

**Effect of Salinity, Photoperiod, Temperature, and
Restricted Food Intake on Growth and Incidence of Sexual Maturation
of Labrador Arctic charr (*Salvelinus alpinus*)**

by

Margaret Jeanette MacPherson

Submitted in partial fulfilment of the requirements
for the degree of Master of Science

at

Dalhousie University
Halifax, Nova Scotia

in co-operation with

Nova Scotia Agricultural College
Truro, Nova Scotia

August 2012

© Copyright by Margaret Jeanette MacPherson, 2012

DALHOUSIE UNIVERSITY
NOVA SCOTIA AGRICULTURAL COLLEGE

The undersigned hereby certify that they have read and recommend to the Faculty of Graduate Studies for acceptance a thesis entitled “Effect of Salinity, Photoperiod, Temperature, and Restricted Food Intake on Growth and Incidence of Sexual Maturation of Labrador Arctic charr (*Salvelinus alpinus*)” by Margaret Jeanette MacPherson in partial fulfilment of the requirements for the degree of Master of Science.

Dated: August 15, 2012

Co-Supervisors: _____

Readers: _____

DALHOUSIE UNIVERSITY
AND
NOVA SCOTIA AGRICULTURAL COLLEGE

DATE: August 15, 2012

AUTHOR: Margaret Jeanette MacPherson

TITLE: Effect of Salinity, Photoperiod, Temperature, and Restricted Food Intake
on Growth and Incidence of Sexual Maturation of Labrador Arctic charr
(*Salvelinus alpinus*)

DEPARTMENT OR SCHOOL: Faculty of Agriculture

DEGREE: MSc CONVOCATION: May YEAR: 2013

Permission is herewith granted to Dalhousie University to circulate and to have copied for non-commercial purposes, at its discretion, the above title upon the request of individuals or institutions. I understand that my thesis will be electronically available to the public.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

The author attests that permission has been obtained for the use of any copyrighted material appearing in the thesis (other than the brief excerpts requiring only proper acknowledgement in scholarly writing), and that all such use is clearly acknowledged.

Signature of Author

Dedication Page

I would like to dedicate this thesis to my mother, Ann Marie. Thank you for all of your help, and for listening when I needed to work out thoughts and ideas.

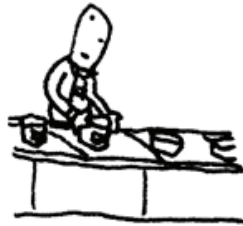
I would like to thank my parents, Arthur and Marilyn, and Crystal, Craig, Shabana, and all of my friends for their support, and the staff of the NSAC Aquaculture Centre (Paul, Mike, and Audrie-Jo) for all of their help.

I would also like to thank Drew, the creator of toothpastefordinner.com, for making me laugh. These are two of my favorite comics:



graduate school: it's like looking directly into the bulb of a high-powered flashlight for two years, only more expensive

Toothpaste For Dinner.com



after graduating college, i had a variety of awful jobs like working in a can factory, and whenever i felt bad, i would keep my spirits high by remembering: well, at least i'm not in grad school

Toothpaste For Dinner.com

TABLE OF CONTENTS

List of Tables	ix
List of Figures	xi
Abstract	xv
List of Abbreviations and Symbols Used	xvi
Acknowledgments	xvii
Chapter 1.0 Introduction	1
Chapter 2.0 Literature Review	4
2.1 Arctic charr Biology.....	4
2.1.1 <i>Arctic charr Life-History</i>	4
2.1.2 <i>Overview of Salmonid Aquaculture</i>	5
2.1.3 <i>Production Cycle of Farmed Salmonids</i>	6
2.1.4 <i>Coastal Zones Research Institute Breeding Program</i>	7
2.2 Hypo-osmoregulatory Ability.....	8
2.2.1 <i>Osmoregulation</i>	8
2.2.2 <i>Smoltification and the Degree of Anadromy</i>	9
2.2.3 <i>Acclimation to Full-Strength Seawater</i>	12
2.2.4 <i>Other Factors Affecting Hypo-Osmoregulatory Ability</i>	13
2.3 Sexual Maturation.....	15
2.3.1 <i>Reproductive Cycle</i>	15
2.3.2 <i>Sexual Maturation in Farmed Salmonids</i>	17
2.4 Photoperiod Manipulation.....	18
2.4.1 <i>Endogenous Circannual Rhythm</i>	18
2.4.2 <i>The Pineal Gland and Melatonin Production</i>	19
2.4.3 <i>Photoperiodic Control of the Timing of Spawning</i>	19
2.4.4 <i>Photoperiodic Control of Sexual Maturation in Virgin Fish</i>	21
2.4.5 <i>Photoperiodic Control of Somatic Growth</i>	22
2.5 Production Efficiency.....	23
2.5.1 <i>Effect of Temperature and Food Availability on Somatic Growth</i>	23

2.5.2 <i>Effect of Temperature and Food Availability on Inhibition of Sexual Maturation</i>	24
2.5.3 <i>Effect of Somatic Growth and Sexual Maturation on Production Efficiency</i>	26
2.6 Summary.....	26
Chapter 3.0 General Materials and Methods	27
3.1 Early Rearing at Shippagan, New Brunswick.....	27
3.2 Pre-experimental Conditions and PIT-tagging.....	27
3.3 Recirculation Systems.....	27
3.4 Photoperiod Regimes.....	28
3.5 Fish Health and Feeding.....	28
3.6 Determination of Sex and Sexual Maturity.....	29
3.7 Determination of Blood Plasma Osmolality.....	30
3.8 Statistical Methods.....	30
Chapter 4.0 Effect of Acclimation to Full-Strength Salinity and Extended Daylength in Autumn on Growth and Survival of Arctic charr	32
4.1 Introduction.....	32
4.2 Objectives and Hypotheses.....	33
4.3 Materials and Methods.....	34
4.3.1 <i>Experimental Design</i>	34
4.3.2 <i>Experimental Procedures</i>	34
4.3.3 <i>Statistical Analysis</i>	35
4.4 Results and Discussion.....	38
4.4.1 <i>Somatic Growth</i>	38
4.4.2 <i>Food Conversion Ratio and Specific Growth Rate</i>	43
4.4.3 <i>Blood Plasma Osmolality</i>	44
4.4.4 <i>Incidence of Mortality</i>	46
4.4.5 <i>Incidence of Sexual Maturation</i>	47
4.4.6 <i>Final Body Weight and Marketable Product</i>	47
Chapter 5.0 Timing of a Long-to-Ambient Photoperiod on Inhibition of Sexual Maturation of Arctic charr	49
5.1 Introduction.....	49

5.2 Objectives and Hypotheses.....	50
5.3 Materials and Methods.....	50
5.3.1 <i>Experimental Design</i>	50
5.3.2 <i>Experimental Procedures</i>	51
5.3.3 <i>Statistical Analysis</i>	53
5.4 Results and Discussion.....	55
5.4.1 <i>Somatic Growth</i>	55
5.4.2 <i>Incidence of Mortality</i>	62
5.4.3 <i>Incidence of Sexual Maturation</i>	63
5.4.4 <i>Final Body Weight and Marketable Product</i>	66
Chapter 6.0 Combined Effect of Temperature, a <i>Long-to-Ambient</i> Photoperiod, and Restricted Food Intake in Winter on the Incidence of Sexual Maturation of Arctic charr	68
6.1 Introduction.....	68
6.2 Objectives and Hypotheses.....	68
6.3 Materials and Methods.....	69
6.3.1 <i>Experimental Design</i>	69
6.3.2 <i>Experimental Procedures</i>	69
6.3.3 <i>Statistical Analysis</i>	71
6.4 Results and Discussion.....	73
6.4.1 <i>Somatic Growth</i>	73
6.4.2 <i>Food Conversion Ratio and Specific Growth Rate</i>	78
6.4.3 <i>Incidence of Mortality</i>	82
6.4.4 <i>Incidence of Sexual Maturation</i>	84
6.4.5 <i>Final Body Weight and Marketable Product</i>	87
Chapter 7.0 General Discussion and Conclusions	90
7.1 General Discussion.....	90
7.2 Conclusions.....	91
7.3 Future Recommendations.....	91
Bibliography	93
Appendix 1: Incident Reports	104
A1.1 Experiment 1.....	104

A1.2 Experiment 2.....	104
A1.3 Experiment 3.....	106

List of Tables

Table 1	<i>P</i> values of the main and interaction effects of salinity, photoperiod, and sexual maturation from Repeated Measures Analysis on mean body weight of Arctic charr from June 1 to December 29, 2005.....	40
Table 2	<i>P</i> values of the main and interaction effects of salinity, photoperiod, sexual maturation, and body size on mean plasma osmolality of Arctic charr in October 2005.....	45
Table 3	<i>P</i> values of the main and interaction effects of salinity, photoperiod, and sex on the incidence of sexual maturation of Arctic charr.....	47
Table 4	Photoperiod treatments December 21, 2005 to April 26, 2006: <i>LDN</i> (simulated <i>Light Dark Natural</i> daylength, Latitude 45 °N), and <i>Long-to-Ambient (LD18:6)</i>	51
Table 5	Arctic charr experimental design with 16 treatment paths, including photoperiod, temperature and salinity treatments.....	51
Table 6	<i>P</i> values of the main and interaction effects of treatment path from Repeated Measures Analysis on mean body weight of Arctic charr from December 21, 2005 to July 5, 2006.....	55
Table 7	<i>P</i> values of the main and interaction effects of treatment path and sexual maturation from Repeated Measures Analysis on mean body weight of Arctic charr from December 21, 2005 to December 12, 2006.....	59
Table 8	<i>P</i> values of the main and interaction effects of photoperiod and sex on the incidence of sexual maturation of Arctic charr.....	63
Table 9	<i>P</i> values of the main and interaction effects of temperature, photoperiod, ration, and sexual maturation from Repeated Measures Analysis on mean body weight of Arctic charr from December 21, 2006 to November 16, 2007.....	74
Table 10	<i>P</i> values of the main and interaction effects of temperature, photoperiod, and ration from Repeated Measures Analysis on mean food conversion ratio of Arctic charr from December 21, 2006 to July 5, 2007.....	79

Table 11	<i>P</i> values of the main and interaction effects of temperature, photoperiod, and ration from Repeated Measures Analysis on mean specific growth rate of Arctic charr from December 21, 2006 to July 5, 2007.....	81
Table 12	<i>P</i> values of the main and interaction effects of temperature, photoperiod, and ration on the incidence of mortality of Arctic charr.....	82
Table 13	<i>P</i> values of the main and interaction effects of temperature, photoperiod, ration, and sex on the incidence of sexual maturation of Arctic charr.....	85

List of Figures

Figure 1	Interaction plot of mean body weight (g) of Arctic charr reared in 10 °C seawater from the four combinations of salinity (20 and 30 ppt) and date (June 1 and July 12) following direct transfer from 10 °C freshwater. Means sharing the same letter are not significantly different at the 5 % level.....	39
Figure 2	Interaction plot of mean body weight (g) of Arctic charr reared in 20 ppt salinity (20 ppt) and 20 increased to 30 ppt salinity (20-30 ppt) from June 1 to December 29, 2005. Salinity gradually increased from 20 to 30 ppt July 16-30, 2005. Within each date, means sharing the same letter are not significantly different at the 5 % level. ns = not significant.....	41
Figure 3	Interaction plot of mean body weight (g) of Arctic charr reared under <i>Constant Long Photoperiod (CLP, LD16:8)</i> and simulated <i>Light Dark Natural (LDN, Latitude 45 °N)</i> from June 1 to December 29, 2005. The <i>CLP</i> treatment began June 21, 2005. Within each date, means sharing the same letter are not significantly different at the 5 % level. ns = not significant.....	42
Figure 4	Interaction plot of mean body weight (g) of sexually immature and maturing Arctic charr from June 1 to December 29, 2005. Within each date, means sharing the same letter are not significantly different at the 5 % level. ns = not significant.....	43
Figure 5	Bar graph of final body weight distribution of Arctic charr on December 29, 2005, sub-divided by sex (females and males) and sexual maturity status (immature and maturing). Immature fish greater than 1 kg, to the right of the vertical line, are of marketable size.....	48
Figure 6	Interaction plot of mean body weight (g) of Arctic charr reared in 16 treatment paths from December 21, 2005 to July 5, 2006. Treatment paths correspond to a combination of four photoperiod treatments (<i>LDN</i> ; and <i>Early</i> , <i>Mid</i> , and <i>Late</i> timing of <i>LD18:6</i> from December 21, 2005 to April 26, 2006), two freshwater temperatures (10 and 5 °C) in May and the transfer from freshwater to 20 ppt seawater at 10 and 5 °C in June. Panels are sub-divided by the four photoperiod regimes, and the timing of the <i>LD18:6</i> is shown by an arrow. Within each date, means sharing the same letter within each panel are not significantly different at the 5 % level. ns = not significant.....	57

Figure 7	Interaction plot of mean body weight (g) of Arctic charr reared in 8 treatment paths from December 21, 2005 to December 12, 2006. Treatment paths correspond to a combination of four photoperiod treatments (<i>LDN</i> ; and <i>Early</i> , <i>Mid</i> , and <i>Late</i> timing of <i>LD18:6</i> from December 21, 2005 to April 26, 2006), two freshwater temperatures (10 and 5 °C) in May and the transfer from freshwater to 20 ppt seawater (5 °C) in June. Panels are sub-divided by the four photoperiod regimes, and the timing of the <i>LD18:6</i> is shown by an arrow. Within each date, means sharing the same letter (lower case) are not significantly different at the 5 % level. Within each treatment over time, means sharing the same letter (upper case) are not significantly different at the 1 % level. ns = not significant.....	60
Figure 8	Interaction plot of mean body weight (g) of sexually immature and maturing Arctic charr from December 21, 2005 to December 12, 2006. Within each date, means sharing the same letter are not significantly different at the 5 % level. ns = not significant.....	62
Figure 9	Bar graph of the incidence of mortality among Arctic charr reared in 16 treatment paths, from December 21, 2005 to December 12, 2006. Treatment paths correspond to a combination of four photoperiod treatments (<i>LDN</i> ; and <i>Early</i> , <i>Mid</i> , and <i>Late</i> timing of <i>LD18:6</i> from December 21 to April 26), two freshwater temperatures (10 and 5 °C) in May and the transfer from freshwater to 20 ppt seawater at 10 or 5 °C in June. Proportions sharing the same letter are not significantly different at the 5 % level.....	63
Figure 10	Bar graph of the incidence of sexual maturity among Arctic charr reared in 5 °C on December 12, 2006. The fish were exposed to four photoperiod treatments (<i>LDN</i> ; and <i>Early</i> , <i>Mid</i> , and <i>Late</i> timing of <i>LD18:6</i> from December 21, 2005 to April 26, 2006). Sexes have been pooled. Proportions sharing the same letter are not significantly different at the 5 % level.....	64
Figure 11	Bar graph of final body weight distribution of Arctic charr reared in 5 °C on December 12, 2006, sub-divided by photoperiod (<i>LDN</i> , and <i>Early</i> , <i>Mid</i> , and <i>Late</i> timing of <i>LD18:6</i> from December 21, 2005 to April 26, 2006) and sexual maturity status (immature and maturing). Sexes have been pooled. Immature fish greater than 1 kg, to the right of the vertical line, are of marketable size.....	66

Figure 12	Interaction plot of mean body weight (g) of Arctic charr from December 21, 2006 to November 16, 2007. The fish were reared in a combination of two temperatures (5 and 10 °C) and two rations (<i>Satiation</i> : fed to apparent satiation throughout the experiment; and <i>Restricted</i> : restricted food intake for 12 weeks, from December 21, 2006 to March 15, 2007). Panels are sub-divided by temperature. Within each date (among both panels), means sharing the same letter (lower case) are not significantly different at the 5 % level. Within each treatment over time, means sharing the same letter (upper case) are not significantly different at the 1 % level.....	76
Figure 13	Interaction plot of mean body weight (g) of immature and maturing Arctic charr reared in 5 and 10 °C from December 21, 2006 to November 16, 2007. Panels are sub-divided by temperature. Within each date (among both panels), means sharing the same letter (lower case) are not significantly different at the 5 % level. Within each treatment over time, means sharing the same letter (upper case) are not significantly different at the 1 % level.....	78
Figure 14	Interaction plot of mean food conversion ratio (FCR) of Arctic charr from December 21, 2006 to July 5, 2007. The fish were reared in a combination of two temperatures (5 and 10 °C), two photoperiods (<i>LDN</i> and <i>Long-to-Ambient</i> , <i>LD18:6</i> , from December 21, 2006 to February 1, 2007), and two rations (<i>Satiation</i> : fed to apparent satiation throughout the experiment; and <i>Restricted</i> : restricted food intake for 12 weeks, from December 21, 2006 to March 15, 2007). The x-axis shows the final date of each data sub-set (~ 6 weeks). Within each date, means sharing the same letter are not significantly different at the 5 % level. ns = not significant.....	80
Figure 15	Interaction plot of mean specific growth rate (SGR, % body weight per day) of Arctic charr from December 21, 2006 to July 5, 2007. The fish were reared in a combination of two temperatures (5 and 10 °C), two photoperiods (<i>LDN</i> and <i>Long-to-Ambient</i> , <i>LD18:6</i> , from December 21, 2006 to February 1, 2007), and two rations (<i>Satiation</i> : fed to apparent satiation throughout the experiment; and <i>Restricted</i> : restricted food intake for 12 weeks, from December 21, 2006 to March 15, 2007). The x-axis shows the final date of each data sub-set (~ 6 weeks). Within each date, means sharing the same letter are not significantly different at the 5 % level.....	82

Figure 16	Incidence of mortality among Arctic charr from December 21, 2006 to November 16, 2007. The fish were reared in a combination of photoperiod (<i>LDN</i> and <i>Long-to-Ambient</i> , <i>LD18:6</i> , from December 21, 2006 to February 1, 2007) and ration (<i>Satiation</i> : fed to apparent satiation throughout the experiment; and <i>Restricted</i> : restricted food intake for 12 weeks, from December 21, 2006 to March 15, 2007). Proportions sharing the same letter are not significantly different at the 5 % level.....	84
Figure 17	Incidence of sexual maturity (immature and maturing) in November 2007 among male and female (M = male, F = female) Arctic charr, reared in a combination of two temperatures (5 and 10 °C) and two photoperiods (<i>LDN</i> and <i>Long-to-Ambient</i> , <i>LD18:6</i> , from December 21, 2006 to February 1, 2007). Proportions sharing the same letter are not significantly different at the 5 % level.....	86
Figure 18	Bar graph of final body weight distribution of Arctic charr on November 16, 2007, sub-divided by temperature (5 and 10 °C), sex (male and female), and sexual maturity status (immature and maturing). Immature fish greater than 1 kg, to the right of the vertical line, are of marketable size.....	88

Abstract

Economic viability of Fraser River, Labrador Arctic charr (*Salvelinus alpinus*) aquaculture in Atlantic Canada may be greatly improved if grow-out could be completed in seawater (30 ppt), while having a low incidence of sexual maturation before harvesting. Growth and survival in seawater was investigated among individually PIT-tagged Arctic charr reared in tanks in the laboratory. Direct transfer from freshwater to brackish water (20 ppt), and then acclimation to 30 ppt was successful. The manipulation of photoperiod, temperature, and food ration can be used as practical applications in aquaculture to arrest maturation; this was investigated in two additional experiments. The most effective photoperiod was *LD18:6* for 6 weeks starting December 21, which reduced maturation to 43% compared to 78% in controls. *Restricted* ration from December 21 through March 15 had no effect on maturation, however, rearing females in 5°C compared to 10°C reduced maturation to 15% compared to >80% in controls.

