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THE EFFECT OF TEMPERATURE ON THE DEVELOPMENT, GROWTH AND SURVIVAL OF ATLANTIC COD (*GADUS MORHUA*) DURING EARLY LIFE-HISTORIES

By

Adrian Jordaan

B.S. Memorial University of Newfoundland, 1998

A THESIS

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Science

(in Marine Biology)

The Graduate School

The University of Maine

December, 2002

Advisory Committee:

Linda J. Kling, Associate Professor of Aquaculture and Fish Nutrition

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THE EFFECT OF TEMPERATURE ON THE DEVELOPMENT, GROWTH AND SURVIVAL OF ATLANTIC COD (*GADUS MORHUA*) DURING EARLY LIFE-HISTORIES

By Adrian Jordaan

Thesis Advisor: Dr. Linda J. Kling

An Abstract of the Thesis Presented
in Partial Fulfillment of the Requirements for the
Degree of Master of Science
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December, 2002

For poikilothermic animals, and in particular those that inhabit aquatic habitats, temperature has a significant effect on all life processes. The purpose of this research was to investigate the contribution of temperature on embryonic development and survival and its effect on vital rates. Of particular interest was what aspect(s) of egg and larval life-histories are most affected by temperature and what consequence temperature effects may have on cumulative mortality.

Three batches of Atlantic cod (*Gadus morhua*) eggs were acquired from a Newfoundland source of adult broodstock held at 4.5-5.5°C. The eggs were divided andacclimated to four constant temperature regimes at approximately 2, 4, 8 and 12°C. Observations on development, growth and survival were made approximately every 4 degree days (mean temperature X number of days). Measurements taken included

development stage for egg and yolk-sac period larvae, standard length of larvae, yolk-sac area, and the mortality of eggs and larvae.

The analysis of size and development stage at hatch shows that the two measures are correlated. During the yolk-sac period, development-based changes in length largely eliminated any early differences in size. There was no relationship between size at hatch and the incidence of first-feeding larvae, but there was a dome-shaped relationship between temperature and the incidence of first-feeding.

Relationships between the environmental temperature and rates of mortality, growth, development and yolk-sac absorption were constructed. Data were collected from two synchronized experiments. Experiment 1 was designed to evaluate the effects of temperature on the development of eggs and then on the growth of larvae. While collecting development and growth information, the yolk-sac size, feeding incidence and the condition of larvae were also noted. Experiment 2 was set up to evaluate the effects of temperature on the mortality of eggs and larvae. Temperature affected egg development and mortality, and larval growth, yolk-absorption, feeding and larval mortality, all known as vital rates. Increasing temperature exponentially decreased the time it took to reach the point at which feeding is initiated. The post yolk-sac growth for all temperatures resulted in a log-normal relationship. From the growth-temperature relationship, the maximum slope, where the growth rate per degree is maximized, and a temperature where growth per day was maximized was calculated. The predicted temperature of maximum growth rate was 7.9°C, and the predicted temperature the growth rate per degree was maximized was 4.2°C. A linear regression model best described the rate of yolk reduction across the different temperatures. The mean survival time during the egg stage showed an

exponential decrease with temperature. The mean survival time for larvae resulted in a log-normal model, again with decreasing survival times with increasing temperature. The results are discussed in reference to normal temperature effects, which cause increasing vital rates with increasing temperature, and negative temperature effects, which changes the relative metabolic cost at different temperatures. Negative effects begin to dominate outside a optimal range of temperatures between 4-8°C. The temperature of maximum growth rate per degree is suggested as the optimal temperature for growth for larval cod in the field.

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TABLE OF CONTENTS

ACKNOWLEDGMENTS....ii

LIST OF TABLES....vii

LIST OF FIGURES....viii

Chapter

1. INTRODUCTION TO PROJECT

    1.1.Outline....1

    1.2. Literature Cited....2

2. EVALUATION OF SIZE AT HATCH, STAGE AT HATCH, EARLY GROWTH AND FEEDING OF ATLANTIC COD (*GADUS MORHUA*) LARVAE CULTURED AT DIFFERENT ENVIRONMENTAL TEMPERATURES....3

    2.1. Abstract....3

    2.2. Introduction....4

    2.3. Materials and Methods....7

        2.3.1. Egg collection and rearing protocol....7

        2.3.2. Sampling....9

        2.3.3. Data collection....10

        2.3.4. Staging table....10

        2.3.5. Statistical Analysis....14

    2.4. Results....15

2.4.1. General........15

2.4.2. Temperature effects........17

2.4.3. The influence of development on size at hatch........19

2.4.4. Influence of egg size........23

2.4.5. Size at hatch and temperature effects on performance........23

2.5. Conclusions........28

2.6. Literature Cited........35

3. THE EFFECT OF TEMPERATURE ON THE RATES OF DEVELOPMENT, GROWTH AND SURVIVAL DURING THE EARLY LIFE HISTORY OF ATLANTIC COD (*GADUS MORHUA*)........42

3.1. Abstract........42

3.2. Introduction........43

3.3. Materials and Methods........47

3.3.1. Feeding........48

3.3.2.Experiment 1: Development and Growth........49

3.3.2.1.Sampling procedure........49

3.3.2.2.Measurements........50

3.3.3.Experiment 2: Mortality........51

3.3.3.1.Egg period mortality........51

3.3.3.2.Larval period mortality........52

3.3.4.Data Analysis........52

3.3.4.1.Experiment 1........52

3.3.4.2.Experiment 2....53

3.3.4.3.General....54

3.4. Results....54

3.4.1.Experiment 1: Development and change in length....55

3.4.2.Experiment 2: Mortality....60

3.5.Discussion....66

3.6.Conclusions and Implications....72

3.7. Literature Cited....72

BIBLIOGRAPHY....78

BIOGRAPHY OF THE AUTHOR....87

LIST OF TABLES

Table 2.1. Explanation of methods used to differentiate between a stage of development (SOD) of 1, 2, 3 and 4 using the jaw structure, yolk-sac and head/gut staging characters (SC)....12

Table 2.2. The mean incubation temperatures (T) and the time to 50% (D50%) and 100% (D100%) hatch reported in both days (d) and degree days (dd; mean temperature×time in days) for each of the three batches of eggs....16

Table 2.3. The stage of development (SOD), size and yolk-sac area at hatch with associated standard errors in parentheses observed in each temperature treatment for batches 1, 2 and 3 (like letter subscripts = no significant difference in Bonferroni corrected pairwise comparisons from 1 way ANOVA for within each batch)....18

LIST OF FIGURES

Figure 2.1. Illustrations of the stage of development (SOD) for cod larvae, to be used in conjunction with Table 2.1 13

Figure 2.2. The standard length of larvae at hatch (circles) and using only larvae that were classified with a position of development of 4 (squares) in relation to incubation temperature 20

Figure 2.3. The standard length at hatch (A) and the accumulated growth (ΔL) of larvae from hatch to stage 4 (B) are presented for mean position of development (SOD) at hatch 21

Figure 2.4. The ratio of development time until 50% hatch to development time until all the larvae were stage 4 at the different development times to stage 4 24

Figure 25. Regression of the residuals from the standard length - stage of development (SOD) at hatch relationship at the different egg diameters 25

Figure 2.6. The incidence of first-feeding larvae over the standard length at hatch (A) and over temperature (B) for batch 1 (circles), batch 2 (squares) and batch 3 (triangles) 26

Figure 3.1. The stage duration (hr) of cod pre-feeding stages, from acclimation to stage 4 larvae, at different temperatures 56

Figure 3.2. The effect of temperature on the growth rate, in mm•day^{-1}, of cod larvae using only data from post yolk-absorption 58

Figure 3.3. The rate of yolk-sac area reduction at the different temperatures 59

Figure 3.4. Feeding incidence as the proportion of larvae with low, medium and high gut fullness from batch 1 at 2 (A), 4 (B), 8 (C) and 12°C (D) over time (days)....61

Figure 3.5. Feeding incidence as the proportion of larvae with low, medium and high gut fullness from batch 2 at 2 (A), 4 (B), 8 (C) and 12°C (D) over time (days)....62

Figure 3.6. Feeding incidence as the proportion of larvae with low, medium and high gut fullness from batch 3 at 2 (A), 4 (B), 8 (C) and 12°C (D) over time (days)....63

Figure 3.7. Kaplan-Meier estimated mean survival time (hr) for the egg stage at the different temperatures 64

Figure 3.8 Kaplan-Meier estimated mean survival time (hr) for the larval stage at the different temperatures 65

Chapter 1

INTRODUCTION TO PROJECT

1.1.Outline

The work reported in the present manuscript was part of a larger project developed to better understand possible interactions between cod stocks on the Scotian Shelf and Georges Bank. It has generally been believed that the Northeast Channel and current patterns at the Scotian Shelf/Georges Bank margin do not allow for mixing of the populations of cod from the two regions. Georges Bank cod spawn from January through June with eggs being released on the Northeast corner of Georges Bank and moving around the bank in a clockwise pattern. Due to the Coriolis effect, water moving south from the Scotian Shelf tends to be pulled around the tip of Nova Scotia and up into the Bay of Fundy. Recently, it has been discovered that large scale fluxes of cold (2°C), fresher water (30‰) from the Scotian Shelf are crossing over the Northeast channel and are being incorporated into the Georges Bank circulation pattern (Bisagni and Smith 1998). More importantly, this water contains significant numbers of cod eggs, indicating that perhaps some Georges Bank cod originate from Canadian waters. One of the goals of the project was to correlate strontium-calcium ratios (Sr/Ca) to the temperature history of laboratory reared cod larvae and use the information to infer the temperature history of larvae collected on Georges Bank and the Scotian Shelf. With this information, the relative contribution from both the Scotian Shelf and Georges Bank to the Georges Bank population of cod could be estimated and a better understanding of the interaction of the two water masses could be realized.

Published literature currently suggests a dome-shaped relationship between size of hatching larvae and the egg incubation temperature (Galloway et al. 1999; Pepin et al. 1997). This is viewed as significant because subtle differences in sizes of larvae are believed to impart large differences in survival probability (Miller et al. 1998). The differences in size are hypothesized to originate through metabolic mechanisms; smaller larvae may have deficiencies in metabolic processes during development which cause less energy to be transferred from the yolk-supply to growth (Pepin et al. 1997). Furthermore, these deficiencies could be carried into the larval stage, reducing growth rates of larvae and exposing them to higher predation risks.

1.2. Literature Cited

Bisagni JJ, Smith PC (1998) Eddy-induced flow of Scotian Shelf water across Northeast Channel, Gulf of Maine. Cont Shelf Res 18: 515-539

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Miller TJ, Crowder LB, Rice JA, Marshall EA (1988) Larval size and recruitment: towards a conceptual framework. Can J Fish Aqua Sci 45: 1657-1670

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Chapter 2

EVALUATION OF SIZE AT HATCH, STAGE AT HATCH, EARLY GROWTH AND FEEDING OF ATLANTIC COD (*GADUS MORHUA*) LARVAE CULTURED AT DIFFERENT ENVIRONMENTAL TEMPERATURES

2.1.Abstract

Temperature has a significant effect on marine fish larvae, particularly through affecting rates of development and the efficiency (growth/energy available) in transferring energy from endogenous and/or exogenous supplies to growth. A dome-shaped relationship between egg incubation temperature and the size of larvae at hatch for Atlantic cod (*Gadus morhua*) is hypothesized to be the result of the latter of the two processes. Yet the rule of equiproportional development (that life history stages occupy a constant proportion of the development time across all temperatures) has never been explicitly tested for developing cod. The purpose of this study was to determine whether the stage of development at hatch influenced the size of larvae at hatch and whether the proportion of larvae that failed to initiate feeding (a primary cause of early mortality in laboratory studies) correlated with the size at hatch and/or temperature. A staging table for yolk-sac larvae was developed and applied to assess the development stage at hatch. Larvae (n=20) were sampled from 50% hatch to first-feeding every 4 degree days for the 4, 8 and 12°C treatments and every day for the 2°C treatment. Data recorded included standard length, development stage, yolk-sac area and whether or not the larvae were feeding. The results indicate that the stage of development at hatch is correlated to larval size. During the yolk-sac period, development-based changes in length largely eliminated any early differences in size. There was no relationship between size at hatch and the

incidence of first-feeding larvae, but did find a dome-shaped relationship between temperature and the incidence of first-feeding. The results suggest that morphology and observations on development should be recorded, by the use of staging tables or brief descriptions of the larvae, in studies of size differences at hatch in larval fish.

2.2.Introduction

Temperature has been implicated as a major factor in both year-to-year fluctuations and long-term trends in fish populations (Bjornstad et al. 1999). There is a strong relationship between temperature and the rate of development (Pepin, 1991; Houde, 1989). When considered with the influence of stage-specific mortality in determining recruitment (Houde, 1997), temperature-induced differences in development times could be a cause for differential survival among thermal regimes. Another mechanism by which temperature may contribute to differences in short-term trends in mortality of cod larvae (*Gadus morhua*) is its influence on the size of larvae at hatch (Pepin et al. 1997; Galloway et al. 1998).

Laboratory studies have shown that higher egg incubation temperature produces smaller hatching larvae, which has been attributed to effects on critical phases of development (Laurence and Rogers, 1976; Blaxter, 1992; Canino, 1994; Pryor and Brown, 1998). Canino (1994) found that lower incubation temperatures produced the largest larvae with largest yolk-sac volumes and highest RNA/DNA ratios for walleye pollock (*Theragra chalcogramma*). Similar results were obtained by Buckley et al. (1990) for winter flounder (*Pleuronectes americanus*). Pepin et al. (1997) showed that Atlantic cod eggs incubated at -1 °C had reduced mean size at hatch (~2.9 mm), and at temperatures of 1 and 3 °C (~3.6 - 3.7 mm) compared to 5 and 7 °C (~4.0 - 4.1 mm). The

mean size at hatch for cod is 4.38 mm, with a range of 3.3-5.71 (Bigelow and Schroeder, 2002). Recent studies suggest a U-shaped relationship between size at hatch and incubation temperature (Chambers, 1997; Pepin et al. 1997; Galloway et al. 1998), as is seen in some meristic characteristics, such as vertebral and fin ray counts (see Blaxter, 1992).

Pepin et al. (1997) suggest an increased metabolic load incurred by embryos incubated at low temperature results in lost growth potential and a smaller length at hatch. This forms the current working hypothesis for smaller sizes at hatch at temperature extremes: as the upper and lower thermal limits are approached, an increased proportion of the maternal-origin yolk supply is spent powering a less efficient metabolism, which results in less energy for growth and hence smaller larvae. However, an unsubstantiated assumption made in studies that observe different sizes at hatch is that all larvae hatch at the same stage of development. The large variation in size of hatching larvae (2.4 - 6.1 mm on the Scotian shelf; Miller et al. 1995) brings into question the validity of this assumption. Slight differences in the timing of hatch may have an influence on the size of larvae. O'Connell (1981) states that size, rather than age, best predicts the state of larval development. Given the possibility that larvae may not hatch at identical stages of development, there are two hypotheses by which temperature may affect the size of hatching larvae: (1) at temperature extremes the yolk-stores are depleted more quickly and/or earlier in development resulting in less available energy for growth and therefore, smaller larvae, and (2) difference in temperature, or the degree of change relative to the original conditions, during incubation cause larvae to hatch at different stages in development (violation of the equiproportional rule) which in turn causes apparent size

differences. An analogy recognized from human biology for the latter hypothesis would be the distinction between low birth-weight and prematurity at birth,.

