks, generally with faster growth occurring in warmer water. The relative contributions of temperature and genotype towards growth in cod however, is not known. Knowledge of this information could be useful in the management of cod stocks and in selecting populations for aquaculture.

This thesis examined growth and energy allocation of cod from different populations, using common environment experiments. In the first experiment, larval cod from the Grand Banks (GB), and the Gulf of Maine (GOM) were reared under identical laboratory conditions to determine the effect of two temperatures on growth. Grand Banks larvae grew faster than GOM larvae at both temperatures tested. This supports the countergradient variation hypothesis, which states that higher latitude populations have greater capacities for growth rates than those at lower latitudes.

The second and third experiments compared the effects of temperature on growth and energy allocation in juvenile cod from the GB and GOM, and juveniles from two inshore bays on the island of Newfoundland. Temperature significantly affected growth rates, food conversion efficiency, and % liver water content, but did not significantly affect

condition factor, hepatosomatic index, or % muscle water content. In contrast to larvae, no differences in growth rates were observed between juvenile GB-GOM cod, or between juveniles from the two inshore sites. This rejects the countergradient variation hypothesis in juvenile cod. However, population differences in other traits (food conversion efficiencies, energy allocation) were found, suggesting genetic differences between the stocks

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LISTS OF ABBREVIATIONS

Chapter One

CnGV - countergradient variation

GV - geographic variation

LA - local adaptation

V_E - environmental variance in a trait

V_G - genotypic variance in a trait

V_P - phenotypic variance in a trait

Chapter Two

ANCOVA - analysis of covariance

ANOVA - analysis of variance

CnGV - countergradient variation

DW - dry weight

ED - eye diameter

F - F statistic

FW - foil weight

GB - Grand Banks

GOM - Gulf of Maine

GSGR - gross length specific growth rate

K - Condition factor

LA - local adaptation

L_F - final length

LFW - larvae + foil weight

L_I - initial length

MH - myotome height

NAFO - Northwest Atlantic Fisheries Organization

p - p value

TL - total length

WW - wet weight

Chapter Three

A - ambient temperature

ANCOVA - analysis of covariance

ANOVA - analysis of variance

b - slope of the regression line

BB - Bonavista Bay

CnGV - countergradient variation

F - F statistic

FB - Fortune Bay

GB - Grand Banks

GFCR - gross food conversion ratio

GOM - Gulf of Maine

GSGR - gross specific growth rate

H- heated temperature

H.S.I. - hepatosomatic index

K - Fulton's condition factor

LA - local adaptation

LAB - lab reared cod

L_T - total length

L_W - liver weight

NAFO - North Atlantic Fisheries Organization

O.S.C. - Ocean Sciences Centre, Logy Bay lab

p - p value

t- t statistic

Time - time in days

T_D - dry tissue weight

T_W - wet tissue weight

W_F - mean final weight of fish

W_{FE} - mean weight of food eaten

W_G - mean weight gain of fish

W_I -mean initial weight of fish

WILD - wild caught cod

W_W - wet weight

% W_L - % water content of liver

% W_M - % water content of muscle

XL_T - mean total length of all fish

Y_c - corrected nutritional index value

Y_O - individuals measured nutritional index value

LIST OF FORMULAE

Chapter One

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

Chapter Two

$DW = LFW - FW$

$K = (DW * TL^{-3}) * 100$

$GSGR = ((Ln\ (L_F) - Ln(\ L_I))^{-28}) * 100$

Chapter Three

$GFCR = W_{FE} * W_G^{-1}$

$GSGR = (\ln W_F - \ln W_I)^{-Time} * 100$

$K = (W_W * L_T^{-3}) * 100$

$H.S.I. = (L_W * W_W^{-1}) * 100$

$\log_{10} Y_C = \log_{10} Y_O - (b^* \log_{10} L_T - b^* \log_{10} XL_T)$

$\%\ Water = 100 - (\ (T_D * T_W^{-1}) * 100)$

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Chapter One

Adaptive geographic variation: A literature review of local adaptation and countergradient variation

1.1 Adaptation

Adaptations are characteristics of organisms that make them better suited to their environment (Brandon, 1990). It is the process of adaptation, by which evolution by natural selection occurs (Brandon, 1990). Adaptations can occur in many traits and can be classified as being either morphological, behavioral, or physiological.

Morphological characteristics are perhaps the most visible of all types of adaptations (Minkoff, 1983). These traits would likely develop after another type of adaptation has occurred (e.g., behavioral). Behavioral traits are often the most flexible and subsequently are usually the first to change under selective pressure (Minkoff, 1983). Whereas many morphological adaptations can be easily identified (e.g., wings, fur, fins, webbed feet, etc.), physiological adaptations are usually more difficult to detect (e.g., heavy metal tolerance in plants, temperature tolerance, growth rates, etc.; Minkoff, 1983).

Temperature adaptation is among the most studied of all physiological traits. The importance of temperature adaptation is evident from its broad range in occurrence. Environmental temperature varies tremendously, and is one of the most important factors affecting physiological processes in organisms (Brett, 1979; Hazel, 1993). Changes in

temperature can occur daily, seasonally, or over a much longer period (climate change). There is also widespread spatial variation in environmental temperature. This may result in substantially different pressures on the physiology of organisms.

1.2 Geographic variation

Species often occur over broad geographic ranges that may encompass large differences in environmental conditions. Within a species distribution, subspecies, populations, or individuals may differ in certain biological characteristics. Geographic variation (GV) can occur in many traits, including: morphology (Schmidt, 1930; Rollefsen, 1934; Barrowclough *et al.*, 1985; Zink and Remsen, 1986; Thorpe, 1989), behavior (Thielcke, 1969; Moynihan, 1979; Arnold, 1981), and physiology (Bullock, 1955; Prosser, 1955; Zhirmunsky, 1959; Vernberg, 1962; Møller, 1968; Garland and Adolph, 1991).

There are three explanations as to how GV occurs: non-genetic phenotypic variation, non-adaptive random genetic drift, and adaptive genetic variation (Levinton, 1983; Minkoff, 1983). Early geneticists thought GV to be purely phenotypic (not heritable) and of no evolutionary value. It is now accepted that both genetic drift and natural selection results in GV (Minkoff, 1983), the importance of each differing among cases.

Throughout a species range, different environmental factors likely affect life history variation through both phenotypic plasticity, and selection on genotypes (see Nicieza *et al.*, 1994b, and references therein). Physiological traits in particular can be influenced by

many factors, such as: temperature, nutritional status, and acclimatization history (Garland and Adolph, 1991).

Through its effect at the molecular level, temperature can influence physiology and subsequently ecology, and is perhaps one of the most important environmental variables influencing GV. Temperature varies greatly with latitude. In certain cases, elevation, wind direction, currents, etc., can result in substantial temperature differences occurring over a small geographical area. As a result, individuals of the same species can be subjected to large temperature differences. Changes in environmental temperature can directly affect the physiology of poikilothermic organisms, which do not regulate internal body temperature. Temperature is in fact, the most important environmental influence on the biology of poikilothermic organisms (Brett, 1979; Prosser, 1986; Cossins and Bowler, 1987; Hazel, 1993).

Discussed here are two patterns of geographic variation in physiological traits that result from variation in temperature, local adaptation and countergradient variation.

1.3 Local adaptation

Local adaptation likely results from fitness benefits in one environment producing tradeoffs in another (Levinton, 1983). Where local adaptation to temperature occurs, biological functions are optimized at temperatures normally encountered in the wild (localized physiological adaptation). Studies have shown local adaptation (LA) in invertebrates (Ament, 1979; Levinton, 1983; Levinton and Monahan, 1983; Lonsdale and

Levinton, 1985), fish (Mitton and Koehn, 1975), amphibians (Berven and Gill, 1983), and reptiles (Niewianowski and Roosenburg, 1993).

Reciprocal transplant or common environment experiments in the laboratory are often used to examine this phenomenon. In cases where LA in growth rate occurs, each population grows fastest in its own environment (or more closely simulated one). In other words, the population is adapted to local conditions. For example, Lonsdale and Levinton (1985) compared growth of copepods (*Scottolana canadensis*) from the east coast of the United States under common laboratory conditions. They found that northern populations grew best at lower temperatures, while southern populations grew best at higher temperatures. This is a classic example of LA in a physiological trait (growth), where performance is best at conditions normally encountered in the wild.

The literature base for this phenomenon is scarce, as detecting it in nature is difficult. I have found no studies where possible tradeoffs in different environments have been adequately investigated to explain how LA occurs. I suggest potential sources of LA are temperature sensitive biochemical pathways, in which specific forms of enzymes function over narrow temperature ranges.

1.4 Countergradient variation

Another geographic pattern in physiological variation is countergradient variation. This phenomenon is indirectly a result of temperature, as it is an adaptation to length of the growing season (Conover and Present, 1990). Conover (1992) defines CnGV as "genetic

variation that compensates for environmental influences on phenotype across an environmental gradient." This phenomenon is not widely recognized but may be widespread in occurrence.

Any phenotype can be expressed using the following equation (from Conover and Schultz, 1995):

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

Where Vp = phenotypic variance in a trait, V_G = genotypic variance in a trait, V_E = environmental variance in a trait, V_{G*E} = variance in the non additive genotype/environment interaction, and Cov(G,E) is the covariance between genotypic and environmental sources of variation. This covariance term "expresses the degree to which genotypes having a measurable effect on phenotype expression are non-randomly distributed among environments that influence the same phenotypic traits" (Conover and Schultz, 1995). Where CnGV occurs, this covariance is negative, and therefore genetics and environment operate in opposite directions on the same trait.

The difference between local adaptation and countergradient variation can be explained using growth rates. If LA occurs, the temperature of maximum growth rate changes, but the maximum growth rate itself does not change. However, if CnGV occurs, the maximum growth rate is altered (Conover, 1992). Faster growth is usually considered to increase fitness. Why all populations do not exhibit the maximum growth rate that is capable for the species, is not clear. It is possible that tradeoffs of higher growth rates (i.e., potential fitness disadvantages) are more significant at lower latitudes.

The first description of countergradient variation was given by Levins (1968; 1969) in *Drosophila melanogaster*. He noticed that what seemed to be similar growth rates at high and low altitudes, were actually quite different in the laboratory, "Although flies caught at cooler, higher latitudes are larger than those taken in the hot lowlands, when they are raised under the same conditions in the laboratory the coastal flies are larger" (Levins, 1969). Larger flies were thought to be less prone to dessication in the hot coastal areas, whereas other environmental factors produced smaller flies.

Conover and Present (1990) made the first description of CnGV in a fish, Atlantic silverside (*Menidia menidia*). Growth and reproduction in this species occur only at water temperatures of 12 °C or higher. As a result, along the eastern seaboard of North America the length of the growing season declines by a factor of 2.5 with increasing latitude (for other examples see Conover, 1990). With all else being equal, the northern fish should be less than half the size of southern fish at the end of the first growing season. However, size at the end of the first growing season is the same for northern and southern silversides (Conover and Present, 1990).

When Conover and Present (1990) placed northern and southern silversides in the laboratory at the same temperature, the northern fish grew faster than southern ones. If the fish were locally adapted to a specific temperature range, the expected result would be increased growth of northern fish at lower temperatures, and increased growth of southern fish at higher temperatures.

There is evidence for countergradient variation in growth rate in other fish species:

arctic char, *Salvelinus alpinus* (DeLabbio *et al.*, 1990); largemouth bass, *Micropterus salmoides* (Williamson and Carmichael, 1990; Phillipp and Whitt, 1991); Atlantic salmon, *Salmo salar* (Nicieza *et al.*, 1994b); mummichog, *Fundulus heteroclitus* (Schultz *et al.*, 1996); and striped bass, *Morone saxatilis* (Conover *et al.*, 1997; Brown *et al.*, 1998). Studies showing CnGV in a number of other characteristics in both plants and animals have been reviewed by Conover and Schultz (1995).

Although not normally recognized as such, metabolic compensation at high latitudes is an example of CnGV. Under such circumstances, when animals are taken from cold (high latitude) environments and placed in warm (low latitude) environments, they have higher metabolic rates than the animals living in the warm environment (Prosser, 1991).

Increased growth rates by animals exhibiting CnGV can be achieved in a number of ways, including: more energy being consumed (increased feeding), more energy being assimilated (increased food conversion efficiency), a change in allocation of energy (more energy going to growth, less to reproduction, metabolism, etc.), or some combination of these (Present and Conover, 1992). It has generally been assumed that physiological efficiencies have been maximized in nature by natural selection (Priede, 1985). If this is true, increased growth rates of animals at higher latitudes cannot be due to increased efficiency. However, Present and Conover (1992) showed that although northern *Menidia menidia* ate 1.7 times more food than those from the south, they were also 1.8 - 2.2 times more efficient at processing it.

The selective pressure for CnGV is thought to be size at the end of the first growing season (Conover, 1992). There are many cases where size-selective winter mortality occurs in fish (Adams *et al.*, 1982; Conover and Ross, 1983; Henderson *et al.*, 1988; Shuter and Post, 1990; Conover, 1992 and references therein). Therefore, small size at the end of the first growing season is selected against. However, why all populations do not evolve the maximum growth rate possible for a species, and thus obtain the maximum size, is not fully understood (Conover and Schultz, 1995).