List of Abbreviations and Symbols Used

<i>TMS</i>	Tricaine methanesulfonate
<i>LDN</i>	Light Dark Natural
<i>CLP</i>	Constant Long Photoperiod
<i>LD</i>	Light Dark
NSAC	Nova Scotia Agricultural College
CZRI	Coastal Zones Research Institute
FCR	Food conversion ratio
SGR	Specific growth rate
Na ⁺ K ⁺ ATPase	Sodium-potassium adenosine triphosphatase
ppt	Parts per thousand
° N	Degrees North
° C	Degrees Celcius
~	Approximately
kg/m ³	Kilogram per meter cubed
20-30 ppt	20 ppt increased to 30 ppt salinity
mOsmol·kg ⁻¹	Milliosmols per kilogram of water

Acknowledgements

I would like to thank Drs. Duston, Astatkie, and Anderson for the opportunity to complete my MSc.

I would especially like to thank Dr. Dian Patterson for all of her help and guidance.

Funding for this research was provided by the Nova Scotia/Canada Agricultural Policy Framework (Science and Innovation Chapter) to J. MacPherson, and also by the Coastal Zones Research Institute through the Atlantic Innovation Fund of the Atlantic Canada Opportunities Agency to Dr. Duston.

Chapter 1.0 Introduction

Arctic charr (*Salvelinus alpinus*) from the Fraser River, northern Labrador, is the principal population used for aquaculture in Canada (Rogers and Davidson 2001). Despite being considered a highly marketable product, the worldwide annual aquaculture production of Arctic charr has remained small compared to Atlantic salmon (*Salmo salar*; Rogers and Davidson 2001). Attempts to culture Arctic charr in low cost cages in seawater have been a commercial failure (poor growth and survival) even though in the wild Arctic charr migrate to seawater during the summer (Delabbio et al. 1990a, Wandsvik and Jobling 1982a). Atlantic salmon and several species of *Oncorhynchus* may undergo substantial changes in their osmoregulatory ability (salinity tolerance) prior to the migration to seawater (Hoar 1988, McCormick and Saunders 1987). By contrast, these changes are not as prominent among the *Salvelinus* species (Hoar 1988). These fish would need to be acclimated to increasing salinity to allow sufficient time for the physiological mechanisms involved in osmoregulation to become activated (McCormick and Saunders 1987). There is a lack of published information on how to successfully culture Arctic charr in seawater (Jobling et al. 1993). Most of the literature focuses on wild populations of Arctic charr, particularly in Norway and Iceland. One of the goals of this MSc research was to determine if acclimating farmed Fraser River Arctic charr to seawater, as opposed to direct transfer from freshwater to seawater, would improve their hypo-osmoregulatory ability, survival, and growth performance for aquaculture purposes.

Two other important problems affecting Arctic charr aquaculture are (i) the high incidence of sexual maturation prior to reaching market size (> 1 kg body weight to be able to harvest fillets), which results in a decrease of somatic growth and a loss of flesh quality and pigmentation (Duston et al. 2003, Hatlen et al. 1996), and (ii) the relatively high variability in growth among individuals reared under the same environmental conditions (Delabbio et al. 1990b, Nordeng 1983). The major factors promoting early sexual maturation are the favorable growing conditions in hatcheries, particularly warm rearing temperatures and high quality diets that are fed to the fish (Nordeng 1983). The goal of Arctic charr aquaculture is to produce immature fish that are greater than 1 kg in body size that have excellent flesh quality and pigmentation to meet consumer demands, and that are of consistent body size at the time of harvesting.

Photoperiod manipulation is one method that has been used to control or arrest the onset of sexual maturation (Bromage et al. 2001). Subjecting Arctic charr to a long photoperiod (18 h light, 6 h dark) for a period of 42 days during the winter months has resulted in a reduction of the incidence of sexual maturity compared to controls subjected to a natural photoperiod (Duston et al. 2003). However, the exact timing of the application of the long photoperiod is uncertain, and the effectiveness of the photoperiod treatment depends on the physiological condition of the fish, as well as sex, age and/or body size, reproductive history and genetic strain (Frantzen et al. 2004a). Two other methods used to arrest sexual maturation include rearing the fish in cold water (Saunders et al. 1983) and restricting food intake (Rowe and Thorpe 1990), both of which may slow somatic growth and divert energy from gonadal growth. A second goal of this MSc research was to define the timing of the application of the long photoperiod, and to evaluate the effectiveness of the three methods to reduce the high incidence of sexual maturation that occurs in farmed Fraser River Arctic charr.

To address these problems, the Atlantic Innovation Fund invested over \$3 million in research and development in the Coastal Zones Research Institute (CZRI) at Shippagan, New Brunswick. This MSc project builds upon work started in 2004 and was part of a 5-year research project entitled “*Commercialization of high pedigreed Arctic charr products.*” One of the major research topics of this project was to sustain a breeding program (CZRI 2002). The project included researchers from across the Maritimes and industrial partners such as *Nova Scotia Arctic Charr* in Millbrook, near Truro, Nova Scotia. At the Nova Scotia Agricultural College (NSAC) Aquaculture Centre, the primary research objective was to assess the hypo-osmoregulatory ability of Arctic charr in seawater. A second objective (established in 2005 after some experimentation) was to investigate the use of a long photoperiod to reduce the high incidence of sexual maturation that was found to occur in Arctic charr.

This MSc project involved three long-term experiments using individually-tagged Fraser River Arctic charr from the CZRI breeding program. All experiments were conducted in the laboratory of the NSAC Aquaculture Centre. Experiment 1 (June – December 2005) assessed the hypo-osmoregulatory ability, survival and growth performance of Arctic charr when acclimated to seawater in the summer. To reduce the

high incidence of sexual maturation among Arctic charr, Experiment 2 (December 2005 – December 2006) attempted to define the timing of the application of a long photoperiod during the winter. Experiment 3 (December 2006 – November 2007) attempted to further reduce the incidence of sexual maturation among Arctic charr using the most effective timing of a long photoperiod established in Experiment 2, in combination with a cold water temperature and a restricted feeding regime in winter. The results of this research will be of benefit to the Arctic charr aquaculture industry in Atlantic Canada, and will enhance the basic knowledge of the biology of Fraser River Arctic charr.

Chapter 2.0 Literature Review

2.1 Arctic charr Biology

Arctic charr belong to the family Salmonidae, which also include trout and salmon (Johnston 2002). They are native to high-Arctic and sub-Arctic habitats, adapted to harsh environments where there is limited food supply and 24 h darkness in winter. Arctic charr can also survive for long periods at low water temperatures in ice covered lakes and rivers (Delabbio 1995). They occur naturally in 18 countries, including Canada and Norway, and are found in North America south to 49 °N and in Northern Europe south to 65 °N (Heasman and Black 1998). In Canada, the two commercially important populations are the migratory (from freshwater to seawater) Fraser River population from northern Labrador (56 °N), and the freshwater population from the Nauyuk River system, Northwest Territories (87 °N; Delabbio et al. 1990b). In Norway, the two commercially important populations are the migratory Hammerfest Arctic charr (70 °N) and the Svalbard Arctic charr (79 °N; Jørgensen and Arnesen 2002).

2.1.1 Arctic charr Life-History

Knowledge of the life-history of wild Arctic charr helps to understand the behaviour and performance of farmed Arctic charr (Delabbio 1995). Arctic charr may spawn several times over their 18 to 20 year life-span (Dempson and Green 1985, Pankhurst 1998). Spawning occurs in freshwater during late October (1 to 3 °C), and eggs (3 to 5 mm diameter) incubate in gravel beds during the winter, hatching as larvae in the spring (Dempson and Green 1985, Jobling et al. 1993). The growth of juveniles during the first two years is relatively slow, averaging a length of 2.5 cm a year (Dempson and Green 1985). Some Arctic charr populations are anadromous, meaning that they are capable of migrating from freshwater to seawater, and then return to freshwater to complete sexual maturation. This migration occurs in the spring when ice breaks up in coastal rivers. First time migrants are 3 to 6 years of age and lengths range from 9 to 20 cm (Dempson and Green 1985). The migration to seawater enables the Arctic charr to feed on abundant resources of fish and invertebrates that are not available in freshwater systems, resulting in rapid growth during the summer (Dempson and Green 1985, Power et al. 2002). Other wild salmonids such as Atlantic salmon undertake

extensive migrations and remain in seawater for at least a year, however, Arctic charr return to freshwater in late summer after spending two months at sea (Klemetsen et al. 2003a). Arctic charr, and other *Salvelinus* species, return to freshwater because they are unable to survive in seawater during the winter period in the high-Arctic and sub-Arctic due to the low water temperature (Klemetsen et al. 2003a, Rounsefell 1958). Wild Arctic charr may undertake several annual migrations to seawater before reaching sexual maturity, in contrast to wild Atlantic salmon that usually mature before returning to freshwater (Klemetsen et al. 2003a, Rounsefell 1958).

2.1.2 Overview of Salmonid Aquaculture

Aquaculture is defined as the farming or husbandry of aquatic animals, plants, or algae (Boghen 1995). The production of aquaculture species may be used to enhance natural populations (Boghen 1995), or it may be used as a nutritional source of protein in the human diet (Heen et al. 1993). The practice of cage-rearing salmonids year-round in seawater began in the late 1960's in Norway, and in 1978 in the Bay of Fundy, Canada (Heen et al. 1993). Atlantic salmon farming is successful because the fish have a year-round tolerance for seawater (CZRI 2002).

Enhancing Arctic charr aquaculture could provide a new species for commercialization, without having to develop new rearing facilities or rearing strategies that were previously established for Atlantic salmon. Early research conducted on Norwegian Arctic charr revealed that the species had good potential for aquaculture and showed rapid growth on commercial feed (Delabbio et al. 1990b, Heasman and Black 1998). Arctic charr aquaculture began in Norway in the 1970's and began in Canada in the mid 1980's (Boghen 1995, Johnston 2002). In New Brunswick, Arctic charr have been cultured experimentally since 1985, commercially since 1987 (McGeachy 1993), and have been cultured experimentally in Nova Scotia in the early 1990's (Murphy 1993). In Canada, the Maritimes region, Quebec and the Yukon produce Arctic charr, with the Maritimes producing the largest number of fish (CZRI 2002).

Even though Arctic charr aquaculture began almost 30 years ago, the worldwide Arctic charr industry has remained small whereas the Atlantic salmon industry is thriving (Rogers and Davidson 2001). This may have occurred because attempts to culture Arctic

charr in seawater (30 parts per thousand (ppt) or higher) during the winter have resulted in poor growth and poor survival rate (Heasman and Black 1998, Rogers and Davidson 2001). The hypo-osmoregulatory ability of Arctic charr is inferior to Atlantic salmon, and over-wintering in seawater may not be feasible (CZRI 2002). In Canada, Arctic charr are typically grown in freshwater supplied from surface or groundwater, or in brackish water (20 ppt; Murphy 1993). The limited amount of research regarding the specific problems of farmed Arctic charr in seawater has played a part in the slow development of the industry (Heasman and Black 1998).

2.1.3 Production Cycle of Farmed Salmonids

Knowledge of the standard production cycle of farmed salmonids such as Atlantic salmon and rainbow trout helps to understand the potential production cycle of farmed Arctic charr. Spawning of farmed salmonids typically occurs in autumn, at a time similar to wild salmonids (Johnston 2002). The eggs may hatch as early as December or January depending on water temperature (1 to 6 °C). Although species dependent, most salmonids are reared in freshwater from egg until their body size is greater than 60 g, at which time they can be transferred to seawater (Saunders 1995). The growth of farmed salmonids from egg to the seawater stage can be compressed into one or two years, as growth under the appropriate rearing conditions is more rapid than in the wild (Delabbio 1995).

The production cycle of growing salmonids from egg to immature one-year-old in freshwater is cost-efficient, but grow-out to market size in freshwater is not efficient in most farms due to space limitations and water demand. A production cycle that may be cost-effective is to grow salmonids to market size in seawater (Fitzgerald et al. 2002). The production cycle of Atlantic salmon involves the transfer from freshwater land-based tanks to sea-cages located in coastal regions, which usually occurs in spring and coincides with a natural increase in growth rate related to the increasing photoperiod (Johnston 2002). The transfer also may occur in summer and autumn (the fish are then termed “out-of-season smolts”), which allows time for a complete harvest of the previous year class (McClure et al. 2007). This prevents the overlapping of two year-classes of fish, which would have a large difference in body size and be at differing stages of sexual

maturation (McClure et al. 2007). Farmed Atlantic salmon typically mature after two winters at sea. As most farms harvest their fish before this time, early onset of sexual maturation is not usually a problem. However, if the Atlantic salmon mature after only one winter at sea, this would be at the time that the fish would be harvested (McClure et al. 2007). For example, farmed Atlantic salmon are typically transferred to sea-cages during April and May and are grown from approximately 80 g to 3 to 5 kg market size in 16 to 24 months (Saunders 1995). The early onset of sexual maturation is a major problem because it negatively affects the growth of the fish and reduces flesh quality and pigmentation (Hatlen et al. 1996).

The potential production cycle of Arctic charr may differ slightly from Atlantic salmon. In Atlantic Canada, Arctic charr are typically grown in freshwater in land-based tanks (Mason 1993, McGeachy 1993) to 1 to 3 kg market size in 24 to 36 months (Johnston 2002). If Arctic charr were to be grown in sea-cages, they should weigh more than 200 g before transfer to seawater, and they should be transferred to seawater in June. This would correspond with the timing of migration of wild Arctic charr (Delabbio et al. 1990b, Dempson and Green 1985). It has also been suggested that Arctic charr need to be acclimated to seawater and cannot tolerate direct transfer (Delabbio et al. 1990b, Duston et al. 2007). For wild Arctic charr, the acclimation to seawater would be associated with a short residency in an estuary in brackish water before the completion of migration (Gulseth et al. 2001a). In a previous experiment at the NSAC, Fraser River Arctic charr were successfully transferred from freshwater to brackish water (20 ppt; Duston et al. 2007). In the current study, Experiment 1 investigated the acclimation of Arctic charr from 20 ppt to 30 ppt during the summer.

2.1.4 Coastal Zones Research Institute Breeding Program

Farmed Fraser River Arctic charr originated from collections of eggs obtained from approximately 80 wild parents during the early 1980's (CZRI 2002). Arctic charr were initially reared in the Rockwood Aquaculture Research Centre in Manitoba, and were distributed to various hatcheries across Canada (CZRI 2002). Arctic charr that are currently being cultivated in Canada were derived from a limited wild gene pool, and as such had a limited amount of genetic variability (CZRI 2002). To develop a

commercially viable strain for aquaculture production in Atlantic Canada, the Coastal Zones Research Institute (CZRI), located in Shippagan, New Brunswick, established a breeding program to select and rear pedigreed Arctic charr families to prevent inbreeding and to provide a constant supply of eggs to the aquaculture industry (CZRI 2002). This breeding program did not directly select for economically important traits.

2.2 Hypo-osmoregulatory Ability

2.2.1 Osmoregulation

Osmoregulation is defined as the fish's ability to regulate and maintain internal osmotic and ionic balance (Jobling 1995). Fish inhabiting freshwater have body fluids that are more concentrated than the surrounding water. Their plasma osmolalities range from 260 to 330 milliosmols per kg of water ($\text{mOsmol}\cdot\text{kg}^{-1}$; Jobling 1995). Therefore, they tend to gain water by osmosis, mostly through the semi-permeable gills. To compensate for the influx of water, freshwater fish must excrete large amounts of dilute urine, or else suffer from cellular lysis. However, valuable ions must be retained in the body and not lost through urine or diffusion, therefore the fish actively pump ions from the surrounding water across the gills (Jobling 1995). This is termed hyper-osmoregulation.

Fish inhabiting seawater have body fluids that are more dilute than the surrounding water, and therefore suffer from dehydration when water is lost from the body by osmosis. Their plasma osmolalities range from 320 to 340 $\text{mOsmol}\cdot\text{kg}^{-1}$, whereas the osmolality of seawater ranges from 800 to 1200 $\text{mOsmol}\cdot\text{kg}^{-1}$ (Jobling 1995). Fish must therefore retain valuable water in the body. They accomplish this by drinking seawater and actively excreting excess monovalent ions such as Na^+ and Cl^- from the body through gill chloride cells, and divalent ions such as Mg^{2+} and SO_4^{2-} through the kidneys, while absorbing fresh water (Jobling 1995). This is termed hypo-osmoregulation. Wild salmonids that migrate from freshwater to seawater must ensure that their internal ion concentrations remain homeostatic during changes in salinity, therefore their osmoregulatory mechanisms must switch between states of ion absorption (hyper-osmoregulation) and ion excretion (hypo-osmoregulation). The blood plasma osmolality of Arctic charr was determined in Experiment 1, when the fish were first

transferred from freshwater to seawater in late spring, and also after being on-grown in seawater for 5 months.

2.2.2 Smoltification and the Degree of Anadromy

Wild anadromous salmonids undertake migrations from freshwater to brackish areas and the open ocean, although all salmonids return to freshwater to spawn (Jobling 1995, Rikardsen and Amundsen 2005). Atlantic salmon and several species of *Oncorhynchus* may undergo substantial changes in their osmoregulatory ability prior to the migration to seawater (Hoar 1988, Jobling 1995, McCormick and Saunders 1987). This change usually occurs several months prior to seawater entry in the spring and is termed smoltification (Hoar 1988, Jobling 1995). The smoltification process is complex and involves physiological, morphological, and behavioural changes, as well as specific environmental factors, such as photoperiod and temperature (Hoar 1988, Jobling 1995). For the physiological changes, the freshwater salmonid fish (termed “parr”) begin to have an increase in the enzyme sodium-potassium adenosine triphosphatase ($\text{Na}^+\text{K}^+\text{ATPase}$), and the chloride cells in their gill lamellae begin to increase in both size and number (Jobling 1995).