Research has demonstrated differences in survival among differing sizes of larval fish (see Miller et al. 1988). Larger larvae are generally equated with increased survival (Bailey and Batty, 1984; Blaxter and Batty, 1985; Blaxter, 1986; Eaton and DiDomenico, 1986; Webb and Weihs, 1986; Butler and Pickett, 1988; Miller et al. 1988; Fuiman, 1989). However, smaller size has been shown to reduce susceptibility to predation in some studies (Litvak and Leggett, 1992; Pepin et al. 1992). Furthermore, prey size selection by predators, which operates directly on size differences among individual prey (Rice et al. 1993), can alter population growth rates (Meeken and Fortier, 1996). The observation that temperature affects larval size is important when viewed in the context that there is a different survival potential for individuals of different sizes.

Laboratory-based studies have been instrumental in discerning the effects of temperature on larvae because the larval stage of marine fish is difficult to sample effectively in the field. Field observations involving the relationship between temperature and size at hatch, egg diameter, and otolith size were made by Miller et al. (1999). Cod eggs were sampled from the Nova Scotian shelf and hatched on board a research cruise. Miller et al. (1999) attempted to develop a predictive relationship between the otolith radius and body size at hatch. This relationship would allow better understanding of the interaction among size at hatch, the size of surviving individuals and size-selection throughout the larval period (see Meeken and Fortier, 1996; Miller et al. 1999). By using otoliths from field captured larvae to back-calculate initial size distributions and comparing these among larvae sampled at different times, it would be possible to observe

differences in the size distribution over time and relate it to survival. However, Miller et al. (1999) found a weak, non-predictive ($r^2 = 0.35$) relationship between otolith size and size at hatch, and a strong negative relationship between size at hatch and temperature and a positive relationship between size at hatch and egg size.

The primary purpose of this research is to use laboratory-based larval rearing techniques in order to determine whether size at hatch and developmental stage at hatch are correlated. This approach tests whether the assumption that larvae are of equal developmental stage at hatch is valid at different temperatures. The results will be discussed in the context of temperature and size effects on survival. In addition, this experiment will be compared to the size at hatch, temperature and egg size relationships presented in Miller et al. (1999). The comparison will be discussed in terms of the possible causes of a weak relationship between otolith size and size at hatch.

2.3. Materials and Methods

2.3.1. Egg collection and rearing protocol

Naturally spawned eggs were collected from the captive broodstock held at the Ocean Sciences Center (OSC), Logy Bay, Newfoundland, Canada. The broodstock were maintained between 5 and 6.5 °C year-round. Three batches of eggs were collected, disinfected and shipped to the Aquaculture Research Center (ARC) on campus at the University of Maine in Orono, ME. Two batches of eggs were transported in 1999 and one in 2000. The transport water temperature and salinity upon arrival at the ARC were 7.7 °C and 31.4‰ in 1999, and 6.1 °C and 31.6‰ in 2000. In neither year was there any ammonia present in the transport seawater. Once at the ARC, each group of eggs was

treated with a 400 ppm gluteraldehyde solution for 10 minutes in order to disinfect the eggs.

Four recirculating seawater systems, each containing four separate 75L tanks, were constructed to rear eggs and larvae. They were randomly assigned temperatures approximating 2, 4, 8 and 12°C. Initially the eggs were divided into four groups and placed in 4L plastic bags with water at equal temperature to that of the water upon arrival. Two bags were allowed to acclimatize to 4°C and two bags to 8°C for 4 hours. Next, one bag was removed from 4°C and placed into 2°C and one was removed from 8°C and placed into 12°C, again for 4 hours. All bags received slight aeration throughout. Each system had one tank stocked with 35 mls of eggs for batch 1, 40 mls of eggs for batch 2 and 35 mls for batch 3.

The 75L tanks were fitted with external stand-pipes and an outflow located centrally in the bottom of the tank. A screened (150μm) 2" pipe acted as the barrier at the outflow. At the base of the screened pipe, perforated Nalgene™ tubing was connected to air supply which served to keep eggs and larvae from impacting the screen and generally maintained even distribution of eggs and larvae in the tank. Water flow rates were maintained at 700 mls/min.

Each temperature treatment was maintained in a separate re-circulating system (See Appendix B for system design) using Honeywell™ T775 temperature controllers modulating the flow of chilled glycol (-12°C) through a heat exchanger. Temperature was recorded every 5 minutes using a temperature logger (Onset™ StowAway tidbit +23°F to +99°F model) placed in every tank. Photoperiod was set on a 16hr-8hr light-dark cycle. The lights were set to reach a maximum of 350 lux, at mid-day,

approximating a sine curve in order to simulate a natural light regime, with no light during the dark period of the cycle. Temperature, salinity and dissolved oxygen were checked daily. Water quality (ammonia and nitrite) was tested every second day. Salinity was maintained between 29.0 and 31.5‰ for all experiments.

Rotifers (*Brachionus* spp.) were supplied at 100% hatch onwards. Feedings were done during the light period, 6 times per day for batch 1 and batch 2 and every hour for batch 3. Prey densities were maintained between 8-10/ml, in excess of levels recommended for growth and survival throughout development (Puvanendran and Brown, 1999).

2.3.2.Sampling

Sampling times were set according to 4 degree-day periods (degree day = mean temperature×number of days), except for the 2 degree treatment which was sampled daily. Thus, larvae were sampled at 06:00, 14:00 and 22:00 for the 12 degree treatments, 10:00 and 22:00 for 8 degree treatment and 22:00 for the 2 and 4 degree treatments. At these sampling times, tanks were observed quickly using a flashlight to ensure a roughly even distribution of eggs/larvae throughout the water. Random samples were taken from the tank, using a 200 ml beaker, until 20 eggs/larvae had been removed. When larvae first made up greater or equal to 9/20 of the sample, it was considered 50% hatch. Larvae were then removed until 20 larvae were sampled.

Subsequent samples were taken on the above schedule, with 20 larvae collected each sample period. 100% hatch was assigned as the time no eggs were seen of the 20 sampled. Sampling was continued on the "4 degree-day" schedule until there were 9 or more fish in the sample with food in their guts, which was considered 50% feeding.

Thereafter, samples were taken once per day at 22:00 for the 12 degree and 8 degree treatment, once every 2 days for the 4 degree treatment and once every 4 days for the 2 degree treatment.

2.3.3.Data collection

Sampled larvae were gently placed by pipette on a 1 ml welled slide, etched with a 1 mm^2 grid and immobilized and euthanized with tricane methanesulfonate (MS-222). Images of the sampled eggs and larvae were recorded using a Flash Point 128™ frame grabber (Integral Technologies) paired with a dissecting microscope. The 1 mm^2 grid served to calibrate the image while calculating measurements. The larvae were then measured to the nearest 0.01mm using Image Pro Plus™ V4.0 (Media Cybernetics). Egg diameters were calculated from eggs just prior to hatch using measurements from two perpendicular axes. One was taken along developing larval body and the other perpendicular to this. Standard length measurements were the longest lengthwise axis, from the end of the notochord to the most anterior part of the head, excluding the lower jaw. The number of larvae holding food in the gut was also noted. The incidence of first feeding was calculated (number of feeding larvae/total number sampled) once a tank had no increase in the number of feeding larvae. Three consecutive evening samples were pooled to calculate the mean incidence of feeding larvae for that treatment.

2.3.4.Staging table

In order to create a staging table, digital images were used in concert with larval stages based on Fridgeirsson (1978) and Hunt von Herbing et al. (1996). It was important that the stages could be quickly identified, provide a relatively accurate description of the larvae and identify ecologically relevant steps during development (yolk absorption, first-

feeding). Three staging characters, believed to reflect the majority of the changes in gross morphology, were used. These were (1) head morphology, (2) yolk-sac morphology and (3) position of head relative to body (Figure 2.1, Table 2.1). For individual larvae, each of the three characters received a value (Table 2.1). The average of the three staging characters was taken to represent the overall "stage of development (SOD)", a continuous variable. The SOD measure used for the remainder of the manuscript is meant as a quantitative measure of development. That is, it describes an individual based on the average of three characters and is because of that different than a "staging table", which is a qualitative assessment of development. Stages will be used to refer to the level (1-5) given to each staging character, while the SOD is the mean of the stages assigned to the three characters. The illustrations in Figure 2.1 represent the last point considered to be at that stage. When a staging character was difficult to discern, appearing to be intermediate, the greater (more developed) stage was chosen. When larvae were observed with eyes that were not fully pigmented they were considered premature. The egg staging table used in this experiment defined the last stage prior to hatch as those with full eye pigmentation. Larvae were classified up to a stage 5 (which is not included in the staging table). Stage 5 was identified using the following characters: (1) the lower jaw projects out past the edge of the head, (2) the swim-bladder can be identified as a small silver sack with black pigmentation on the dorsal edge and (3) the yolk-sac is extremely reduced. Beyond stage 5, larvae grew at different rates and the high variation in morphology among and within individuals made staging large numbers of individuals impractical.

Table 2.1. Explanation of methods used to differentiate between a stage of development (SOD) of 1, 2, 3 and 4 using the jaw structure, yolk-sac and head/gut staging characters (SC).

SC	SOD = 1 if	SOD = 2 if	SOD = 3 if	SOD = 4 if
Jaw	Rounded, mouth covered by oropharyngeal membrane	Rectangular with flattened surface ventral to eye, mouth may be visible	Sharp jaw angle located under eye, mouth ventral	Jaw angle posterior to eye, mouth equal with plane of most anterior part of head
Yolk-sac	Integument surrounding yolk sac attached to anterior part of developing jaw structure, yolk-sac in contact with head	Integument connected to head but now on posterior edge, yolk-sac no longer in contact with head, space created between head and yolk-sac within surrounding integument	Integument connected to posterior of head, yolk-sac reduced, the space visible in Stage 2 is now minimal	Separation of integument from head by structures on ventral surface of larvae, yolk-sac smaller than gut
Head / gut	Deflected downwards / straight constricted tube, therefore, eye is located beneath plane of gut	Intermediately deflected / straight tube inflated with presence of rectal valve, therefore dorsal edge of eye is approximately at middle of gut	Little or no deflection, rectal valve has formed a clear constriction in intestine, therefore dorsal edge of eye approximately at dorsal edge of gut	No deflection / Intestine well convoluted (not seen). Larvae should be feeding and prey should be present in gut.

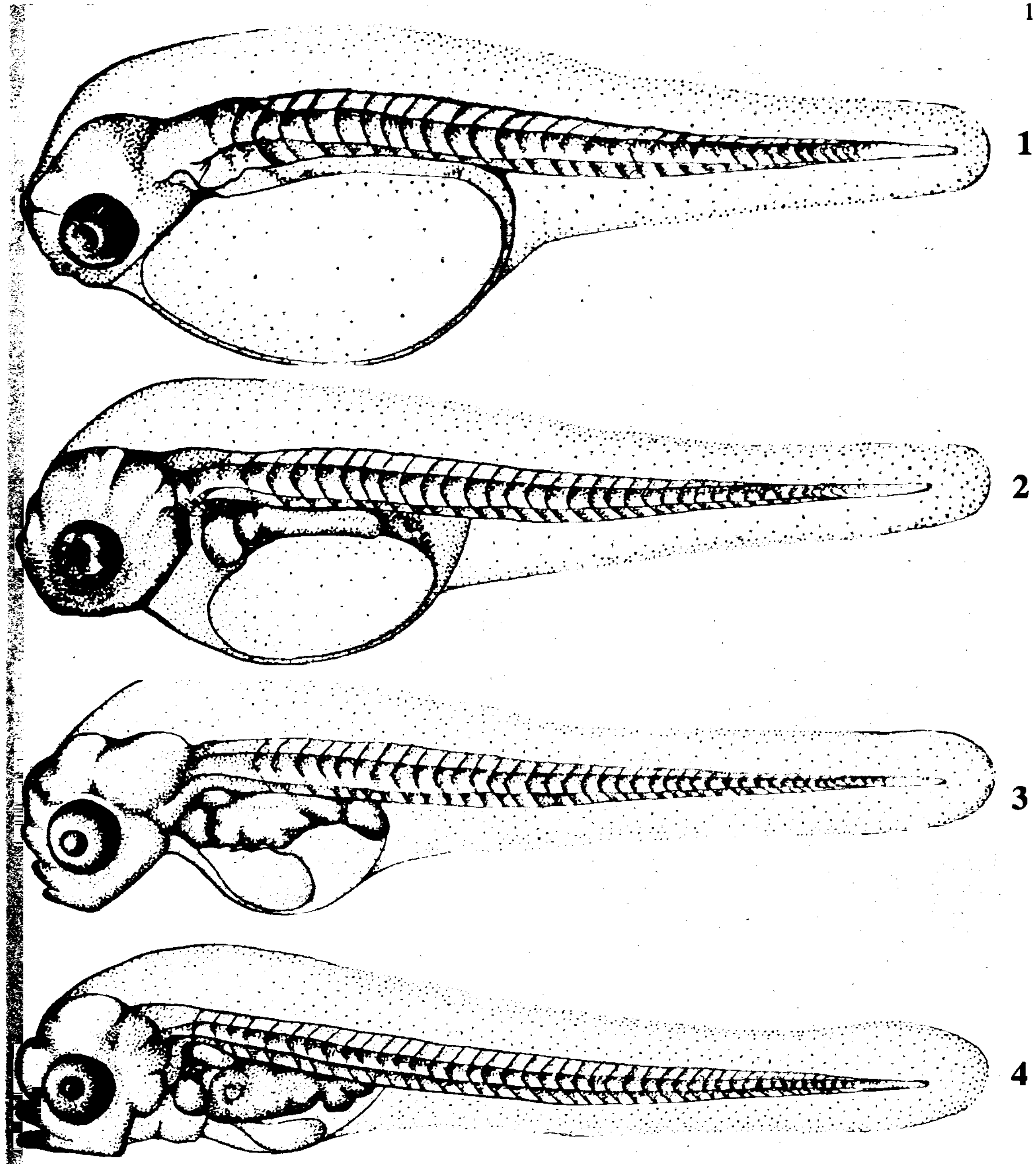


Figure 2.1. Illustrations of the stage of development (SOD) for cod larvae, to be used in conjunction with Table 2.1.

Represented are the last point where a larva would be classified with a SOD of 1, 2, 3 and 4 for all staging characters. Larval illustrations represent a SOD of 1, 2, 3 and 4 from top to bottom.

Scientific illustration methods outlined in Weitzman and Dalen (1989) and Wood (1979) were used. Accurate pen and ink illustrations were created with the aid of the 1 mm^2 microscopic grid and dividers.

2.3.5.Statistical Analysis

The data for standard length, stage and yolk-sac area at hatch were first compared using a one-way analysis of variance (ANOVA), testing for temperature effects within each batch. The mean standard length and SOD at hatch for each tank, calculated in the ANOVA analyses, were then tested to determine whether correlations were present among size at hatch, the stage of development at hatch and temperature. The mean size at hatch for each tank was also tested once all larvae had achieved an equal stage to determine the relationship between temperature and size, independent of stage. For this, all the larvae that were given a SOD classification of 4 during analysis of pictures were pooled and a mean size (SL4) was calculated for each tank.

To determine whether there was any relationship between the development stage at hatch and subsequent growth, the accumulated growth ΔL) from hatch until all larvae were stage 4 were also correlated. ΔL was calculated for each batch and temperature treatment by subtracting the mean size at hatch for each tank from the mean size calculated from all larvae whose SOD was classified as 4 (SL4). The proportion of time to 50% hatch over the time until all larvae were stage 4 will also be used to determine if the larvae are hatching at an earlier stage.