1.5 Significance of local adaptation and countergradient variation

Local adaptation and CnGV are situations where similar phenotypes may have quite different genotypes (Conover and Schultz, 1995). This is important because it is virtually undetectable in the field. Therefore, much genetic variation probably goes unnoticed, and populations that seem similar may actually be genetically different. Local adaptation and CnGV are good examples of the evolutionary adaptiveness of growth. They also suggest how a selective pressure can alter a physiological process.

These phenomena are examples of how populations of organisms can have specific adaptations for an environment. If that population is removed from the area, reintroductions from another population of the same species may not be successful. This point is important for the introduction of foreign populations for recreational fisheries (see Phillipp and Whitt, 1991).

Local adaptation and CnGV may also indicate that climate change (i.e., global

warming) may have different effects on populations of the same species. For example, if winters along the eastern seaboard of North America were shorter, northern Atlantic silversides would obtain larger sizes at one year of age than their southern counterparts.

There is also a significance of LA/CnGV to aquaculture. Traditionally, fish farmers would select a 'strain' of fish that grows best in the wild. By taking broodstock from this population the farmer would hope to obtain best possible growth rates for his/her animals. If the species exhibited CnGV, the farmer would benefit from taking a different strain (possibly the slowest growing one in the wild). This strain may outperform others in terms of growth rates, food conversion efficiency, and size at maturity when reared at the same conditions. The fast-growing strain in the wild may result solely from increased temperature (or longer growing season). For example, Williamson and Carmichael (1990) showed that northern *Micropterus salmoides* grew faster, resisted net stress better, tolerated ammonia better, ate pellet food more readily, tolerated low temperatures and low oxygen levels better, and had better food conversion efficiency than a southern strain. However, locally adapted populations may be the only ones that are capable of surviving in certain environments.

This thesis investigated the effect of different temperatures on growth rates, food conversion efficiencies, and energy allocation in different cod stocks. Common environment experiments were used to estimate the relative contributions of environmental and genetic effects towards observed population differences in wild northwest Atlantic cod *(Gadus morhua* L.).

Chapter two

Growth of larval Atlantic cod (*Gadus morhua* L.) from the Grand Banks and Gulf of Maine under common laboratory environments

2.1 Introduction

Many species of fishes exhibit geographic variation in growth rates (Boehlert and Kappenman, 1980; Shepherd and Grimes, 1983; Conover and Present, 1990; Delabbio *et al.*, 1990; Torrissen *et al.*, 1993). This is often caused by variation in environmental factors, such as food supply and temperature. Temperature is the primary environmental influence on poikilothermic organisms (Prosser, 1986), and often varies in a geographic pattern. Studies involving temperature can be particularly useful when examining geographic variation in growth rates.

In addition to environmental effects on growth, different populations of a species may have different genetic capacities for growth rates. In some species of invertebrates, growth rate is adapted to local conditions (Ament, 1979; Levinton, 1983; Lonsdale and Levinton, 1985). Here, the temperature (or presumably any environmental variable) at which the maximum growth rate occurs differs among populations. As a result, each population grows fastest under conditions most commonly encountered in its natural environment. This is easily illustrated in reciprocal transplant experiments, where one population is placed in the environment of another (Niewianowski and Roosenburg, 1993).

Where local adaptation (LA) occurs, each population grows best in its own environment.

Another pattern of geographic variation in the genetic capacity for growth rate is countergradient variation (CnGV) (Phillipp and Whitt, 1991; Conover and Schultz, 1995; Schultz *et al.*, 1996). Here, the capacity for growth rate varies with latitude in a countergradient manner. Whereas the temperature of maximum growth rate differs in LA, in CnGV the maximum growth rate itself is different among populations (Conover and Present, 1990). As a result, under common conditions, higher latitude populations always have faster growth rates than those at lower latitudes. Countergradient variation is believed to be an adaptation to a shorter growing season at higher latitudes, unlike LA, which is an adaptation to temperature (Conover and Schultz, 1995).

Atlantic cod (*Gadus morhua* L.) are found in the western north Atlantic, from Baffin Island (~ 63° N) to Cape Hatteras (~ 35° N; Scott and Scott, 1988). A large temperature gradient occurs over this area, resulting from a cold current (Labrador Current) flowing south, and a warm current (Gulf Stream) flowing north (Drinkwater, 1996). This results in both warmer overall mean temperatures, and a longer growing season in the south. A large amount of variation exists in growth of cod throughout this area, generally with faster growth occurring in warmer water (Brander, 1994; Brander, 1995; Krohn *et al.*, 1997; Shackell *et al.*, 1997). In addition to temperature, food abundance, population density, and other factors likely play a role in determining growth rates of cod.

As with any species, genetic differences in the capacity for growth rate in cod are

difficult, even if possible, to detect from field studies. This is because growth rate is a result of the interaction between genotype and environment. Common environment and reciprocal transplant experiments eliminate environmental variability, and are used to infer a genetic contribution to observed growth rates. There have been several common environment experiments conducted on different populations of cod (Gamble and Houde, 1984; Godø and Moksness, 1987; Blom *et al.*, 1994; van der Meeren *et al.*, 1994; Svåsand *et al.*, 1996; Hunt von Herbing *et al.*, 1996; Puvanendran and Brown, 1998). However, to my knowledge only two studies (Hunt von Herbing *et al.*, 1996; Puvanendran and Brown, 1998) have examined growth of northwest Atlantic cod under common environments.

Hunt von Herbing *et al.* (1996) compared larval cod originating from the east coast of Newfoundland, and the Scotian Shelf (off Nova Scotia). They found that Newfoundland cod grew faster than Nova Scotia cod at 5 °C and 10 °C. However, their study was focused on physiological development, and they did not suggest that their results were an indication of countergradient variation in growth rate. In addition, the experiments were conducted at different times of the year, using wild zooplankton as prey. Therefore, the results may have been confounded by different diets. Similarly, the study by Puvanendran and Brown (1998), was not designed to specifically test for population differences. Both studies compared a fall-winter spawning stock to a spring-summer spawning stock, which may compromise latitudinal comparisons.

Fish experience more dramatic changes in their biology during the larval stage than at any other time in their life cycle. Most marine fish larvae are poorly developed upon

hatching, after which substantial morphological, physiological, behaviourial, and corresponding ecological changes occur. Larval fish growth is influenced by many variables, including the development of the digestive system, prey type, and feeding behavior (Blaxter, 1986; Noakes and Godin, 1988). Selective pressures for LA and CnGV are thought to be potential disadvantages of small size (Conover, 1992), and therefore faster growing fish have increased fitness. Specific growth rates of larval fish are higher than in juveniles and adults. Therefore, one would expect that evolved differences in growth rates would be most pronounced in the larval stage. Thus, intrinsic differences in the capacity for growth rate would likely be easiest to detect in this stage.

This study compared growth rates of larval cod from two populations under identical conditions, at two temperatures. The objective was to determine if there are intrinsic differences in the capacity for growth rate. If both populations have similar growth rates under identical conditions, it would suggest that no intrinsic differences in growth capacity exist, and the variation observed in the wild would be likely due to environmental factors. It is hypothesized that CnGV in growth rate exists in Northwest Atlantic cod, and therefore I predict that the more northern of the two populations will have faster growth rates than the southern population at all temperatures.

2.2 Materials and methods

2.2.1 Collection of gametes and egg incubation

Cod oocytes and sperm were stripped from spawning adults at sea. Collections

were made from the Grand Banks (GB, 45° N, 55° W) in the Northwest Atlantic Fisheries Organization's (NAFO) division 3Ps, and the Gulf of Maine (GOM, 42° N, 70° W) in NAFO division 5Y (Figure 2.1), two distinct populations (Brander, 1995). The GB sample was collected on April 28/98 and consisted of gametes from two females and seven males. Oocytes from the first female were fertilized with milt from four males, and the other three males were used to fertilize oocytes from the second female. Fertilized embryos were pooled a short time later. The GOM sample was collected on June 28/98 and consisted of gametes from three females and nine males. Gametes from all 12 individuals were mixed together.

Embryos were brought to Memorial University of Newfoundland's Ocean Science Centre, near St. John's. Embryos from both populations arrived before formation of the blastula. Incubation of embryos was conducted in 300 litre conical tanks at a temperature of 8 ± 1 °C standard deviation.

2.2.2 Experimental setup

The following protocols were used for both populations. Larvae were placed under experimental conditions at 100% hatch. Triplicate 30 litre glass aquaria (black) were set up at 7 °C and 12 °C, for experimental tanks, and temperature was controlled using Neslab units. Stocking density was 40 larvae per litre of water (1200 per tank). Each aquarium (flow through design) was provided with filtered seawater at a rate of 50 ml per minute. Light intensity was 1500 lux at the water's surface. Lights were on 24

hours per day.

Larvae were fed cultured rotifers (*Branchionus plicatilis*) at a prey density of 4000 per litre. Light aeration ensured prey were equally distributed throughout the tanks. Twice daily, three 5 ml aliquots were taken from each tank to determine prey concentration. Prey were then adjusted to the desired density. At day 21, *Artemia fransisco* nauplii were added to all tanks. A mixture of rotifers and *Artemia* (total prey density 4000/litre) were supplied to the larvae for one week thereafter. Subsequently, only *Artemia* were given and densities were adjusted as for rotifers. This feeding regime resulted in food always being present in the tanks. It was based on previous growth experiments on larval cod (Puvanendran and Brown, 1999).

2.2.3 Sampling

Ten larvae were arbitrarily chosen from each tank at the start of the experiment (30 per treatment), and five (15 per treatment) weekly thereafter, up to six weeks post-hatch. Each larva was killed using MS-222, placed on a depression slide, videotaped under a dissecting microscope, and discarded. Larval images were analyzed using an image analysis system. Total length (TL), myotome height (MH, at anus), and eye diameter (ED) were recorded to the nearest 0.1 mm for each larva. On week six, total length (TL; 0.1 mm) of larvae was measured using a dissecting microscope. These larvae were then placed on separate pre-weighed pieces of aluminum foil for determination of dry weight (DW, described below), MH and ED were not recorded.

Dry weights were obtained by placing larvae (rinsed with distilled water) on pre-weighed foils in a 60 °C oven for 24 hours. Dried larvae and foils were weighed to the nearest microgram. Original foil weight (FW) was then subtracted from larvae + foil weight (LFW) to obtain larval dry weight (DW), using the formula

$$DW = LFW - FW.$$

2.2.4 Data analysis

Relationships between TL / DW, TL / ED, and TL / MH were first compared for differences among treatments (each population at each temperature) using analysis of covariance (ANCOVA), and then were analyzed using linear regression. Condition factors of both populations were calculated at each temperature using the formula:

$$K = (DW * TL^{-3}) * 100.$$

Population comparisons using condition factors can be biased if condition factors are associated with fish length. Therefore, condition factors were first tested for association with length using ANCOVA, before being statistically compared for population and temperature effects in a 2-way analysis of variance (ANOVA).

Comparisons of growth rates between populations and temperatures were carried out by ANCOVA. The growth model contained terms representing the effects of population, temperature, age, and all interactions, with age as the covariate term. An alpha of 0.05 was set as the acceptance level for the test. Each datum in the analysis was the average length of the five (ten at week 0) fish sampled per tank per week, which gave a

sample size of N = 60 (12 tanks * 5 sampling dates, see below). This was a conservative approach, and eliminated any confounding effects of tanks. Gross specific growth rates were calculated for each population at both temperatures from hatch to four weeks of age (see below), using the formula

$$GSGR = ((Ln\ (L_F) - Ln\ (L_I))^{*-28}) * 100$$

where GSGR = gross length specific growth rate, Ln (L_I) = average of the initial ln lengths of fish from each tank, Ln (L_F) = average of the final ln lengths of fish from each tank.

2.3 Results

The relationship of dry weight to total length was Ln DW = -8.156 + 3.502 Ln TL, $r^2 = 0.94$ (Figure 2.2). Conditions factors did not significantly differ among populations at either temperature (ANOVA; $F_{1,51} = 0.09$, $p = 0.771$). However, condition factors were significantly higher for both populations at 12 °C than 7 °C (ANOVA; $F_{1,51} = 15.80$, $p < 0.001$; Figure 2.3). ANOVA comparisons of condition factor and total length of larvae showed no significant relationship ($p > 0.05$ for both populations) at either temperature.

The slopes of the relationship of eye diameter to total length were not significantly different between GB and GOM larvae at either temperature (ANCOVA; $F_{1,289} = 0.81$, $p = 0.368$), the regression equation being ED = -0.183 + 0.097 TL, $r^2 = 0.96$ (Figure 2.4a). There was a significant interaction between GB and GOM larvae and temperature for the relationship between MH and TL, indicating different slopes. The regression analysis was then carried out at each temperature. At 12 °C no significant difference in slopes of GB

and GOM larvae was found (ANCOVA; $F_{1,155} = 2.29$, $p < 0.132$), and the regression equation was MH = -0.507 + 0.152 TL, $r^2 = 95.9$. There was a significant difference in slopes of MH and TL relationships between the two populations at 7 °C (ANCOVA; $F_{1,131} = 21.95$, $p < 0.001$). The regression equation for GB larvae was MH = -0.392 + 0.129 TL, $r^2 = 94.9$, while that of GOM larvae was MH = -0.128 + 0.0862 TL, $r^2 = 81.2$ (Figure 2.4b). The yolk-sac, representing the source of endogenous energy, was absorbed by all fish at one week of age.