To determine if the fish are ready to be transferred from freshwater to seawater for on-growing, a seawater challenge test is often performed with a sample of fish (Jobling 1995). The seawater challenge test is an abrupt change in salinity, from freshwater to full-strength seawater (> 30 ppt) for a period of 24, 48, or 96 h. The fish are then blood sampled and plasma osmolality is analyzed at the specified time interval, and any mortalities that occurred throughout the test are recorded (Jobling 1995). Testing and monitoring both the $\text{Na}^+\text{K}^+\text{ATPase}$ activity and the blood plasma osmolality of Atlantic salmon parr that are undergoing the smoltification process, along with observations of morphological and behavioural changes, ensures a low incidence of mortality when the fish are transferred to seawater (Jobling 1995, McCormick and Saunders 1987). If the Atlantic salmon are not transferred (as a farming practice) or do not migrate (among wild fish) to seawater during the critical 6-8 week period in the spring (Bjerknes et al. 1992), their hypo-osmoregulatory ability decreases and reverts back to the freshwater state, a term called “desmoltification” (Hoar 1976).

The marine phase of wild salmonids corresponds to rapid increases in growth, as the migration to seawater enables the fish to feed on abundant resources that are not available in freshwater systems (Hoar 1988). This rapid growth also occurs when farmed salmonids are transferred from the freshwater rearing facility to seawater cages for on-growing. However, when a fish is transferred directly from freshwater to seawater, its ability to osmoregulate may not occur immediately, and could take many hours or even days to develop (Eddy 1981, McCormick and Saunders 1987). The first response, usually within the first few hours, is an increase in the drinking rate of seawater and an initial dehydration of the entire body. Some fish may be able to regain osmotic and ionic balance, although many freshwater fish cannot tolerate even low salinities, and usually die of dehydration (Eddy 1981). For smolting salmonids, osmoregulatory adaptation is complete approximately 10 days after transfer to seawater. However, appetite and growth may be suppressed for up to 30 days in Atlantic salmon smolts (Usher et al. 1991). The drinking of seawater and the processing of salts most likely affects appetite and the digestion of food (Usher et al. 1991). Stagg et al. (1989) suggest that an important part of smolting is the ability to regulate the dehydration of gill tissues, which precedes the excretion of salts from the gill chloride cells.

Among the *Salvelinus*, the physiological, morphological, and behavioural changes associated with smoltification are not as prominent and it has been suggested that they undergo a partial smoltification (Hoar 1988). The *Salvelinus* have a “modest” rise in hypo-osmoregulatory ability during the spring, and as such appear to be less likely to smolt than the *Salmo* (Hoar 1976). The *Salvelinus* have a lower degree of anadromy than the *Salmo* and the *Oncorhynchus* (Rounsefell 1958).

The degree of anadromy of salmonids is based upon several criteria, such as: (1) the extent of the migrations in the sea, (2) the duration of the stay in the sea, (3) the state of maturity attained while at sea, (4) spawning habits and habitat, (5) mortality after spawning, and (6) the occurrence of freshwater forms of the fish. The number 10 was given as a weight to the category signifying the most anadromous condition, while the #0 was given as a weight signifying the least anadromous condition (Rounsefell 1958):

- (1) *Salvelinus alpinus* has been weighted as #4 in terms of their extent of migrations to the sea. *S. alpinus* undertake migrations close to the coastline,

usually in estuarine conditions, for a short period of time. By contrast, *Salmo salar* is weighted as #8, as they migrate long distances and do not normally migrate far offshore.

- (2) With respect to the duration of the stay in the sea, *S. alpinus* are weighted as #2, as the majority of the young fish enter the sea after the first winter although some remain in freshwater. The young fish that enter the sea typically return to freshwater before the onset of sexual maturation, and may undertake several migrations to the sea before attaining maturation. By contrast, *S. salar* is weighted as #5, as the young fish normally migrate to sea after their first winter and remain in the sea until they mature.
- (3) *S. alpinus* gonads may vary from immature to slightly maturing while at sea (#2). The upstream migration in freshwater may include young fish and adults, and the migration upstream may be independent of the time of spawning. By contrast, two *Oncorhynchus* species (*O. gorbuscha* and *O. keta*) have gonads that are very advanced in seawater before returning to freshwater (#10).
- (4) In terms of spawning and habitat, *S. alpinus* typically spawn in lakes (#2), whereas *Salmo* spawn in streams in flowing water (#7).
- (5) Spawning does not affect survival in *S. alpinus* (#0), compared to *Oncorhynchus* (but not *O. mykiss*) that all die soon after spawning (#10).
- (6) The freshwater forms of *S. alpinus* are very common and some young anadromous fish remain in freshwater (#3), similar to the freshwater forms of *S. salar* (#5).

In addition, the degree of anadromy varies greatly among anadromous populations of Arctic charr (about 1300 worldwide), due to their varied life-history (Klemetsen et al. 2003a). Latitude has an effect on the degree of anadromy of North American Salmonidae, and within each species, the degree of anadromy tends to be greater towards the northern part of their geographic range (Rounsefell 1958, Swanson et al. 2010). This may explain why Norwegian Arctic charr (Latitude > 70 °N) appear to

have a higher degree of anadromy than Fraser River Arctic charr (Latitude 56 °N). This is explained in more detail in the following section.

2.2.3 Acclimation to Full-Strength Seawater

In salmonids that do not truly smoltify, the changes in gill chloride cell function and other physiological components have not occurred prior to exposure to seawater. The fish would need to be acclimated to increasing salinity to allow sufficient time for the physiological mechanisms involved in osmoregulation to become activated (McCormick and Saunders 1987). Unlike farmed Atlantic salmon smolts that can be directly transferred from freshwater to seawater at a specific time in the spring, Fraser River Arctic charr may need to be transferred to brackish water (20 ppt) and then acclimated to full-strength seawater (30 ppt). This would be similar to wild Arctic charr who remain in a brackish estuary for some time before migrating to full-strength seawater (Gulseth et al. 2001a). The transfer to brackish water followed by a gradual increase in salinity has proven a successful means to acclimate Atlantic salmon that were not in the smolt stage to full-strength seawater (Duston and Knox 1992).

The Fraser River is 116 km long and flows east from the border of Quebec/Labrador where it empties into Nain Bay (Dempson and Green 1985). The seaward migration of Fraser River Arctic charr takes place in spring (late April or early May) when the ice begins to break up in the river, and therefore the actual time of migration is variable and depends on local environmental conditions such as water temperature, total snowfall amount, and the thickness of the ice cover (Dempson and Green 1985, Klemetsen et al. 2003a, Power et al. 2000). In contrast to Fraser River Arctic charr, Norwegian Arctic charr display seasonal changes in hypo-osmoregulatory ability, re-smoltifying every spring as they prepare to return to seawater (Aas-Hansen et al. 2003). Their smoltification is comparable to that of Atlantic salmon, enabling them to tolerate direct transfer to full-strength seawater and maintain appetite and growth (Jørgensen et al. 2007). Norwegian Arctic charr often experience salinities above 25 ppt on their first day entering seawater, in contrast to Fraser River Arctic charr (Jørgensen et al. 2007). The coastal waters of Norway are typically free of ice, due to the warm waters of the North Atlantic drift, one of the branches from the tail of the Gulf Stream.

Norwegian Arctic charr may also spend time in an estuary during the winter months, rather than migrate to freshwater rivers and lakes, indicating that these Arctic charr may at least be partly adapted to cold temperatures in brackish and perhaps full-strength seawater (Jensen and Rikardsen 2008).

Most of the literature provides knowledge of the hypo-osmoregulatory ability of wild Arctic charr, in particular Norwegian and Icelandic Arctic charr. A lack of published work on farmed Fraser River Arctic charr identifies the need to determine their hypo-osmoregulatory ability for on-growing in seawater. The benefits of growing Arctic charr in brackish water as opposed to either freshwater or seawater need to be defined as well, given the availability of intermediate salinities in eastern Canada (Le François et al. 2002, CZRI 2002).

2.2.4 Other Factors Affecting Hypo-Osmoregulatory Ability

In Norway in the 1980's, Arctic charr were grown in sea-cages and exhibited good growth during the summer, however, high mortality during the winter occurred due to a decrease in hypo-osmoregulatory ability (Wandsvik and Jobling 1982a). This is a serious problem for the aquaculture industry, and growing Arctic charr year-round in seawater may only be feasible under certain environmental conditions. The freezing point of seawater is $-1.8\text{ }^{\circ}\text{C}$, below the freezing point of the fluids of fish tissue, $-0.6\text{ }^{\circ}\text{C}$ (Skuladottir et al. 1990). Rainbow trout held in seawater at temperatures near $0\text{ }^{\circ}\text{C}$ experience problems with the functioning of their digestive tracts, and it has been suggested that the drinking of seawater is decreased or ceases completely at low temperatures (Belkovskiy et al. 1991, Finstad et al. 1988). Atlantic salmon held at $1\text{ }^{\circ}\text{C}$ suffered from an increase in plasma osmolality and a reduction in tissue moisture content, due to a decrease in drinking rate (Lega et al. 1992). The hypo-osmoregulatory ability of brown trout (*Salmo trutta*) was also compromised at low seawater temperatures (Thomsen et al. 2007). Interestingly, wild Arctic charr from the Nauyuk river system in Canada have been found in the ocean during the time of spring migration, when there was ice on the sea and the seawater temperature was below $-1\text{ }^{\circ}\text{C}$ (Klemetsen et al. 2003a). This suggests that Arctic charr may be able to survive in cold seawater, although maybe

for only a short period of time if the fish are only “tolerant of seawater” rather than “fully adapted to seawater” (Wandsvik and Jobling 1982b).

Although low winter temperatures can be detrimental in seawater, cold rearing temperatures in freshwater during spring may be critical to the development of hypo-osmoregulatory ability and associated smolt characteristics in salmonids. As farmed Arctic charr juveniles in Atlantic Canada are typically reared in 9-12 °C freshwater (McGeachy 1993), the development of any hypo-osmoregulatory ability may be inhibited at this temperature. Elevated spring temperatures in freshwater (greater than 10 °C) may impair the osmoregulatory ability of Norwegian Arctic charr (Aas-Hansen et al. 2003) and Atlantic salmon smolts by affecting the activity of the enzyme $\text{Na}^+\text{K}^+\text{ATPase}$ (16 °C; Duston et al. 1991). By contrast, elevated temperatures (up to 10 °C) during winter can increase somatic growth (Bottengård and Jørgensen 2008, Duston and Saunders 1995).

Independent of smoltification, an important factor affecting hypo-osmoregulatory ability is body size (Bjerknes et al. 1992, Handeland and Stefansson 2001). Hypo-osmoregulatory ability increases as body size increases, due the decline in surface area for ion and water transport with respect to mass (Hoar 1976). Atlantic salmon must attain a critical body size (greater than 50 g) in order to successfully complete smoltification (Fitzgerald et al. 2002). By comparison, non-smolting species may need to have a larger body size when transferred to seawater. Fraser River Arctic charr from the CZRI breeding program (> 400 g) directly transferred from freshwater to 30 ppt (both at 10 °C) suffered 15 % mortality and very poor growth (Duston et al. 2007). By contrast, Arctic charr transferred to 20 ppt exhibited good growth and survival, comparable to fish reared in freshwater and 10 ppt (Duston et al. 2007). This supports the hypothesis that there is a critical body size (> 200 g) for survival in full-strength seawater (Delabbio et al. 1990b).

Independent of temperature, a naturally increasing photoperiod in the spring and summer improves hypo-osmoregulatory ability, while the naturally decreasing photoperiod in the fall and winter leads to a reduction in hypo-osmoregulatory ability (Staurnes 1993). The change in hypo-osmoregulatory ability reflects the natural migratory pattern of wild Arctic charr, from freshwater during winter and spring, to seawater for the summer months, and back to freshwater in autumn to over-winter (Staurnes 1993).

The complex interactions between temperature, body size, and photoperiod affect the ability of Arctic charr to survive in seawater. To better define this relationship, Experiments 1, 2 and 3 examined the effects of temperature and photoperiod on growth performance and survival of Arctic charr reared in seawater.

2.3 Sexual Maturation

2.3.1 Reproductive Cycle

Arctic charr typically spawn once per season in the autumn, but are capable of spawning more than once in their lifetime. This reproductive strategy is termed iteroparity (Pankhurst 1998). Fish reproduce as soon as they are able to do so ‘developmentally’, meaning at the earliest opportunity (Thorpe 1995). For wild Arctic charr, the age at first maturity is usually greater than 5 years (Dempson 2001). Females in the wild do not usually spawn every year as they need to allow for the sufficient recovery of body weight (Johnston 2002).

The annual reproductive cycle is synchronized with seasonal changes in photoperiod, temperature, and food resources. Synchronized spawning ensures that both males and females mature simultaneously, and also ensures that the eggs hatch at a time when survival is optimal (Bromage et al. 2001). The development of male and female sex organs is a gradual process, beginning approximately 12 months before spawning occurs (Taranger et al. 1999). Knowledge of the timing of the developmental processes within the reproductive cycle helps to better understand how photoperiod manipulation can be used to arrest the onset of sexual maturation.

There are three main phases of ovarian development in salmonids: pre-vitellogenesis, vitellogenesis, and final oocyte maturation. In addition, there are two processes of vitellogenesis that occur at the same time in the same year: vitellogenesis of oocytes that will develop and ovulate during that year, and the slow growth of oocytes that will develop the next spawning year (Bourlier and Billard 1984). The ovaries of immature females contain pre-vitellogenic oocytes (Pankhurst 1998). Endogenous vitellogenesis commences early in January, and it is characterized by the formation of yolk vesicles and the appearance of oil droplets in the oocytes. By the end of March, yolk granules appear in the oocyte, which commences exogenous vitellogenesis (Davies

et al. 1999). At this time, the oocyte diameter increases from 500-1000 μm to approximately 5 mm by June, due to the incorporation of vitellogenin (Sumpter et al. 1984). The gonadal steroid oestradiol-17 β stimulates the synthesis of vitellogenin from the liver, and during the spring, plasma concentrations of oestradiol-17 β and testosterone increase slowly at approximately the same rate (Sumpter et al. 1984). Vitellogenin is a high-energy glycolipo-phosphoprotein which provides essential nutrients for future alevins (Pankhurst 1998).

Final oocyte maturation begins upon completion of exogenous vitellogenesis. The volume of the oocyte continues to increase, resulting in a dramatic increase in the weight of the ovaries and the gonadosomatic index (GSI) between September and the time of spawning (Sumpter et al. 1984). The gonadosomatic index is the ratio of fish gonad weight to body weight. In most salmonids, a GSI value greater than 30 % during summer is indicative of final maturation occurring in the autumn (Peterson and Harmon 2005). Peak levels of oestradiol-17 β normally occur one to two months before ovulation in the autumn, whereas peak levels of testosterone occur at the time of ovulation (Frantzen et al. 1997). Prostaglandins stimulate ovulation, which occurs when the follicles rupture and release the oocytes (ova) into the peritoneal cavity (Pankhurst 1998). The ova may remain in the peritoneal cavity for several days before being released to the environment for external fertilization, which is termed oviposition (Stacey 1984).

The three main phases of testicular development include spermatogenesis, spermiogenesis, and spermiation. Sperm cells, termed spermatogonia and spermatocytes, increase in number during early spring and summer (Takashima and Yamada 1984). Spermiogenesis occurs during the autumn just prior to spawning, and involves the spermatids differentiating into mature spermatozoa (sperm). Spermiation is described as the hydration of the seminal fluid-spermatozoa suspension (milt), which is then released from the male (Pankhurst 1998). The principle steroid in male reproduction is testosterone and its derivative 11-keto-testosterone. 11-keto-testosterone stimulates the development of secondary sexual characteristics, such as the hooked jaw and reddish body color, while testosterone is associated with the regulation of spermatogenesis (Pankhurst 1998).

2.3.2 Sexual Maturation in Farmed Salmonids

Early sexual maturation is disadvantageous in commercial salmonid farming, as maturation results in a decreased growth rate compared to immature fish, when energy is diverted from somatic growth into gonadal growth (Aksnes et al. 1986). Farmed Atlantic salmon that are maturing experience a decrease in appetite during the summer, while immature Atlantic salmon continue to feed (Kadri et al. 1997). It has been suggested that this reduction in appetite is similar to the cessation of feeding in wild Atlantic salmon that are migrating up-river to spawn, and that the cessation of feeding may be independent of rearing temperature (Kadri et al. 1997).

The major factors promoting early sexual maturation among farmed salmonids are the favorable growing conditions in hatcheries, particularly warm rearing temperatures and high quality diets (Nordeng 1983). Under culture, the growth of Arctic charr is greatly accelerated; a fish often attains a size in one year that would take several years in the wild (Delabbio 1995). If sexual maturation could be delayed or arrested by photoperiod manipulation, somatic growth could be maximized, allowing the fish to reach market size (> 1 kg) before maturing. This could be of considerable advantage to the aquaculture industry (Duston et al. 2003, Jobling and Baardvik 1991).

Sexual maturation negatively affects the flesh quality of farmed salmonids. In maturing Atlantic salmon, the fat content in the flesh decreases from 12 to 5 %, the water content increases from 66 to 74 %, and the protein content decreases from 22 to 19 %. The texture becomes watery and tough, and there are significant changes in the odour and flavour (Aksnes et al. 1986). Flesh pigmentation is an important measure of market acceptability, with pale flesh being of inferior quality (Christiansen and Wallace 1988, Jobling et al. 1993). The desirable red pigmentation is due to the incorporation of dietary astaxanthin (Aas et al. 1997). During sexual maturation, astaxanthin is mobilized from the flesh into the developing ova of females and the skin of males, thus reducing flesh quality (Hatlen et al. 1996). Both the somatic growth and flesh quality may be improved by arresting the onset of sexual maturation using photoperiod manipulation or several other techniques that can be easily used on a fish farm.

The onset of sexual maturation also affects the hypo-osmoregulatory ability of farmed salmonids. Maturing male Atlantic salmon experienced high mortality when

subjected to a salinity tolerance test (Bjerknes et al. 1992). Maturing brook charr (*Salvelinus fontinalis*) reared in seawater during the spawning season (September/October) experienced high mortality compared to immature fish, who did not suffer any mortality. In addition, maturing males performed poorly compared to both immature and maturing females (LeFrançois et al. 1997). It has been suggested that because gonadal growth was inhibited in immature fish, that there were more energy reserves available for osmoregulation, an energy demanding process (LeFrançois et al. 1997). Reduced gill Na⁺-K⁺ATPase activity was recorded in maturing female brook charr reared in seawater during the spawning season (LeFrançois and Blier 2000). To assess the effect of sexual maturation on hypo-osmoregulatory ability, the growth and survival of immature and maturing male and female Arctic charr reared in seawater tanks during the spawning season (September through November) was investigated in Experiments 2 and 3.

2.4 Photoperiod Manipulation

2.4.1 Endogenous Circannual Rhythm

Understanding how spawning is synchronized and timed in salmonids and other commercially important species such as Atlantic cod (*Gadus morhua*; Davie et al. 2007) allows one to understand how photoperiod manipulation can be successfully used in aquaculture to (1) produce a year-round supply of eggs and (2) control or arrest the onset of sexual maturation.