The importance of egg size on the size at hatch will be included by using the residuals from the standard length at hatch versus the stage of development relationship. If the egg diameter affects the size of the larvae independent of developmental influence,

the result should be a positive relationship between the residuals and the observed egg diameters.

The incidence of first feeding larvae was compared to the size at hatch and incubation temperature in order to determine whether size or temperature correlate with the performance of larvae at feeding.

Linear and non-linear models were solved by means of the least-squares method. The normality of the residuals was tested using the Kolmogorov-Smirnov test and homogeneity of the variance was tested using the Levene Median test. The significance level, α, was set at 0.05. Both normality and constant variance as well as the type II error (β) were monitored and are reported when significant. Multiple comparisons within the ANOVA model, testing where significant differences occurred between size, stage and yolk-sac area within each batch, were made using Bonferroni corrected alpha values (α'). α' was calculated by dividing 0.05 by the number of comparisons (k) for each measurement (Sokal and Rohlf, 1995), in this case 4 temperatures (k=4). All reported p-values are Bonferroni adjusted. The ANOVA analysis was completed using SYSTAT™ (ver 9.0) and the regression analyses using Sigmaplot™ 2001 for Windows™ (ver 7.0).

2.4.Results

2.4.1.General

The incubation statistics are presented in Table 2.2. Upon arrival to the ARC facility, Batch 2 arrived at a later stage in development, which is reflected in shorter incubation times compared to batch 1 and 3 for all temperature treatments. Egg diameters (n=20 for each batch) were 1.38, 1.27 and 1.19 mm for batches 1, 2 and 3, respectively. In all batches, there was a consistent trend of decreasing time to 50 and 100% hatch with

Table 2.2. The mean incubation temperatures (T) and the time to 50% (D50%) and 100% (D100%) hatch reported in both days (d) and degree days (dd; mean temperature×time in days) for each of the three batches of eggs.

	Batch 1			Batch 2			Batch 3		
Treatment	T (°C) ± SD	D50% (d / dd)	D100% (d / dd)	T (°C) ± SD	D50% (d / dd)	D100% (d / dd)	T (°C) ± SD	D50% (d / dd)	D100% (d / dd)
2	1.9 ± 0.11	31 / 59	32 / 61	2.1 ± 0.05	15 / 32	17 / 36	0.93 ± 0.25	34 / 32	36 / 33
4	4.0 ± 0.07	21 / 84	23 / 92	3.9 ± 0.05	12 / 47	14 / 55	3.5 ± 0.17	20 / 70	23 / 81
8	8.1 ± 0.09	11.5 / 93	11.5 / 93	8.0 ± 0.13	6 / 48	7.5 / 60	8.1 ± 0.05	9.5 / 77	10 / 81
12	12.5 ± 0.09	6.3 / 79	7.3 / 91	12.5 ± 0.12	4 / 50	4.3 / 54	12.4 ± 0.05	6 / 74	6.3 / 79

increasing temperature. This trend was not seen when time was expressed as degree-days. There was no consistent trend in time interval between 50% and 100% hatch, which might indicate a difference in the hatching period between treatments. The time interval ranged from 0 days in the 8°C treatment (batch 1) to 3 days for 4°C treatment (batch 3). Thus, it is not believed that using either 50% or 100% hatch as the "time of hatching" is incorrect. The time of hatch is seen as somewhat arbitrary. Premature larvae (non-pigmented eyes) were present earlier than 50% hatch, but all larvae at 50% hatch had a mean stage of development (SOD) of at least 1. Larvae that reached a mean SOD of 5 and were not feeding began to show signs of starvation.

2.4.2.Temperature effects

Within batch variation was tested with one-way ANOVAs. There were significant temperature treatment effects on the stage of development (SOD) and standard length at hatch for batch 1 and 3 ($p < 0.001$), but not for batch 2 (Table 2.3). For batch 1 and 3, larvae in the warmer temperature treatments had a smaller mean SOD and standard length at hatch (Table 2.3). Batch 2 showed no trend in either size at hatch or SOD at hatch across the temperature treatments (Table 2.3). Combining data from all three batches, the relationship between the SOD at hatch and temperature (T) was: $SOD = 2.3(0.14) - 0.053(0.02)*T$ ($r^2 = 0.46$, df = 11, p-value =0.015). The lack of differences in size and SOD at hatch for batch 2 (Table 2.3) resulted in a low statistical power (β) at $\alpha = 0.05$ of 0.70 for the SOD at hatch-temperature regression and reduced r^2 values. From these analyses, incubation temperature significantly affected yolk-sac area for all batches ($p < 0.004$; Table 2.3). Batch 2 showed no trend in the yolk-sac area across the

Table 2.3. The stage of development (SOD), size and yolk-sac area at hatch with associated standard errors in parentheses observed in each temperature treatment for batches 1, 2 and 3 (like letter subscripts = no significant difference in Bonferroni corrected pairwise comparisons from 1 way ANOVA for within each batch).

	Batch 1			Batch 2			Batch 3		
Treatment	SOD	SL at hatch (mm)	YS area (mm^2)	SOD	SL at hatch (mm)	YS area (mm^2)	SOD	SL at hatch (mm)	YS area (mm^2)
2	2.1 (0.2)^{b}	4.44 (0.26)^{b}	0.53 (0.15)^{a}	1.9 (0.2)^{a}	4.12 (0.19)^{a}	0.60 (0.10)^{b}	2.5 (0.3)^{b}	4.44 (0.17)^{c}	0.37 (0.14)^{b}
4	2.3 (0.4)^{c}	4.48 (0.20)^{b}	0.36 (0.07)^{c}	1.8 (0.4)^{a}	4.04 (0.24)^{a}	0.54 (0.09)^{ab}	2.5 (0.3)^{b}	4.31 (0.25)^{c}	0.42 (0.17)^{b}
8	2.1 (0.2)^{b}	4.41 (0.12)^{b}	0.47 (0.05)^{b}	1.9 (0.3)^{a}	3.95 (0.11)^{a}	0.42 (0.18)^{a}	1.7 (0.3)^{a}	3.71 (0.15)^{b}	0.55 (0.19)^{b}
12	1.3 (0.2)^{a}	3.96 (0.13)^{a}	0.66 (0.19)^{a}	1.9 (0.2)^{a}	4.04 (0.14)^{a}	0.53 (0.13)^{ab}	1.7 (0.4)^{a}	3.99 (0.20)^{a}	0.79 (0.32)^{a}

temperature treatments. Batches 1 and 3 had the largest yolk-sac areas associated with the smallest larvae at the earliest stages of development.

The regression of the standard length at hatch (from Table 2.3) against incubation temperature resulted in a weak significant negative relationship (Figure 2.2). In order to eliminate the effect of the different SOD at hatch, only stage 4 larvae were regressed against the incubation temperature (Figure 2.2). By utilizing the mean size of stage 4 larvae (SL4) in the regression analysis of standard length and incubation temperature, the explained variance was increased by 42% while the slope of the regression was reduced by 22% (Figure 2.2). The trend evident in both these analyses is that, at comparable points in development, larvae from warmer temperature treatments were slightly smaller in size.

2.4.3. The influence of development on size at hatch

There was a significant positive relationship between the standard length at hatch and the SOD at hatch (p-value = 0.0063; Figure 2.3A). There was also a significant negative relationship between the SOD at hatch and the ΔL, or accumulated growth from hatch to stage 4 (p-value = 0.0018; Figure 2.3B). In other words, larvae that hatch at later development stages undergo the least change in standard length from hatch until the time of first-feeding.

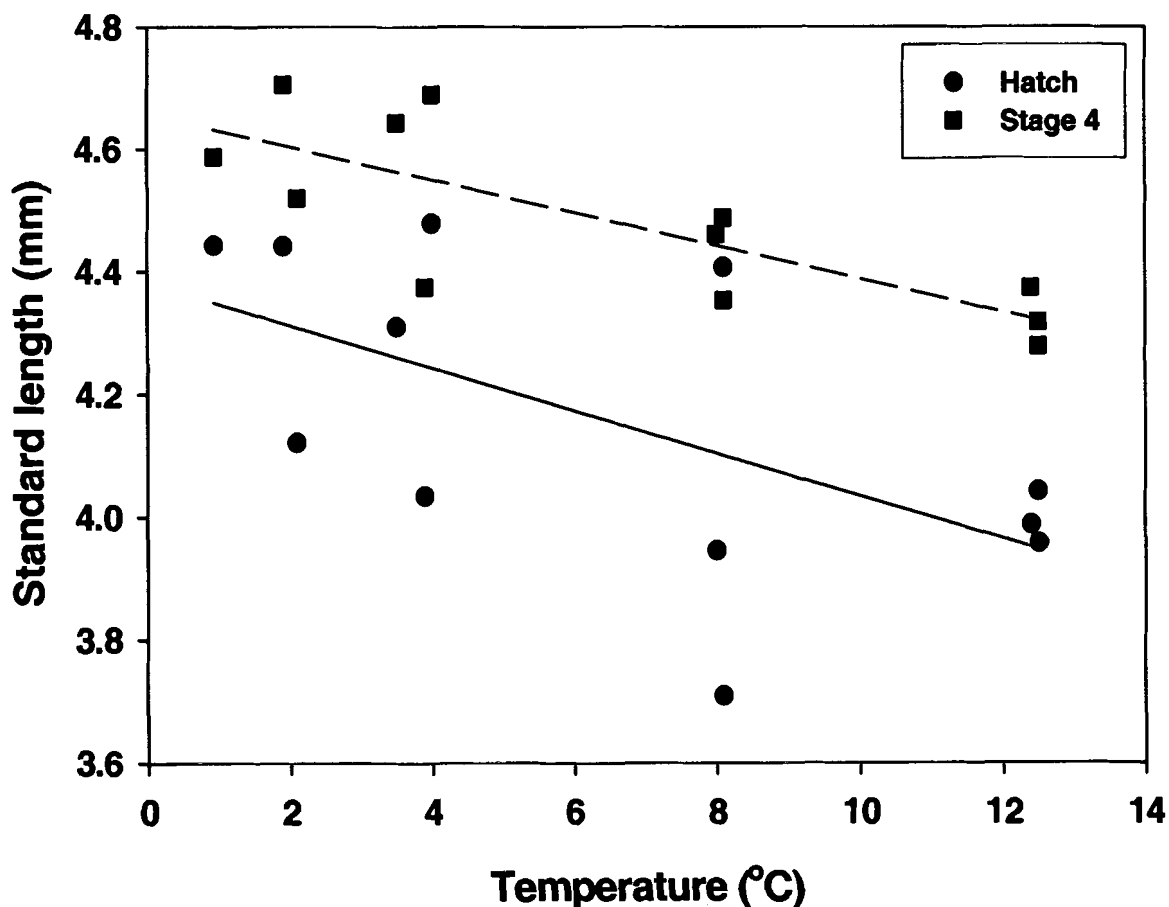
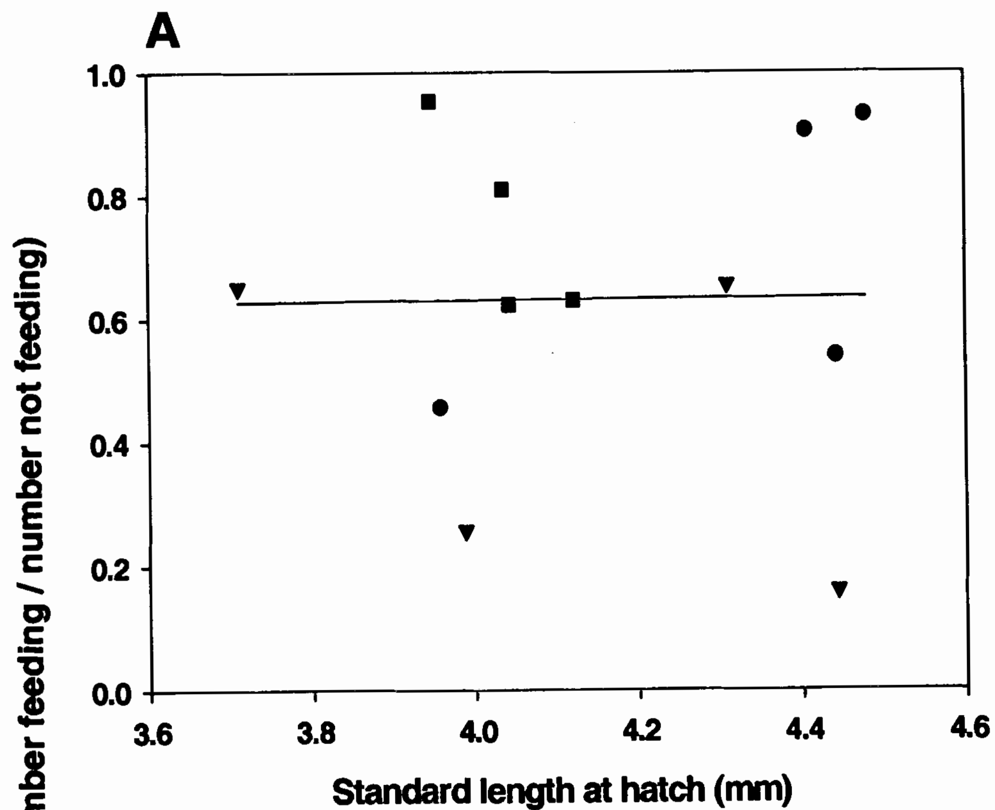


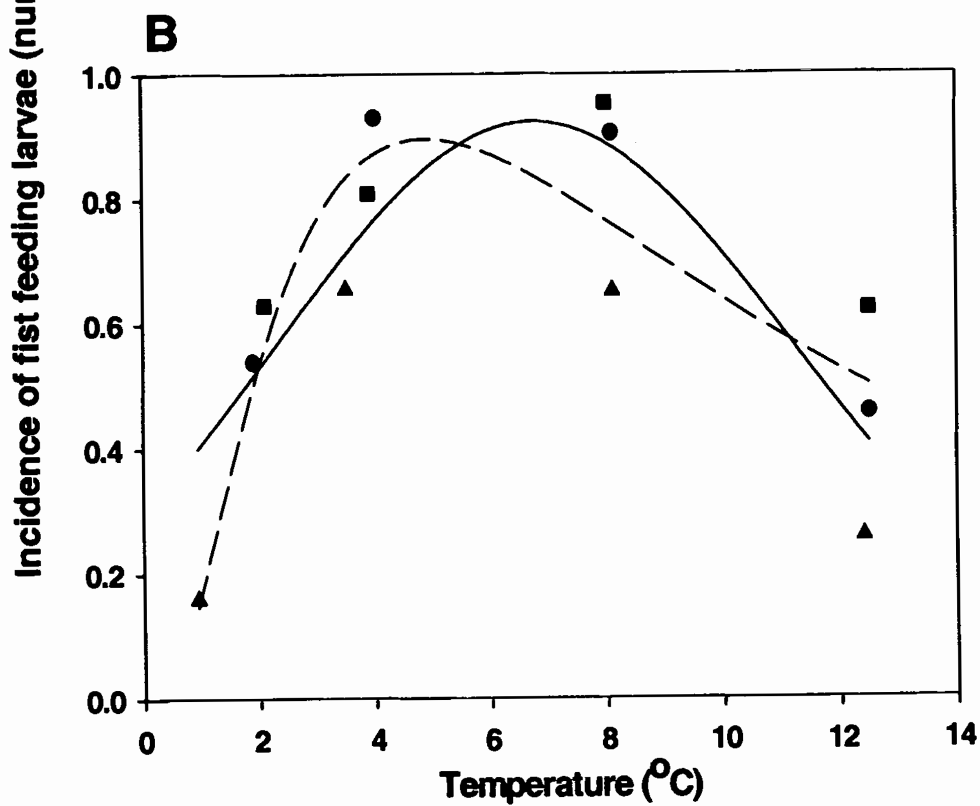
Figure 2.2. The standard length of larvae at hatch (circles) and using only larvae that were classified with a stage of development of 4 (squares) in relation to incubation temperature.