Growth rates were compared from hatch (week 0) to week four. Survival to week four was similar for all treatments and averaged 13%. Data from week four to week six was not included in the growth rate analysis because larvae had begun metamorphosis at this time. This likely resulted in increased mortality, which led to different stocking densities among tanks. In the ANCOVA model, the interaction terms; population-temperature, and population-temperature-age were not significant ($p > 0.05$) and hence were subsequently removed from the analysis. Total length increased with age at both temperatures (Figure 2.5), although fish at 12 °C grew significantly faster than those at 7 °C (ANCOVA: $F_{1,54} = 218.60$, $p < 0.001$; Figure 2.6). Grand Banks cod had significantly faster growth rates than GOM cod at 7 °C and 12 °C (ANCOVA: $F_{1,54} = 25.83$, $p < 0.001$; Figures 2.5 and 2.6).

Figure 2.1: Northwest Atlantic Ocean, showing approximate northern and southern limits of the distribution of Atlantic cod, and location of collection sites Grand Banks (GB) and Gulf of Maine (GOM).

Figure 2.2: Relation of Ln dry weight to Ln total length of Grand Banks (GB) and Gulf of Maine (GOM) cod (at six weeks post hatch), reared under identical laboratory conditions at 7 °C and 12 °C. Each symbol represents one cod larva.

Figure 2.3: Condition factor of Grand Banks (GB) and Gulf of Maine (GOM) larval cod (at six weeks post hatch). Reared under identical laboratory conditions from hatch at 7 °C and 12 °C. Each bar is the mean of the tank means, (n = 3 per sample), error bars = standard error.

Figure 2.4: Relation of (a) eye diameter, (b) myotome height at anus, to total length of larval cod from the Grand Banks (GB) and Gulf of Maine (GOM). Reared under identical laboratory conditions from hatch to five weeks of age at 7 °C and 12 °C. Each symbol represents one fish.

Figure 2.5: Total length (mm) at age (weeks post-hatch) of Grand Banks (GB) and Gulf of Maine (GOM) cod, reared under identical laboratory conditions at 7 °C and 12 °C. N = 3 (tank averages) per sample, vertical bars = standard error.

Figure 2.6: Gross length specific growth rate (% length increase per day) from hatch to four weeks of age of Grand Banks (GB) and Gulf of Maine (GOM) cod, reared under identical laboratory conditions at 7 °C and 12 °C. N = 3 (tank averages) per sample, error bars = standard error.

2.4 Discussion

The significantly higher condition factors of larvae at 12 °C indicate that fish from both populations were heavier at length at 12 °C than those at 7 °C. Myotome height was used as a means of measuring body depth. Similarity of slopes at 12 °C indicates that body depth increased with length in an isometric relationship for the two populations. However, body depth of GB larvae increased to a greater extent with length than GOM larvae at 7 °C. Eye diameter increase with length was isometric for both populations.

Larval cod from both populations grew faster at 12 °C than 7 °C. Faster growth at higher temperatures is well documented for larval and post-larval cod (e.g., Campana and Hurley, 1989; Brander, 1994; Brander, 1995; Hunt von Herbing *et al.*, 1996; Krohn *et al.*, 1997; Shackell *et al.*, 1997). Brander (1994) reported that over the first four years of life of cod, each 1 °C increase in environmental temperature results in a 29% increase in size.

In the wild, GOM cod grow faster than those on the GB (Campana *et al.*, 1995). Brander (1994) reported that the average weight of four year old cod on Georges Bank (near the Gulf of Maine) is 3.47 kg, while on the southern Grand Banks it is 0.85 kg. The faster growth rate of GB cod in this study may have been due to increased food consumption, increased food conversion efficiency, or both. Present and Conover (1992) found that the increased growth of northern populations of larval Atlantic silversides (*Menidia menidia*) was due to the fact that they ate more food, and were more efficient at processing it. Better food conversion efficiency in northern populations was also found by Nicieza *et al.* (1994a) in Atlantic salmon (*Salmo salar*).

It is premature to conclude that the increased growth rate observed in GB cod is genetic in origin. Gametes were collected from wild adults and parental effects (e.g., maternal investment) may have been present. Grand Banks larvae were larger at hatch than GOM larvae, which may have been a result of different egg sizes. Unfortunately, this comparison was not made as egg diameters were not obtained. Kjesbu (1989) reported that a single female cod may have eggs of different sizes from successive batches, throughout a single spawning season. Therefore, one must be careful when relying on sizes of eggs to suggest individual variations in maternal investment. Parental effects were unlikely to be significant in this study, as growth was compared well beyond the absorption of the yolk-sac, a maternal influence.

Rearing animals for successive generations under laboratory conditions is the best method to quantify the genetic origin of growth rates. Any differences in parental investment due to environmental variation would be "bred out" (Garland and Adolph, 1981). This is not feasible to do with cod, due to the relatively long life cycle. However, collecting gametes from wild caught adults is an acceptable method for conducting common environment experiments (Battaglia, 1957; Antonovics *et al.*, 1971; Lonsdale and Levinton, 1985; Garland and Adolph, 1991; Conover *et al.*, 1997). In addition, due to the small sample size of adults in this study, it is not known if the samples taken accurately represent the whole population. There were however, seven possible crossings in the GB sample, and 27 in the GOM sample, which should have provided a reasonable genetic mix.

The results of this study show that the faster growth in the wild of the more

southerly population (GOM) is likely due to environmental variability between the two areas. The GOM experiences higher yearly average temperatures (50m, 6.4 °C) than the GB (50m, 1.8 °C; deYoung *et al.*, 1994), which likely causes the difference in growth rate. In fact, the negative effect that colder temperatures on the GB have on growth of larval cod is reduced by their capacity for faster growth rates. Although GB larvae grew faster than GOM larvae at both temperatures, this difference was greatest at 7 °C. This may indicate that GB cod are better adapted to cold water temperatures. Other studies also suggest greater capacities for growth rate from higher latitudes (or colder environments) in adult (Brander, 1995), juvenile (Suthers and Sundby, 1996), and larval cod (Hunt von Herbing *et al.*, 1996).

Faster growth rates in northern populations have been found in other species of fish: arctic char, *Salvelinus alpinus* (DeLabbio *et al.*, 1990); largemouth bass, *Micropterus salmoides* (Williamson and Carmichael, 1990; Phillipp and Whitt, 1991); Atlantic salmon, *Salmo salar* (Nicieza *et al.*, 1994b); mummichog, *Fundulus heteroclitus* (Schultz *et al.*, 1996); and striped bass, *Morone saxatilis* (Conover *et al.*, 1997). The phenomenon has been termed countergradient variation, and is believed to be an adaptation to a shorter growing season (Conover and Present, 1990).

Countergradient variation results in higher latitude populations growing faster than those at lower latitudes, when reared at the same temperature. Although growth rate of the more northerly population of Atlantic cod was significantly faster in this study, the substantial differences in growth rate reported by Conover and Present (1990) in Atlantic

silversides were not present. The two populations used in this study represent areas near the southerly limit of cod distribution (GOM), and one near the middle (GB). An unsuccessful attempt was made to collect gametes from cod near the northern limit of their distribution. If countergradient variation is present throughout the distribution of cod in the northwest Atlantic, the greatest differences in growth rate would be seen by comparing populations from both extremes of the distribution. This is an area open to future research.

The results of this study support the findings of Hunt von Herbing *et al.* (1996), where larval Newfoundland cod grew faster than those from Nova Scotia. Faster growth of Newfoundland cod was correlated with a more developed intestinal tract than cod from Nova Scotia cod, and this may have resulted in the faster growth rates. In both studies, the northern population outgrew the southern, suggesting that northwest Atlantic cod larvae likely exhibit CnGV in growth rate. This may be an adaptation to the colder annual water temperatures, and shorter growing seasons in northern areas.

In recent years, many cod stocks in the northwest Atlantic have been severely depleted, with the largest decline taking place off northeastern Newfoundland. If in fact the observed growth rates in this study are genetic in origin, and are representative of the whole population, it could have significant implications for the recovery of cod stocks. The capacity for faster growth rates in cod from northern populations may indicate that cod from southern areas would not be able to rebuild stocks to the north. For example, larvae from southern populations might reach a smaller size at the end of the first growing

season in northern environments. Size - selective winter mortality has been reported in many fish species (Conover and Ross, 1983; Henderson *et al.*, 1988; Schuter and Post, 1990; Conover, 1992). If this occurs in young-of-the year cod, offspring of cod from southerly areas may be less likely to survive in areas farther north, as they might be smaller in size.

The capacity for faster growth in the northern population is also significant for the development of cod aquaculture. Traditionally, one would choose a stock with the fastest growth rates to culture. However, if countergradient variation is present, northern populations (possibly slowest growing in the wild) may be the best suited for domestication (Williamson and Carmichael, 1990).

If the selective pressure for CnGV in growth rate of fishes is size - selective winter mortality, it is expected that this would be greatest in the earliest life history stages. Therefore, examination of growth of juvenile cod from different populations is necessary before this phenomenon can be adequately addressed in this species. This is the focus of Chapter three in this thesis.

The results of my study seem to indicate that environmental factors in the northwest Atlantic which likely result in slower growth of larval cod in northern areas, have also resulted in an adaption for a higher capacity for growth in northern cod. There is mounting evidence that CnGV in growth rate is a common phenomenon in larval fish. This is somewhat surprising in species such as Atlantic cod, where eggs and larvae drift freely in the water column and limits on gene flow would be expected to be low. As more

common environment experiments are conducted, occurrences of CnGV or other patterns in geographic variation could indicate that more genetic variation among fish populations may exist than what was previously believed.

Chapter Three

Effect of temperature on growth and energy allocation in four populations of juvenile Atlantic cod (*Gadus morhua* L.)

3.1 Introduction

Fish from different populations often vary in morphology, growth rates, age and size at maturity, and spawning seasons (Colby and Nepszy, 1981; Templeman, 1981; Beacham, 1983; Taylor, 1991; Brander, 1994). Environmental variability is usually assumed to be the main factor contributing to these differences. Factors such as temperature, food quantity, food quality, inter and intra-specific competition, etc., may influence fish life histories. However, in field situations it is difficult to determine which factors have the greatest effects. Furthermore, genetic-based differences are virtually impossible to detect (Garland and Adolph, 1991; Conover and Schultz, 1995).

Common environment and reciprocal transplant experiments can be used to estimate the relative contribution of environmental and genetic factors towards different life history traits (Garland and Adolph, 1991; Conover *et al.*, 1997). These experiments are designed to eliminate the variability in environmental influences on phenotypic expression, and thus make inferences about genotypic differences. However, no experimental design is perfect. This is due to past history, parental contributions to observed variance, and laboratory selection on different genotypes (Garland and Adolph, 1991). Thus, results

obtained from such studies must be interpreted with caution.

Experiments on amphibians have shown that much of the observed geographic variation in these animals may be due to environmental differences (Berven, 1982; Riha and Berven, 1991). For example, Berven (1982) showed that larval development of wood frogs (*Rana sylvatica*) from high and low elevations was largely due to temperature. In such circumstances, reciprocally transplanted animals develop life history traits similar to those of individuals in their transplanted environment. In many cases, geographic variation in fishes is likely to be primarily due to environmental variability. However, common environment studies of fish have demonstrated genetic differences in the capacity for growth rate (Conover and Present, 1990; Delabbio *et al.*, 1990; Schultz *et al.*, 1996), food conversion efficiency (Williamson and Carmichael, 1990, Present and Conover, 1992; Nicieza *et al.*, 1994a), low oxygen tolerance (Williamson and Carmichael, 1990), and morphology (Robinson and Wilson, 1996).

Atlantic cod (*Gadus morhua* L.) are an ecologically and commercially important groundfish in the northwest Atlantic, and have been extensively studied throughout this century. Growth rate and age at maturity varies widely among cod stocks. Generally, growth rates are higher and age at maturity is lower in warmer water (May *et al.*, 1965; Loeng, 1989; Brander, 1995). For example, the average weight of a four year old cod off Labrador (average temperature 2 °C) is 0.6 kg, whereas a cod of the same age in the Celtic sea (average temperature 11 °C) is 7.3 kg (Brander, 1994).

Cod range from Baffin Island (~ 63° N) to Cape Hatteras (~ 35° N) in the

northwest Atlantic (Scott and Scott, 1988). Water temperatures in this area are influenced by two currents. The Gulf Stream is a warm current that flows north from the Gulf of Mexico before crossing the Atlantic Ocean south of Newfoundland. In contrast, the cold Labrador current flows south along the Labrador and Newfoundland coast before moving offshore to mix with the Gulf Stream (Drinkwater, 1996). As a result, cod near the southern end of their distribution may experience much warmer water temperatures, and longer growing seasons than those to the north. In addition, these currents may result in substantial temperature differences occurring over small geographical distances, such as the northeast and south coast of Newfoundland (Narayanan *et al.*, 1996).