The synchronization and accurate timing of spawning in seasonal breeders is hypothesized to be mediated by an endogenous (internal) oscillator or clock. The endogenous control of reproductive development ensures that even under constant environmental conditions, an iteroparous salmonid will exhibit a ‘free-running’, approximately annual rhythm of sexual maturation (Duston and Bromage 1986). This endogenous rhythm has been shown to free-run with a ‘circannual periodicity’ in female rainbow trout under constant photoperiod regimes such as LD6:18, continuous light (LD24:0), or long days (LD18:6), when reared under constant temperature for two to four consecutive reproductive cycles (Duston and Bromage 1986, 1987, 1991). Therefore, the successful spawning of rainbow trout maintained under constant short and long days

suggests that sexual maturation is not entirely dependent on seasonal changes in daylength (Duston and Bromage 1987).

Instead, the seasonally-changing daylength acts as a 'zeitgeber' or coordinating mechanism, providing a series of photoperiodic cues that synchronizes or 'entrains' the endogenous rhythm of reproduction and spawning (Duston and Bromage 1986). Experiments using female rainbow trout have shown that the photoperiod most stimulatory to ovarian development is a long day (≥ 16 h light per day) or a naturally increasing daylength until spring or early summer, then followed by a constant short day (≤ 8 h light per day; Bromage and Duston 1986, Duston and Bromage 1987). A long day is perceived as a signal to initiate the development of male and female sex organs, while a short day is perceived as a signal to initiate final maturation (Bromage et al. 1984).

2.4.2 The Pineal Gland and Melatonin Production

Photoperiodic signals are received by the retina, the pineal gland, and possibly by photoreceptors within the brain (Amano et al. 2000). The pineal gland synthesizes melatonin, and the highest levels of melatonin occur during the night, while levels fall to basal during the day (Bromage et al. 2001). The duration of the increase in melatonin is proportional to the length of the night. This occurs at all times throughout the year, revealing that melatonin provides information on the daily and seasonally changing light-dark cycle in Atlantic salmon (Bromage et al. 2001). It has been postulated that the rhythms of circulating melatonin may potentially be used by Atlantic salmon (Randall et al. 1995) and Arctic charr (Strand et al. 2008) to time daily and seasonal events such as sexual maturation.

2.4.3 Photoperiodic Control of the Timing of Spawning

Artificial photoperiod regimes employed on fish farms may be used to advance or delay the timing of sexual maturation as compared to controls on an ambient photoperiod, or they may be used to synchronize the time of spawning of individual fish (Bromage and Duston 1986). The use of constant long days (*LD18:6* or *LD16:8*) or continuous light (*LD24:0*) regimes has resulted in the advance of maturation in rainbow trout by 8 weeks when compared to fish maintained under ambient photoperiod (Duston and Bromage

1986). Exposing rainbow trout to constant short days (*LD8:16*) after the summer solstice (June 21) and also after the winter solstice (December 21) advanced the timing of spawning (Bromage and Duston 1986).

Exposing trout to constant long days of *LD16:8* from February to June 21, then a drastic reduction to constant short days of *LD8:16* from June 21 onwards advanced spawning by 3 to 4 months compared to controls (Bromage and Duston 1986). In addition, subjecting trout to a mere 6 weeks of *LD18:6* from February to May, then a direct reduction to *LD6:18* thereafter (a *Long-to-Short* regime), resulted in a 12 week advance in spawning (Bromage et al. 1984, Bromage and Duston 1986).

An important concept in the use of a *Long-to-Short* photoperiod regime is that the abrupt reduction in photoperiod provides an important cue for the advancement of the time of spawning, and also has an additional effect of synchronizing the time of spawning of individual trout to within 6 weeks between the first and last ovulating female (Duston and Bromage 1987). In addition, it has been established that the absolute size of the reduction in daylength has only a marginal effect on gonadal development, and it is actually the direction of the change in photoperiod which provides the cue for normal reproductive development (Bromage and Duston 1986, Duston and Bromage 1987).

In terms of delaying the time of spawning, subjecting trout to constant *LD18:6* days after the summer solstice causes a delay in maturation, as well as subjecting trout to constant *LD6:18* after the winter solstice (Bromage and Duston 1986). Therefore, the advance or delay of sexual maturation in rainbow trout depends on the timing of the ‘photoperiodic zeitgeber’.

Long days earlier in the year, particularly if followed by short days, would be expected to lead to corrective phase advances of the rhythm because it will have been perceived as ‘running behind real time’. Similarly, short days early in the year or long days after the summer solstice would both be perceived as the clock being ‘ahead of real time’ and would be followed by phase delays in the rhythm (Bromage et al. 2001). Although advanced spawning results in a decrease in oocyte size (Duston and Bromage 1988), the fecundity of the broodstock and the quality and fertility of the eggs are not affected by the photoperiod manipulation, providing that spawning is not advanced more than 2 to 3 months (Bourlier and Billard 1984, Bromage et al. 1984). As a result, the

finfish aquaculture industry has utilized the manipulation of artificial photoperiods to modify the speed of maturation and the time of spawning, allowing for the year-round production of eggs (Bromage et al. 1984).

2.4.4 Photoperiodic Control of Sexual Maturation in Virgin Fish

In addition to modifying the speed and timing of maturation, photoperiod manipulation has been used to control sexual maturation in virgin fish. The current hypothesis for the commencement of sexual maturation is that two requirements must be achieved. As gonadal development and maturation requires a considerable amount of energy, virgin fish may need to attain a critical size or energy status at a certain phase of the circannual cycle to proceed with sexual maturation. For the second requirement, the endogenous clock needs to be at a specific ('gate open') phase of this circannual rhythm (Duston and Bromage 1988). Therefore, the 'decision' to mature is proposed to be a gated rhythm, and the fish must pass through this 'gate' or 'window of opportunity' to be able to mature (Bromage et al. 2001).

Since photoperiod manipulation is an easy and convenient method to employ on a fish farm, research should be conducted to establish the most effective photoperiod regime to reduce the incidence of early sexual maturity. Research on rainbow trout (Duston and Bromage 1988), Atlantic salmon (Taranger et al. 1999), Fraser River Arctic charr (Duston et al. 2003), and Atlantic cod (Davie et al. 2007), has shown that sexual maturation can be arrested or inhibited by a photoperiodic advancement of the timing of an annual 'critical period'. Subjecting Arctic charr to a long day (*LD18:6*) for 42 days starting February 3, then an abrupt change to short day (*LD6:18*) on March 16 resulted in a decreased incidence of sexual maturation (Duston et al. 2003). The proportion of males that matured was 45 %, as compared to 83 % in the controls maintained under simulated natural photoperiod (45 °N). In addition, the proportion of females that matured was 20 % compared to 50 % in the controls (Duston et al. 2003). The effectiveness of this *Long-to-Short* regime was first established in an experiment involving rainbow trout (Duston and Bromage 1988). The *Long-to-Short* regime early in the year resulted in a phase advance in the timing of spawning. It has been postulated that salmonids exposed to the photoperiodic advance early in the year had not achieved the necessary body size or

energy status, and were unable to mature during that reproductive cycle (Duston et al. 2003, Taranger et al. 1999). Reductions in the incidence of sexual maturity in Arctic charr were also achieved using a *Long-to-Ambient* regime, where 66 % of males and 32 % of females matured (Duston et al. 2003). Although the *Long-to-Short* regime was shown to be the most effective in reducing the incidence of sexual maturation in Arctic charr, most fish farms may not be able to achieve a daylength shorter than the natural seasonal daylength. Therefore, *Long-to-Ambient* may be the most appropriate regime.

The timing of the application of the *Long-to-Ambient* regime is critical, and the hypothesis is that a further reduction in the incidence of sexual maturation may be achieved if the photoperiod treatment occurs earlier in the year (winter solstice, December 21), before the onset of gonadal development. The exposure to a longer photoperiod in winter arrests oogenesis at the pre-vitellogenesis stage and inhibits the circulation of melatonin (Peterson and Harmon 2005, Taranger et al. 1999). However, the response to a specific photoperiod regime may vary throughout the year depending on the physiological condition of the fish, as well as sex, age and/or body size, reproductive history and genetic strain (Frantzen et al. 2004a). The timing of the application of the *Long-to-Ambient* regime was investigated in Experiment 2, in an attempt to arrest the onset of sexual maturation among individually-tagged Arctic charr.

2.4.5 Photoperiodic Control of Somatic Growth

Photoperiod manipulation may be used to enhance growth of juvenile salmonids in the freshwater stage. The growth of farmed salmonids from egg to the seawater stage can be compressed into one or two years, as growth under the appropriate rearing conditions is more rapid than in the wild (Delabbio 1995). One of the goals is to produce under-yearling out-of-season smolts, to ensure a constant supply of fish for consumers (Handeland and Stefansson 2001). Photoperiod regimes such as continuous (*LD24:0*) have been used to enhance the growth of juvenile Atlantic salmon in freshwater (Handeland and Stefansson 2001). Long photoperiods may also be used in summer and autumn to enhance growth under a naturally decreasing photoperiod, as a longer daylength allows the fish to feed for a longer period of time (Tveiten et al. 1996) and stimulates growth hormone secretion (McCormick et al. 1995). A previous study at

NSAC showed that the growth of Fraser River Arctic charr in 20 ppt deteriorated in November despite a constant rearing temperature of 10 °C (Duston et al. 2007). To address the problem of a seasonal deterioration in growth, the fish in Experiment 1 were reared at the same temperature as Duston et al. (2007), and a long photoperiod (*LD16:8*) from June 21 onwards was applied to assess growth performance and hypo-osmoregulatory ability in autumn. *LD16:8* was chosen as the natural photoperiod in Truro, NS (Latitude 45 °N) on June 21 was just over 15 hours long.

2.5 Production Efficiency

2.5.1 Effect of Temperature and Food Availability on Somatic Growth

When food is not limiting, the two most important factors affecting the growth rate of farmed Arctic charr in freshwater are temperature and body size (Jobling 1983). Optimal growth and food conversion ratio of Arctic charr in freshwater occurs between 10 and 13 °C (Wandsvik and Jobling 1982b). At low freshwater temperatures, Arctic charr show the greatest resistance to freezing compared to other salmonids such as Atlantic salmon and rainbow trout (Fletcher et al. 1988), and Arctic charr exhibited higher growth rates than farmed rainbow trout between 0.3 and 5 °C (Brännäs and Wiklund 1992, Jobling et al. 1993). Immature Arctic charr over-wintering in freshwater lakes are able to feed despite the cold water (less than 1 °C; Klemetsen et al. 2003b). By contrast, sexually mature charr do not appear to feed when over-wintering in freshwater lakes (Rikardsen et al. 2003).

Hatchery producers in Atlantic Canada typically maximize year-round growth by rearing Arctic charr in fresh groundwater at approximately 10 °C. The transfer to seawater in the spring would result in a thermal challenge, as seawater temperatures in Atlantic Canada are approximately 5 °C (Peterson and Harmon 2005, Saunders et al. 1983). Atlantic salmon smolts transferred from 6 °C freshwater to 2, 4, and 6 °C seawater had reduced food intake during the first few weeks after transfer, but appetite and growth resumed thereafter (Arnesen et al. 1998). The hypo-osmoregulatory ability of the fish was not compromised at even the lowest temperature, suggesting that the reduction in appetite and growth was related to the cold temperature (Arnesen et al. 1998). As Arctic charr are less tolerant to direct transfer from freshwater to seawater compared to Atlantic

salmon, an acclimation from 10 to 5 °C freshwater in spring may improve the transfer to 5 °C seawater. This was investigated in Experiment 2.

Temperature also affects the food conversion ratio (FCR), which is the ratio of the amount of food consumed to the gain in biomass. Food conversion ratio is an important calculation used by fish farmers to express the performance of fish and feed, as the cost of feed is the biggest expense while growing fish to market size (Sinnott 2002). An FCR with a ratio ranging from 1:1 to 1.2:1 is indicative of best-performing individuals that can efficiently convert food to flesh, thus reducing production costs (Sinnott 2002). Another calculation used by fish farmers is the specific growth rate (SGR), which is a measure of the percentage of body weight increase per day (Sinnott 2002). In Experiments 1 and 3, food offered per tank was recorded to allow for calculations of FCR and SGR.

Wild Arctic charr often exhibit seasonal changes in food intake and growth in association with the increasing and decreasing temperature and photoperiod; food intake and growth are high during spring and summer, while suppression occurs in autumn and winter (Damsgård et al. 1999, Rikardsen et al. 2003, Sæther et al. 1996). Long-term fasting in Arctic charr is natural, and corresponds to the food availability in the high-Arctic (Tveiten et al. 1996). Wild Arctic charr rely on endogenous energy reserves throughout the winter months (Aas-Hansen et al. 2003). However, these energy reserves may be depleted by as much as 46 % by spring (Dutil 1986). These seasonal changes in food intake are also observed in farmed Arctic charr that were offered food throughout the year (Tveiten et al. 1996). In the current study, food offered per tank was recorded to monitor the seasonal changes in food intake of the Arctic charr.

2.5.2 Effect of Temperature and Food Availability on Inhibition of Sexual Maturation

There are a few ways to attempt to control the onset of sexual maturation. The first and foremost is by photoperiod manipulation. However, other methods can be used in combination with photoperiod manipulation. These include slowing growth rate by restricting food intake during the winter period when the ‘decision’ to mature is being made (Rowe and Thorpe 1990). Rearing in cold water during the winter period also slows growth rate (Saunders et al. 1983). Sexual maturation may be arrested due to insufficient acquisition of energy reserves during this critical time, which are required for

the development of gonads the following autumn (Rowe and Thorpe 1990, Saunders et al. 1983). In addition, spawning may be delayed due to slow oocyte growth and development; low temperatures in winter reduce the rate of sequestration and incorporation of yolk into the oocytes (Jobling 1995).

The restriction of food in Atlantic salmon parr, particularly during the late winter (April), has been shown to affect the incidence of sexual maturation the following autumn (Rowe and Thorpe 1990). In contrast, in male Atlantic salmon smolts, maturation as grilse (a term for the maturation of salmon after only one winter at sea) was not related to a restricted food intake during the winter (Duston and Saunders 1999). In fact, the changes in growth from the previous summer/autumn in seawater (over one year before final maturation) were more associated with the onset of sexual maturation than reduced growth in winter (less than one year before final maturation; Duston and Saunders 1999). In females, however, food restriction during the winter significantly affected the incidence of sexual maturation (Duston and Saunders 1999). Similar results were found in female Atlantic salmon when they were exposed to a restricted ration within the December to April period (Thorpe et al. 1990). Clearly, there are significant differences among male and female Atlantic salmon. Perhaps the effects of a food restriction were more pronounced in females due to the higher energy requirement for the development of oocytes (Johnston 2002).

In contrast to Atlantic salmon, in repeat-spawning Arctic charr, the proportion of sexually maturing fish was independent of the length of time of the fasting (3 to 9 months; Frantzen et al. 2004b). The fish in this experiment were very large, almost 800 g, and perhaps these fish had better energy reserves compared to small, virgin fish. Sexual maturation in small Arctic charr (< 200 g) was significantly reduced by restricting food intake during the autumn and winter, one-year prior to final maturation (Imsland and Gunnarsson 2011). Perhaps the long-term fasting that occurs during over-wintering reduces the effectiveness of a restricted ration in large Arctic charr (Tveiten et al. 1996). Experiment 3 investigated the combined effects of a cold temperature, a *Long-to-Ambient* photoperiod regime, and a restricted ration in early winter on the growth performance and the incidence of sexual maturation among individually-tagged male and female Arctic charr.

2.5.3 Effect of Somatic Growth and Sexual Maturation on Production Efficiency

Other factors affecting somatic growth are aggression and competition for food by dominant individuals within a population or tank. Competition may be reduced or avoided by carefully feeding to apparent satiation at every meal, and juveniles on a fish farm are often sorted or graded according to body size to improve growth for subordinate fish (Brännäs et al. 2002). In terms of production efficiency, a tank containing individuals of varying sizes is difficult to harvest all at once as only a number of individuals will have reached market size, increasing production costs for multiple harvests (Jobling et al. 1993, Johnston 2002). Experiments 1, 2 and 3 examined the variation in final body weights of individually-tagged Arctic charr, and the total biomass of both marketable (immature fish > 1 kg) and non-marketable fish (all maturing fish and immature fish < 1 kg) was calculated with respect to the treatments.

2.6 Summary

The literature provides knowledge about the life-history of wild Fraser River Arctic charr, which helps to understand the behaviour and performance of farmed fish. To maximize aquaculture production of Fraser River Arctic charr, a safe protocol for introducing the fish to seawater needs to be established. An experiment examining the acclimation of Fraser River Arctic charr to seawater would be beneficial to determine the extent of their hypo-osmoregulatory ability for on-growing in seawater, and how it impacts their survival and subsequent growth performance.

To maximize aquaculture production of Fraser River Arctic charr, the high incidence of sexual maturation that occurs before harvesting needs to be addressed. An experiment to determine the exact timing of the application of a long photoperiod would be beneficial to help arrest or reduce the high incidence of sexual maturation. In combination with photoperiod manipulation, the use of cold water temperature and restricted food intake may be effective at reducing the incidence of sexual maturation. An experiment combining photoperiod, temperature, and restricted food intake would be beneficial to evaluate the effectiveness of these treatments to arrest or reduce the incidence of sexual maturation among farmed Fraser River Arctic charr.

Chapter 3.0 General Materials and Methods

3.1 Early Rearing at Shippagan, New Brunswick

Fish were reared from egg to one year old (greater than 100 g) under simulated natural photoperiod in 10 to 13 °C freshwater. During this time, fish were graded twice, at 10 g and 250 g, by discarding the fish that were less than the median weight. NSAC received the larger and better performing individuals. CZRI has provided fish that were identified by a combination of fin-clippings (at 10 g body size) and hot-brands (at 250 g body size) to identify different families and generations. Clipped fins were either adipose, pelvic or pectoral on either left or right side of the fish. Hot-brands were either front, centre, or back of left side of the fish.

3.2 Pre-experimental Conditions and PIT-tagging

At least one month prior to the start of each of the three experiments, 0+ to one-year-old Arctic charr ($n = 600$) were transferred by truck from Shippagan to the NSAC Aquaculture Centre. The fish were held in a flow-through tank supplied with well water (9.5 – 10 °C) under simulated natural photoperiod. In all experiments, food was withheld from the fish 24 h prior to being anesthetized. Fish were anesthetized by removing them from the tank in groups of 4 or 5 and submersing them in a water bath that contained 0.1 g L⁻¹ tricaine methanesulfonate (*TMS*; Syndel Laboratories Ltd. AquaLife TMS). Once anesthetized (2 – 5 min in the water bath), the fish were identified by injecting an electronic Passive Integrated Transponder (PIT) tag either into the body cavity (Experiment 1) or into the musculature on the right flank posterior to the dorsal fin (Experiments 2 and 3). The location of the injection was changed due to the difficulty of relocating the tags within the body cavity. Each PIT-tag (12 mm long, 2 mm wide) consisted of a unique 10-digit alpha-numeric code which was read by scanning over the exterior of the fish with a PIT-tag reader (AVID Power Tracker™ V, Calgary, AB).