Relationship for standard length at hatch (SL) over the incubation temperature (T) given by linear regression, with standard error of the estimates in parentheses: SL = 4.38 (0.11) - 0.03 (0.15)*T (r^2 = 0.36, df = 11, p-value = 0.0391). The standard length using all observed stage 4 larvae (SL4) against the incubation temperature resulted in the regression equation, with the standard error of the estimates in parentheses: SL4 = 4.66 (0.05) - 0.03 (0.007)*T (r^2 = 0.62, d.f = 11, p-value = 0.002).

Figure 2.3. The standard length at hatch (A) and the accumulated growth (ΔL) of larvae from hatch to stage 4 (B) are presented for mean stage of development (SOD) at hatch.

For A: Shown are results of linear regression of standard length (SL) against SOD, with standard error of estimate in parentheses: $SL = 3.09(0.31) + 0.54(0.16)*SOD$ ($r^2 = 0.54$, d.f. = 11, p-value = 0.0063). **For B:** Shown is the linear regression of accumulated growth (ΔL) from hatch until all larvae had reached stage 4 against the mean SOD, with standard error of estimate in parentheses: dashed line, $\Delta L = 1.01(0.16) - 0.35(0.08)*SOD$ ($r^2 = 0.64$, d.f. = 11, p-value = 0.0018).


A
1.0
0.8
0.6
0.4
0.2
0.0
3.6
3.8
4.0
4.2
4.4
4.6
Standard length at hatch (mm)
Incidence of fist feeding larvae (number feeding / number not feeding)


B
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
12
14
Temperature (°C)

2.5. Conclusions

The results of this study suggest that temperature induced differences in size at hatch largely originate from differences in development stage, or the SOD, at hatch for Atlantic cod. Therefore, larvae with a small size at hatch are actually at an earlier stage in their development. Moreover, from the time of hatch these small individuals increased in size more than larger individuals (later stage of development), resulting in a reduction in the slope of the temperature-size relationship by the time of first-feeding (equal stage of development). The growth after hatch served to counteract size at hatch effects and reduce the size variation among individuals by first-feeding. Prior to first-feeding, developing larvae (pre and post-hatch) are dependent on endogenous energy stores. The observations that size variation at hatch is largely due to differences in stage at hatch and that, by first-feeding, size variation is much reduced indicates less depletion in these endogenous reserves than would be estimated based solely on observations of size at hatch. When comparing stage-specific development rates, the stages of development being compared must adhere to the equiproportional rule (Peterson, 2001). The equiproportional rule states that the duration of life history stages must be a constant proportion of the embryonic development time, across all temperatures (Peterson, 2001). This assumption was also not supported when using time to hatch to define a life-history period. Of the two proposed mechanisms by which temperature may affect the size of hatching larvae, quickly depleted yolk-stores and less energy available for growth at temperature extremes versus differences in development stage and thus size at hatch, this study's results provide evidence in support of the latter hypothesis.

The ability to initiate feeding is heavily influenced by morphological or biochemical flaws. Sub-lethal effects, such as growth differences, dominate thereafter as energetic efficiency exerts a larger influence on larvae (Rombough, 1997). The functional inability of larvae to feed in the extreme temperature treatments (2°C and 12°C) in the present study reflects an accumulation of flaws during development. The larvae from the extreme temperatures were commonly observed with locked jaws and persistent constrictions in the gut (pers. obs.) that negated the ability to feed and eventually caused death by starvation. After larvae progress into the feeding period (stage 4), net energy gain or growth is a function of the rates of ingestion and assimilation and the expenditure necessary to capture prey.

The lack of correlation between successful first-feeding and size at hatch can be explained by the presence of small under-developed larvae at higher temperatures, larger well-developed larvae at lower temperatures and the failure of large numbers of larvae at both temperature extremes to feed. Because only a restricted range of temperatures allowed for a high frequency of feeding larvae, observed temperature effects on size likely occur beyond the range of temperatures that allow for normal development. This supports the inclusion of some measure of post-embryo fitness when conclusions regarding environmental effects on egg stages are being considered. Subtle changes in the observed viability during egg development can translate into large effects when the larvae are pressed to actively forage.

In the present study, the predicted maximum values for the incidence of first-feeding, 4.9 and 6.8°C, are very close to the range of temperatures experienced by the broodstock (parents), 5.0 to 6.5°C. Since the maternal temperature history is believed to

influence the upper lethal thermal limit of larval fish (Rombough, 1997), the maternal temperature history, in relation to the temperature experienced by eggs and larvae, may have a significant effect on relationships observed at hatch. This is particularly important if the maternal temperature prior to the release of gametes gives larvae different abilities in the post-fertilization environment. Although the correlation suggests a relationship between parental temperature history and the larval thermal optima is possible, there is also the indication that recruitment is better in temperatures closer to the middle of the species temperature range (Sundby 2000) between 4-9°C (Brander 1995). Genetic and environmental effects have not been thoroughly investigated and there is evidence to support both genetic-based differences in juvenile growth and conversion efficiency (Purchase and Brown 2000) between Gulf of Maine and Grand Bank stocks. There is also evidence of no difference between Norwegian coastal cod and northeast Arctic cod stock larval and early juvenile growth (Otterlei et al. 1999).

The influence of differing spawning events is evident in the difference in the response of batch 2 compared to batches 1 and 3. Batch 2 had no change in the size at hatch and stage at hatch across the incubation temperatures, while batches 1 and 3 did. As previously discussed, batch 2 was collected and subsequently exposed to temperature treatments at a later point in development and as a result had shorter incubation times under the experimental treatments. The two possible causes of the limited effect of temperature on size and stage of larvae in batch 2 are (1) larvae from different spawning events (different parents, genetic material) have different responses to temperature change or (2) the eggs were collected past a critical period in development where temperature influences sizes at hatch. The possibility of either case suggests a large

temperature influence on individual survival during early life. Larvae from different spawning events that encounter different environmental conditions may have dramatically different outcomes with regard to early life-history traits. The results highlight the importance of continued study of temperature effects during early stages of development in fish.

The differential influence of temperature on different egg batches could have contributed to the fact Miller et al (1999) only generated a weak relationship between size of hatching larvae and the otolith size. In that study, the use of a common incubation temperature after collecting eggs from varying temperature regimes (from different sampling stations and depths, see Miller at al. (1999) for details) could have contributed to increased variation in the size of hatching larvae. Differences in the temperature change encountered in the time between sampling and incubation could have biased observed temperature effects because the direction and magnitude of temperature change was not constant between individuals. The influence of development stage on size would be increased by the presence of premature larvae (those lacking eye pigmentation). This would help explain observations of extremely small larvae such as those reported by Pepin et al. (1997) and the wide range of sizes reported by Miller et al. (1995).

Our data show that the egg diameters must be taken into account in studies of the early life stages of cod. Miller et al. (1999) showed that egg diameter significantly affects size at hatch and obtained the following correlation between standard length at hatch (SL) and egg diameter (ED): $SL = 0.656 + 2.55*ED$ ($n=128$, $r^2= 0.345$, p-value = 0.0001). By using the egg diameters from the present study and the relationships between egg size and standard length defined in Miller et al. (1999) the predicted standard length for batches 1,

2 and 3 were 4.19 mm, 3.89 mm and 3.68 mm, respectively. The relationship given by Pepin (1991) is: $SL = 2.89ED^{0.89}$ ($r^2 = 0.62$, $n = 187$, p-value < 0.01). It is important to note that this equation was the result of a cross-species meta-analysis, which in conjunction with data from this experiment, resulted in predicted lengths at hatch of 3.86 mm, 3.57 mm and 3.36 mm for batches 1, 2 and 3, respectively. These relationships generate a few points. First, that over the range of egg sizes reported in this study cod appear to hatch at a size slightly larger (~10%) than the egg size predicted from many species. Second, the values derived from the Pepin (1991) equation were 92.2%, 91.7% and 91.4% the values derived from Miller et al. (1999) for batches 1, 2 and 3 respectively, showing the consistent predictive ability of both studies. The Miller et al. (1999) model is likely reasonably predictive despite only accounting for 35% of the total variance in that study. The reason for the high unexplained variance of that study is because they were not able to account for differences in the stage of developmental at hatch, which is potentially a much larger and more unpredictable cause of differences in size at hatch.

The findings of this study bring to light several considerations for future research on larval fish. In the present study, development strongly influenced the size of larvae at hatch. Reporting the morphology of hatching individuals is therefore necessary to explain differences in larval size at hatch and the growth experienced in early life. Consequently, the timing of sampling will strongly influence any initial measurements of standard length. It is suggested that standard length measurements be coupled with some description of the morphology of larvae, either by using a staging table or by brief description (ie: head deflected downward, eyes fully pigmented, etc). Caution should be

taken when making generalizations about yolk-sac absorption rates and differences in length. The ecological relevance of differences in these types of measures should be scaled back if based on one sample at hatch. In addition, it could be instructive if attempts were made to relate the maternal temperature history and temperature changes during development to observed survival. All temperature changes from egg collection through incubation and rearing should be reported, where possible, in larval fish studies.

Much of the research on larval fish is motivated by the ultimate goal of predicting fluctuations in population abundance for the purpose of fisheries management. For this reason, it is critical to consider the results of a study on growth and ontogeny in terms of survival of larval fish. Factors that influence survival at the time around hatch are particularly important since extremely high mortality rates have been associated with the yolk-sac period of marine fish (Hewitt at al. 1985; Taggart and Leggett, 1987). It is surmised that the primary source of this mortality is predation (Leggett and Deblois, 1994). High predation rates may be drastically reduced over the course of the first few days post-hatch because of rapid increases in length and/or behavioral changes associated with ontogeny (Bailey and Batty, 1984; Blaxter and Batty, 1985; Blaxter, 1986; Eaton and DiDomenico, 1986; Webb and Weihs, 1986; Butler and Pickett, 1988; Fuiman, 1989). This suggestion matches the present observation that cod larvae that hatch with the head deflected downwards, in the form of stage 1 larvae, then experience initial rapid growth. In this study, the elevation of the larval fish head during the transition from stage 1 to 2 primarily determined the apparent rapid growth in length of larvae during early development (see Figure 2.1). It is therefore more accurate to interpret the observed

initial increase in length as rapid ontogenetic change. These changes are part of normal yolk-sac period development.

Length differences of larvae at hatch could easily be misinterpreted as differences in actual size rather than stage-based morphology differences. This misinterpretation might lead to the assumption that smaller individuals are at a competitive disadvantage, needing to catch up to larger individuals by consuming more prey and transferring it to somatic tissue growth. However, the reality is that small larvae will catch up in length, to a large degree, through ontogenetic changes. Hunt von Herbing et al. (1996) suggested that early cod growth is controlled by intrinsic genetic factors, progressing to more environmentally determined growth as the larvae age. These intrinsic factors are involved in the process of early length differences between temperatures where larvae hatch at different development stages and then develop and increase in length by first feeding. After yolk-sac absorption, temperature will become an important modifier of individual growth rates (Campana and Hurley, 1989). These subtle points would be overlooked in a study of growth alone.

The probability of capture of larvae is primarily dependent on the relative sizes of predator and prey (Folkvord and Hunter, 1986; Pepin et al. 1987; Miller et al. 1988). Vulnerability to a specific predator should follow a domed-shaped relationship over a range of prey sizes (Bailey and Houde, 1989). If within a predator prey window, the developmental stage at hatch of larvae may be as critical to survival as size. Particularly, the predation of larvae during the yolk-sac stage is likely extremely high (Hewitt at al. 1985; Taggart & Leggett, 1987; Leggett and Deblois 1994). The responsiveness of larvae to physical contact, the maximum escape speed and mean escape speed all increase after

hatch until, or just subsequent to, yolk absorption (Yin and Blaxter, 1987). Ontogenetic based changes in morphology, principally at first feeding, correlate with rising activity levels (Skiftesvik, 1992) suggesting a correlation between ontogenetic and behavioral traits. Hatching at a very early stage with a large yolk-sac may leave an individual larva highly susceptible to predators. The results of this study link the traits of early developmental stage and small size at the time of hatch. Differences among individuals at hatch could impart different abilities to escape. The effects of size and developmental stage, and their interaction, may have subtle and synergistic influences on survival. An interesting question would be whether there is an optimal size and stage of development for larvae to hatch in terms of survival. and whether there is a relationship between optimal stage/size and maximum escape speeds. Further study of the interaction among ontogeny, behavior, and performance, especially in relation to parental condition and genetics, would increase the understanding of early life-history mortality and the role of early life-history traits in determining survival.

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Chapter 3

THE EFFECT OF TEMPERATURE ON THE RATES OF DEVELOPMENT, GROWTH AND SURVIVAL DURING THE EARLY LIFE HISTORY OF ATLANTIC COD (*GADUS MORHUA*)

3.1.Abstract

In this study, results of the effects of temperature on the vital rates of laboratory reared cod eggs and larvae are compared with information available in the literature regarding the effects of temperature on recruitment. Three batches of Atlantic cod (*Gadus morhua*) eggs were incubated at temperatures of 2, 4, 8 and 12°C. Observations were made every 4 degree-days for the 4, 8 and 12 degree treatments, and every day for the 2 degree treatment. Data were collected from two synchronized experiments. Experiment 1 was designed to evaluate the effects of temperature on the development of eggs and then on the growth of larvae. While collecting development and growth information, the yolk-sac size, feeding incidence and the condition of larvae were also noted. Experiment 2 was set up to evaluate the effects of temperature on the mortality of eggs and larvae. Temperature differentially influenced all vital rates, leading to the conclusion that only relatively large changes in temperature (+/- ~4°C) affect survival through disruption of normal morphological development, while smaller changes in temperature (+/- ~1°C) affect the growth of larvae. Indirect effects through growth are likely to be more important in influencing survival in the field than direct effects (lethal effects), because small changes in growth rates have been shown to be detrimental to recruitment over time. The age at which individuals experience changes in temperature and the degree of

the change (absolute and rate) will be important in determining the magnitude of the thermal effect on survival.

3.2.Introduction

The complex relationships among environmental variability, basic biological processes and recruitment of fish stocks are poorly understood despite a significant investment in research directed towards environment-recruitment relationships (Sundby 2000). Temperature has been implicated as a major factor in both year-to-year fluctuations and long-term trends in fish populations (Bjornstad et al. 1999). The effects of environmental factors, temperature in particular, are critical during the egg and larval periods (Rombough 1997; Pepin 1991; Houde 1989; Houde and Zastrow 1993) during which mortality ranges from 5-30% day^{-1} (Batty and Blaxter, 1992). The rates of growth, survival. and development (duration of life stages), have been referred to as "vital rates" because of the important role they play in influencing cumulative mortality and recruitment of fish populations (Lo et al. 1995; Pepin 1991). It is imperative to understand the effects of temperature on these important rates.