Most of the life history variation in cod has been attributed to environmental variability such as temperatures differences. In recent years however, researchers have found genetic differences among cod stocks, and gene flow may be more limited than previously believed (Bentzen *et al.*, 1996; Ruzzante *et al.*, 1996). Few studies have addressed the relative contribution of genotypic and environmental influences on life history traits in cod. To my knowledge, there have only been two studies (in addition to Chapter Two) which have compared growth rates of different populations of larval cod from the northwest Atlantic under common environments (Hunt von Herbing *et al.*, 1996; Puvanendran and Brown, 1998). I am aware of no study that has examined juvenile cod from this area. Knowledge of the contribution of environmental and genetic influences on life history traits in cod could be helpful in identifying stocks, improving year class prediction models, estimating the effects of climate change, and selecting superior stocks

for aquaculture.

This study examines the contribution of temperature towards growth rates and energy allocation (towards energy reserves) in young of the year juvenile cod from four northwest Atlantic populations. Based on previous work with larval cod (Hunt von Herbing *et al.*, 1996, Chapter Two), it is hypothesized that the capacity for growth rates and food conversion efficiency increase with increasing latitudes.

3.2 Materials and Methods

3.2.1 Collection of fish

Juvenile cod for this study were obtained by two methods. The first group was raised from eggs in the laboratory (LAB). Gametes were obtained from two different populations of cod by stripping spawning adults at sea. Collections were made on the Grand Banks (GB; 46° N, 55° W), in the North Atlantic Fisheries Organizations (NAFO) division 3Ps, and in the Gulf of Maine (GOM; 42° N, 70° W), in the NAFO division 5Y, as described in Chapter Two (Figure 3.1). Incubation and early larval rearing was conducted in 300 litre conical tanks using standard protocols for the rearing of cod (Puvanendran and Brown, 1999). Young juveniles were transferred to 3000 litre tanks, where they were weaned onto food pellets, and kept until transfer to the experimental setup.

Juvenile cod from two other populations were collected from two inshore bays on the island of Newfoundland (WILD), using a beach seine. The first sample was collected

in the NAFO division 3L from Newman Sound (48° N, 53° W) in Bonavista Bay (BB), on November 15/98. The other sample was taken in the NAFO division 3Ps from Connaigre Bay (47° N, 55° W) in Fortune Bay (FB), on November 24/98 (Figure 3.1).

Upon arrival at the Ocean Sciences Centre facilities in Logy Bay, Newfoundland (47° 35' 20" N, 52° 40' 55" W; O.S.C.), a sub-sample of the wild cod from each collection site was taken for determination of total length (cm), wet weight (g), Fulton's condition factor, and hepatosomatic index, as a means of comparing initial size and nutritional status (see procedure below). All fish were weaned onto pellet food (same diet as hatchery reared fish) and acclimated to the experimental setup for more than two weeks. The LAB reared fish were also transferred to the experimental setup at this time.

3.2.2 Experimental setup

All fish were kept in two rectangular 2000 litre raceways for the experiments. Each raceway was divided into 10 areas using framed netting. These areas served as experimental "tanks", each of which was supplied with aeration and included 10 fish from a single population. Three tanks in each raceway contained cod from the GOM (n =30) and GB (n=30), while two tanks contained cod from BB (n=20) and FB (n=20), (N =200; Appendix One).

In Experiment I, raceway #1 received filtered heated seawater while raceway #2 received filtered unheated seawater (Ambient), which was approximately 1 - 2 °C above ambient temperatures at the O.S.C. In Experiment II, both raceways received water of the

same temperature (~ 6 °C), intermediate to those in Experiment I. During the time between the two experiments, temperatures were gradually brought together (increased in ambient group, decreased in heated group). Temperatures and dissolved oxygen were monitored throughout both experiments (see Figure 3.2 for temperature profiles) and did not differ among tanks in each raceway. Lighting was provided by flourescent tubes, and photoperiod was adjusted fortnightly to approximate day-lengths at 44 °N (intermediate latitude for all populations). Twilight was provided using an incandescent bulb which came on ½ hour before the main lights and remained on ½ hour after the main lights. Light intensity was measured at the water's surface, and was 1500 lux under full lighting, and two lux during twilight.

All fish were fed pellet food to satiation daily, and the amount eaten by each tank of fish was recorded. The average weight of food eaten by each fish per tank was used for determination of gross food conversion efficiency, which was calculated using gross food conversion ratio (GFCR):

$$GFCR = W_{FE} * W_G^{-1},$$

where W_{FE} is mean weight of food eaten, and W_G is mean weight gain of fish.

3.2.3 Sampling

Sampling of cod was done at the start of the Experiment I (Dec. 09/98), at week 5, and week 14. All fish were anaesthetized using 2-phenoxy ethanol (0.125ml / litre of water), and measured for total length (L_T, to the nearest 0.1 cm), and wet weight (W_W, ±

0.01 g). For Experiment II, this was done at the start of the experiment (March 30/99), at week 4, and at week 8. Growth was plotted as L_T and W_W increase over time. Gross specific growth rates (Busacker *et al.*, 1990) were calculated based on the increase in average fish weight per tank using the formula:

$$GSGR = (\ln W_F - \ln W_I)^{\cdot Time} * 100,$$

where W_F = mean final ln fish weight, W_I is mean initial ln fish weight, and Time is time in days.

In addition to an initial sample, at the end of the Experiment I, a sample of fish from each experimental tank were killed using 2-phenoxy ethanol. Condition was calculated using Fulton's condition factor (K; Ricker, 1975):

$$K = (W_W * L_T^{-3}) * 100,$$

where W_W is wet weight (grams), and L_T is total length (cm). Tissue water content was determined using a standardized sample of epaxial muscle (striated white muscle in dorsal muscle mass) and the whole liver. The tissues were first weighed wet ($T_W \pm 0.01$ g), and then dried to constant weight in a 60 °C oven, for determination of dry weight (T_D, $\pm$ 0.001 g). Tissue water content (liver = % W_L, muscle = % W_M) and hepatosomatic index (H.S.I.) was calculated respectively as:

$$\% \text{ Water} = 100 - ((T_D * T_W^{-1}) * 100),$$

and

$$H.S.I. = (L_W * W_W^{-1}) * 100,$$

where L_W = liver weight. The same procedure was done on all surviving fish at the end of

Experiment II. Condition factor, H.S.I., % W_L, and % W_M were all used as measures of nutritional status (see discussion).

3.2.4 Data analysis

3.2.4.1 Comparisons

Comparisons were made between fish of LAB-WILD origin, and between GB-GOM and FB-BB populations. Although both WILD populations originated from higher latitudes than the LAB fish, latitudinal comparisons of cod from a WILD population were not made with cod from a LAB population, due the possibility of WILD/LAB origin being a confounding factor. An alpha of 0.05 was set as the significance level of all tests.

3.2.4.2 Initial samples

Samples of FB and BB cod taken at time of collection, and initial measures of K, H.S.I., % W_L, and % W_M at the start of Experiment I were analysed using t-test. Measures of nutritional status were tested for association with L_T of fish using analysis of covariance (ANCOVA; see below).

3.2.4.3 Experiment I and Experiment II

Comparisons of GSGR, GFCR K, H.S.I., % W_L, and % W_M were made between LAB-WILD cod, GB-GOM cod, and FB-BB cod. Gross SGR and GFCR were analysed using 3-way analysis of variance (ANOVA). The variables in the model were origin,

temperature, sampling period, and all interaction terms. Condition factor, H.S.I., % W_L, and % W_M were analysed in a 2-way hierarchical ANOVA. Tank effects were nested in the design, but were removed if found not to be significant. The variables in these models were origin, temperature, and origin-temperature interaction. Transformation and randomization were not required to meet the assumptions of the tests, as residuals were found to be homogeneous and normal in distribution. Graphical representations of nutritional indices are based on the mean of the replicate (tank) means for each treatment. Means, standard errors, and range of the treatment means are presented in Appendix B.

Due to possible bias in population comparisons, if significant relationships (at alpha 0.05) between measured indices of nutritional status and length of the fish were found, data were standardized using the procedure outlined by Widdows (1985). Data were first log transformed and the regression equations between the measured nutritional index and T_L for each population, at each temperature was calculated. The slopes of these regression equations were then used in the following standardization equation:

$$\log_{10} Yc = \log_{10} Yo - (b*\log_{10} L_T - b*\log_{10} XL_T),$$

where Yc is the corrected nutritional index value for the mean total length of all fish (XL_T), b is the slope of the regression line, and Yo and L_T are the individuals measured index value and total length respectively. Corrected values were converted back to linear scale, and statistically compared using the analyses described above.

3.3 Results

3.3.1. Initial samples

The sub-sample of the wild fish collected in November 1998 from FB and BB showed no significant differences in L_T ($t_{19} = 0.97$, $p = 0.34$), W_W ($t_{21} = 0.77$, $p = 0.45$), or K ($t_{23} = -1.09$, $p = 0.29$) between the two populations. However, BB cod had significantly higher H.S.I. than FB cod ($t_{33} = 4.84$, $p < 0.001$; Figure 3.3).

Samples were taken of the four populations at the start of Experiment I to determine initial nutritional status (Figure 3.4). Analysis of K, H.S.I., % W_L, and % W_M showed no significant relationship ($p > 0.05$) with L_T for any of the populations. Laboratory reared fish (GB and GOM) had significantly higher K ($t_{20} = -3.99$, $p < 0.001$), and H.S.I. ($t_{18} = -6.58$, $p < 0.001$), and significantly lower % W_L ($t_{19} = 7.69$, $p < 0.001$), and % W_M ($t_9 = 6.83$, $p < 0.001$) than wild caught fish (FB and BB). Bonavista Bay cod had significantly higher K ($t_{11} = -2.42$, $p = 0.034$), and % W_M ($t_{10} = -2.60$, $p = 0.026$) than FB cod, but there were no significant differences in H.S.I. ($t_{13} = -1.95$, $p = 0.073$), or % W_L ($t_{13} = -0.45$, $p = 0.66$) between the two populations. Gulf of Maine cod did not have significantly different K ($t_1 = 2.28$, $p = 0.26$), or H.S.I. ($t_1 = 0.28$, $p = 0.83$) than GB cod (Figure 3.4). Due to limited samples sizes, % W_L and % W_M could not be statistically compared for GB and GOM cod at the start of the experiment.

3.3.2 Experiment I

Gross specific growth rate, gross food conversion ratio

In Experiment I, different populations of cod were kept at heated and ambient water temperatures for 14 weeks. Mortality was low in all groups (< 5 % total) and populations were not statistically compared. Total length and W_w increased in all treatments (each population at each temperature) during the experiment (Figure 3.5). Gross SGR of LAB and WILD cod was significantly higher, and GFCR significantly lower, at heated temperatures than ambient temperatures (GSGR; ANOVA, $F_{1,48} = 729.95$, $p < 0.001$; Figure 3.6; GFCR, ANOVA, $F_{1,48} = 227.67$, $p < 0.001$; Figure 3.7). The interaction terms, sampling period*origin and sampling period*temperature were significant for LAB-WILD comparisons. Therefore the models were broken down by temperature and sampling period, and comparisons were analysed using 1-way ANOVA. Juvenile cod of WILD origin grew significantly faster than those of LAB origin during the period between weeks 5 to 14, and 0 to 14 under heated water, but there was no significant difference from week 0 to 5, or during any time period under ambient water temperatures (Table 3.1). In contrast, GFCR of WILD cod was significantly higher than LAB cod from week 0 to 5 under heated temperatures, and all sampling periods under ambient water temperatures (Table 3.2).

Gross specific growth rates were not significantly different for GB-GOM cod at either temperature (ANOVA; $F_{1,24} = 1.72$, $p = 0.202$), but GOM cod had significantly higher GFCR than GB cod at both temperatures (ANOVA; $F_{1,24} = 12.54$, $p = 0.002$;

Figure 3.6). There was a significant interaction effect on GSGR and GFCR between FB-BB origin and sampling period. The models were subsequently broken down and comparisons were analysed using 2-Way ANOVA at each sampling period. Bonavista Bay cod grew significantly faster than those from FB from week 0 to 5, but there was no significant difference from week 5 to 14, or week 0 to 14, or for GFCR at either temperature (Table 3.3).

Condition factor

The variables K, H.S.I., % W_L, and % W_M at the end of Experiment I (Figure 3.8) were significantly ($p < 0.05$) associated with total length, and the data were standardized as described in the Material and Methods section. Slopes used in the standardization equations are presented in Appendix C. Condition factor was not significantly different for juvenile cod held under heated and ambient water temperatures. There was a significant interaction on K between the variables LAB-WILD origin and temperature, and the model was therefore broken down. No significant difference between LAB-WILD cod was found at heated temperatures, but LAB cod had significantly higher K than WILD cod at ambient temperatures. Although plots of the mean of tank means suggest similar K for GB-GOM cod (Figure 3.9a), hierarchical ANOVA showed that Gulf of Maine cod had significantly higher K than GB cod at both temperatures (Table 3.4). A significant interaction between FB-BB fish comparisons and temperature was found, and the two populations were compared at each temperature. No significant difference in K of FB-BB

fish was present at heated temperatures, but BB cod had significantly higher K than FB cod at ambient temperatures (Table 3.4).