3.3 Recirculation Systems

Four identical recirculation systems were available for experiments, in which temperature, salinity, and photoperiod were regulated (Aquabiotech Inc. Multi-tank

REBF Systems, Coaticook, QC). Each recirculation system included bio-filtration, solid separation by sediment traps and a sand filter, and the removal of dissolved organic matter by foam fractionation. Each system (4675 L) was able to recycle up to 82 % of the water on a daily basis; the freshwater makeup flow rate was 200 ml/min and the seawater makeup flow rate was 400 ml/min, for a total of 864 L per day. Filtered seawater was trucked to the NSAC Aquaculture Centre from the National Research Council of Canada – Halifax’s Marine Research Station (Ketch Harbour, NS), and was stored in underground tanks. Water temperature was regulated by a pump / chiller unit (0.5 to 30 °C). Air enriched with oxygen was injected into the water via an air tube injector, and carbon dioxide was degassed through the biofilter. A recirculation system was comprised of eight insulated fiberglass tanks, each with a diameter of 1 m and a working volume of approximately 495 L. Each tank was covered with a light-proof canopy (Hinspergers Poly Industries Ltd., Truro, NS). Light (50 lux) was provided by a single 60 watt incandescent bulb housed inside each canopy, 60 cm from the water surface. Photoperiod (with no twilight period) was regulated using a direct digital control system (Delta Controls) with Orcaview 3.30 software.

3.4 Photoperiod Regimes

Three photoperiod regimes were used within the study: (1) simulated natural daylength, also known as *Light Dark Natural (LDN, Latitude 45 °N)*, in Experiments 1, 2, and 3; (2) summer solstice daylength, also known as *Constant Long Photoperiod (CLP, 16 h Light/8 h Dark or LD16:8)*, in Experiment 1; and (3) *Long-to-Ambient (LD18:6 for 6 weeks then LDN)*, in Experiments 2 and 3.

3.5 Fish Health and Feeding

Fish health was monitored daily. Oxygen concentration (% saturation), salinity (ppt), temperature (°C), and pH (7.0 to 8.0) were recorded daily. Oxygen saturation in all tanks was maintained > 90 %. Oxygen concentration was measured in at least two tanks (randomly chosen) within each recirculation system before feeding the fish in the morning. pH was maintained by manual addition of sodium bicarbonate. Total ammonia-nitrogen and nitrite concentrations were recorded either daily, weekly or bi-

weekly, depending on what stage/condition the biofilters in the recirculation system were in. The frequency of recording total ammonia-nitrogen and nitrite concentrations was higher in the weeks after the fish were first introduced to the recirculation systems. The frequency decreased when the concentrations stabilized and were within normal range (i.e. the biofilters were active with nitrifying bacteria).

Fish were hand-fed a commercial salmon diet to apparent satiation (i.e. feeding was stopped when uneaten food was observed on the bottom of the tank) two to three times per day. The two diets were: (1) Signature Salmon™, Shur-Gain, Truro, NS (42 % crude protein, 26 % fat, and 4 % fibre) or (2) Skretting™, St. Andrews, NB (44 % crude protein, 24 % fat, and 1.5 % fibre). The Signature Salmon™ diet was used while the fish were in freshwater, and the Skretting™ diet was used while the fish were in seawater (Experiments 1 and 2). However, the diet was changed to Signature Salmon™ (Experiment 3) due to the high incidence of diarrhea that occurred the previous year while the fish were in seawater. The pellet size was gradually increased from 3.5 to 7.5 mm to fit the growing fish during the experimental period. Food offered per tank was recorded in order to calculate food conversion ratio ($FCR = \text{food fed} / \text{biomass gain}$). The fish were cared for in accordance to the local Animal Care and Use Committee guidelines that follow the Canadian Council on Animal Care Codes of Practice (CCAC 2005).

Any mortalities were promptly removed from the tank, PIT-tag number recorded, measured for body size, dissected to determine sex and sexual maturity status, and gonad weights (g) recorded. Mortalities were visually inspected to attempt to determine the cause of death. Any previous data for the mortalities were removed from the master data file. Only fish which survived to the end of the experiment, and had a complete data set were used in the data analysis.

3.6 Determination of Sex and Sexual Maturity

Sex and sexual maturity was first assessed by visual inspection on sampling days throughout all three experiments. Females were considered to be fully mature when they ovulated, i.e. when the eggs could be extruded from the body cavity by applying gentle

pressure on the abdomen. Males were considered to be fully mature or “running” when milt could be extruded from the body cavity by applying gentle hand pressure.

Sex and sexual maturity of all fish were determined by dissection when the fish were euthanized (overdose of *TMS*) at the end of each experiment. Fish were classified as “immature” if the male gonads appeared to be thread-like, and if female gonads only contained primary oocytes. In all three experiments, the term “maturing” refers to all fish that had gonads in some stage of development (i.e. were not immature) and all fish that had fully matured.

3.7 Determination of Blood Plasma Osmolality

Non-lethal blood samples were collected from a representative sample of fish from each treatment combination in Experiment 1 to determine blood plasma osmolality. Fish were anesthetized with *TMS*, and a blood sample was drawn from the *ductus Cuvier* under the right gill operculum using a heparinized 19 gauge needle and 1 mL syringe. Blood samples were stored on ice and then centrifuged (3000 rpm for 6 min). Blood plasma was collected and stored at -20°C until analyzed. Analysis of the plasma samples was performed in duplicate 50 μl samples using a Freezing Point Osmometer ($\mu\text{Osmette 5004}$, Precision Systems Inc., Natick, MA). A reference solution (290 $\text{mOsm kg}^{-1}\text{ H}_2\text{O}$, Clinicontrol™) was used after every twelve 50 μl samples.

3.8 Statistical Methods

The effect of the treatment combinations on the mean body weight of the fish was analyzed as repeated measures using the MIXED procedure of SAS as multiple-factor factorials, with individual fish as the experimental unit (SAS Institute 2008). Covariance structures were determined to be either compound symmetry, autoregressive order 1, or unstructured. When required, transformations of mean body weight and food conversion ratio were performed to satisfy the normality and constant variance assumptions of the ANOVA (Littell et al. 1998). The *P*-values in all factorial tests were estimated using the Restricted Maximum Likelihood method. Least squares means of the treatment combinations from the highest order significant interaction effects were computed, and their letter groupings were generated to identify the means that were significantly

different at either a 5 % (within each sampling date) or a 1 % (within each treatment over time) level of significance.

The incidence of mortality and sexual maturity were both analyzed as either single factor or multiple-factor factorials using the CATMOD procedure of SAS with the contrast statement (SAS Institute 2008). The response variables % mortality and % sexual maturation were calculated and their letter groupings were generated at the 5 % level of significance.

In each experiment, immature fish with a round weight > 1 kg were categorized as marketable. The biomass of marketable fish was determined as the sum of the body weight (kg) of the all of the immature fish that were over 1 kg round weight at the termination of each experiment. Maturing fish with a round weight > 1 kg were categorized as non-marketable, and their biomass was determined in the same manner as the immature fish. In addition, all other fish (both immature and maturing) with round weights less than 999 g were included in the total biomass of non-marketable fish.

Chapter 4.0 Effect of Acclimation to Full-Strength Salinity and Extended Daylength in Autumn on Growth and Survival of Arctic charr

4.1 Introduction

A major problem inhibiting Arctic charr aquaculture is that the Fraser River stock directly transferred from freshwater to 30 ppt seawater suffer either high mortality or very poor growth compared to Atlantic salmon (Duston et al. 2007). Cultured Arctic charr may need to be transferred to brackish water (20 ppt) and then acclimated to full-strength seawater (30 ppt), mimicking wild fish which remain in a brackish estuary for some time before migrating to full-strength seawater (Gulseth et al. 2001a).

The initial laboratory trial in 2004 at NSAC (as part of the AIF funded project) transferred Arctic charr from freshwater to ≤ 20 ppt. They exhibited good growth and survival, whereas fish reared in 30 ppt grew poorly (Duston et al. 2007). Following on from this trial, the objective of the current study was to determine if Fraser River Arctic charr could grow efficiently at 30 ppt if preceded by direct transfer from freshwater to 20 ppt, then acclimation to 30 ppt.

In the 2004 trial, the growth of Arctic charr in 20 ppt was good from June to August, but deteriorated in November despite a constant rearing temperature of 10 °C (Duston et al. 2007). To test the hypothesis if this decrease in growth was due to the seasonal decrease in daylength, photoperiod was included as an experimental factor in the present trial. The use of an artificial photoperiod such as *LD16:8* may be used in summer and autumn to enhance growth under a naturally decreasing photoperiod, as a longer daylength allows the fish to feed for a longer period of time (Tveiten et al. 1996) and stimulates growth hormone secretion (McCormick et al. 1995). The fish in the current study were reared in tanks at the same temperature as Duston et al. (2007; 10 °C), and a long photoperiod (*LD16:8*) from June 21 – December 29 was compared to a control on simulated natural daylength.

The onset of sexual maturation in autumn affects the hypo-osmoregulatory ability of most salmonids. Reduced gill enzyme activity ($\text{Na}^+ \text{-K}^+ \text{ATPase}$) was recorded in maturing female brook charr (*Salvelinus fontinalis*) reared in seawater during the spawning season (LeFrançois and Blier 2000). Farmed salmonids that are sexually maturing experience a decrease in appetite during the summer, while immature salmonids

continue to feed (Kadri et al. 1997). The slow growth of Arctic charr in autumn during the spawning season may be attributed to the onset of sexual maturation and the negative effects of sexual maturation on hypo-osmoregulatory ability. Small, slow-growing fish may be also be immature fish that are unable to osmoregulate, as body size is an important factor affecting hypo-osmoregulatory ability (Bjerknes et al. 1992, Handeland and Stefansson 2001). To assess the effect of sexual maturation on hypo-osmoregulatory ability, blood plasma osmolality and growth of immature and maturing male and female Arctic charr reared in seawater tanks at constant 10 °C during the spawning season (September through November) was investigated. In addition, blood plasma osmolality was determined for both fast and slow-growing fish.

4.2 Objectives and Hypotheses

The objectives of this experiment were to (i) investigate the growth performance and survival of Arctic charr when transferred directly from freshwater to full-strength seawater (30 ppt), (ii) investigate the growth performance and survival of Arctic charr when acclimated from intermediate (20 ppt) to full-strength seawater (30 ppt), and (iii) investigate how a long photoperiod (*CLP*; *LD16:8*) from June onwards affects growth, survival, and hypo-osmoregulatory ability of Arctic charr in autumn.

It was hypothesized that Arctic charr would be osmotically stressed in June, after the direct transfer from freshwater to 30 ppt, and that they would not be osmotically stressed when transferred from freshwater to 20 ppt. The acclimation from 20 ppt to 30 ppt was hypothesized to result in a better growth performance and survival compared to the direct transfer from freshwater to 30 ppt. It was hypothesized that *CLP* throughout the summer and autumn would improve the growth performance of Arctic charr, and also that sexually maturing Arctic charr would be osmotically stressed in October when reared under simulated natural photoperiod (*LDN*), but not when reared under *CLP*. In addition, it was hypothesized that slow-growing Arctic charr would be osmotically stressed during this time.

4.3 Materials and Methods

4.3.1 Experimental Design

The experiment was a 3x2 factorial design with three replicates. The two factors were salinity (20 ppt, 20 increased to 30 ppt (20-30 ppt), and 30 ppt) and photoperiod (*LDN* and *CLP* from June 21 onwards).

4.3.2 Experimental Procedures

Arctic charr (Fraser River, Labrador stock, 1+ year-old, ~ 626 g) from 12 full-sib families (from the F3¹ generation of class 2003) of a pedigreed breeding program by the CZRI were used in the study. F3¹ means the second family from the third generation. In March 2005, 600 fish were transferred by truck from Shippagan to the NSAC Aquaculture Centre and were held in two tanks (1200 L) with a flow-through supply of well-water (10 °C). On April 20, each fish was anesthetized with *TMS* and PIT-tagged in the body cavity. On May 21, an electrical panel failure resulted in a loss of water supply to one of the tanks, and 120 fish died.

Prior to the start of the experiment, the salinity of two recirculation systems was adjusted to 20 ppt and a third system to 30 ppt using a combination of natural and artificial seawater (Instant Ocean® Sea Salt, Dynamic Aqua-Supply Ltd., Surrey, BC). Salinity was then controlled by adjusting the ratio of freshwater: seawater make-up water. The experiment commenced June 1 and ended December 29, 2005. At this time, all fish were anesthetized, PIT-tag recorded, and measured for body size at 6-week intervals. On June 1, the fish (n = 476) were sorted by family (n = 12) into twelve, 250 L insulated tanks containing freshwater supplied with oxygen (> 90 % saturation). Fish from each family were then randomly distributed among three recirculation systems (6 tanks each) held at 10.0 °C (± 0.5 °C).

In total there were n = 157 to 161 fish per recirculation system with n = 25 to 28 fish per tank (~ 16.4 kg/m³). Within each recirculation system, the photoperiod for three tanks was *LDN*. For the other three tanks, the photoperiod was *LDN* from June 1 to June 21 then *CLP* from June 21 onwards. On July 16, the freshwater make-up was switched off in one recirculation system, resulting in salinity increasing progressively, reaching 30 ppt on July 30. A system malfunction on August 6 resulted in the loss of all fish (n =

153) from the 30 ppt treatment (for the incident report, see Appendix 1.1). Dead fish were removed from the system, PIT-tag recorded, measured for body size, and dissected to determine sex and sexual maturity status. The decision was made to continue the experiment as data could still be collected from the fish in the 20 and 20-30 ppt treatments.

Non-lethal blood samples were taken on two occasions throughout the trial: (1) on June 17, from a random sample of fish from both the 20 and 30 ppt treatments, and (2) on October 18, from two size classes of fish (slow-growing: < 700 g, fast-growing: > 1000 g). The size classes were chosen based on a review of individual growth rates from June 1 to October 4. The experiment was terminated December 29 when all fish were euthanized by *TMS* overdose. PIT-tag numbers were recorded and each fish was measured for body size and dissected to determine sex and sexual maturity status.

4.3.3 Statistical Analysis

A system malfunction on August 6 resulting in the loss of all fish in the 30 ppt treatment necessitated that the analysis of somatic growth be divided into two components:

(1) the effect of salinity on body weight of the fish ($n = 436$) within the first 6 weeks in seawater was analyzed as repeated measures using the MIXED procedure of SAS as a two-factor factorial, with individual fish as the experimental unit (SAS Institute 2008). The factors were salinity (20 and 30 ppt) and measurement date (June 1 and July 12). The most appropriate covariance structure was determined to be compound symmetry (Littell et al. 1998). The statistical model for the two-factor factorial in this analysis was expressed as

$$Y_{ijk} = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} was the response variable (body weight), μ was the overall mean, τ_i was the effect of date ($i = 1, 2$), β_j was the effect of salinity ($j = 1, 2$), the number of replicates was denoted by k ($k = 1, 2, \dots, n_{ijk}$), and ε_{ijk} was the error term;

(2) the effect of salinity and photoperiod on body weight of the fish ($n = 289$) that were directly transferred from freshwater to 20 ppt was analyzed as repeated measures using the MIXED procedure of SAS as a four-factor factorial, with individual fish as the experimental unit (SAS Institute 2008). The factors were salinity (20 ppt and 20-30 ppt), photoperiod (*LDN* and *CLP*), sexual maturity (immature and maturing), and measurement date (June 1, July 12, August 23, October 4, November 18, and December 29). The most appropriate covariance structure was determined to be autoregressive order 1, and mean body weight required a square root transformation to satisfy the normality and constant variance assumptions (Littell et al. 1998). The statistical model for the four-factor factorial in this analysis was expressed as

$$Y_{ijklm} = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + \delta_k + (\tau\delta)_{ik} + (\beta\delta)_{jk} + (\tau\beta\delta)_{ijk} + \gamma_l + (\tau\gamma)_{il} + (\beta\gamma)_{jl} + (\tau\beta\gamma)_{ijl} + (\delta\gamma)_{kl} + (\tau\delta\gamma)_{ikl} + (\beta\delta\gamma)_{jkl} + (\tau\beta\delta\gamma)_{ijkl} + \varepsilon_{ijklm}$$

where Y_{ijklm} was the response variable (body weight), μ was the overall mean, τ_i was the effect of date ($i = 1, 2, \dots, 6$), β_j was the effect of salinity ($j = 1, 2$), δ_k was the effect of photoperiod ($k = 1, 2$), γ_l was the effect of sexual maturation ($l = 1, 2$), the number of replicates was denoted by m ($m = 1, 2, \dots, n_{ijklm}$), and ε_{ijklm} was the error term.

Mean plasma osmolality in June was analyzed as a one-way ANOVA using the MIXED procedure of SAS, with salinity (20 and 30 ppt) as the factor and individual fish ($n = 12$) as the experimental unit (SAS Institute 2008). Mean plasma osmolality in October was analyzed as a four-factor factorial using the MIXED procedure of SAS, with salinity (20 and 20-30 ppt), photoperiod (*LDN* and *CLP*), sexual maturity (immature and maturing), and body size (slow and fast-growing fish) as factors and individual fish ($n = 38$) as the experimental unit (SAS Institute 2008). The statistical model for the one-way ANOVA in this analysis was expressed as

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

where Y_{ij} was the response variable (blood plasma osmolality), μ was the overall mean, τ_i was the effect of salinity ($i = 1, 2$), the number of replicates was denoted by j ($j = 1, 2, \dots$,

n_{ij}), and ε_{ij} was the error term. The statistical model for the four-factor factorial in this analysis was expressed as

$$Y_{ijklm} = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + \delta_k + (\tau\delta)_{ik} + (\beta\delta)_{jk} + (\tau\beta\delta)_{ijk} + \gamma_l + (\tau\gamma)_{il} + (\beta\gamma)_{jl} + (\tau\beta\gamma)_{ijl} + (\delta\gamma)_{kl} + (\tau\delta\gamma)_{ikl} + (\beta\delta\gamma)_{jkl} + (\tau\beta\delta\gamma)_{ijkl} + \varepsilon_{ijklm}$$

where Y_{ijklm} was the response variable (blood plasma osmolality), μ was the overall mean, τ_i was the effect of salinity ($i = 1, 2$), β_j was the effect of photoperiod ($j = 1, 2$), δ_k was the effect of sexual maturation ($k = 1, 2$), γ_l was the effect of body size ($l = 1, 2$), the number of replicates was denoted by m ($m = 1, 2, \dots, n_{ijklm}$), and ε_{ijklm} was the error term.

Food conversion ratio (FCR = food fed/biomass gain) and specific growth rate (SGR = $[(\ln W_T - \ln W_t) / (T - t)] \times 100$), where W_T and W_t are mean body weights (g) at times T and t , respectively, and $(T - t)$ is the time between measurements) from June 1 to July 12 were both analyzed as a one-way ANOVA using the MIXED procedure of SAS, with tank ($n = 6$ for both FCR and SGR) as the experimental unit (SAS Institute 2008). The factor was salinity (20 and 30 ppt). The statistical models for both of the one-way ANOVAs were expressed as

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

where Y_{ij} was the response variable (FCR or SGR), μ was the overall mean, τ_i was the effect of salinity ($i = 1, 2$), the number of replicates was denoted by j ($j = 1, 2, \dots, n_{ij}$), and ε_{ij} was the error term.