Generalizations based upon multiple species of fish larvae can shed some light on the effects of temperature on mortality, growth and development. Cross-species meta-analyses, using data derived from surviving larvae (at the time of field-sampling), indicate instantaneous growth rates (G) and instantaneous mortality rates (Z) are correlated (Pepin 1991; Houde and Zastrow 1993). Both G and Z increase by a factor of 0.01 per degree Celsius, as predicted by separate regression analyses against temperature (Houde and Zastrow 1993). This balanced response to temperature maintains a constant ratio of G/Z across latitudes (Houde 1989) or temperatures (Pepin 1991). Taken together,

these principles suggest that: (1) temperature induced changes in growth (mass) of individuals is offset by mortality (numbers), (2) across species, there is a strong relationship between the rates of growth and mortality, and that temperature influences these rates in an equal and antagonistic way. Similarly, rates of growth and ingestion are closely related (Jobling 1997), and across species there is an equal increase in G and ingestion rate (I) with temperature at a rate expected by enzyme kinetics ($Q_{10} \sim 2.0$; Houde and Zastrow 1993). The equal effect of temperature on G and I results in no temperature-induced change in growth efficiency (G/I) (Houde 1989, Houde and Zastrow 1993).

Despite these generalizations, there is variation among different species as well as over the geographical range of an individual species in the effects of temperature on mortality, growth and development. The equal change in G and I with temperature, observed in meta-analyses across many species, does not occur within a population of a single species. In the case of a single species, changes in the relative metabolic costs at different temperatures decouples ingestion and growth, resulting in a higher temperature of maximum ingestion than temperature of maximum growth (Jobling 1997). Furthermore, metabolic maintenance requirements of ectotherms increase with increasing temperature. Consequently, a reduction in food supply reduces the temperature at which the best growth performance is observed.

There are differences in the response of local cod populations to the thermal environment. Sundby (2000) observed that cod stocks from the colder part of the species range tend to have increased recruitment in warmer than average years, while those from warmer parts of the range have increased recruitment in cooler than average years.

Ouellet et al. (1997) advocates a weak but significant negative cold-water effect on recruitment dynamics in the southern St. Lawrence. This echoes the statement by Galloway et al. (1998) that cod survival is lower in cold years in Norwegian waters. Some of these local effects can be attributed to the complex interaction of more than one environmental variable, for example temperature and food availability. A link between copepod biomass and temperatures associated with the North Atlantic Subpolar Gyre (Sundby 2000) suggests a possible confounding influence of food and temperature. Galloway et al. (1998) also proposes a dependence on the interaction between zooplankton and temperature as a cause of lower survival in Norwegian cod.

On a local scale, the interaction of these variables can be quite complex. On Georges Bank, there was an observed dome-shaped relationship between larval growth and water temperatures during 1984-85 (Campana and Hurley 1989) and 1992-94 (Buckley et al. In Review). Buckley et al. (In Review) argue that food limitation is a mechanism for reduced growth at higher temperatures. Pressures from food limitation are accompanied by complicated and dynamic oceanography in the productive Georges Bank ecosystem. For example, Flierl and Wroblewski (1984) showed that Gulf Stream warm core rings are associated with reductions in larval abundance on Georges Bank. They developed a model to estimate losses of larvae on Georges Bank based on advection, predation, metamorphosis and physiological death. They were unable to quantify the losses due to each variable because of the general unavailability of critical information such as physiological temperature effects. Large scale fluxes of cold (< 2°C), fresher water (30‰) from the Scotian shelf also cross-over the Northeast channel and are incorporated into the Georges Bank circulation pattern (Bisagni and Smith, 1998). The

Scotian shelf water contains large numbers of gadid eggs (Bisagni and Smith 1998), suggesting that eggs and larvae from Browns Bank could be incorporated into Georges Bank, if the changes in the thermal environment do not act as a barrier to dispersal. Bisagni and Smith (1998) also suggest an interaction between Gulf Stream warm core rings and Scotian shelf water fluxes, which means that temperature effects may be compounded due to the presence of both extremes within a relatively small area.

A better understanding of the effects of temperature on the growth and assimilation efficiency of larvae is required to grasp the significance of meso-scale oceanographic events on individual-scale larval survival. Laboratory studies of larvae of an individual species can serve to simplify some of the interactions in inherently complex natural systems such as Goerges Bank. At the most basic level, the temperature ranges within which a species can survive should be determined. In laboratory experiments, cod have been incubated and hatched at temperatures from -1.5 to 12°C, while in the wild the temperature range associated with cod spawning is -1.5 to 9.2° (Galloway et al. 1998). The midpoint of the tolerance range for cod embryos is 6 to 6.5°C (given in Rombough 1997). Studies have demonstrated, using mortality based on the LT_{50}, that thermal tolerance range of cod embryos is -1.8 to 12.0°C (Johansen and Krogh 1914; Valerio et al. 1992). The lower thermal limit determined for larvae is -1.35°C, which runs counter to the norm of increasing thermal tolerance ranges throughout development (Blaxter 1992). Once larvae hatch, the temperature range over which they can persist increases significantly and culturing is commonly accomplished at temperatures between 10 and 12°C. By altering the thermal regime after hatch, researchers have observed increasing

growth rates up to temperatures of 14-16°C for larvae from Norwegian and Arctic stocks (Otterlei et al. 1999).

The effects of temperature during early life can be studied through two general approaches: (1) differences in state variables - morphology, either in meristic (eg: vertebral counts) or morphometric characters (eg: size at hatch), and (2) observation of rate processes over time (eg: growth and mortality rates). This study employed the second approach, investigating the effects of temperature on rates of mortality, growth and development. Cod eggs and larvae were cultured in the laboratory at four different temperatures (approximately 2, 4 8 and 12°C). Two experiments were conducted, one on development and growth of eggs/larvae and the other on mortality of the eggs and larvae at the different temperatures. This research is meant to complement earlier work on differences in morphology (Chapter 1) by comparing the effects of temperature on the vital rates of cod eggs (development, mortality) and larvae (growth, yolk-sac absorption, mortality) and relating them to some observed recruitment relationships.

3.3.Materials and Methods

Naturally spawned eggs were collected from the captive broodstock held at the Ocean Sciences Center (OSC), Logy Bay, Newfoundland, Canada. The broodstock were maintained at 5-6.5°C year-round. Three groups of eggs were shipped in a cooler to the Aquaculture Research Center (ARC) on campus at the University of Maine in Orono, Maine, United States. Two groups of eggs were transported in 1999 and one in 2000. The transport water temperature and salinity were 7.7°C and 31.4‰ in 1999, and 6.1°C and 31.6‰ in 2000. In neither year was there any detectable ammonia in the transport seawater. Once at the ARC laboratory, each batch of eggs was disinfected in 400 ppm

gluteraldehyde solution. The eggs were placed in 4L plastic bags, with slight aeration, filled with seawater equal to the temperature upon arrival.

Four recirculating seawater systems, each containing four separate 75L tanks, were used to rear eggs and larvae. They were randomly assigned temperatures approximating 2, 4, 8 and 12°C. Half of the bags (n=8 for each batch) were moved to the 4 and 8°C treatments, and allowed to acclimatize for four hours. Half of the 4 and 8°C bags were moved to 2 and 12°C tanks, and again all the bags were left for four hours. Each tank was stocked with 35 mls of eggs for batch 1, 40 mls for batch 2 and 35 mls for batch 3.

The temperature was maintained in the separate re-circulating systems using Honeywell™ T775 temperature controllers modulating the flow of chilled glycol (-12°C) through heat exchangers. The temperature controls were connected to an alarm set to trip if the temperature changed by 1°F. Temperature was recorded every 5 minutes using a temperature logger (Onset™ StowAway tidbit +23°F to +99°F model). Photoperiod was set on a 16hr-8hr light-dark cycle. The lights were set to reach a maximum of 350 lux at mid-day and approximated a sine curve, with no light during the dark period of the cycle. Water samples were taken on a daily basis to ensure temperature and salinity stability and regular basis for water quality (ammonia and nitrite).

3.3.1.Feeding

Prey densities were high enough (8-10/ml) to guarantee growth and survival throughout development (Puvenendran and Brown 1999). Feedings were done 6 times per day for batch 1 and batch 2 and every hour for batch 3. Rotifers (*Brachionus* spp.) were supplied from hatching until larvae attained 7mm standard length. From then until

10mm standard length, the larvae were offered a mix or rotifers and *Artemia* nauplii, thereafter receiving solely *Artemia* nauplii. Rotifers and *Artemia* were enriched with of DHA Selco™ and Algamac 2000™ for 12 hours prior to feeding for batch 1 and 2. For batch 3 a combination of Algamac 2000™ and Innovative Aquaculture™ Algae Enrichment Formula enriched for 12 hours was used. Feedings were initiated the day following 100% hatch.

3.3.2.Experiment 1: Development and Growth

3.3.2.1.Sampling procedure

Sampling times were set according to a 4 degree-day period (mean temperature×time in days), except the 2°C treatment. This meant that the larvae were sampled at 06:00, 14:00 and 22:00 for the 12 degree treatments, 10:00 and 22:00 for 8 degree treatment and 22:00 for the 2 and 4 degree treatments. This sampling regime was maintained from incubation to 50% feeding for batches 1 and 2 and from hatch to 50% feeding for batch #3. After this, the sampling was continued on a 12 degree day basis for all batches.

During the egg period, all tanks were checked on a daily basis at the above sampling times. A flashlight was used to quickly scan the tanks. The presence of hatching fish and the distribution of the eggs/larvae in the tank was noted. When hatching commenced, larvae were randomly taken from the tank until 20 eggs/larvae had been removed. If there were at least 9 larvae, then it was considered 50% hatch. Larvae were then removed until there were 20 larvae total sampled.

Subsequent samples were continued on the above schedule, collecting 20 larvae each sample period. The time marking 100% hatch was when there were no eggs

remaining. When there were 10 or more fish in the sample with food in there gut, it was considered the time of 50% feeding. Sampling was continued on the "4 degree-day" schedule until 50% feeding. Thereafter, sampling was done only at 22:00 to allow for comparison of gut fullness after a day of feeding. These samples were taken once per day for the 12 degree and 8 degree treatment, once every 2 days for the 4 degree treatment and once every 4 days for the 2 degree treatment.

Once sampled, the 20 eggs/larvae were gently placed by pipette on a 1 ml welled slide, with 1 mm^2 grid, by pipette and anesthetized with tricane methanesulfonate (MS-222). Photographic images of the sampled larvae were taken with a digital camera paired with a dissecting microscope. The image was saved to the computer using a Flash Point 128™ frame grabber (Integral Technologies).

3.3.2.2.Measurements

The 1 mm grid, served to calibrate measurements and each image was checked by measuring a 1 mm square to ensure accuracy in the calibration settings. The standard length of each larva was measured to the nearest 0.01mm using Image Pro Plus™ V4.0 (Media Cybernetics). Standard length was measured from the tip of the snout to the end of the notochord. Yolk-sac area, calculated from the 2-dimensional images, was also measured in Image Pro Plus™ by outlining each larval yolk-sac and using the area command. This assumes that changes in area are representative of changes in volume.

Stages of development for eggs and larvae were based on Fridgeirsson (1978) and Hunt von Herbing et al. (1996). The larval staging table is described in Chapter 1.

Feeding condition, starvation and presence of deformities were determined for each larva. Feeding condition was assigned by ranks based on ingestion of

microparticulate diets, given by Baskerville-Bridges and Kling (1999). Starvation was judged using criteria assigned by Jordaan and Brown (In Review) and Yin and Blaxter (1986). These are (1) loss of pigmentation, (2) collapsing gut, (3) shrinking myotomes. All three of these had to be satisfied for a larva to be considered starving. Deformities were of 3 types: (1) significant bends or curves of body (crooked larvae), (2) the jaw frozen open (lock-jaw), bloating of body cavity (edema). The first condition can be observed in both eggs and larvae, while the latter two conditions can only be observed in post-hatch larvae.

3.3.3.Experiment 2: Mortality

3.3.3.1.Egg period mortality

Mortality was observed using 2.5 L tanks (n=5 for batches 1 and 2, n=10 for batch 3). The 2.5 L tanks were placed in the two empty 75 L tanks of each recirculating system and were held in place by a frame of PVC piping. Each 2.5 L tank contained a central screened outflow and air supply as well as water inflow delivered to the surface of the tank, which was scaled to mimic the conditions of the rearing tanks. Still, the differences between these mortality tanks and the rearing tanks used for development and growth makes a direct comparison between the mortality and growth questionable. Therefore the two measure will be separated in the following discussion into Experiment 1 and 2, respectively.

Each 2.5 L tank was stocked with 100 eggs at the same time that experiment 1 was initiated. Eggs and larvae, when alive, are buoyant. Mortality counts were completed by stopping the air supply, and allowing a few minutes for the eggs (and later larvae) to settle out. A siphon was established with a ¼ inch Nalgene™ tubing fitted with a solid

plastic extension of same diameter. The solid plastic end was used to remove all the material that settled out, the dead eggs were counted and the air-flow restored. The siphoned eggs were checked periodically to ensure that they were dead, this revealed that in some cases extremely deformed eggs (no chance of hatching) sunk out of the water but that live eggs did not.

Mortality counts were done on a 4 degree day basis, except for the 2°C treatment (daily) and were recorded as the number found dead. This results in mortality counts being taken every 8 hours for the 12°C treatment, every 12 hours for the 8°C treatment and every 24 hours for the 2°C and 4°C treatments. At the conclusion of the experiments, the tanks were cleaned out and the number of living and dead eggs counted.

3.3.3.2.Larval period mortality

At 100% hatch, all the 2.5 liter tanks were removed, cleaned and replaced. The tanks were restocked using larvae. The same sampling methodology was used as for egg mortality. The sampling schedule followed the rearing tank schedule, having a reduced sampling effort after 50% feeding.

3.3.4.Data Analysis

3.3.4.1.Experiment 1

There were only two measures of development rate, because batch 3 was spared from sampling in order to have enough larvae for for larval analysis, the egg development rate was substituted by the time required for all larvae to reach stage 4, for which there was three batches worth of data. The development time will be referred to as the stage duration (D). Stage 4 larvae were used instead of the hatching period to define life stages because hatching has been shown to occur at different stages of development (Chapter 1).

The growth rate, reported as a change in length (mm) instead of mass, was modeled by linear regression models to calculate an average increase in length per day for each batch at each temperature. Yolk-sac area was transformed to a linear measure by calculating the square root. This was done to improve the performance of the data with regard to assumptions of normality and constant variance while the transformed yolk-sac measurement was regressed against time, returning a rate of yolk-reduction, for each temperature and batch. All the above analyses resulted in one rate measure for each batch and temperature (n=12 total).

The index of gut fullness, which was initially divided into a 6-step classification system, was reduced to a 3-step system (high, medium and low levels of feeding). The low feeding level indicated no feeding or only very small remnants of prey in the gut. The high feeding level would correspond to larvae that were full of prey, such as would be expected under conditions of optimal feeding. The medium level was intermediate to the two. The number of starving larvae and the number of larvae classified with certain levels of gut fullness was divided by the number of larvae sampled to achieve indexes of performance.