Hepatosomatic index

Hepatosomatic index was not significantly different for juvenile cod reared under heated and ambient water temperatures. Lab reared fish however had significantly higher H.S.I. than WILD fish at both temperatures (Table 3.5). There was a significant interaction effect on H.S.I. for both population comparisons and temperature, and the populations were subsequently compared at each temperature. Gulf of Maine cod had significantly higher H.S.I. than GB cod at heated temperatures, but there was no significant difference at ambient temperatures. In contrast, H.S.I. of FB-BB cod was not significantly different at heated temperatures, but BB cod had significantly higher H.S.I. than FB cod at ambient temperatures (Figure 3.9b, Table 3.5).

% liver water content

Juvenile cod reared under ambient water temperatures had significantly higher % W_L than those at heated temperatures. A significant interaction between % W_L comparisons of LAB-WILD origin and temperature was present (Figure 3.9c, Table 3.6). When tested at each temperature, WILD cod had significantly higher % W_L than LAB cod at heated temperatures, and significantly lower % W_L at ambient water temperatures. There was no significant difference in % W_L of GB-GOM cod, or FB-BB cod at either

temperature.

% muscle water content

Muscle water content was not significantly different for cod reared under heated and ambient water temperatures, or of LAB-WILD origin. A significant interaction effect on % W_M was present between GB-GOM cod and temperature (Figure 3.9d, Table 3.7). Cod from the GOM had significantly higher % W_M than those from the GB at heated temperatures, but there was no significant difference under ambient temperatures. There was no significant difference in % W_M of FB-BB cod at either temperature.

Table 3.1: ANOVA results for comparisons of gross specific growth rates of juvenile cod of LAB-WILD origin. Fish were reared at heated and ambient water temperatures for 14 weeks. Where a significant difference was present ($p < 0.05$), the treatment with higher values is indicated.

Tank temperature	Week 0-5	Week 5-14	Week 0-14
Heated	$F_{1,8} = 0.23$, $p = 0.645$	$F_{1,8} = 20.21$, $p < 0.001$, WILD	$F_{1,8} = 40.00$, $p < 0.001$, WILD
Ambient	$F_{1,8} = 3.50$, $p = 0.098$	$F_{1,8} = 0.02$, $p = 0.901$	$F_{1,8} = 0.35$, $p = 0.572$

Table 3.2: ANOVA results for comparisons of gross food conversion ratios for juvenile cod of LAB-WILD origin. Fish were reared at heated and ambient water temperatures for 14 weeks. Where a significant difference was present ($p < 0.05$), the treatment with higher values is indicated.

Tank temperature	Week 0-5	Week 5-14	Week 0-14
Heated	$F_{1,8} = 8.35$, $p = 0.020$, WILD	$F_{1,8} = 1.23$, $p = 0.300$	$F_{1,8} = 0.32$, $p = 0.590$
Ambient	$F_{1,8} = 34.54$, $p < 0.001$, WILD	$F_{1,8} = 8.97$, $p = 0.017$, WILD	$F_{1,8} = 19.72$, $p = 0.002$, WILD

Table 3.3: ANOVA results for comparisons of gross specific growth rates (GSGR) and gross food conversion ratios (GFCR) for juvenile cod from Fortune and Bonavista Bays. Fish were reared at heated and ambient water temperatures for 14 weeks. Where a significant difference was present ($p < 0.05$), the comparison with higher values is indicated.

Response variable	Week 0-5	Week 5-14	Week 0-14
GSGR	$F_{1,4} = 15.00$, $p < 0.001$, BB	$F_{1,4} = 0.11$, $p = 0.756$	$F_{1,4} = 1.60$, $p = 0.275$
GFCR	$F_{1,4} = 1.71$, $p = 0.261$	$F_{1,4} = 0.00$, $p = 0.973$	$F_{1,4} = 0.30$, $p = 0.612$

Table 3.4: Results of ANOVA comparisons for condition factor of LAB-WILD, GB-GOM, and FB-BB juvenile cod, reared for 14 weeks at heated and ambient water temperatures. When a significant interaction ($p < 0.05$) with temperature was present, comparisons were made at each temperature. Where a significant difference was present ($p < 0.05$), the treatment with higher values is indicated.

Comparison	Both temperatures	Heated temperature	Ambient temperature
LAB-WILD		$F_{1,87} = 3.59$, $p = 0.062$	$F_{1,95} = 18.27$, $p < 0.001$, LAB
GB-GOM	$F_{1,108} = 13.96$, $p < 0.001$, GOM		
FB-BB		$F_{1,34} = 1.40$, $p = 0.244$	$F_{1,36} = 12.71$, $p < 0.001$, BB
Temperature	$F_{1,182} = 2.64$, $p = 0.107$		

Table 3.5: Results of ANOVA comparisons for hepatosomatic index of LAB-WILD, GB-GOM, and FB-BB juvenile cod, reared for 14 weeks at heated and ambient water temperatures. When a significant interaction ($p < 0.05$) with temperature was present, comparisons were made at each temperature. Where a significant difference was present ($p < 0.05$), the treatment with higher values is indicated.

Comparison	Both temperatures	Heated temperature	Ambient temperature
LAB-WILD	$F_{1,83} = 4.60$, $p = 0.035$, LAB		
GB-GOM		$F_{1,21} = 16.36$, $p < 0.001$, GOM	$F_{1,26} = 0.61$, $p = 0.441$
FB-BB		$F_{1,12} = 0.14$, $p = 0.711$	$F_{1,18} = 14.58$, $p = 0.001$, BB
Temperature	$F_{1,83} = 1.83$, $p = 0.180$		

Table 3.6: Results of ANOVA comparisons for % liver water content of LAB-WILD, GB-GOM, and FB-BB juvenile cod, reared for 14 weeks at heated and ambient water temperatures. When a significant interaction ($p < 0.05$) with temperature was present, comparisons were made at each temperature. Where a significant difference was present ($p < 0.05$), the treatment with higher values is indicated.

Comparison	Both temperatures	Heated temperature	Ambient temperature
LAB-WILD		$F_{1,37} = 8.36$, $p = 0.006$, WILD	$F_{1,46} = 5.31$, $p = 0.026$, LAB
GB-GOM	$F_{1,43} = 1.50$, p 0.228		
FB-BB	$F_{1,32} = 0.09$, $p = 0.771$		
Temperature	$F_{1,83} = 14.19$, $p < 0.001$, Ambient		

Table 3.7: Results of ANOVA comparisons for % muscle water content of LAB-WILD, GB-GOM, and FB-BB juvenile cod, reared for 14 weeks at heated and ambient water temperatures. When a significant interaction ($p < 0.05$) with temperature was present, comparisons were made at each temperature. Where a significant difference was present ($p < 0.05$), the treatment with higher values is indicated.

Comparison	Both temperatures	Heated temperature	Ambient temperature
LAB-WILD	$F_{1,83} = 0.94$, $p = 0.334$		
GB-GOM		$F_{1,17} = 6.61$, $p = 0.020$, GOM	$F_{1,22} = 0.76$, $p = 0.392$
FB-BB	$F_{1,32} = 0.00$, $p = 0.947$		
Temperature	$F_{1,83} = 0.94$, $p = 0.334$		

Figure 3.1: Northwest Atlantic Ocean, showing approximate northern and southern limits of the distribution of Atlantic cod (*Gadus morhua* L.), and location of collection sites Grand Banks (GB), Gulf of Maine (GOM), Fortune Bay (FB), and Bonavista Bay (BB).

Figure 3.2: Daily temperatures (°C) of heated and ambient raceways used in juvenile cod study from the start of Experiment I (Week 0; Dec 9) to the end of Experiment II (Week 24; May 25). Experiment I ended on Week 14, Experiment II began on Week 16.

Figure 3.3: (a) Total length, (b) wet weight, (c) Conditon factor, and (d) hepatosomatic index of 0-group juvenile cod collected from Fortune Bay (FB) and Bonavista Bay (BB) in November 1998. N = 14 for FB, 40 for BB, shown are median (solid line), mean (dotted line), 25 th and 75 th percentiles (box), 10 th and 90 th percentiles (vertical bars), and 5 th and 95 th percentiles (circles).

Figure 3.4: (a) Fulton's condition factor, (b) hepatosomatic index, (c) % liver water content, and (d) % muscle water content of Grand Banks (GB), Gulf of Maine (GOM), Fortune Bay (FB), and Bonavista Bay (BB) juvenile cod at the start of Experiment I. Error bars = standard error, sample size shown above error bars.

Figure 3.5: (a) Total length, and (b) wet weight of juvenile cod from the Grand Banks (GB), Gulf of Maine (GOM), Fortune Bay (FB), and Bonavista Bay (BB), reared at heated (H) and ambient (A) water temperatures for 14 weeks. Each symbol is the mean of the tank means for each treatment (n = 3 for GB and GOM, 2 for FB and BB), vertical bars = standard error.

Figure 3.6: Gross weight specific growth rate (% increase per day) of juvenile Atlantic cod from the Grand Banks (GB), Gulf of Maine (GOM), Fortune Bay (FB), and Bonavista Bay (BB), reared at (a) heated and (b) ambient water temperatures for 14 weeks. Each bar is the mean of the tank means (n = 3 for GB and GOM, 2 for FB and BB), error bars = standard error.

Figure 3.7: Gross food conversion ratio (food eaten / weight gained) of juvenile Atlantic cod from the Grand Banks (GB), Gulf of Maine (GOM), Fortune Bay (FB), and Bonavista Bay (BB), reared at (a) heated and (b) ambient water temperatures for 14 weeks. Each bar is the mean of the tank means (n = 3 for GB and GOM, 2 for FB and BB), error bars = standard error.

Figure 3.8: (a) Fulton's condition factor, (b) hepatosomatic index, (c) % liver water content, and (d) % muscle water content of Grand Banks (GB), Gulf of Maine (GOM), Fortune Bay (FB), and Bonavista Bay (BB) juvenile cod, reared at heated and ambient water tempertures for 14 weeks. Samples were taken at the end of Experiment I. Each bar is the mean of the tank means (n = 3 for GB and GOM, 2 for FB and BB), error bars = standard error.

Figure 3.9: Standardized (a) Fulton's condition factor, (b) hepatosomatic index, (c) % liver water content, and (d) % muscle water content of Grand Banks (GB), Gulf of Maine (GOM), Fortune Bay (FB), and Bonavista Bay (BB) juvenile cod, reared at heated and ambient water tempertures for14 weeks. Samples taken at the end of Experiment I. Each bar is the mean of the tank means (n = 3 for GB and GOM, 2 for FB and BB), error bars = standard error.

3.3.3 Experiment II

Gross specific growth rate, gross food conversion ratio

Mortality was low in all treatments (3 % total) and populations were not statistically compared. Cod kept under heated and ambient temperature in Experiment I were kept at common temperatures (~ 6 °C) in Experiment II (Figure 3.2; Week 16 - Week 24). Total length and W_w increased for all treatments during the experiment (Figure 3.10). Gross SGR of LAB and WILD cod was significantly higher for fish that were held at ambient temperatures in Experiment I, than those kept at heated temperatures (ANOVA; $F_{1,48} = 421.96$, $p < 0.001$; Figure 3.11). The interaction term origin*temperature was significant for the LAB-WILD comparisons, and the model was broken down by temperature. Juvenile cod of WILD origin, which had been held under ambient temperatures in Experiment I, grew significantly faster than those of LAB origin (ANOVA; $F_{1,24} = 147.34$ $p < 0.001$) in Experiment II. However, there was no significant difference between LAB-WILD cod kept under heated temperatures in Experiment I, during Experiment II (ANOVA; $F_{1,24} = 1.41$, $p = 0.246$). Gross FCR was not significantly affected by temperature history (ANOVA; $F_{1,48} = 0.01$, $p = 0.938$), or LAB-WILD origin (ANOVA; $F_{1,48} = 2.74$, $p = 0.104$; Figure 3.12).

There was a significant interaction effect on GSGR between GB-GOM origin and sampling period (Figure 3.11). The model was subsequently broken down and the comparison was analysed using 2-Way ANOVA at each sampling period. Grand Banks cod from both temperature treatments grew significantly faster than those from the GOM

from week 0 to 4 (ANOVA; $F_{1,8} = 13.00$, $p = 0.007$), while GOM cod grew significantly faster than GB cod from week 4 to 8 (ANOVA; $F_{1,8} = 7.26$, $p = 0.027$). However, there was no significant difference in growth rates of the two populations from week 0 to 8 (ANOVA; $F_{1,8} = 0.10$, $p = 0.760$). The interaction term for GSGR of FB-BB cod and temperature was significant, and the analysis was subsequently carried out at each temperature. There was no significant difference in GSGR of FB-BB cod from heated temperatures (ANOVA; $F_{1,6} = 1.98$, $p = 0.209$), but BB cod under ambient temperatures in Experiment I grew faster than FB cod (ANOVA; $F_{1,6} = 40.09$, $p < 0.001$). Gross FCR was not significantly different for GB-GOM cod (ANOVA; $F_{1,24} = 2.04$; $p = 0.166$). However, fish from BB had higher GFCR than those from FB, although this was only slightly significant (ANOVA; $F_{1,12} = 4.84$, $p = 0.048$; Figure 3.12).