The p -values in all factorial tests were estimated using the Restricted Maximum Likelihood method. Least squares means of the treatment combinations from the highest order significant interaction effects were computed, and their letter groupings were generated to identify the means that were significantly different at a 5 % level of significance.

The incidence of mortality and sexual maturity were both analyzed using the CATMOD procedure of SAS with the contrast statement (SAS Institute 2008). Mortality within the first 8 weeks in seawater was analyzed with three levels of salinity (20, 20-30,

and 30 ppt). Mortality from August through December for the 20 and 20-30 ppt treatments ($n = 5$ and $n = 11$, respectfully) was not statistically analyzed. Reporting of mortality excluded the fish from the August 6 incident. Sexual maturity in December was analyzed as a three-factor factorial with salinity (20 and 20-30 ppt), photoperiod (*LDN* and *CLP*), and sex (male and female) as factors. The response variables % mortality and % sexual maturation were calculated, the proportions were further compared using the analysis of contrasts, and their letter groupings were generated at the 5 % level of significance.

Immature fish with a round weight > 1 kg were categorized as marketable. The biomass of marketable fish was determined as the sum of the body weight (kg) of the all of the immature fish that were over 1 kg round weight on December 29. Maturing fish with a round weight > 1 kg were categorized as non-marketable, and their biomass was determined in the same manner as the immature fish. In addition, all other fish (both immature and maturing) with round weights less than 999 g were included in the total biomass of non-marketable fish. The biomass of marketable and non-marketable fish was also determined with respect to sex.

4.4 Results and Discussion

4.4.1 Somatic Growth

Direct transfer from freshwater to 30 ppt on June 1 greatly inhibited growth compared to the direct transfer to 20 ppt ($p < 0.0001$). The initial mean weight (621 g) in 30 ppt only increased 66 g compared to 141 g among Arctic charr in 20 ppt by July 12 (Fig. 1). After more than 8 weeks in seawater (at the time of the loss of the 30 ppt treatment on August 6), their mean weight was 686 g whereas the fish in 20 ppt were estimated to be 815 g. The fish in the 20 ppt treatment were not weighed on August 6 when the 30 ppt fish were lost, therefore their weight was estimated by examining the growth trajectory in Figure 2.

The weight of the 30 ppt fish in the current experiment increased significantly in the first six weeks after transfer. By contrast, a previous study in the NSAC Aquaculture Centre showed that Arctic charr performed poorly when transferred directly from freshwater to 30 ppt (Duston et al. 2007). Although it has been suggested that a critical

body size of 200 g may be required for Fraser River Arctic charr to successfully survive long-term in seawater (Delabbio et al. 1990b), perhaps the 200 g difference in initial body size of the fish in this experiment (600 g) compared to the fish (400 g) in Duston et al. (2007) was a contributing factor. By contrast, Norwegian Arctic charr less than 100 g were successfully transferred from freshwater to 33 ppt (at 7 °C) from March through June, and exhibited a hypo-osmoregulatory ability comparable to Atlantic salmon (Jørgensen et al. 2007). Due to the sudden mortality of the population in 30 ppt on August 6, it cannot be confirmed whether growth would have improved over time. A follow-up experiment is recommended, to determine if the fish transferred to 30 ppt would achieve a better growth performance throughout the summer and autumn.

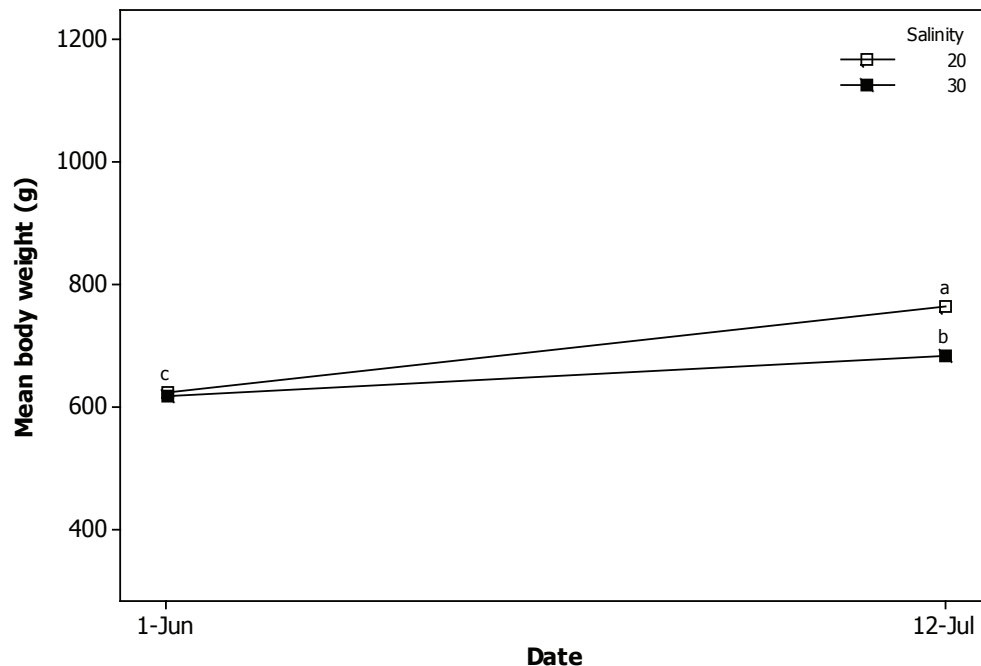


Fig. 1. Interaction plot of mean body weight (g) of Arctic charr reared in 10 °C seawater from the four combinations of salinity (20 and 30 ppt) and date (June 1 and July 12) following direct transfer from 10 °C freshwater. Means sharing the same letter are not significantly different at the 5 % level.

When comparing growth in 20 and 20-30 ppt from June to December, there was no effect of salinity ($p = 0.7970$; Table 1). Although it appears as though the Date*Salinity interaction was significant ($p = 0.0011$; Table 1), all p -values within the multiple means comparison within each date (comparing 20 to 20-30 ppt) were greater

than 0.2, and therefore there was no significant difference among the treatments within each date (Fig. 2). The significant differences occurred over time, within each of the salinity treatments.

Table 1

P values of the main and interaction effects of salinity, photoperiod, and sexual maturation from Repeated Measures Analysis on mean body weight of Arctic charr from June 1 to December 29, 2005.

Source of variation	<i>P</i> values
Date	< 0.0001
Salinity	0.7970
Date x Salinity	0.0011
Photoperiod	0.3691
Date x Photoperiod	0.0016
Salinity x Photoperiod	0.2374
Date x Salinity x Photoperiod	0.3777
Maturation	0.7746
Date x Maturation	< 0.0001
Salinity x Maturation	0.6461
Date x Salinity x Maturation	0.1641
Photoperiod x Maturation	0.2553
Date x Photoperiod x Maturation	0.2828
Salinity x Photoperiod x Maturation	0.3352
Date x Salinity x Photoperiod x Maturation	0.8932

To successfully culture Fraser River Arctic charr in seawater, the results show that the fish should be transferred from freshwater to 20 ppt and then be acclimated through a progressive increase in salinity to 30 ppt. Similarly, a previous study on Canadian Arctic charr (Nauyuk River system and Fraser River) used step-wise increments in salinity to acclimate to 32 ppt over a period of two weeks, to “simulate the natural salinities experienced by wild charr during spring migration” (Delabbio et al. 1990b). In contrast, Norwegian populations do not appear to require an acclimation period, and are often exposed to high salinities (~25 ppt) within a day of seaward migration (Jørgensen et al. 2007).

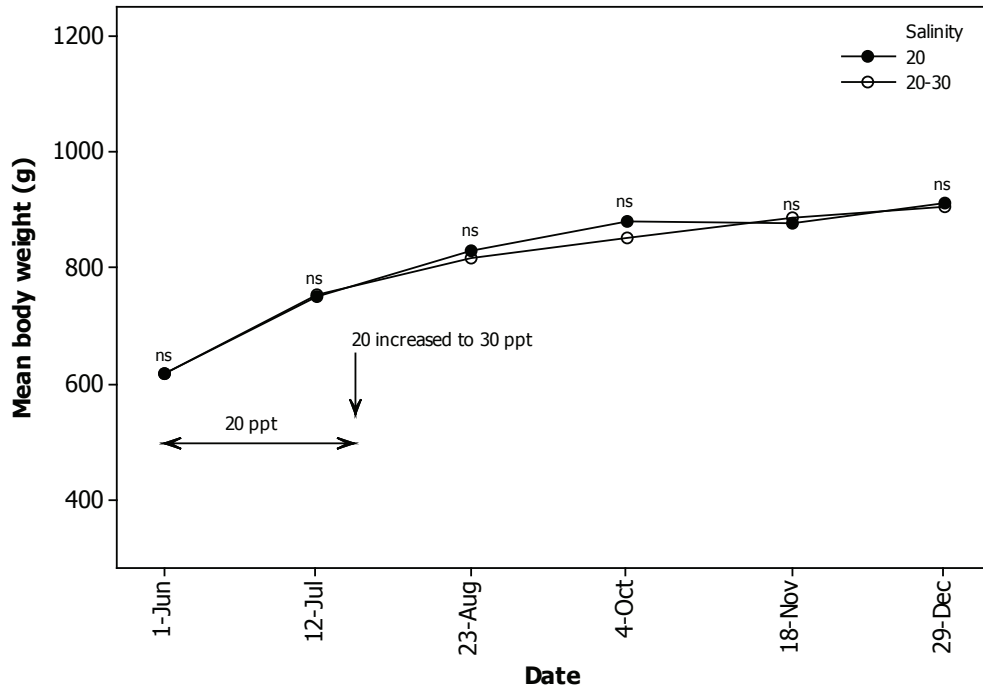


Fig. 2. Interaction plot of mean body weight (g) of Arctic charr reared in 20 ppt salinity (20 ppt) and 20 increased to 30 ppt salinity (20-30 ppt) from June 1 to December 29, 2005. Salinity gradually increased from 20 to 30 ppt July 16-30, 2005. Within each date, means sharing the same letter are not significantly different at the 5 % level. ns = not significant.

Although salinity had no effect on the growth of the 20 and 20-30 ppt fish from June through December, there was a significant effect of both photoperiod and sexual maturation on mean body weight. The four-way interaction effect (Date*Photoperiod*Salinity*Maturation) was not significant ($p = 0.8932$), however, the effect of photoperiod and maturation changed over time (Date*Photoperiod and Date*Maturation, $p = 0.0016$ and $p < 0.0001$, respectively; Table 1). Mean body weight was only affected by photoperiod on December 29, with Arctic charr reared under *CLP* having a significantly higher mean body weight (948 g) than those reared under *LDN* (873 g; Fig. 3). This is in contrast to the plateau in body weight that was observed in Arctic charr reared in 10 °C under *LDN* September through December (Duston et al. 2007).

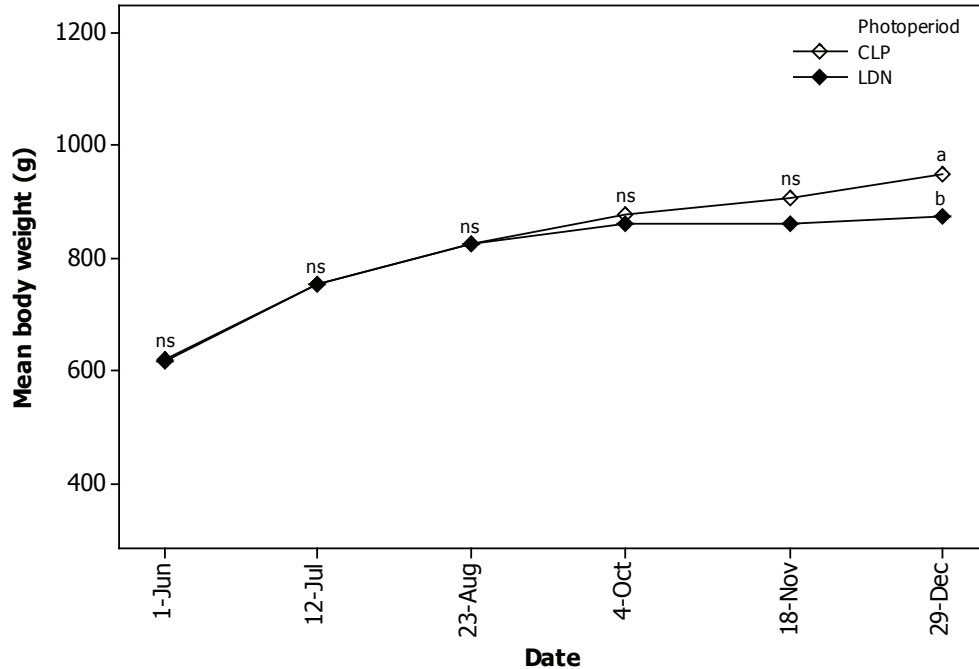


Fig. 3. Interaction plot of mean body weight (g) of Arctic charr reared under *Constant Long Photoperiod* (CLP, LD16:8) and simulated *Light Dark Natural* (LDN, Latitude 45 °N) from June 1 to December 29, 2005. The CLP treatment began June 21, 2005. Within each date, means sharing the same letter are not significantly different at the 5 % level. ns = not significant.

Sexually maturing fish had a significantly higher mean body weight in July compared to immature fish (791 g and 716 g respectively; Fig. 4). Although mean body weight was not significantly different from August through October, the immature fish were slightly larger than the maturing fish in November, and were over 100 g larger than maturing fish by December (980 g and 843 g, respectively; Fig. 4). The growth of the maturing fish in this experiment was very similar to the growth of the Arctic charr in Duston et al. (2007), in that the weight of the maturing fish only increased by approximately 200 g from June to December. The growth of the immature fish in this experiment was also very similar to the fish grown in freshwater and 10 ppt in Duston et al. (2007), with the fish attaining a mean weight of approximately 1000 g by December. A decline in weight of both maturing and immature Norwegian Arctic charr from late summer onwards was noted by Tveiten et al. (1996), who postulated that Arctic charr cease feeding in autumn once they have achieved a threshold body condition to sustain them through spawning and over-wintering. This was observed in the maturing Arctic charr in this experiment, but was not observed in the immature Arctic charr. Perhaps the

difference in rearing temperature (4 °C) in Tveiten et al. (1996) compared to this experiment (10 °C), and the significant difference in natural photoperiods between Norway (Latitude 70 °N) and Nova Scotia (Latitude 45 °N) had an effect on the growth of the Arctic charr.

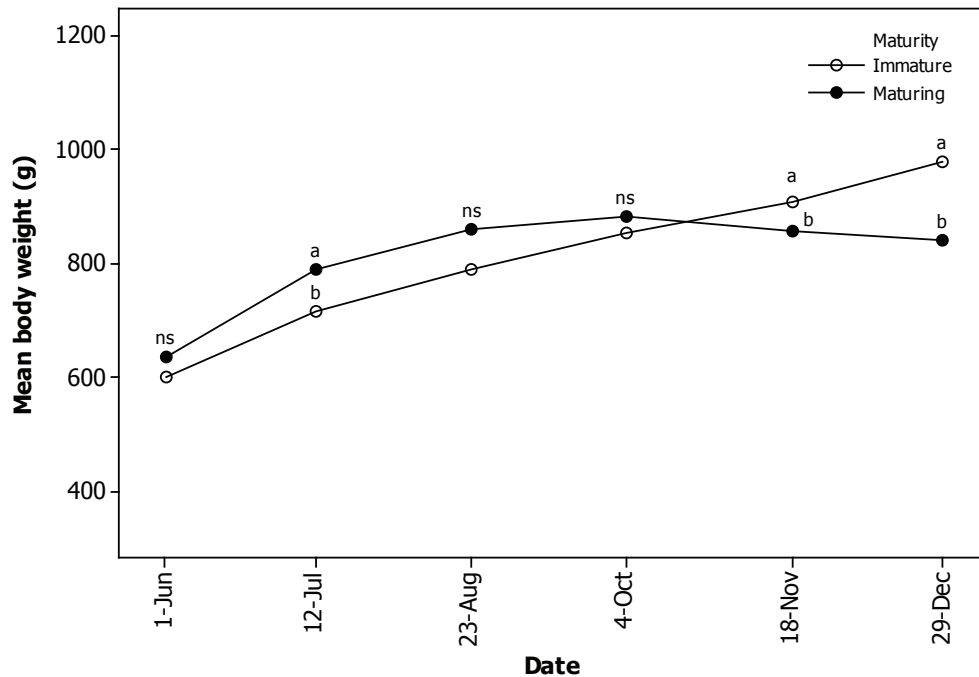


Fig. 4. Interaction plot of mean body weight (g) of sexually immature and maturing Arctic charr from June 1 to December 29, 2005. Within each date, means sharing the same letter are not significantly different at the 5 % level. ns = not significant.

4.4.2 Food Conversion Ratio and Specific Growth Rate

Fish reared in 20 ppt had a better FCR and higher SGR than fish in 30 ppt after direct transfer to seawater (June 1 to July 12). FCR was 1.22 vs. 1.97 (20 ppt < 30 ppt, $p = 0.0064$), and SGR was 0.73 vs. 0.39 % d⁻¹ (20 ppt > 30 ppt, $p = 0.0004$). In Duston et al. (2007), the FCR of fish directly transferred from freshwater to 30 ppt was greater than 3.0 in August and September, but improved by October. The 200 g difference in body size between the fish in Duston et al. (2007) and fish in this experiment (400 g vs. 600 g, respectively) may have contributed to the fish in this experiment having a better FCR of 1.97 after 6 weeks in 30 ppt.

During the brief feeding period in summer, the SGR of wild Arctic charr can be extremely high, almost 1.5 % per day (Johnson 1980). In this experiment, however, an SGR of 1.5 % d⁻¹ was not achieved. An SGR of 0.73 % d⁻¹ was achieved after 6 weeks in 20 ppt, lower than the SGR in freshwater (over 0.8 % d⁻¹), but similar to the SGR in 10 and 20 ppt (0.7 % d⁻¹; Duston et al. 2007). An SGR of 0.39 % d⁻¹ in 30 ppt was similar to ~ 0.45 % d⁻¹ in Duston et al. (2007), supporting the hypothesis that food intake was suppressed in 30 ppt.

Due to the loss of the 30 ppt treatment on August 6, FCR and SGR could not be calculated after July 12, as the fish in 20 and 20-30 ppt were not weighed until August 23. If there happens to be a loss of one treatment, a recommendation would be to weigh the fish in the remaining treatments soon afterwards, to be able to statistically analyze all data at that time point. As there was no difference in the growth of the fish when reared in 20 and 20-30 ppt (Fig. 2), and very little difference when reared under *CLP* and *LDN* (Fig. 3), or among immature and maturing fish (Fig. 4), FCR or SGR analysis was not performed for the June – December grow-out period.