3.3.4.2.Experiment 2

Mortality data did not meet the assumptions of constant variance or normality of residuals, even when natural log-transformed and regressed against time. Therefore, the non-parametric Kaplan-Meier (K-M) or product limit estimator (Lee 1992) was used to generate a survivorship curve. Individual mortalities are recorded as deaths (failures) associated with the hour of removal. Hours were used instead of days because of the different sampling times in the temperature treatments (3 times per day for the 12 degree

treatment). The surviving eggs/larvae at the conclusion of the experiment were right-censored, which identifies the larvae as alive at the time of sampling. The K-M also estimates a mean survival time in hours for each tank. This mean survival time was used to model the temperature effect on survival which allows for good comparison with the development times. This analysis resulted in an estimated mean survival time for each batch and temperature (n=12 total)

3.3.4.3.General

Where assumptions of normality of residuals and constant variance (Sokal and Rohlf 1995) held, the rate data (change in length, yolk-sac reduction) and the duration data (stage duration, mortality) were modeled over the range of temperatures, using regression analysis in Sigmaplot™ 2001 for Windows™ (ver 7.0). All linear and non-linear models were solved by means of the least-squares method. When more predictive, non-linear regression results are reported. Suitable non-linear regression models were determined by consulting Hoerl (1954). The normality of the residuals was tested using the Kolmogorov-Smirnov (K-S) test and homogeneity of the variance was tested using the Levene Median test.

3.4.Results

Temperature affected egg development and mortality, and larval growth, yolk-absorption, feeding and mortality. Because there were slight differences between the temperature treatments of each batch, temperature will be treated as a continuous variable and batches as replicates. This results in 4 temperatures and 3 batches, n=12, used in the analyses of vital (mortality, growth, and development) rates. The only exception to this is for the egg mortality-temperature curve. High mortality during transport of batch 3

resulted in the decision to favor data collection during the larval phase, so no egg mortality experiment was run for that batch, resulting in only n=8 data points for the analysis.

3.4.1.Experiment 1: Development and change in length

Stage duration (D), the time of acclimation to the time when all larvae were stage 4, is plotted against temperature in Figure 3.1. Batch 2 had slightly shorter development times compared to batch 1 and 3 because it arrived later in development. The resulting regression equation of D in regards to temperature (T), with standard error of the estimates in parentheses is

$D = 1079.6(78.9)^{-0.16(0.02)\times T}$ (DF = 11, F = 115.73, $r^2 = 0.92$, P<0.0001). Increasing temperature exponentially decreased the time it took to reach stage 4, the point at which feeding is initiated.

When the post yolk-sac growth for all temperatures was modeled to a log-normal relationship, a strong significant relationship resulted (Figure 3.2A, $r^2 = 0.77$, p = 0.0013). Only non-starving individuals were used in the analysis. The following points summarize the resulting growth-temperature relationship: there is an inflection point in the growth-temperature relationship, seen as the maximum slope when the first-derivative of the growth-temperature curve is plotted (Figure 3.2B); there is also a temperature (Tmax) where growth rate was maximum (Gmax). The predicted temperature of Gmax, where the slope of the growth-temperature relationship is 0, was 7.9°C, and the predicted temperature of maximum slope was 4.2°C.

The rate of yolk-sac reduction with temperature is shown in Figure 3.3. The rate of loss of the yolk-sac approximates a linear relationship, with increasing rates of yolk-

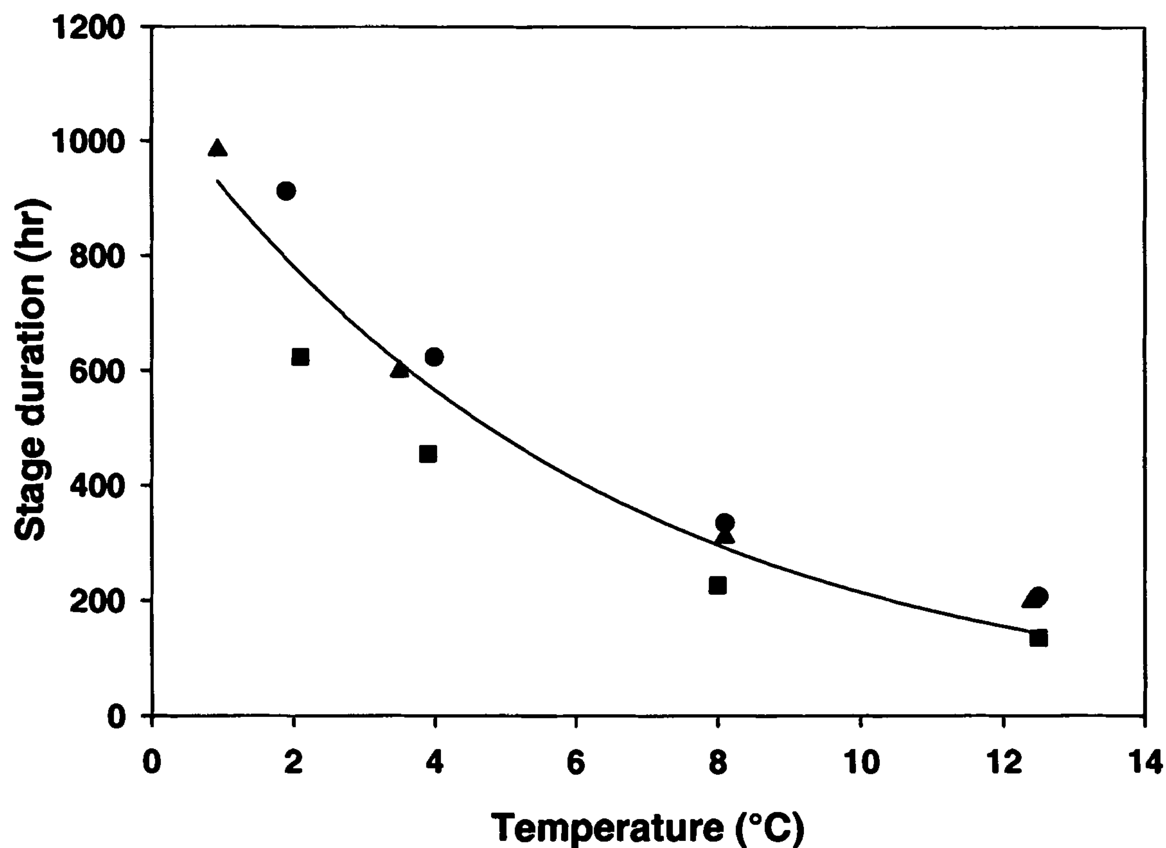
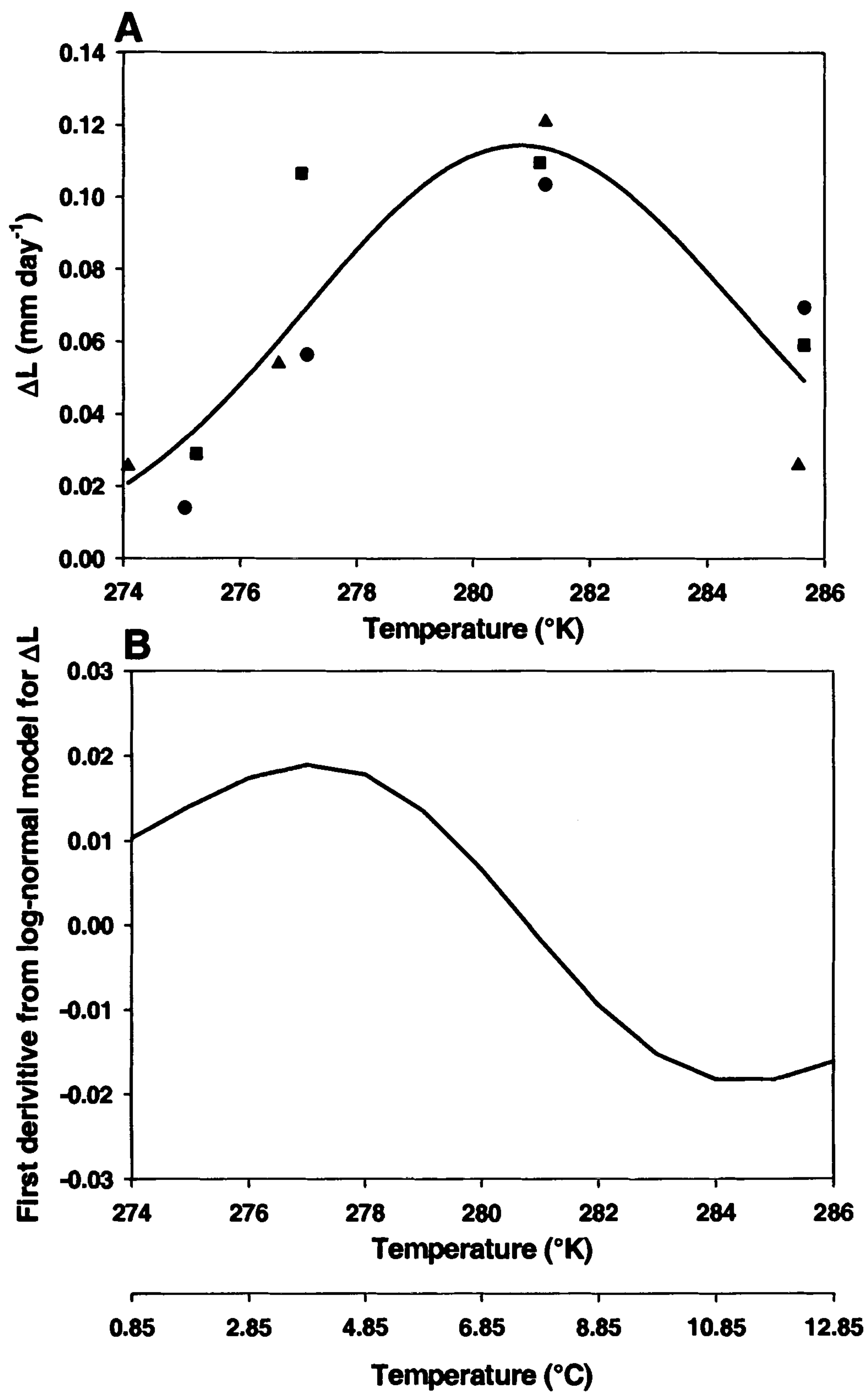


Figure 3.1. The stage duration (hr) of cod pre-feeding stages, from acclimation to stage 4 larvae, at different temperatures.

The resulting relationship is an exponentially decreasing time spent during the egg stage with increasing temperature. The stage duration (D) in regards to temperature (T), with standard error of the estimates in parentheses is: $D = 1079.6(78.9)^{-0.16(0.02)\times T}$ (DF = 11, F = 115.73, $r^2 = 0.92$, $P<0.0001$). Shown are data from batch 1 (circles), batch 2 (squares) and batch 3 (triangles).


A
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
ΔL (mm day-1)
274
276
278
280
282
284
286
Temperature (°K)
B
0.03
0.02
0.01
0.00
-0.01
-0.02
-0.03
First derivitive from log-normal model for ΔL
274
276
278
280
282
284
286
Temperature (°K)
0.85
2.85
4.85
6.85
8.85
10.85
12.85
Temperature (°C)

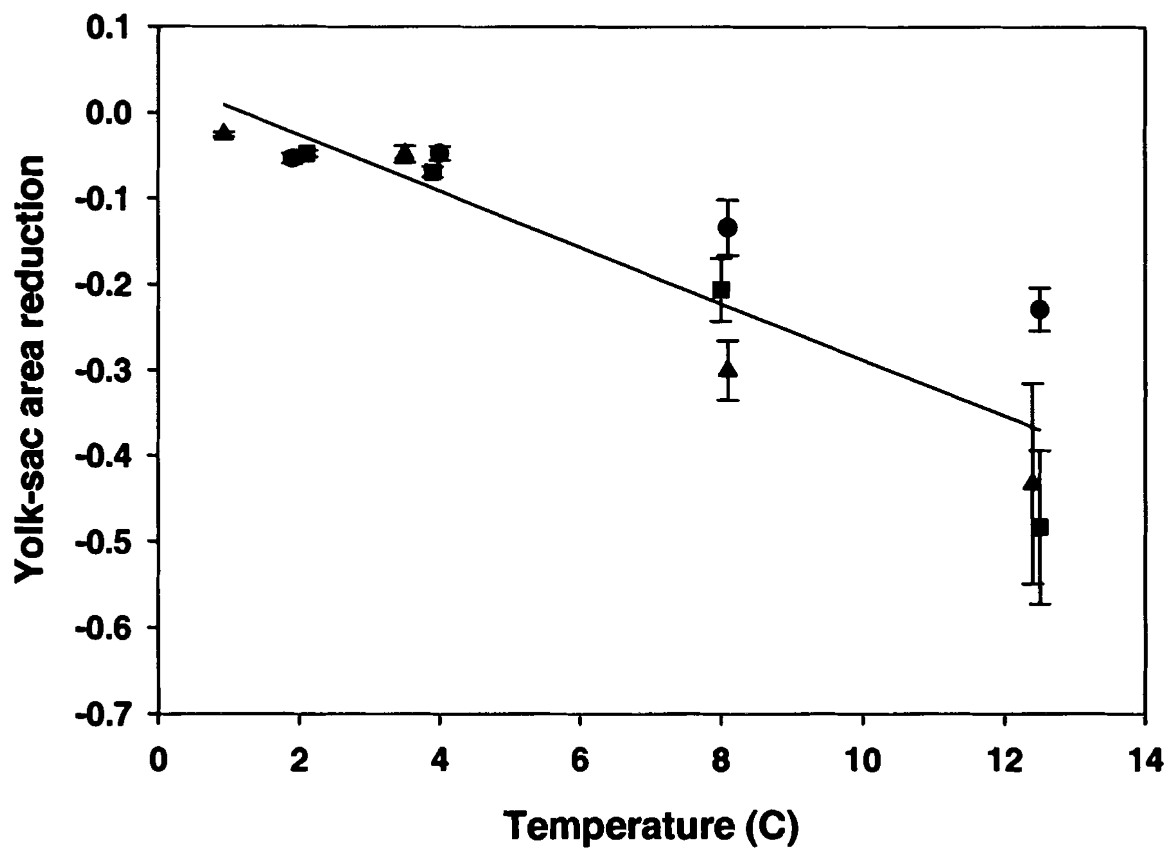


Figure 3.3. The rate of yolk-sac area reduction at the different temperatures.

The linear relationship describing the rate of yolk reduction (Y) over the different temperatures (T), with the standard error of the estimate in parentheses is:
$Y = 6.04\ (0.04) - 0.033(0.005)T$ ($DF = 11$, $F = 39.25$, $r^2 = 0.797$, $P<0.0001$).

sac reduction with increasing temperature. The linear model of the rate of yolk reduction (Y) over the different temperatures (T), with the standard error of the estimate was: $Y = 6.04\ (0.04) - 0.033(0.005)T$ ($DF = 11$, $F = 39.25$, $r^2 = 0.797$, $P<0.0001$). Increasing variation with increasing temperature resulted in violation of the assumption of a constant variance, therefore the discussion of yolk reduction will be limited.

The gut fullness of the larvae was dramatically altered by the temperature treatment (Figure 3.4 for batch 1, Figure 3.5 for batch 2, Figure 3.6 for batch 3). In the 2°C and 12°C treatments, the incidence of larvae at low and high feeding levels made up approximately the same proportion of larvae. That is, always about 50% of the larvae were barely or not feeding. In the intermediate temperatures, it is clear that feeding was initiated. However, there is a drop in the feeding levels in the time period around 15 days post hatch. This period of lowered feeding appears to match observations by Jordaan and Brown (In Review), who suggest that factors in the rearing environment have a greater effect on survival during this important period. The feeding incidence indicates that the range of temperatures that were considered optimal is in the 4-8°C range.