Condition factor

The variables K, H.S.I., % W_L, and % W_M at the end of Experiment II (Figure 3.13) were significantly ($p < 0.05$) associated with L_T, and were standardized as described in the Materials and Methods section. Exposure to different temperatures in Experiment I did not significantly affect K of LAB or WILD cod in Experiment II. Plots of the tank means suggest that WILD cod previously kept at ambient temperatures had higher K than LAB cod subjected to the same temperature regime (Figure 3.14a). However, hierarchical ANOVA showed that LAB cod had higher K at both temperature treatments. Gulf of Maine cod from both temperature regimes had significantly higher K than GB cod,

whereas there was no significant difference in K of FB-BB cod from either temperature treatment (Table 3.8).

Hepatosomatic index

The hepatosomatic index was not significantly affected by previous temperature regime. Comparisons of LAB-WILD cod and FB-BB cod also showed no significant differences in H.S.I. (Figure 3.14b, Table 3.9). There was a significant interaction in the effect of GB-GOM origin with temperature and the model was broken down. After eight weeks at a common temperature, the H.S.I. of juvenile cod kept under heated temperatures in Experiment I was significantly higher for GOM than GB fish. However, the H.S.I. of juvenile cod kept under ambient temperatures in Experiment I, was significantly higher for GB than GOM cod in Experiment II.

% liver water content

After eight weeks of common temperatures in Experiment II, the % liver water content of cod kept under ambient temperatures was significantly higher than those kept under heated temperatures in Experiment I. However, % W_L was not significantly different for LAB-WILD, GB-GOM, or FB-BB comparisons (Figure 3.14c, Table 3.10).

% muscle water content

Temperature exposure in Experiment I did not significantly affect % muscle water

content in Experiment II. There was also no significant difference in % W_M of LAB-WILD, or GB-GOM cod. A significant interaction effect between the FB-BB population comparison and past temperature regime was present, and the analysis was subsequently conducted at each temperature treatment. No significant difference in % W_M of FB-BB cod from either temperature treatment was found (Figure 3.14d, Table 3.11).

Table 3.8: Results of ANOVA comparisons for condition factor of LAB-WILD, GB-GOM, and FB-BB juvenile cod, reared at a common temperature for eight weeks. Where a significant difference was present ($p < 0.05$), the treatment with higher values is indicated.

Comparison	Both temperature treatments from Experiment I
LAB-WILD	$F_{1,91} = 17.53$, $p < 0.001$, LAB
GB-GOM	$F_{1,52} = 14.17$, $p < 0.001$, GOM
FB-BB	$F_{1,35} = 4.12$, $p = 0.050$
Temperature	$F_{1,91} = 0.05$, $p = 0.827$

Table 3.9: Results of ANOVA comparisons for hepatosomatic index of LAB-WILD, GB-GOM, and FB-BB juvenile cod, reared at a common temperature for eight weeks. Heated refers to fish initially exposed to heated temperatures, and ambient refers to fish initially exposed to ambient temperatures for 14 weeks. When a significant interaction ($p < 0.05$) with temperature was present, comparisons were made at each temperature. Where a significant difference was present ($p < 0.05$), the treatment with higher values is indicated.

Comparison	Both temperatures	Heated temperature	Ambient temperature
LAB-WILD	$F_{1,90} = 0.0$, $p = 0.955$		
GB-GOM		$F_{1,28} = 12.78$, $p = 0.001$, GOM	$F_{1,23} = 4.41$, $p = 0.047$, GB
FB-BB	$F_{1,35} = 0.58$, $p = 0.450$		
Temperature	$F_{1,90} = 0.31$, $p = 0.545$		

Table 3.10: Results of ANOVA comparisons for % liver water content of LAB-WILD, GB-GOM, and FB-BB juvenile cod, reared at a common temperature for eight weeks. Where a significant difference was present ($p < 0.05$), the treatment with higher values is indicated.

Comparison	Both temperature treatments in Experiment I
LAB-WILD	$F_{1,90} = 1.08$, $p = 0.302$
GB-GOM	$F_{1,51} = 2.87$, $p = 0.962$
FB-BB	$F_{1,35} = 1.63$, $p = 0.210$
Temperature	$F_{1,90} = 6.93$, $p = 0.010$, Ambient

Table 3.11: Results of ANOVA comparisons for % muscle water content of LAB-WILD, GB-GOM, and FB-BB juvenile cod, reared at a common temperature for eight weeks. Heated refers to fish initially exposed to heated temperatures, and ambient refers to fish initially exposed to ambient temperatures for 14 weeks. When a significant interaction ($p < 0.05$) with temperature was present, comparisons were made at each temperature.

Comparison	Both temperatures	Heated temperature	Ambient temperature
LAB-WILD	$F_{1,90} = 0.01$, $p = 0.931$		
GB-GOM	$F_{1,52} = 1.79$, $p = 0.187$		
FB-BB		$F_{1,18} = 2.99$, $p = 0.101$	$F_{1,18} = 3.35$, $p = 0.085$
Temperature	$F_{1,90} = 3.43$, $p = 0.067$		

Figure 3.10: (a) Total length, and (b) wet weight of juvenile cod from the Grand Banks (GB), Gulf of Maine (GOM), Fortune Bay (FB), and Bonavista Bay (BB), reared at a common temperature for eight weeks. (H) refers to fish initially kept at heated temperatures, and (A) refers to fish intially kept at ambient temperatures for 14 weeks. Each symbol is the mean of the tank means (n = 3 for GB and GOM, 2 for FB and BB), vertical bars = standard error.

Figure 3.11: Gross specific growth rate (% increase per day) of juvenile Atlantic cod from the Grand Banks (GB), Gulf of Maine (GOM), Fortune Bay (FB), and Bonavista Bay (BB), reared at a common temperature for eight weeks. (a) refers to fish initially kept at heated temperatures and (b) refers to fish initially kept at ambient temperatures for 14 weeks. Each bar is the mean of the tank means (n = 3 for GB and GOM, 2 for FB and BB), error bars = standard error.

Figure 3.12: Gross food conversion ratio (food eaten / weight gained) of juvenile Atlantic cod from the Grand Banks (GB), Gulf of Maine (GOM), Fortune Bay (FB), and Bonavista Bay (BB), reared at a common temperature for eight weeks. (a) refers to fish initially kept at heated temperatures, and (b) refers to fish initially kept at ambient temperatures for 14 weeks. Each bar is the mean of the tank means (n = 3 for GB and GOM, 2 for FB and BB), error bars = standard error.

Figure 3.13: (a) Fulton's condition factor, (b) hepatosomatic index, (c) % liver water content, and (d) % muscle water content of Grand Banks (GB), Gulf of Maine (GOM), Fortune Bay (FB), and Bonavista Bay (BB) juvenile cod, reared at a common temperature for eight weeks. Heated refers for fish initially kept at heated temperatures, and ambient refers to fish intially kept at ambient temperatures for 14 weeks. Each bar is the mean of the tank means, error bars = standard error.

Figure 3.14: Standardized (a) Fulton's condition factor, (b) hepatosomatic index, (c) % liver water content, and (d) % muscle water content of Grand Banks (GB), Gulf of Maine (GOM), Fortune Bay (FB), and Bonavista Bay (BB) juvenile cod, reared at a common temperature for eight weeks. Heated refers to fish intially kept at heated temperatures, and ambient refers to fish initially kept at ambient temperatures for 14 weeks. Each bar is the mean of the tank means, error bars = standard error.

3.4 Discussion

This study examined the effects of different temperatures on growth and energy allocation in different populations of juvenile cod. The results show that physiological responses to temperature may be different among populations.

Juvenile cod from all populations grew faster under heated than ambient water temperatures in Experiment I. The slow growth of all populations under ambient water temperatures suggests that wild 0-group cod in northern areas would grow little over the winter months. Ambient temperatures during the experiment were actually warmer than those occurring around Newfoundland at this time of year. In addition, the experimental fish were fed to satiation daily, which is likely more food than is consumed in the wild. Faster growth of cod at higher temperatures is well documented (Campana and Hurley, 1989; Brander, 1994; Brander, 1995; Hunt von Herbing *et al.*, 1996; Krohn *et al.*, 1997; Shackell *et al.*, 1997). Brander (1994) found that over the first four years of life, each 1 °C increase in water temperature results in a 29% increase in size.

Although common environment experiments are an accepted method for estimating genetic differences among populations, it is premature to conclude that population differences found in this study are completely genetic in origin. Non-genetic parental influences, such as size of the yolk-sac in newly hatched larvae, can affect progeny. However, these types of influences are unlikely to have persisted in the GB and GOM samples, as they were kept for a full year under identical conditions. This should have been enough time to mitigate non-genetic parental effects, and therefore differences

among these groups are likely to be genetic in origin. Non-genetic influences may be more important for the two wild caught populations (FB and BB), as they may have been exposed to substantially different environmental factors before capture. Therefore, observed differences in phenotypes may or may not have a genetic basis.

Although the WILD caught cod in my study would likely have originated from many different parents, the LAB reared fish were obtained from a restricted gene pool. As the sampled fish may not have adequately represented the whole population, the results have to be viewed with some caution. In addition, laboratory selection on different genotypes can occur (Garland and Adolph, 1991). However, similar selective pressures would likely occur for all groups, thus comparisons can be made.

Although cod from southern (warmer) areas grow faster than those from northern (colder) environments in the wild (Brander, 1994; Campana *et al.*, 1995), overall there was no significant difference (under common conditions) in weight specific growth rates between juvenile cod from the GB and GOM, or FB and BB in this study. This suggest that environmental variability is likely responsible for most of the observed differences in growth rates between these stocks in the wild.

The capacity for growth rate has been found to be higher in larval cod from northern areas (Hunt von Herbing *et al.*, 1996; Chapter Two). Faster growth rates of higher latitude populations is termed countergradient variation (CnGV), and is believed to be an adaption to shorter growing seasons (Conover and Present, 1990; Conover, 1992). Due to selective pressures against small size, higher latitude populations must

grow faster than those in lower latitudes, when conditions allow for growth. In extreme cases of CnGV, the phenomenon may result in similar phenotypes occurring over broad environmental conditions (Conover, 1990). However, if less prevalent, one would expect that CnGV may only "buffer" latitudinal differences. It is generally assumed that size-selective mortality is highest during the earliest periods of life, and therefore it has been predicted that CnGV would be most significant in the larval stage (Conover, 1992). This hypothesis has been supported in this thesis, as larval GB cod had higher capacities for growth rate than those from the GOM (Chapter Two), but juveniles (same sibling group as larvae) had similar growth rates.

In Experiment I, the two wild populations (FB and BB) grew faster than the lab cod (GB and GOM) at heated temperatures, but there was no difference at ambient temperatures. This difference in growth rates may have been a result of compensatory growth. This occurs when animals are able to compensate for periods of poor growing conditions, such as depressed food rations (Kim and Lovell, 1995; Nicieza and Metcalfe, 1997) or low temperatures (Nicieza and Metcalfe, 1997), by increasing growth rates when conditions become favorable. WILD fish may have experienced colder water temperatures or poor feeding conditions than LAB cod prior to capture. When placed under heated temperatures with unlimited food, compensatory growth may have occurred. At ambient temperatures metabolic rates may have been too low for this to take place. This was supported in Experiment II, as WILD cod held at ambient temperatures in Experiment I grew significantly faster than LAB cod in Experiment II.

Whereas compensatory growth would have occurred under heated temperatures in Experiment I, it was not until the temperature was increased in Experiment II, that those fish that had been previously kept at ambient temperatures were able to "catch up."

In a similar manner, cod held at ambient temperatures in Experiment I had faster growth rates than those kept under heated temperatures, when both were placed at an intermediate temperature in Experiment II. These results however do not prove compensatory growth occurred, as there was no separate control group. Cod kept at ambient temperatures in Experiment I grew faster at an intermediate temperature (in Experiment II) than those previously held at heated temperatures. However, one would need to know growth rates of cod at intermediate temperatures for both experiments (a third group in Experiment I) in order to confirm compensatory growth. Nevertheless, the fish kept under ambient temperatures in Experiment I, had similar growth rates at the intermediate temperature in Experiment II (~ 6 °C), as the fish under heated temperatures had in Experiment I (~ 13 °C).

In Experiment I, food conversion efficiency was similar between LAB reared and WILD caught cod under heated temperatures, but WILD cod had poorer food conversion efficiencies than LAB cod at ambient temperatures. However, there was no significant difference in GFCR for LAB-WILD cod of either temperature treatment in Experiment II. I cannot speculate on a possible reason for this difference.

Although growth rates were not significantly different between GB and GOM cod in Experiment I, GOM cod were less efficient at converting food eaten to body mass than

GB cod. There was however no significant difference in food conversion efficiency between the two populations in Experiment II. Northern populations have been shown to have increased food conversion efficiency compared southern populations in Atlantic silversides, (Present and Conover, 1992), and Atlantic salmon, *Salmo salar* (Nicieza *et al.*, 1994a). Higher latitude populations may evolve improved food conversion efficiencies in order to better exploit those limited periods when temperatures allow for rapid growth (Nicieza *et al.*, 1994a).