4.4.3 Blood Plasma Osmolality

In June, Arctic charr directly transferred from freshwater to 30 ppt did not exhibit signs of osmoregulatory stress when compared to those transferred to 20 ppt ($p = 0.2106$). Mean plasma osmolalities in 20 ppt (328 mOsmol·kg⁻¹) and in 30 ppt (337 mOsmol·kg⁻¹) were within the normal range (320 – 340 mOsmol·kg⁻¹; Jobling 1995). By contrast, the mean plasma osmolality of the fish in 30 ppt was significantly higher (~335 mOsmol·kg⁻¹) than the fish in 20 ppt (~315 mOsmol·kg⁻¹) in August (Duston et al. 2007). Although there was a significant difference among those plasma osmolalities, the values in Duston et al. (2007) were quite similar to the values in this experiment. The large body size of the fish in this experiment and in Duston et al. (2007) was most likely a contributing factor. Independent of smoltification, an important factor affecting hypo-osmoregulatory ability is body size (Bjerknes et al. 1992, Handeland and Stefansson 2001). This appears to be particularly important for Fraser River Arctic charr. For example, small Fraser River charr (~140 g) were osmotically-stressed throughout their 9 month seawater trial (June – April; Delabbio et al. 1990a), whereas Norwegian Arctic

charr (~100 g) transferred to 35 ppt were not osmotically stressed, and displayed hypo-osmoregulatory ability comparable to Atlantic salmon (Halvorsen et al. 1993). The difference in hypo-osmoregulatory ability among different strains of the same salmonid species may be due to genetic variability. Smoltification may be linked to genetics, and through a breeding program, fish with better hypo-osmoregulatory ability could be selected for grow-out in seawater (Strand et al. 2007).

In October, mean plasma osmolalities were only slightly elevated in the slow-growing fish (353 mOsmol·kg⁻¹) compared to the fast-growing fish (328 mOsmol·kg⁻¹, $p = 0.0346$; Table 2). Mean plasma osmolality was also slightly elevated in fish reared in 20-30 ppt (354 mOsmol·kg⁻¹) compared to those reared in 20 ppt (326 mOsmol·kg⁻¹, $p = 0.0147$; Table 2). Surprisingly, the mean plasma osmolality of the sexually maturing fish was not significantly different from immature fish ($p = 0.6797$; Table 2). This is in contrast to Staurnes (1993), who reported that the onset of sexual maturation in salmonids has been shown to inhibit hypo-osmoregulatory ability.

Table 2

P values of the main and interaction effects of salinity, photoperiod, sexual maturation, and body size on mean plasma osmolality of Arctic charr in October 2005.

Source of variation	<i>P</i> values
Salinity	0.0147
Photoperiod	0.4329
Salinity x Photoperiod	0.8132
Maturation	0.6797
Salinity x Maturation	0.0539
Photoperiod x Maturation	0.6425
Salinity x Photoperiod x Maturation	0.9496
Body size	0.0346
Salinity x Body size	0.0978
Photoperiod x Body size	0.9134
Salinity x Photoperiod x Body size	0.2190
Maturation x Body size	0.2042
Salinity x Maturation x Body size	0.0860
Photoperiod x Maturation x Body size	0.3047
Salinity x Photoperiod x Maturation x Body size	N/A ¹

¹Not enough degrees of freedom

In addition, there was no effect of photoperiod ($p = 0.4329$) on mean plasma osmolality in October, although it has been shown that hypo-osmoregulatory ability

decreases with a decreasing photoperiod in autumn (Gulseth et al. 2001b). The results in this experiment show no evidence of *CLP* ameliorating the hypo-osmoregulatory ability of Arctic charr compared to *LDN*. Perhaps a constant rearing temperature of 10 °C delayed the decline of hypo-osmoregulatory ability in autumn, compared to wild Arctic charr which normally exhibit seasonal changes in hypo-osmoregulatory ability, food intake and growth in association with the decreasing temperature and photoperiod (Rikardsen et al. 2003, Sæther et al. 1996).

4.4.4 Incidence of Mortality

The incidence of mortality during the first eight weeks (June to August) in seawater was independent of salinity ($p = 0.0923$). Mortality in 30 ppt was 6 % (9 of 161), in 20-30 ppt was 3 % (4 of 155) and in 20 ppt was 1 % (2 of 160). Two main issues caused mortality in the fish. The first was the occurrence of a distended abdomen, in which the swim bladder of the fish was inflated. The second was the onset of sexual maturation in some of the fish. In October, there was a high occurrence of fish with cloudy eyes, although this did not result in mortality. Pathology was performed by a veterinarian, although no causative agent (water quality, gas pressure in the recirculation system, or a pathogen) was found. A low incidence of mortality during this 7 month experiment (212 days) is in contrast to Delabbio et al. (1990a), who suggested that Fraser River Arctic charr do not fully adjust to seawater after 245 days. The results are also in contrast to the Arctic charr in Duston et al. (2007), that experienced a significantly higher mortality in 30 ppt (16.3 %) compared to 20 ppt (3.3 %). Again, perhaps the 200 g difference in initial body size of the fish in the current study (600 g) compared to the fish (400 g) in Duston et al. (2007) was a contributing factor.

During the remainder of the experiment (August through December), the incidence of mortality was 8 % within 20-30 ppt and 3 % within 20 ppt. At low seawater temperatures, the inability to osmoregulate may be caused by a decrease or cessation of drinking by the fish (Belkovskiy et al. 1991). In the present study, temperature was constant 10 °C, which may account for the low incidence of mortality and relatively normal plasma osmolalities that were observed.

4.4.5 Incidence of Sexual Maturation

The incidence of sexual maturation in December was high, 74 % in females compared to 47 % in males ($p < 0.0001$; Table 3), similar to the incidence of sexual maturation found in Duston et al. (2003), but much higher than 5 % in females and 22 % in males in Duston et al. (2007).

There was no effect of salinity ($p = 0.8900$) nor photoperiod ($p = 0.0579$) on the incidence of sexual maturation in this experiment (Table 3). However, the timing of completion of sexual maturation among females was affected by photoperiod. In December, there were no ovulating females (0 of 50) reared under *CLP*, compared to 38 % (13 of 34) reared under *LDN*. Among males, the timing of spermiation was not quantified, as spermiation in males is not a discrete event like ovulation in females. A delay of sexual maturation is often found when a constant long photoperiod is applied after the summer solstice (Bromage and Duston 1986, Taranger et al. 1998). In this experiment, there was no attempt to control the high incidence of sexual maturation, as the experiment started in June after the ‘decision’ to mature had been made.

Table 3

P values of the main and interaction effects of salinity, photoperiod, and sex on the incidence of sexual maturation of Arctic charr.

Source of variation	<i>P</i> values
Salinity	0.8900
Photoperiod	0.0579
Salinity x Photoperiod	0.1213
Sex	<0.0001
Salinity x Sex	0.4406
Photoperiod x Sex	0.8715
Salinity x Photoperiod x Sex	0.3289

4.4.6 Final Body Weight and Marketable Product

There was a large variation in body weight among Arctic charr reared under the same environmental conditions, with final body weights in December ranging from 200 to over 1900 g (Fig. 5). At the end of the trial, there was 80.9 kg of high-value marketable product and a total of 187.3 kg of non-marketable product. With respect to sex, only 10.6 % (12 of 113) of females and 27.8 % (49 of 176) of males were of

marketable size, generating 16.3 and 64.6 kg of biomass, respectively. Overall, only 20.0 % (58 of 289) of the fish were of marketable size, which may result in a large economic loss to the farmer (Hatlen et al. 1996, McClure et al. 2007).

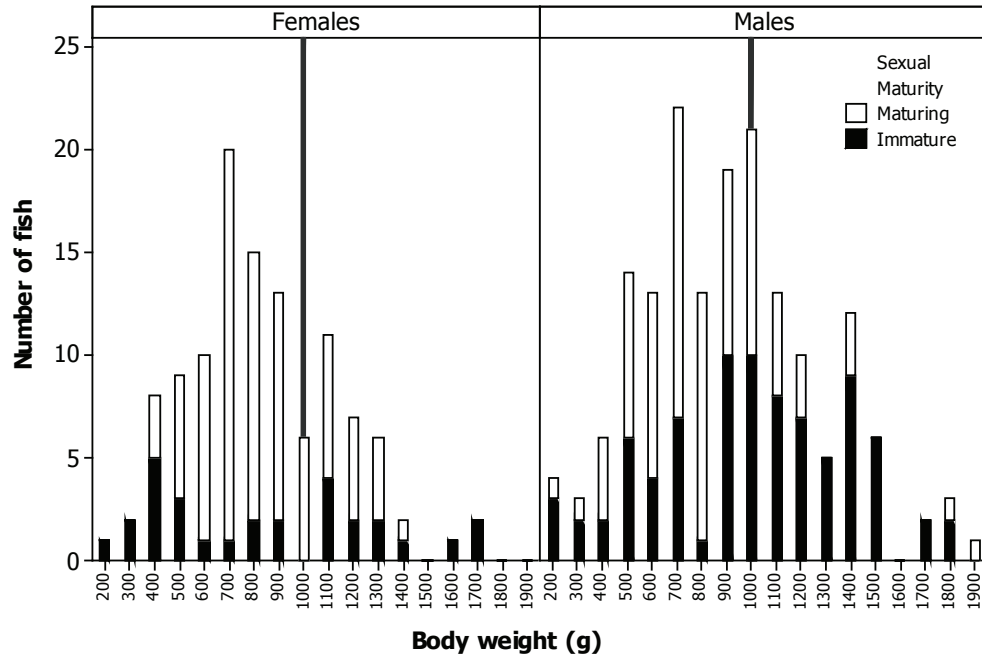


Fig. 5. Bar graph of final body weight distribution of Arctic charr on December 29, 2005, subdivided by sex (females and males) and sexual maturity status (immature and maturing). Immature fish greater than 1 kg, to the right of the vertical line, are of marketable size.

Chapter 5.0 Timing of a *Long-to-Ambient* Photoperiod on Inhibition of Sexual Maturation of Arctic charr

5.1 Introduction

Two important problems limiting the commercial viability of Arctic charr aquaculture in Atlantic Canada are the high incidence of early sexual maturation and the relatively high variability in growth among individuals reared under the same environmental conditions (Delabbio et al. 1990b, Nordeng 1983). The goal is to produce immature fish that are greater than 1 kg in body size that have excellent flesh quality and pigmentation to meet consumer demands, and that are of consistent body size at the time of harvesting.

Subjecting Arctic charr to a *Long-to-Ambient* photoperiod regime (18 h light, 6 h dark) for a period of 42 days starting February 3 resulted in a modest reduction of the incidence of sexual maturity compared to controls on a natural photoperiod (Duston et al. 2003). The present study tests the hypothesis that a further reduction in the incidence of sexual maturation may be achieved if the photoperiod treatment occurs earlier in the year (winter solstice, December 21), before the onset of gonadal development. The objective of the current study was to quantify the effect of the *Long-to-Ambient* photoperiod regime, starting on either December 21, February 1, or March 15, on the incidence of sexual maturation among individually-tagged Fraser River Arctic charr. The variation in final body weights of individually-tagged Arctic charr was also investigated, and the total biomass of both marketable (immature fish > 1 kg) and non-marketable fish (all maturing fish and immature fish < 1 kg) was calculated with respect to the treatments.

Arctic charr producers in Atlantic Canada typically maximize year-round growth by rearing fish in fresh groundwater at approximately 10 °C. If the seawater acclimation process is commercialized, then the transfer to seawater in the spring would result in a thermal challenge, as seawater temperatures in Atlantic Canada are approximately 5 °C (Peterson and Harmon 2005). The acclimation from 10 to 5 °C in freshwater was investigated in the current study, to determine if it would facilitate the transfer and acclimation to 5 °C seawater. These objectives led to a complex experimental design with 16 treatment levels or “paths”.

5.2 Objectives and Hypotheses

The objectives of this experiment were to (i) define the optimum timing of the *Long-to-Ambient* photoperiod regime for reducing the incidence of sexual maturation among Arctic charr, and (ii) determine the effects of the treatment paths on the growth performance and survival of Arctic charr.

It was hypothesized that the application of a *Long-to-Ambient* photoperiod regime on December 21 would be the most effective at reducing the incidence of sexual maturation, compared to the application on either February 1 or March 15. The acclimation from 10 to 5 °C in freshwater was hypothesized to facilitate the transfer to 5 °C seawater in June. In addition, it was hypothesized that the number of sexually maturing fish would decrease and the number of immature fish of marketable quality and size would increase as a result of the treatments.

5.3 Materials and Methods

5.3.1 Experimental Design

The experimental design had 16 treatment levels or “paths” constructed from treatment combinations of three factors (n ~ 32 fish per path). The factors of interest were photoperiod (4 levels), temperature in freshwater (2 levels), and temperature in seawater (2 levels). The photoperiod treatments with 2 replicate tanks (n ~ 65 per tank) occurred between December 2005 and April 2006 (Table 4); the change in freshwater temperature occurred in May, and the transfer to 20 ppt seawater occurred in June (Table 5). Although the levels of the factors looked like a factorial design, it was a single factor experiment with 16 levels or paths, as the switch to the two temperatures occurred after the fish graduated from the photoperiod treatments.

Table 4

Photoperiod treatments December 21, 2005 to April 26, 2006: *LDN* (simulated *Light Dark Natural* daylength, Latitude 45 °N), and *Long-to-Ambient (LD18:6)*.

Treatment	Photoperiod
<i>LDN</i>	<i>LDN</i> December 21, 2005 to December 14, 2006
<i>Early</i>	<i>LD18:6</i> from December 21 to February 1, then back to <i>LDN</i>
<i>Mid</i>	<i>LDN</i> from December 21 to January 31, then <i>LD18:6</i> from February 1 to March 15, then back to <i>LDN</i>
<i>Late</i>	<i>LDN</i> from December 21 to March 14, then <i>LD18:6</i> from March 15 to April 26, then back to <i>LDN</i>

Table 5

Arctic charr experimental design with 16 treatment paths, including photoperiod, temperature and salinity treatments.

Path	Photoperiod treatment	Freshwater temperature (°C)	Seawater temperature (°C)
	Dec 2005 to Apr 2006	May 2006	June 2006
1	<i>LDN</i>	10	10
2	<i>LDN</i>	10	5
3	<i>LDN</i>	5	10
4	<i>LDN</i>	5	5
5	<i>Early</i>	10	10
6	<i>Early</i>	10	5
7	<i>Early</i>	5	10
8	<i>Early</i>	5	5
9	<i>Mid</i>	10	10
10	<i>Mid</i>	10	5
11	<i>Mid</i>	5	10
12	<i>Mid</i>	5	5
13	<i>Late</i>	10	10
14	<i>Late</i>	10	5
15	<i>Late</i>	5	10
16	<i>Late</i>	5	5

¹n ~ 32 fish per path

²Changes in rearing conditions occurred Dec 2005 to June 2006

³For codes, see Table 4.

5.3.2 Experimental Procedures

Arctic charr (Fraser River, Labrador stock, 0+ year-old, ~ 191 g) from 8 full-sib families (from the F3¹¹ generation of class 2004) of a pedigreed breeding program by the CZRI were used in the study. F3¹¹ means the third family from the third generation. In November 2005, 600 fish were transferred by truck from Shippagan to the NSAC Aquaculture Centre and were held in a 1200 L tank with a flow-through supply of well-

water (10 °C). On December 20, the first group of 80 fish was anesthetized with *TMS*, graded by fork length (225 – 275 mm), PIT-tagged, and body weight (± 1.0 g), fork length (± 1.0 mm) and PIT-tag number recorded. The fish were then randomly distributed in groups of ten to 8 tanks within a recirculation system. After the first group of 80 fish, the grade of fork length was adjusted to 240 – 275 mm. Fish with distorted body shapes were not used in the experiment. In total, there were 516 fish with $n = 64$ to 65 fish per tank (~ 12.2 kg/m³). The experiment commenced December 21, 2005 and ended December 14, 2006. At this time, all fish were anesthetized, PIT-tag number recorded, and measured for body size at 6-week intervals.

The experiment was conducted in two phases: freshwater phase from December 21 to June 1 and seawater phase from June 1 to December 14. Arctic charr ($n \sim 16$ per tank) in *LDN* and constant 10 °C treatment path served as controls (Table 4). The remaining photoperiod treatments were on *LDN* except for a six-week period when the photoperiod was *LD18:6*. The timing of the *LD18:6* differed between treatments: Dec 21, 2005 to Feb 1, 2006 (*Early*); Feb 1 to Mar 15 (*Mid*); and Mar 15 to Apr 26 (*Late*, Table 4). After the *LD18:6* photoperiod ended, the photoperiod was returned to *LDN*. In addition, half of the fish from each of the 8 tanks were relocated to a separate freshwater recirculation system (10 °C) to reduce stocking density (from ~ 40.8 to 22.7 kg/m³). The water temperature in the original recirculation system was gradually decreased from 10 °C by approximately 1 °C per day until the temperature reached 5 °C (May 2 to May 9). In total, there were 16 experimental tanks (eight at 5 °C and eight at 10 °C), with approximately 32 fish per tank, and 2 replicate tanks per treatment path.

On June 1, the fish were measured for body size and then transferred directly from freshwater (5 °C or 10 °C) to 20 ppt (5 °C or 10 °C; two separate recirculation systems). Half of the fish from each of the 16 experimental tanks experienced a ± 5 °C change in temperature, while the other half did not experience a change in temperature (Table 5). In mid-July, the salinity in each of the two recirculation systems was gradually increased to 30 ppt over a period of two weeks using a combination of natural and artificial seawater (Instant Ocean® Sea Salt, Dynamic Aqua-Supply Ltd., Surrey, BC). This salinity was maintained until the end of the experiment, December 14, 2006. On July 20, 69 % (177 of 258) fish from the 10 °C seawater treatment died (for the incident

report, see Appendix 1.2). The decision was made to continue the experiment, as the surviving fish recovered their appetite and appeared normal, and data could still be collected from the 5 °C seawater treatment. The surviving fish were routinely monitored for any health problems and were maintained in the recirculation system until the end of the experiment. At the end of the experiment December 12-14, all fish were then euthanized by *TMS* overdose, PIT-tag recorded, measured for body size, and dissected to determine sex and sexual maturity status.