3.4.2.Experiment 2: Mortality

The Kaplan-Meier (K-M) estimated mean survival time during the egg stage was regressed against the environmental temperature using an exponential model for the larvae (Figure 3.7, $r^2 = 0.913$, $DF = 7$, $F = 62.7$, $P = 0.0002$).

The K-M estimated mean survival time was regressed against the environmental temperature using a log-normal model for the larvae (Figure 3.8, $r^2 = 0.93$, $DF = 11$, $F = 62.10$, $P < 0.0001$). The resulting trend “flips” from an exponential fit, expected if the

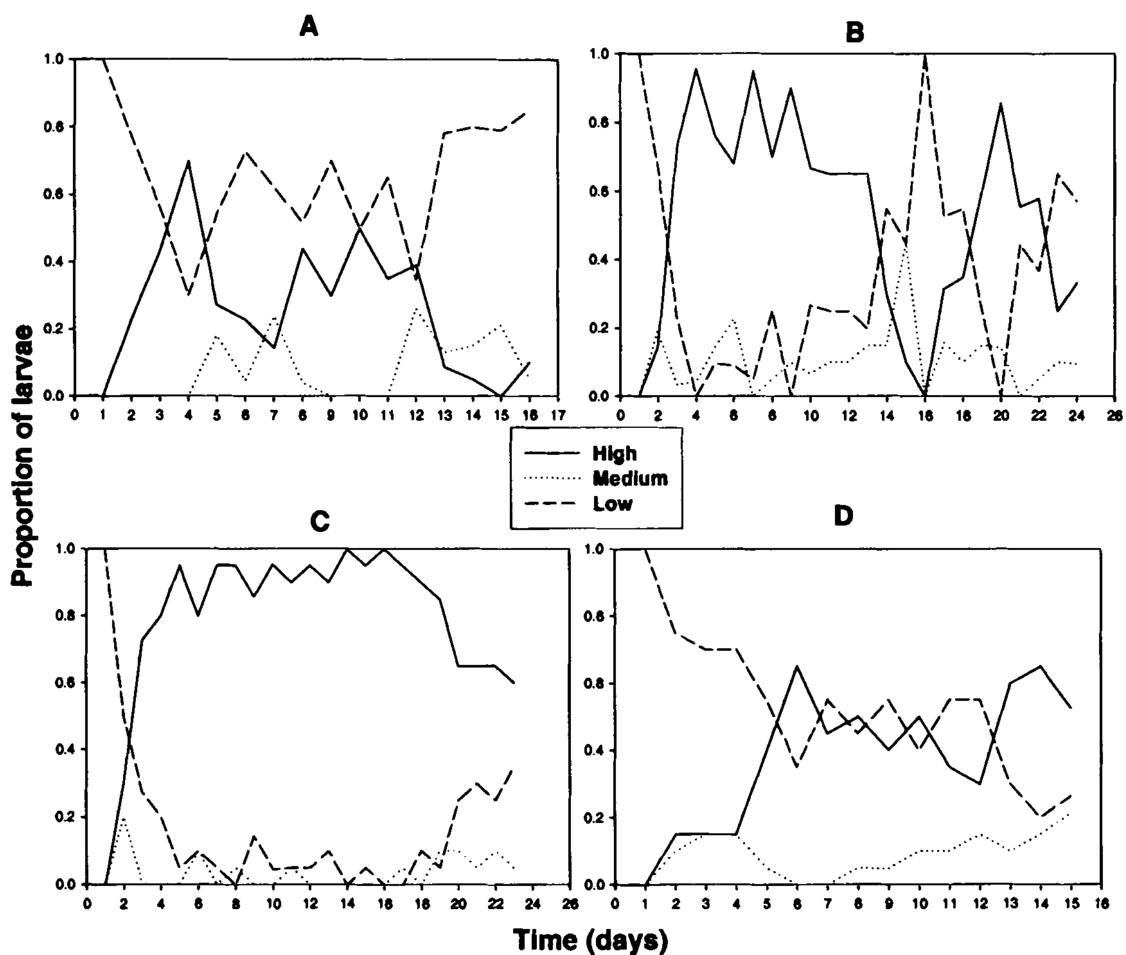


Figure 3.4. Feeding incidence as the proportion of larvae with low, medium and high gut fullness from batch 1 at 2 (A), 4 (B), 8 (C) and 12°C (D) over time (days).

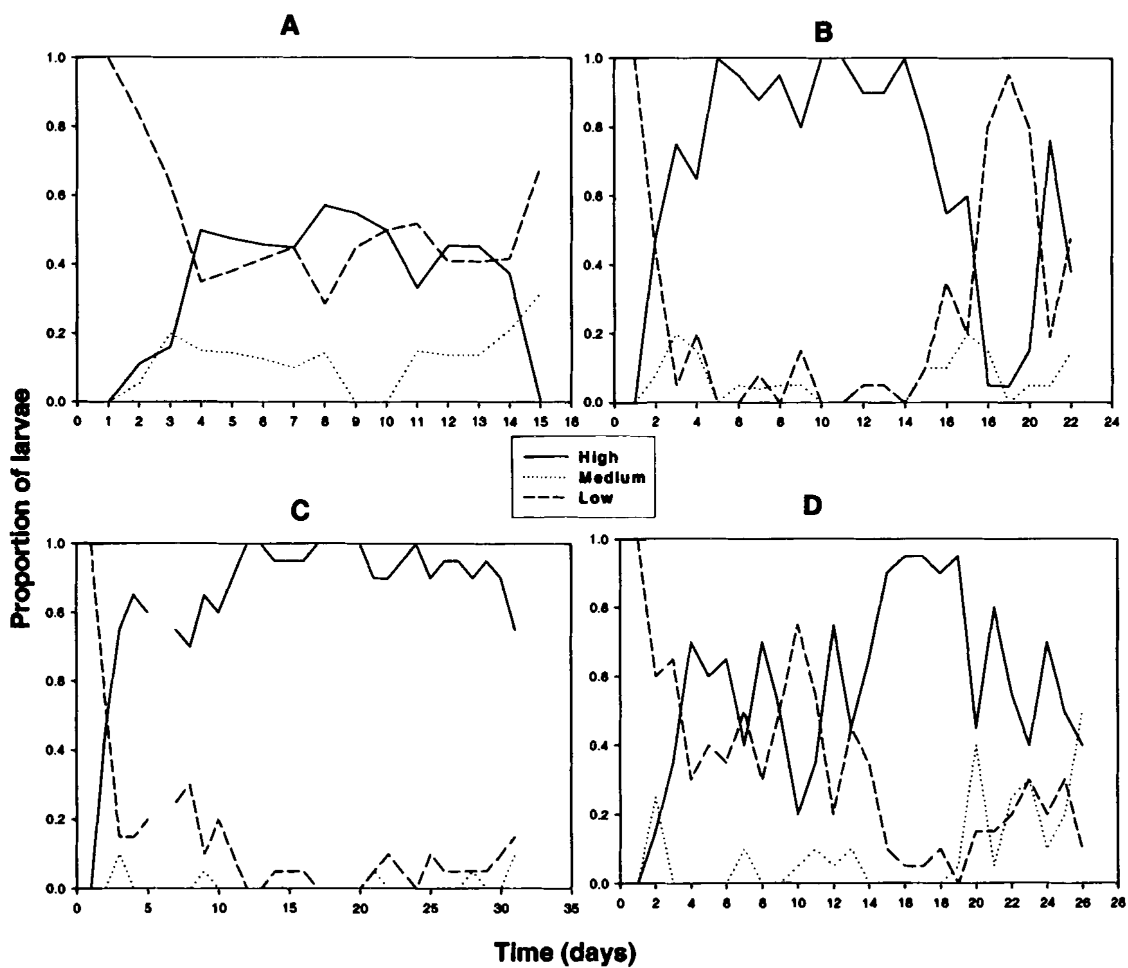


Figure 3.5. Feeding incidence as the proportion of larvae with low, medium and high gut fullness from batch 2 at 2 (A), 4 (B), 8 (C) and 12°C (D) over time (days).

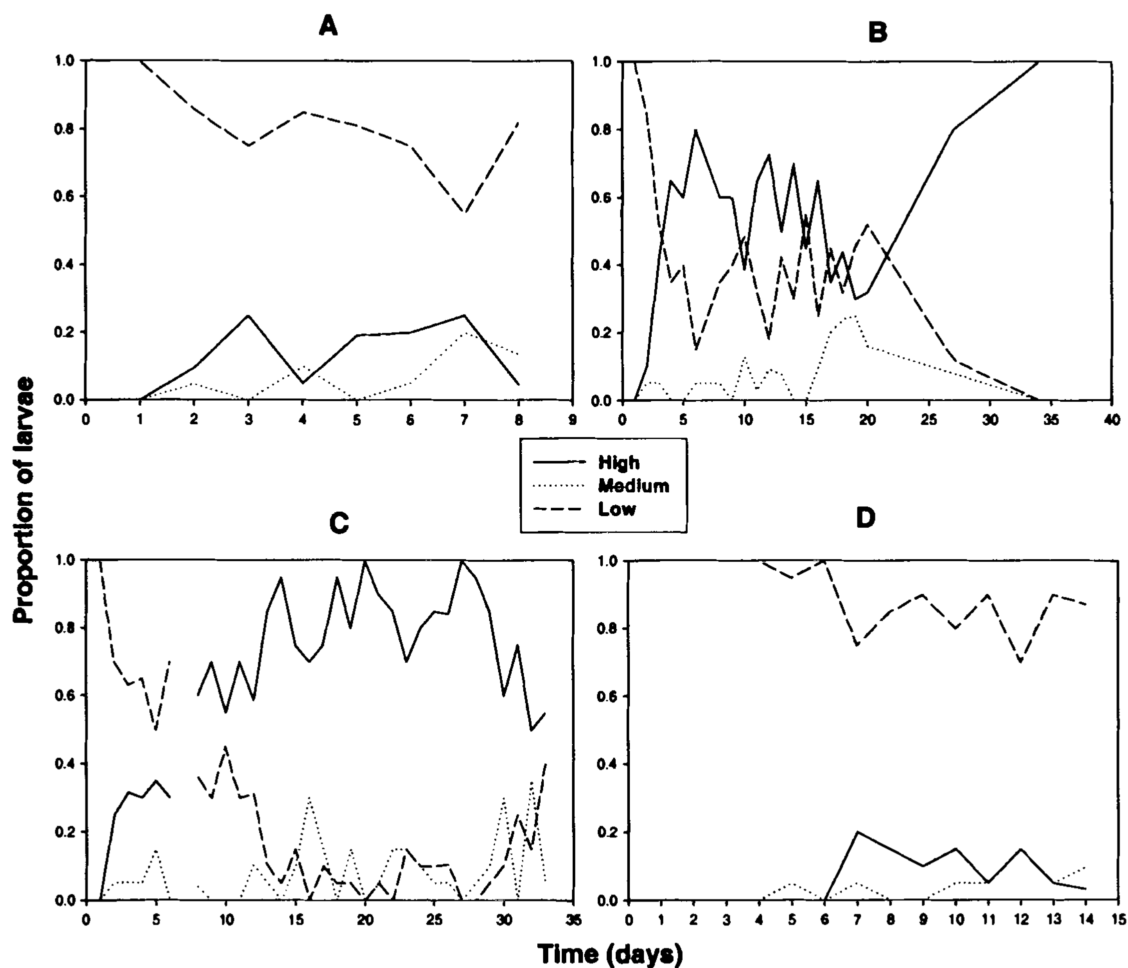


Figure 3.6. Feeding incidence as the proportion of larvae with low, medium and high gut fullness from batch 3 at 2 (A), 4 (B), 8 (C) and 12°C (D) over time (days).

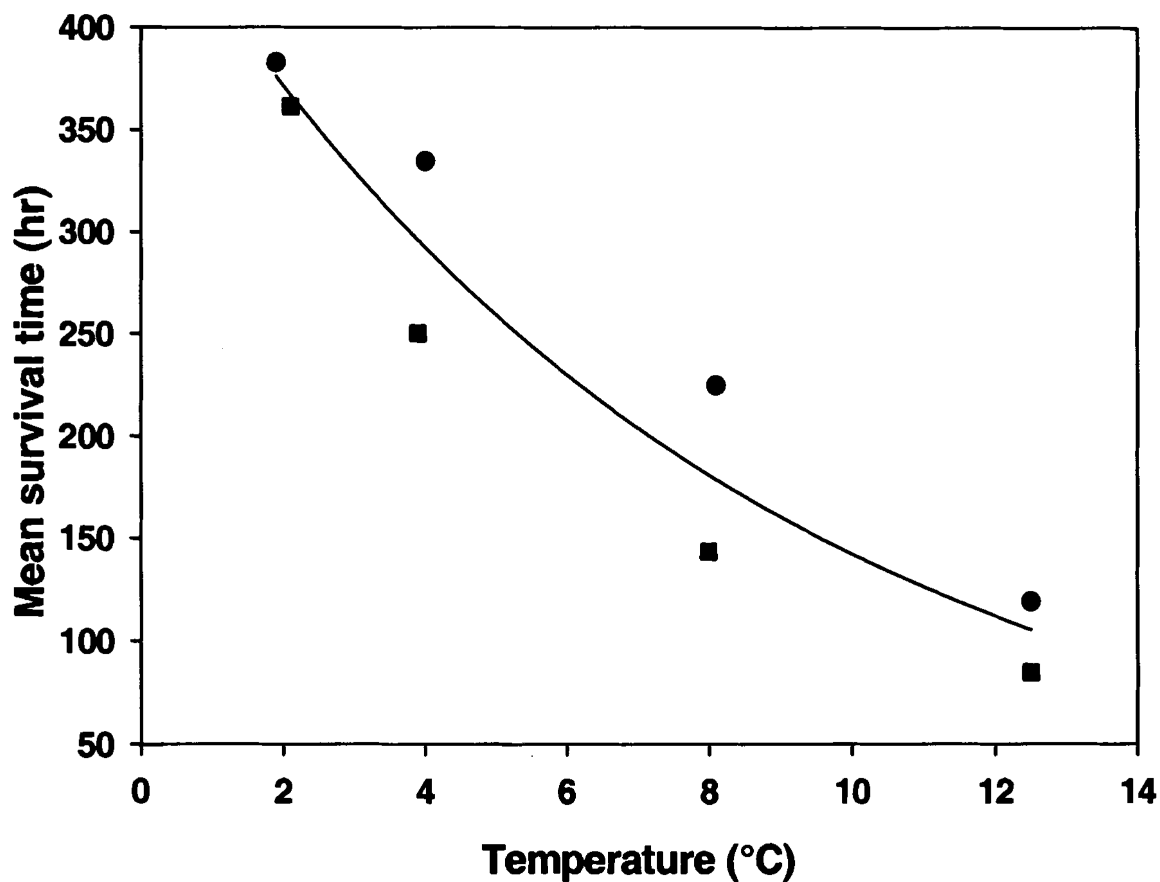


Figure 3.7. Kaplan-Meier estimated mean survival time (hr) for the egg stage at the different temperatures.

The resulting equation of survival time (M) over temperature (T) is $M = 472.16(41.2)^{-11.97(0.02)\times T}$ ($r^2 = 0.913$, DF = 7, F = 62.7, P =0.0002). Circles = batch 1, squares = batch 2.