Nutritional status of fish can be assessed by numerous means. In cod, measurements such as Fulton's condition factor, hepatosomatic indices, and percent water content of liver and muscle tissue have been shown to be good predictors of energy reserves (Lambert and Dutil, 1997; Grant and Brown, 1998). Condition factors are a means of comparing body weight at length. Higher K means that fish are heavier at length, and are assumed to be "healthier." Similarly, the hepatosomatic index is useful in comparing liver size at weight. Much of the energy storage in cod is lipid in the liver (Lambert and Dutil, 1997). Therefore, fish with higher H.S.I. likely have more lipid reserves. The water content of the liver can also be used to measure lipid storage (Love, 1970). As lipids are metabolized, they are replaced by water. Therefore, higher liver water content is associated with lower lipid reserves. For example, liver energy content can range from 30 to 5 kJ/g, for 20% to 80% liver water content respectively (Lambert and Dutil, 1997). The other nutritional measurement used in this study was % water content in contractile muscle. In many species of fish, protein in the muscle is used as an

energy source (Love, 1970; Lambert and Dutil, 1997). In a manner similar to lipids in the liver, as proteins are metabolized from the muscle, are replaced by water. Muscle energy content in cod ranges from 5 kJ/g for muscle of 78% water, to 1.5 kJ/g for muscle of 90% water (Lambert and Dutil, 1997). Therefore, calculation of muscle water content can be a useful means of measuring protein reserves.

Upon collection, 0-group juvenile cod from Bonavista Bay had higher hepatosomatic indices than similar sized fish from Fortune Bay, indicating that BB fish had larger livers relative to body weight. Higher H.S.I. is associated with increased energy storage (see above), and hepatosomatic index is known to be affected by diet. Grant and Brown (1999) found that 0-group cod in Trinity Bay, Newfoundland develop higher H.S.I. when consuming *Calanus finmarchicus*. Due to the fact that samples were only collected once in my study, and stomach content was not recorded, clear conclusions of population differences in H.S.I. of wild 0-group juvenile cod from these areas cannot be made.

Although all cod were kept under identical conditions for 14 weeks in Experiment I, and a further eight weeks in Experiment II, differences in indices of nutritional status were present at the end of the experiments. LAB cod had higher K than WILD cod at the start of Experiment I. After 14 weeks of common conditions this was still evident under ambient temperatures, but under heated conditions there was no significant difference. This indicates that the WILD cod "caught up" to LAB cod in a similar way to GSGR, and this increase in K may be another example of compensatory growth. Gulf of Maine cod

had higher condition factors than GB cod throughout the study, and are heavier at length than GB cod. Cod from BB had higher K than those from FB at collection, and this was still evident after the 14 week period under ambient temperatures in Experiment I. However, under heated water, and for both temperature treatments (from Experiment I) in Experiment II, there was no significant difference in H.S.I. between the two populations, again supporting compensatory growth of FB cod.

Although H.S.I. was not significantly different for GB-GOM cod at ambient temperatures, GOM fish had higher H.S.I. at heated water temperatures in Experiment I. Unexpectedly, in Experiment II, GOM cod kept under heated temperatures in Experiment I had significantly higher H.S.I. than GB cod, but the opposite was true for the ambient temperature group. Since GOM cod are found in warmer water than GB cod, this suggests that GOM fish are better adapted for lipid storage in warmer water, and the opposite is true for GB cod. Comparisons of H.S.I. for FB-BB fish were similar to those of K for both experiments. It is likely that FB "caught up" to BB fish under heated temperatures, but metabolic rates were too low for this to occur under ambient temperatures in Experiment I. Furthermore, the observed H.S.I. values likely caused the observed differences in K.

Cod often develop corpulent livers in captivity (Grant and Brown, 1998). This was prevalent in this study as LAB cod had higher H.S.I. than WILD cod in Experiment I. Presumably, by the end of Experiment II, WILD cod had been kept under "good growth conditions" long enough to also develop corpulent livers. In the wild, H.S.I. often changes

throughout the year (Lambert and Dutil, 1997; Grant and Brown, 1999). Water temperature did not significantly affect H.S.I. during either experiment, suggesting that some factor other than temperature (e.g., diet) is likely responsible for the variations observed in the wild.

The only index that was significantly affected by water temperature was % water content of the liver. Fish reared under heated temperatures had lower % W_L than those under ambient temperatures. This difference was still significant after eight weeks at a common temperature in Experiment II. Increase in water content is associated with decrease in stored lipids in the livers of cod (Lambert and Dutil, 1997). Therefore, these results indicate that although H.S.I. was not significantly different, cod reared under heated conditions had more lipid reserves than those kept at ambient temperatures.

In Experiment I, LAB reared cod had lower % W_L than WILD fish at heated temperatures, but the opposite was true under ambient water. Since lower water content and increased liver size are associated with increased lipid storage, the % W_L and H.S.I. results at heated temperatures show that LAB cod stored more lipids. However, I do not know why LAB fish would have larger livers than WILD fish at ambient temperatures, but higher % W_L. The similarity between % W_L of GB-GOM and FB-BB cod indicates that the populations had similar lipid reserves per gram of liver. These results indicate that where larger livers (higher H.S.I.) were present (e.g., BB at ambient temperature), the increase in liver weight was not due to increased water content, and therefore more lipid storage had occurred.

In cod, contractile muscle protein is thought to be used as an energy source only after liver lipid reserves are exhausted (Lambert and Dutil, 1997). A "jellied" muscle condition can occur in cod when water content in the muscle is high, and is often associated with adults during spawning. It is believed that the fish take muscle protein and use it as energy for reproduction, and therefore the muscle water content increases (Roff, 1982). Exact causes and mechanisms behind the formation of jellied muscle are not known. The results of this study suggest that temperature (at least over the observed range) does not likely play a direct role in determining % W_M of young cod, as fish under the two temperature regimes had similar levels. Unlike lipid storage, where estimates using H.S.I. and % W_L showed LAB fish storing more lipids than WILD fish, there was no significant difference in % W_M in LAB-WILD cod. GB cod had lower % W_M (higher protein levels) than GOM cod at heated temperatures, but there was no significant difference under ambient temperatures in Experiment I, or for either group in Experiment II. Thus, in terms of energy storage, GB-GOM cod did similar things at ambient temperatures, but at heated temperatures GB cod stored more energy than GOM cod as muscle protein, while GOM cod stored more energy than those for the GB as lipids in the liver.

Reaction norms are often used to compare environmental effects on different populations. Schmaulhausen (1949) defines the norm of reaction as "phenotypic expression of a genotype in different environments." Variation in plasticity between genotypes in relation to a range of specified environments can be analyzed by testing for a

significant genotype-environment interaction in an ANOVA (Thompson, 1991). A significant interaction may result from differences in the direction, or slope of the reaction norm.

Reaction norms are presented for nutritional indices of GB-GOM , and FB-BB cod in Experiment I (Figures 3.15; 3.16) and Experiment II (Figures 3.17; 3.18). The interaction term population-temperature was not significantly different for K, or % W_L of GB-GOM cod in either experiment, or for % W_M in Experiment II. However, H.S.I. population comparisons of GB-GOM cod in both experiments, and % W_M in Experiment I were significantly associated with temperature. The FB-BB population comparisons also showed significant interaction effects between population and temperature for K, H.S.I., and % W_L, in Experiment I, and % W_M in Experiment II. However there was no significant interaction effect with temperature for % W_M in Experiment I, or K, H.S.I., and % W_L in Experiment II. Due to possible past influences on observed phenotypes of FB and BB cod (see above), conclusions based on differences in reaction norms of these populations are limited. However, results for GB and GOM cod indicate genotypic differences in response to temperature for H.S.I., and % W_M .

Population differences in growth rates were not found to differ significantly in this study. Therefore, the hypothesis that the capacity for growth rate increases with latitude was rejected for juvenile cod. However, GB cod did have better food conversion efficiency than GOM cod, supporting the CnGV model. Nutritional indices did not clearly fit either the LA or CnGV models.

This study has identified effects of temperature on growth and energy allocation towards energy reserves in young of the year juvenile cod. In addition, differences in growth rates of the populations in the wild, were shown not to be due to higher genetic capacities for growth rates in the southern populations. Apparent population differences in nutritional indices were present, but should be interpreted with caution until a more intensive study can be completed. These results may be useful in better managing cod stocks, as temperatures affect different stocks differently. Comparisons made between juvenile cod reared under laboratory conditions from egg, to those of newly settled juveniles caught in the wild, may give insights into effects of captivity on young cod, and may be useful for aquaculture of the species.

Figure 3.15: Reaction norms of standardized (a) Fulton's condition factor, (b) hepatosomatic index, (c) % liver water content, and (d) % muscle water content of Grand Banks (GB), and Gulf of Maine (GOM) juvenile cod, reared at heated and ambient temperatures for 14 weeks. Each symbol is the mean of the tank means (n = 3), vertical bars = standard error.

Figure 3.16: Reaction norms of standardized (a) Fulton's condition factor, (b) hepatosomatic index, (c) % liver water content, and (d) % muscle water content of Fortune Bay (FB), and Bonavista Bay (BB) juvenile cod, reared at heated and ambient temperatures for 14 weeks. Each symbol is the mean of the tank means (n = 2), vertical bars = standard error.

Figure 3.17: Reaction norms of standardized (a) Fulton's condition factor, (b) hepatosomatic index, (c) % liver water content, and (d) % muscle water content of Grand Banks (GB), and Gulf of Maine (GOM) cod, reared at a common temperature for eight weeks. Heated refers to fish initially kept at heated temperatures, and ambient refers to fish initially kept at ambient temperatures for 14 weeks. Each symbol is the mean of the tank means (n = 3), vertical bars = standard error.

Figure 3.18: Reaction norms of standardized (a) Fulton's condition factor, (b) hepatosomatic index, (c) % liver water content, and (d) % muscle water content of Fortune Bay (FB), and Bonavista Bay (BB) cod, reared at a common temperature for eight weeks. Heated refers to fish initially kept at heated temperatures, and ambient refers to fish initially kept at ambient temperatures for 14 weeks. Each symbol is the mean of the tank means (n =2), vertical bars = standard error.

Chapter Four

Summary and suggestions for future research

4.1 Summary

Geographic variation in life history traits is well documented in many organisms. These differences are often considered to be purely environmental based, but genetic differences have been found (Conover and Present, 1990; Williamson and Carmichael, 1990; Robinson and Wilson, 1996). Through common environment experiments, the capacity for growth rate in many organisms has been shown to vary in a geographic pattern. Growth rates may be adapted to local conditions (Levinton, 1983), or vary with latitude in a countergradient manner (Conover and Present, 1990).

Cod in the northwest Atlantic exhibit marked differences in life history traits, including growth rates and size at maturity (May *et al.*, 1965; Brander 1994). These differences have been repeatedly cited as occurring as a result of differences in environmental variables (mainly water temperatures) among the stocks. However, investigations into the effects of water temperature on different stocks of cod under common environments are rare.

Common environment experiments are often used to address the relative contribution of different environmental factors and genetics to observed phenotypes. This approach was used in this study, to make latitudinal comparisons between groups of cod.

In the first experiment, growth of larval cod from the Grand Banks (GB) was compared to that of those from the Gulf of Maine (GOM). Whereas GOM cod grow faster than those from the GB in the wild (Brander, 1994; Campana *et al.*, 1995), under common conditions the GB larvae grew faster. This result supported a well studied hypothesis, termed countergradient variation. This hypothesis states that higher latitude populations have higher capacities for growth rates, due to adaptation to shorter growing seasons.

To further investigate temperature effects on different cod stocks, two other experiments were conducted using juvenile cod. One group (GB and GOM) was collected as eggs in the wild and reared under identical laboratory conditions until the start of the experiments. These were sibling fish to those used in the larval experiment. Two other populations (FB and BB) of "wild" cod comprised the second group. These fish were collected as juveniles from two inshore bays on the island of Newfoundland. Latitudinal comparisons were not made between the populations reared in the laboratory to those collected from the wild as juveniles, due to the possibility of LAB/WILD origin being a confounding factor. Due to recent interest in cod aquaculture, LAB reared fish were compared to WILD fish.

The results from the juvenile experiments suggest that much of the observed variation in life history traits (particularly growth rates) among these stocks is based on environmental differences. However, substantial population differences in energy allocation were found. Different reaction norms for several nutritional indices between GB and GOM cod may indicate adaptation to different environments.

Although sibling animals were used, differences in the capacity for growth rates were present for Grand Banks and Gulf of Maine larvae, but not juveniles. This suggests that pressures selecting for faster growth of northern populations are more important for larval than juvenile fish.

4.2 Suggestions for future research

A more intensive study is required to fully investigate the contribution of environmental and genetic factors towards life history variation in cod stocks. To determine prominent trends, comparisons of representative stocks throughout the entire distribution of cod should be made. In addition, a wide range of temperatures (and possibly other environmental variables) should be used. Finally, in order to ensure results are representative of the whole stock, gametes should be taken from as many adults as possible, and the genetic variability among progeny identified.