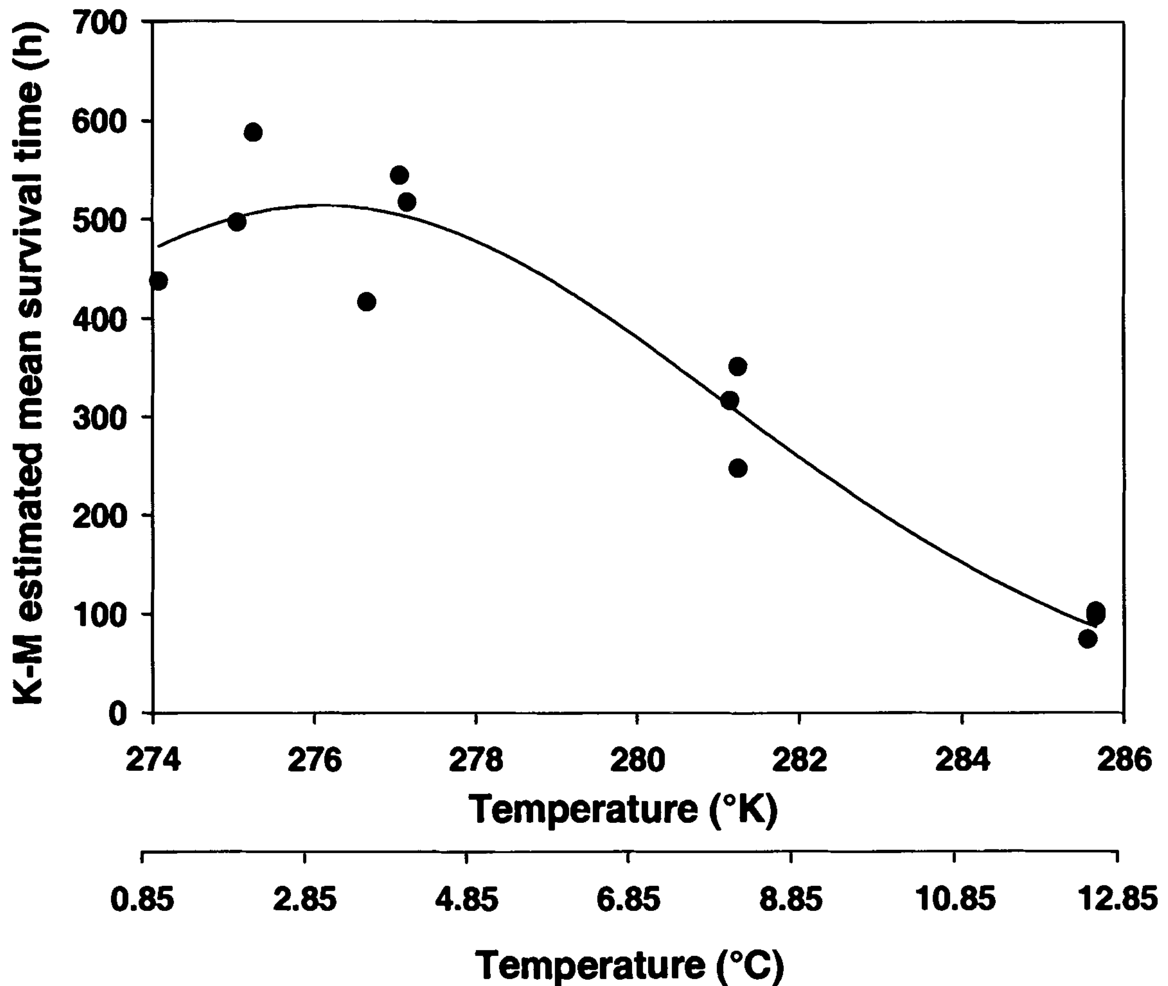


Figure 3.8. Kaplan-Meier estimated mean survival time (hr) for the larval stage at the different temperatures.

The resulting log-normal regression equation, with the standard error of the estimates in parentheses, is: $M = 514.26(22.5) * \exp(-0.5 * (\ln(T/276.11(0.72))/0.018(0.0024))^2)$, where M = survival time (hr) and T = temperature in degrees Kelvin , $r^2 = 0.93$, DF =11, $F = 62.10$, $P < 0.0001$)

larvae died at a rate equal to that of development, to a relationship with a peak value contained within the temperature range. The influence of temperature was more pronounced during the larval period than the egg period, seen by the move to a log-normal model for larvae from a linear model for eggs.

3.5.Discussion

The regression result of the development-temperature analysis reflects a typical time to hatch-temperature relationship (Blaxter 1992), which is a standard exponential curve (Figure 2.1). The effect of temperature on development is best appreciated by comparing the exponential curve to a tangent to the curve at 6°C, which demonstrates a linear response. The predicted exponential curve has increased development times at the extreme temperature (greater than 8°C, less than 4°C), compared to the tangent line.

The growth-temperature relationship also reflects the expected relationship (Jobling 1993; 1997). There are two processes that result in the log-normal growth-temperature relationship. The first process is the "normal" temperature effect, which acts through changes in chemical reaction rates, known to be important in mediating larval fish growth rates (Blaxter 1992). The second process is a "negative" temperature effect resulting in deleterious effects on the relative metabolic rates (Jobling 1997). These deleterious effects occur outside some optimal range of temperatures, approximately 4-8°C determined from the feeding incidence in this study. The growth-temperature curve is best viewed from the perspective of the first derivative of the log-normal relationship (Figure 2.2B). The first derivative describes the response of the log-normal curve to the temperature. The curve illustrates a temperature where slope is maximized (4.2°C) which indicates the greatest change in growth per unit temperature. Below and above this

temperature, larvae experienced reduced growth rate per unit of temperature change. The maximum slope under ad libitum feeding corresponds to where the greatest potential of growth rate per degree and where any "negative" effects are minimized. The maximum growth per day (Gmax) occurs where the first-derivitive of the growth-temperature curve is equal to 0. At Gmax, which is at 7.9°C in the present study, the two processes (negative and normal temperature effects) are equal. At higher temperatures, the rate of growth begins to decline as "negative" temperature effects begin to dominate over "normal" temperature effects.

The enzymatic rate-temperature relationship is the primary force behind growth rate-temperature relationships (Blaxter 1992). Increasing enzymatic reaction rates occur with increasing temperature because of an increase in the kinetic energy of the products (Eckert et al. 1988). This process increases the rates of development and growth with increasing temperature. Counteracting this "normal" temperature effect is the "negative" effect of changes in protein conformation, particularly around enzyme active sites (where binding occurs) (Hochachka and Somero 2002). Other possible negative effects on growth could be differential effects on enzymes involved in determining protein turnover rates, and factors involved in determining prey capture success. Eventually, changes in the enzyme tertiary structure become extreme and the enzymes permanently unfold, known as protein denaturation, rendering enzyme systems inactive (Eckert et al. 1988; Hochachka and Somero 2002). The denaturation of enzymes is a lethal aspect of temperature on ectotherms and is responsible for setting the upper thermal lethal limit.

The results of Otterlei et al. (1999) and Steinarsson and Björnsson (1999) show a maximum growth per day at temperatures higher than reported in this study. It is believed

that there are two factors responsible for this discrepancy. First is a lack of data between 8-12°C, which did not allow an exact calculation of Gmax. It is believed that if there were more temperature treatments and a more complete model, the temperature of Gmax could be slightly higher. This would have shifted both the temperature of Gmax and maximum slope towards higher temperatures. Second is the acclimation of egg stages rather than larvae. Since younger stages are more susceptible to temperature effects (Blaxter 1992), the acclimation of eggs is likely responsible for restricting the temperature response to a narrower range of temperatures. By acclimating larval stages of cod, Otterlei et al. (1999) were able to increase the temperature of Gmax to between 14-16°C. Rearing of Newfoundland stock cod is often accomplished at temperatures around 12°C once the larvae have hatched.

There are multiple ecological and biological factors that influence the growth rate-temperature relationship. Although the individual components involved in shaping the relationship are informative, fisheries biologists have focused on the use of the maximum growth per day as a proxy identifying the optimal temperature (Otterlei et al. 1999, Steinarsson and Björnsson 1999). The temperature of maximum growth per day is not the temperature where the growth is most efficient. Jobling (1993; 1997) shows clearly the relationship among the ingestion rate, growth rate and conversion efficiency with changing temperature. The temperature at which the ingestion rate, growth rate and conversion efficiency are maximized is different. The maximum ingestion rate occurs at the highest temperature, whereas the maximum growth rate occurs at a temperature slightly lower than that. The conversion efficiency is maximized at the lowest temperature relative to the other two terms (Jobling 1997). If a larva is growing

inefficiently there will be two possible deleterious effects. The two parallel possibilities are: (1) individuals will have slowed growth ingesting equal quantities of prey per volume use of the endogenous supply, (2) an individual without slowed growth must ingest more prey per unit of growth. In the first case individuals will tend to have longer stage durations and incur higher risks (e.g. predation) because of an extended period during stages of life experiencing high mortality (Houde 1997). Because organ development is dependent on size, not age (O'Connell, 1981), and age is a poor predictor of metamorphosis (Policansky, 1982), the size of individuals tends to influence the time of metamorphosis more than age (Chambers and Leggett 1987). In the second case, larvae will have to search and capture more for prey, thus exposing themselves to higher potential for predation mortality (Skiftesvik 1992) through both increased encounter rates with predators and additional expenditure of energy (reduced growth). Note that in each case larvae are exposed to higher risk of predation, which is viewed as the most important source of mortality during early life (Bailey and Houde 1989). The two mechanisms are the result of increasing metabolic costs and both result in increased risks, but the responses operate on different temporal scales. The temperature resulting in maximum growth per day may not be the temperature that results in long-term survival. Lankford et al. (2001), using Atlantic silversides (*Menidia menidia*), provide experimental evidence suggesting that maximization of the rates of ingestion and growth may incur fitness costs in the form of increased predation risk. This further demonstrates that the temperature for maximum growth per day may not be the optimal temperature for survival, as suggested by Otterlei et al. (1999) and Steinarsson and Björnsson (1999).

The extremely tight energy budget of larvae compared to adults (Weiser 1991) may preclude investing the energy into maintaining plasticity (being eurythermal). Furthermore, stochastic bi-directional variation in the thermal environment, due to destruction of vertical water-column stability in storm events and mixing of water masses, may prevent the evolution of a concrete mechanism for dealing with thermal stress where the larvae are often captives of the physical environment. Growth is the most important consideration for an organism facing a maximum chance of survival of 0.54 in a million (Haedrich and Fisher 1998).

As with the growth-temperature relationship, there are two processes involved in shaping the mortality-temperature relationships in this study. The first process, as it was for growth, is the "normal" temperature effect caused by changes in rate processes due to temperature (Hochachka and Somero 2002). This is the cause of varying development times in ectothermic organisms such as fish larvae (Blaxter 1992). If the rate of death reflects the rate of development, the expected mortality-temperature relationship would approximate the development-temperature relationship. For example, individual larvae that do not initiate feeding will experience mortality as the population transitions from endogenous to exogenous feeding. The timing of this mortality is dependent on the development rate or duration of the embryonic stage. The point-of-no-return (PNR) is strongly related to temperature (Pepin 1991), which is a reflection of this "normal temperature" effect. This process does not affect cumulative mortality in specific life stages (stage-specific mortality), which Pepin (1991) provides an example of in noting that larval mortality rates are significantly affected by temperature ($P < 0.001$), while cumulative mortality is not.

The second process involved in altering the mean survival time is cumulative mortality or the total number of individuals that die at each temperature with, as would be expected, increasing numbers of dying larvae increasing the mortality rate. Larvae from the 2 and 12°C treatments in this study had poor feeding levels compared to the 4 and 8°C treatments. The mean time of survival reported in the mortality-temperature relationships will reflect this as a decrease in the mean survival time. As temperature becomes deleterious to developing cod, the second process compounds the temperature effect (first process). During the egg stage, the relationship did not move strongly away from the development-temperature pattern. The relationship is more linear, which is caused by higher levels of mortality particularly in the period just subsequent to transfer into the systems, and at the time of hatching. The influence of the second process was much more clear during the larval period as larvae were more affected by temperature than eggs in regards to effects on survival time.

The egg stage mortality-temperature curves were not much different than the development curves, although they were slightly more linear. For the larvae however, the curves had a much more dome-shaped appearance compared to the development-temperature relationship. Within a range of temperatures near the center of the tolerance range (4-8°C), there is little effect of cumulative mortality. As temperatures away from the optimal range are approached, the influence of temperature on rate processes, the normal temperature effect, is confounded by additional cumulative mortality (mortality independent of rate processes). Larval mortality was strongly influenced by temperature because sub-lethal effects became lethal once the larvae became active swimmers where prey capture is necessary for survival. For larvae, prey capture requires active foraging

and this constitutes a bottleneck to early survival in the temperature extremes, seen by higher numbers of non-feeding larvae in the extreme temperature treatments. Furthermore, intrinsic (genetic) factors may dominate prior to yolk-absorption moving to extrinsic (environmental) factors as the fish begin to feed (Hunt von Herbing et al. 1996), which supports observations that larvae are more influenced by temperature.

3.6.Conclusions and Implications

Sundby (2000) that recruitment tends to be better in warmer years in the cold part of the geographic range of cod and in cooler years in the warmer part of the geographic range. It appears, therefore, that although populations can exist over a fairly broad range of temperatures, cumulative survival occurs over a smaller range of temperatures somewhere in the middle of the species thermal range. Without a quantitative genetic study it is not possible to determine the contribution of environment and genetics in shaping these responses. The relative contribution (genetic versus environmental) to any variation in performance of larvae will be necessary information for predicting effects of climate change on stocks of fish. But simple experiments relating the most efficient growth to environmental factors should help researchers understand mechanisms that result in short and long-term fluctuations in survival. I suggest that the temperature resulting in the fastest growth per day, per unit temperature (maximum slope of the growth rate-temperature relationship) is a better proxy for the optimal temperature for cod survival than the temperature resulting in maximum growth per day.

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BIOGRAPHY OF THE AUTHOR

Adrian was born in Calgary, Alberta during a snowstorm on the 20th of January, 1972. During the early years of his life, his parents began unknowingly training him for a career in biology through many camping trips across the Canadian Rocky mountains to the Pacific coast. Then, in 1986, his family moved to Newfoundland on the East coast of Canada. The salty air suited him well and there was no shortage of snow. After high school at Holy Heart of Mary, he began attending Memorial University of Newfoundland in St. John's. During his studies, he was lucky to be taken into the aquaculture and fisheries research laboratory of Dr. Joe Brown, where he was quickly given many responsibilities. In this environment he felt at home and completed an honours degree in Biology.

While under the impression he was going to stay in Newfoundland for a masters degree, he was sent the description of a project here at the University of Maine with Dr. Linda Kling and Dr. David Townsend. The strength of the research and the presence of significant amounts of snow persuaded Adrian to move "south of the border". During his 3^+ years in Maine, he has completed this thesis, worked in Acadia National Park on an inventory of tide pool/estuarine fishes, participated on a research cruise, organized activities with the School of Marine Sciences social committee and designed the Aquaculture website. He plans to continue on with a PhD at the University of Maine and eventually an academic position at an institution with strong research, teaching and at least an occasional snowstorm. Adrian is a candidate for the Master of Science degree in Marine Biology from The University of Maine in December, 2002.