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Appendix A

Experimental tank setup for Capture Three. Two 2000 litre raceways each had 10 "tanks" which were separated by fly screen. Each tank contained 10 fish, from either the Grand Banks (GB), Gulf of Maine (GOM), Fortune Bay (FB), or Bonavista Bay (BB). One raceway received heated filtered seawater and one raceway received ambient filter seawater.

APPENDIX B

Appendix B-1: Total length (cm), wet weight (g), and standardized Fultons condition factor (K), hepatosomatic index (H.S.I.), liver water content (% W_L), and muscle water content (% W_M) for Grand Banks (GB), Gulf of Maine (GOM), Fortune Bay (FB), and Bonavista Bay (BB) cod reared under identical conditions for 14 weeks at heated (H) and ambient (A) temperatures. Each value is mean ± S.E. (range) of the treatment, N = total length, wet weight, and K sample size, n = H.S.I., % W_L, and %W_M sample size.

Treatment	Total length	Wet weight	K	H.S.I.	% W_L	% W_M
GB (H) N = 27 n = 12	17.5 ± 0.3 (13.0 - 20.6)	46.89 ± 2.57 (15.20 - 74.90)	0.8 ± 0.0 (0.6 - 1.0)	7.0 ± 0.4 (4.0 - 10.0)	29.4 ± 0.9 (25.1 - 31.6)	80.0 ± 0.2 (79.2 - 81.1)
GB (A) N = 30 n = 14	13.2 ± 0.3 (9.8 - 16.9)	18.33 ± 1.57 (5.38 - 40.36)	0.7 ± 0.0 (0.6 - 1.0)	8.0 ± 0.5 (5.0 - 12.6)	35.2 ± 1.3 (25.1 - 39.8)	80.8 ± 0.3 (79.2 - 82.6)
GOM (H) N = 26 n = 11	13.5 ± 0.3 (11.0 - 16.6)	23.13 ± 2.06 (7.98 - 48.19)	0.8 ± 0.0 (0.6 - 1.0)	10.2 ± 0.7 (7.9 - 15.8)	27.5 ± 0.9 (25.1 - 31.6)	80.7 ± 0.4 (79.2 - 83.4)
GOM (A) N = 29 n = 14	10.2 ± 0.2 (8.3 - 13.0)	9.08 ± 0.58 (3.51 - 17.12)	0.8 ± 0.0 (0.6 - 1.0)	8.6 ± 0.4 (6.3 - 12.6)	34.2 ± 1.6 (25.1 - 50.1)	80.5 ± 0.2 (79.1 - 82.2)
FB (H) N = 18 n = 8	15.8 ± 0.4 (11.7 - 18.1)	33.02 ± 2.58 (11.83 - 59.83)	0.8 ± 0.0 (0.6 - 1.0)	8.2 ± 0.4 (6.3 - 10.0)	30.8 ± 0.8 (25.1 - 31.6)	81.0 ± 0.2 (80.4 - 81.9)
FB (A) N = 19 n = 9	10.3 ± 0.2 (8.6 - 12.9)	7.84 ± 0.54 (4.69 - 13.95)	0.6 ± 0.0 (0.6 - 0.8)	6.1 ± 0.4 (4.0 - 7.9)	32.6 ± 1.3 (25.1 - 39.8)	80.7 ± 0.2 (79.9 - 81.7)
BB (H) N = 18 n = 8	14.7 ± 0.5 (9.4 - 18.1)	27.37 ± 2.97 (6.42 - 50.56)	0.8 ± 0.5 (0.6 - 0.8)	8.0 ± 0.5 (6.3 - 10.0)	32.8 ± 1.6 (25.1 - 39.8)	80.8 ± 0.3 (79.5 - 82.1)
BB (A) N = 20 n = 10	9.4 ± 0.4 (6.6 - 13.8)	6.96 ± 0.91 (2.08 - 18.46)	0.7 ± 0.0 (0.6 - 1.0)	8.0 ± 0.3 (6.3 - 10.0)	30.3 ± 0.8 (25.1 - 31.6)	80.9 ± 0.3 (79.8- 82.4)

Appendix B-2: Total length (cm), wet weight (g), and standardized Fultons condition factor (K), hepatosomatic index (H.S.I.), liver water content (% W_L), and muscle water content (% W_M) for Grand Banks (GB), Gulf of Maine (GOM), Fortune Bay (FB), and Bonavista Bay (BB) cod, at a common temperature for eight weeks. (H) refers to fish initially kept at heated temperatures and (A) refers to fish initially kept at ambient temperatures for 14 weeks. Each value is mean ± S.E. (range) of the treatment, N = sample size.

Treatment	Total length	Wet weight	K	H.S.I.	% W_L	% W_M
GB (H) N = 15	20.1 ± 0.5 (16.7 - 24.0)	68.43 ± 5.65 (33.13 - 112.87)	0.8 ± 0.0 (0.7 - 0.9)	6.7 ± 0.3 (5.1 - 8.7)	26.4 ± 0.7 (22.5 - 32.0)	79.5 ± 0.1 (78.3 - 80.2)
GB (A) N = 13	16.5 ± 0.5 (13.7 - 19.7)	35.20 ± 3.22 (21.62 - 58.04)	0.8 ± 0.0 (0.7 - 0.8)	8.7 ± 0.3 (7.3 - 10.4)	26.6 ± 1.0 (22.7 - 36.1)	79.0 ± 0:1 (78.3 - 79.5)
GOM (H) N = 15	15.7 ± 0.6 (12.7 - 19.7)	35.19 ± 4.52 (14.38 - 74.57)	0.8 ± 0.0 (0.6 - 1.0)	8.7 ± 0.4 (5.4 - 11.2)	24.4 ± 0.7 (21.5 - 29.0)	79.3 ± 0.2 (78.1 - 80.3)
GOM (A) N = 14	12.9 ± 0.4 (10.8 - 15.6)	18.77 ± 1.90 (10.01 - 33.16)	0.8 ± 0.0 (0.7 - 0.9)	6.5 ± 0.9 (2.3 15.5)	26.0 ± 0.8 (22.4 - 32.2)	78.9 ± 0.2 (78.1 - 80.5)
FB (H) N = 10	19.2 ± 0.4 (16.5 - 21.0)	56.98 ± 4.54 (32.73 - 83.92)	0.8 ± 0.0 (0.6 - 0.9)	7.8 ± 0.5 (5.4 - 11.6)	24.3 ± 0.7 (20.2 - 27.6)	79.3 ± 0.1 (78.4 - 80.0)
FB (A) N = 10	14.8 ± 0.4 (12.5 - 16.6)	25.07 ± 1.76 (14.67 - 32.87)	0.8 ± 0.0 (0.7 - 0.8)	7.1 ± 0.2 (6.0 - 8.1)	27.6 ± 0.7 (23.8 - 31.4)	80.3 ± 0.9 (75.3 - 86.2)
BB (H) N = 10	17.6 ± 0.7 (13.3 - 21.8)	43.40 ± 6.17 (11.08 - 85.22)	0.7 ± 0.0 (0.5 - 0.8)	7.9 ± 0.3 (6.2 - 9.9)	26.3 ± 0.6 (23.0 - 29.3)	81.0 ± 0.9 (78.9 - 88.7)
BB (A) N = 9	12.2 ± 0.3 (10.8 - 14.0)	13.80 ± 1.13 (9.74 - 19.88)	0.7 ± 0.0 (0.7 - 0.8)	7.8 ± 0.9 (4.9 - 15.1)	27.6 ± 0.6 (24.4 - 30.8)	76.1 ± 2.1 (61.3 - 87.0)

APPENDIX C

Appendix C-1: Regression parameters describing the relationships between log transformed condition factors and total lengths (cm) of Grand Banks (GB), Gulf of Maine (GOM), Fortune Bay (FB), and Bonavista Bay (BB) cod kept under heated and ambient water temperatures for 14 weeks. The regression equation is $y = a + bx$, with $y = \log_{10} K$, a = intercept, b = slope, and $x = \log_{10} L_T$.

Temp	Heated				Ambient			
Pop	GB	GOM	FB	BB	GB	GOM	FB	BB
a	-0.37	-0.82	-0.44	-0.38	-0.55	-0.22	0.153	0.025
b	0.228	0.663	0.294	0.242	0.378	0.118	-0.29	-0.125

Appendix C-2: Regression parameters describing the relationships between log transformed heptosomatic indices and total lengths (cm) of Grand Banks (GB), Gulf of Maine (GOM), Fortune Bay (FB), and Bonavista Bay (BB) cod kept under heated and ambient water temperatures for 14 weeks. The regression equation is $y = a + bx$, with $y = \log_{10}$ H.S.I., a = intercept, b = slope, and $x = \log_{10} L_T$.

Temp	Heated				Ambient			
Pop	GB	GOM	FB	BB	GB	GOM	FB	BB
a	-0.44	-0.37	0	-1.01	-0.59	0.461	2.31	0.9
b	1.18	1.24	0.833	1.74	1.35	0.415	-1.56	0

Appendix C-3: Regression parameters describing the relationships between log transformed % water contents and total lengths (cm) of Grand Banks (GB), Gulf of Maine (GOM), Fortune Bay (FB), and Bonavista Bay (BB) cod kept under heated and ambient water temperatures for 14 weeks. The regression equation is $y = a + bx$, with $y = \log_{10} \%W_L$, a = intercept, b = slope, and $x = \log_{10} L_T$.

Temp	Heated				Ambient			
Pop	GB	GOM	FB	BB	GB	GOM	FB	BB
a	2.1	1.83	2.6	2.89	3.1	1.69	1.62	1.37
b	-0.59	-0.37	-1	-1.26	-1.42	-0.15	-0.11	0.111

Appendix C-4: Regression parameters describing the relationships between log transformed % muscle water content and total lengths (cm) of Grand Banks (GB), Gulf of Maine (GOM), Fortune Bay (FB), and Bonavista Bay (BB) cod kept under heated and ambient water temperatures for 14 weeks. The regression equation is $y = a + bx$, with $y = \log_{10} \%W_M$, a = intercept, b = slope, and $x = \log_{10} L_T$.

Temp	Heated				Ambient			
Pop	GB	GOM	FB	BB	GB	GOM	FB	BB
a	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9
b	0	0	0	0	0	0	0	0

Appendix C-5: Regression parameters describing the relationships between log transformed condition factorsand total lengths (cm) of Grand Banks (GB), Gulf of Maine (GOM), Fortune Bay (FB), and Bonavista Bay (BB) cod kept under heated and ambient water temperatures for 14 weeks, and then at a common intermediate temperature for eight weeks (data analysed here). The regression equation is $y = a + bx$, with $y = \log_{10} K$, a = intercept, b = slope, and $x = \log_{10} L_T$.

Temp	Heated				Ambient			
Pop	GB	GOM	FB	BB	GB	GOM	FB	BB
a	-0.41	-0.71	-0.25	-0.9	-0.15	-0.1	0.06	-0.1
b	0.25	0.523	0.125	0.61	0.03	0	-0.14	0

Appendix C-6: Regression parameters describing the relationships between log transformed heptosomatic indices and total lengths (cm) of Grand Banks (GB), Gulf of Maine (GOM), Fortune Bay (FB), and Bonavista Bay (BB) cod kept under heated and ambient water temperatures for 14 weeks, and then at a common intermediate temperature for eight weeks (data analysed here). The regression equation is $y = a + bx$, with $y = \log_{10}$ H.S.I., a = intercept, b = slope, and $x = \log_{10} L_T$.

Temp	Heated				Ambient			
Pop	GB	GOM	FB	BB	GB	GOM	FB	BB
a	-0.53	0	0.285	-0.1	0.419	1.89	1.04	0.8
b	1.13	0.814	0.5	0.827	0.436	-0.94	-0.16	0.06

Appendix C-7: Regression parameters describing the relationships between log transformed % water contents and total lengths (cm) of Grand Banks (GB), Gulf of Maine (GOM), Fortune Bay (FB), and Bonavista Bay (BB) cod kept under heated and ambient water temperatures for 14 weeks, and then at a common intermediate temperature for eight weeks (data analysed here). The regression equation is $y = a + bx$, with $y = \log_{10} \%W_L$, a = intercept, b = slope, and $x = \log_{10} L_T$.

Temp	Heated				Ambient			
Pop	GB	GOM	FB	BB	GB	GOM	FB	BB
a	2.11	2.01	1.58	1.93	2.73	1.99	1.65	1.81
b	-0.58	-0.1	-0.17	-0.43	-1.1	-0.48	-0.18	-0.306

Appendix C-8: Regression parameters describing the relationships between log transformed % muscle water content and total lengths (cm) of Grand Banks (GB), Gulf of Maine (GOM), Fortune Bay (FB), and Bonavista Bay (BB) cod kept under heated and ambient water temperatures for 14 weeks, and then at a common intermediate temperature for eight weeks (data analysed here). The regression equation is $y = a + bx$, with $y = \log_{10} \%W_M$, a = intercept, b = slope, and $x = \log_{10} L_T$.

Temp	Heated				Ambient			
Pop	GB	GOM	FB	BB	GB	GOM	FB	BB
a	1.93	1.92	1.9	1.93	1.91	1.9	1.96	2.03
b	0	0	0	0	0	0	0	-0.126