5.3.3 Statistical Analysis

A system malfunction on July 20 resulting in the loss of 69 % of the fish (177 of 258) in the 10 °C recirculation system necessitated that the analysis of somatic growth be divided into two components:

(1) the effect of treatment path on body weight was analyzed as repeated measures using the MIXED procedure of SAS as a two-factor factorial, with individual fish ($n = 411$) as the experimental unit (SAS Institute 2008). The factors were path (16 levels; Table 5) and measurement date (December 21, February 2, March 16, April 26, June 1, and July 5). Sexual maturation was not included as a factor because it was too early to determine the maturation status of the fish in July (the gonads were not fully developed at this time). The statistical model for the two-factor factorial in this analysis was expressed as

$$Y_{ijk} = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} was the response variable (body weight), μ was the overall mean, τ_i was the effect of date ($i = 1, 2, \dots, 6$), β_j was the effect of treatment path ($j = 1, 2, \dots, 16$), the number of replicates was denoted by k ($k = 1, 2, \dots, n_{ijk}$), and ε_{ijk} was the error term;

(2) the effect of treatment path and sexual maturation on body weight was analyzed as repeated measures using the MIXED procedure of SAS as a three-factor factorial, with individual fish ($n = 235$) as the experimental unit (SAS Institute 2008). The factors were path (8 levels), sexual maturity (immature and maturing), and measurement date (December 21, February 2, March 16, April 26, June 1, July 5,

September 6, October 4, November 9, and December 12). Sex was not included as a factor (fish were not separated by sex) due to the complexity of the treatment paths. This analysis only included fish in 5 °C (Paths 2, 4, 6, 8, 10, 12, 14, and 16). The 10 °C fish (Paths 1, 3, 5, 7, 9, 11, 13, and 15) were omitted from this analysis, due to the incident that occurred in July. In both analyses, the most appropriate covariance structure was determined to be unstructured, and mean body weight required a square root transformation to satisfy the normality and constant variance assumptions (Littell et al. 1998). The statistical model for the three-factor factorial in this analysis was expressed as

$$Y_{ijkl} = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + \delta_k + (\tau\delta)_{ik} + (\beta\delta)_{jk} + (\tau\beta\delta)_{ijk} + \varepsilon_{ijkl}$$

where Y_{ijkl} was the response variable (body weight), μ was the overall mean, τ_i was the effect of date ($i = 1, 2, \dots, 10$), β_j was the effect of treatment path ($j = 1, 2, \dots, 8$), δ_k was the effect of sexual maturation ($k = 1, 2$), the number of replicates was denoted by l ($l = 1, 2, \dots, n_{ijkl}$), and ε_{ijkl} was the error term.

The first part of the body weight analysis examined the changes in mean body weight from December 2005 through July 2006, in an attempt to (1) observe whether growth during winter was a predictor of sexual development, and (2) determine if a thermal challenge from 10 to 5 °C June 1 would affect the growth performance of the fish. The second part of the body weight analysis examined the changes in mean body weight of the remaining fish in the 5 °C treatment, from December 2005 through December 2006.

The P -values in all factorial tests were estimated using the Restricted Maximum Likelihood method. Least squares means of the treatment combinations from the highest order significant interaction effects were computed, and their letter groupings were generated to identify the means that were significantly different at either a 5 % (within each date) or a 1 % (within each treatment over time) level of significance.

The incidence of mortality and sexual maturity were both analyzed using the CATMOD procedure of SAS with the contrast statement (SAS Institute 2008). Mortality throughout the trial (excluding the 10 °C fish from the incident on July 20) was analyzed

with treatment path (16 levels) as the factor. The purpose of this analysis was to determine if a thermal challenge in June, from 10 °C to 5 °C, in combination with a direct transfer from freshwater to 20 ppt seawater would affect the incidence of mortality. Sexual maturity (5 °C fish only) in December was analyzed as a two-factor factorial with photoperiod (*LDN*, *Early*, *Mid*, and *Late*) and sex (male and female) as factors. The treatment effect of sex was not significant, therefore the sexes were pooled and the proportions were further compared using the analysis of contrasts. The response variables % mortality and % sexual maturation were calculated and their letter groupings were generated at the 5 % level of significance.

Immature fish (in 5 °C treatment paths) with a round weight > 1 kg were categorized as marketable. The biomass of marketable fish (in 5 °C treatment paths) was determined as the sum of the body weight (kg) of the all of the immature fish that were over 1 kg round weight on December 12. Maturing fish with a round weight > 1 kg were categorized as non-marketable, and their biomass was determined in the same manner as the immature fish. In addition, all other fish (both immature and maturing) with round weights less than 999 g were included in the total biomass of non-marketable fish. The biomass of marketable and non-marketable fish was also determined for each photoperiod treatment.

5.4 Results and Discussion

5.4.1 Somatic growth

Mean body weight from December 21, 2005 to July 5, 2006 was significantly affected by the interaction effect of treatment path and measurement date ($p < 0.0001$; Table 6).

Table 6
P values of the main and interaction effects of treatment path from Repeated Measures Analysis on mean body weight of Arctic charr from December 21, 2005 to July 5, 2006.

Source of variation	<i>P</i> values
Date	< 0.0001
Path	0.0049
Date x Path	< 0.0001

There was no significant difference in mean body weight from December 21 through April 26, with the exception of the fish in the *Early* photoperiod treatment on April 26 (Fig. 6). Under the *Early* photoperiod treatment, fish transferred from 5 °C freshwater to 5 °C seawater (Path 8) had a significantly lower mean body weight (574 g) compared to the other three treatment Paths (Paths 5, 6, and 7; 681 g, 650 g, and 674 g, respectfully). Significant differences in mean body weight were mainly observed June 1 and July 5, after the change in temperature from 10 to 5 °C (May 2-9) and the transfer from freshwater to 20 ppt seawater (June 1; Fig. 6).

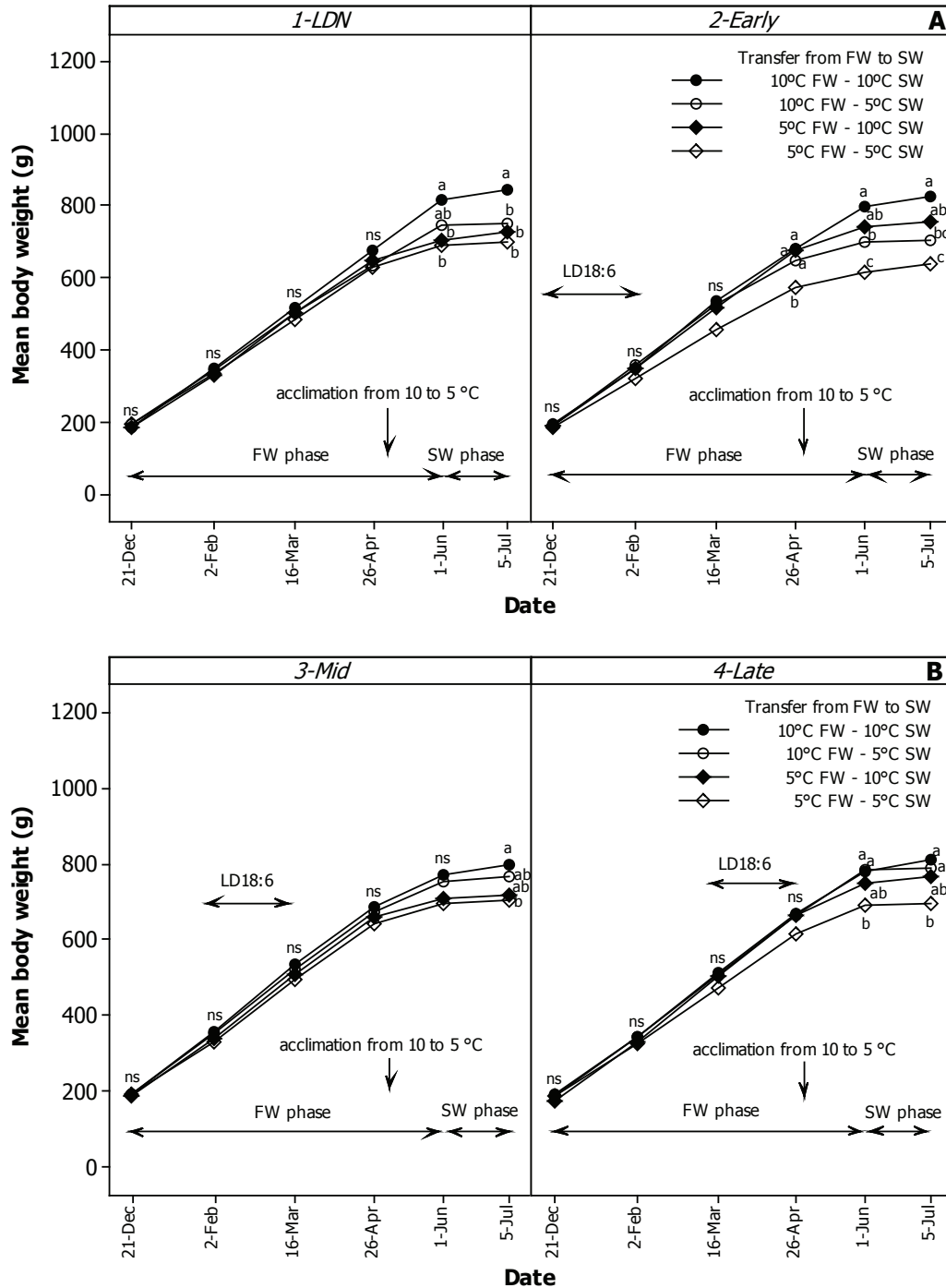


Fig. 6. Interaction plot of mean body weight (g) of Arctic charr reared in 16 treatment paths from December 21, 2005 to July 5, 2006. Treatment paths correspond to a combination of four photoperiod treatments (*LDN*; and *Early*, *Mid*, and *Late* timing of *LD18:6* from December 21, 2005 to April 26, 2006), two freshwater temperatures (10 and 5 °C) in May and the transfer from freshwater to 20 ppt seawater at 10 and 5 °C in June. Panels are sub-divided by the four photoperiod regimes, and the timing of the *LD18:6* is shown by an arrow. Within each date, means sharing the same letter within each panel are not significantly different at the 5 % level. ns = not significant.

The mean body weight of the fish in Duston et al. (2003) was similar to the body weight of the fish in the current study in February and March. However, significant differences were observed from late-April onwards in both studies. Duston et al. (2003) found that growth between immature and maturing male and female Arctic charr differed from April through November. As the analysis of weight in the present study ended July 5 due to the incident that occurred in the 10 °C treatment (Paths 1, 3, 5, 7, 9, 11, 13, and 15), the changes in body weight in the current study could not be compared with Duston et al. (2003).

From the analysis of weight December through March, there was no indication of which fish in the four photoperiod treatments would mature. Jobling and Baardvik (1991) suggested that body size in early winter may be a poor predictor of maturity, as fish in a hatchery are most often derived from several sibling groups. The Arctic charr in the present study and in Duston et al. (2003) were hatchery-reared, while the Arctic charr in Adams and Huntingford (1997), for example, were hatched from eggs collected from the wild. Perhaps due the large size of the fish in the present study (> 600 g) and in Duston et al. (2003; 300-500 g), compared to the small size of the Arctic charr in Adams and Huntingford (1997; 1.2 to 15.3 g), differences in growth during early winter were not detectable.

As this fish in the present study were quite large, 200 g at the start of the trial, it may be predicted that a large proportion of fish would mature, regardless of photoperiod treatment. The fish may have accumulated enough energy by the start of the experiment (December) to have initiated sexual maturation, and as Frantzen et al. (2004a) stated: “even under conditions in which the critical period or ‘gate open’ position of the circannual rhythm might have been advanced by photoperiodic manipulation”.

Short-term growth in seawater (June 1 – July 5) is also shown in Figure 6. Under *LDN*, the fish in 10 °C freshwater to 10 °C seawater had the highest mean body weight on July 5 compared to the other treatments. Under the *Early* photoperiod, the fish acclimated to 5 °C, and then transferred to 5 °C seawater had the lowest mean body weight compared to the other treatments (Fig. 6).

Norwegian Arctic charr (2 years old, ~135 g) transferred from freshwater to 20 ppt at constant 8 °C in both December and June exhibited good growth and low

mortality, indicating that they were able to adapt completely to brackish water (Arnesen et al. 1993a). In a similar experiment, Arnesen et al. (1993b) directly transferred Arctic charr (150 g) to 35 ppt seawater in April at constant 8 °C, and the fish also experienced good growth and no mortality after 30 days. The direct transfer of large Fraser River Arctic charr (400 g) from freshwater to 20 ppt on June 30 did not affect growth compared to freshwater and 10 ppt controls, at least among immature fish (Duston et al. 2007). Another study, transferring Arctic charr from 8 to 1 °C at 15 ppt, showed that plasma osmolalities were elevated compared to those that remained in 8 °C (Finstad et al. 1989). In Finstad et al. (1989), the fish were first acclimated to 15 ppt before the thermal challenge, in contrast to the present study where the fish were directly transferred from freshwater to brackish water. No growth data were reported in Finstad et al. (1989), however, it may be possible to infer that there was a slight elevation in the plasma osmolality of the fish in the present study after the thermal challenge. In the current study, the transfer from 10 to 5 °C did not appear to negatively affect the growth of the fish by July 5.

The three-way interaction effect (Date*Path*Maturation) was not significant ($p = 0.5735$) for the mean body weight of fish from December 21, 2005 to December 12, 2006 (Table 7). However, there was a significant interaction effect between date, and path and maturation (Date*Path and Date*Maturation, $p = 0.0057$ and $p < 0.0001$, respectively; Table 7).

Table 7

P values of the main and interaction effects of treatment path and sexual maturation from Repeated Measures Analysis on mean body weight of Arctic charr from December 21, 2005 to December 12, 2006.

Source of variation	<i>P</i> values
Date	< 0.0001
Path	0.6257
Date x Path	0.0057
Maturation	0.3244
Date x Maturation	< 0.0001
Path x Maturation	0.6735
Date x Path x Maturation	0.5735

Mean body weight was not significantly different among the treatment paths, with the exception of Path 6 (525 g) and Path 8 (478 g) within the *Early* photoperiod on March 16 (Fig. 7). However, this is quite possibly an artifact, and may not have any biological significance, as the *p*-value (0.0497) indicates it was barely significant statistically. There were no significant differences in body weight between treatment paths from December 2005 to December 2006. In terms of somatic growth, it does not appear to be necessary to acclimate Arctic charr from 10 to 5 °C in freshwater before the transfer to 5 °C seawater.

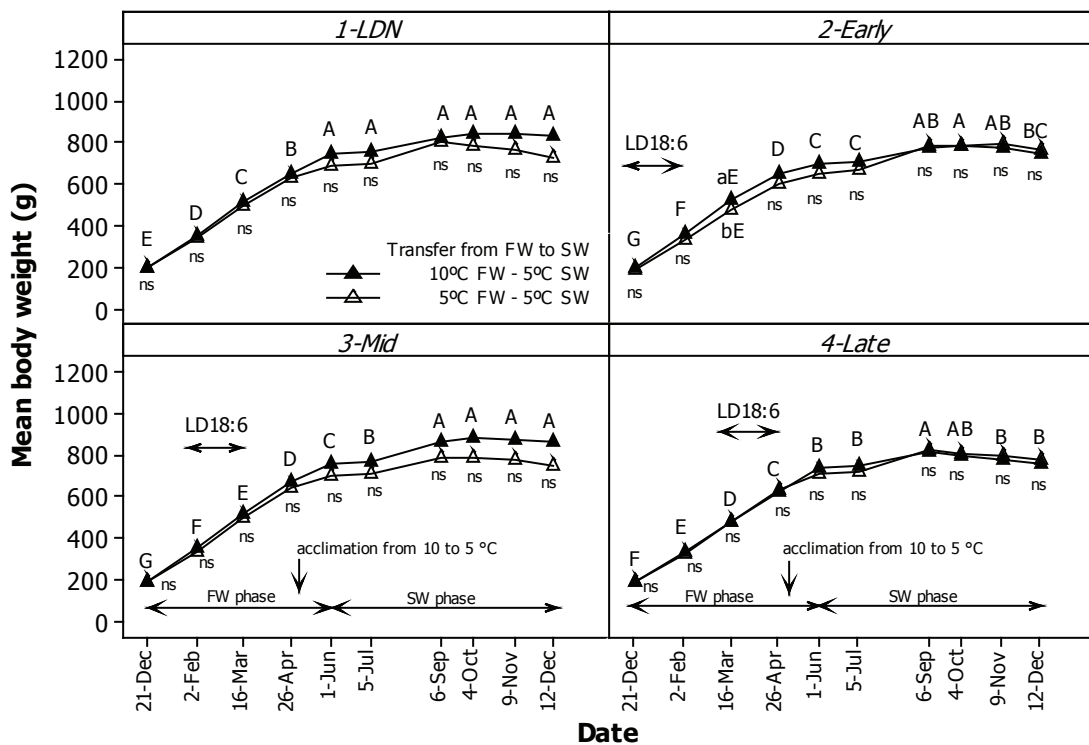


Fig. 7. Interaction plot of mean body weight (g) of Arctic charr reared in 8 treatment paths from December 21, 2005 to December 12, 2006. Treatment paths correspond to a combination of four photoperiod treatments (*LDN*; and *Early*, *Mid*, and *Late* timing of *LD18:6* from December 21, 2005 to April 26, 2006), two freshwater temperatures (10 and 5 °C) in May and the transfer from freshwater to 20 ppt seawater (5 °C) in June. Panels are sub-divided by the four photoperiod regimes, and the timing of the *LD18:6* is shown by an arrow. Within each date, means sharing the same letter (lower case) are not significantly different at the 5 % level. Within each treatment over time, means sharing the same letter (upper case) are not significantly different at the 1 % level. ns = not significant.

Mean body weight of the fish within each treatment path from June through December was affected by the 5 °C water temperature (Fig. 7). This was expected, as water temperature affects the metabolism of the fish. Mean body weight increased significantly from December 21 to June 1 in all treatments. However, the fish in *LDN* did not increase in mean body weight from June 1 through December 12 (Fig. 7). The fish in the *Early* and *Late* photoperiod treatments did not increase in mean body weight from June to July, as opposed to the *Mid* photoperiod that did increase in mean body weight. While the fish in the *Early* and *Late* photoperiods increased in mean body weight by September, the *Mid* photoperiod treatment did not increase from September 6 through December 12 (Fig. 7). Overall, growth during the autumn in 5 °C seawater appeared to be leveling-off.

Mean body weight of sexually maturing fish was greater than immature fish, from March 16 through July 5 only (Fig. 8). Although there was no significant difference in mean body weight from September through December when the fish were in 30 ppt, mean body weight of both the immature and maturing fish appeared to be leveling-off during this time (Fig. 8). A similar growth trend was found in Experiment 1, and in immature fish (in 20 ppt only) and maturing fish in Duston et al. (2007). Whereas the immature fish in Experiment 1 attained a final mean body weight of ~ 1000 g, the fish in this experiment were only ~ 800 g, even though in June the fish were ~ 100 g larger than in Experiment 1. This was expected, as the rearing temperature in this experiment was 5 °C from June onwards, compared to 10 °C in Experiment 1. Differences in mean body weight of immature and maturing Arctic charr from October through December were observed in Duston et al. (2007), particularly among fish reared in freshwater, 10 and 20 ppt (constant 10 °C), and Experiment 1 (no effect of salinity, constant 10 °C), but was not observed in this experiment, where fish were reared at constant 5 °C since June 1.

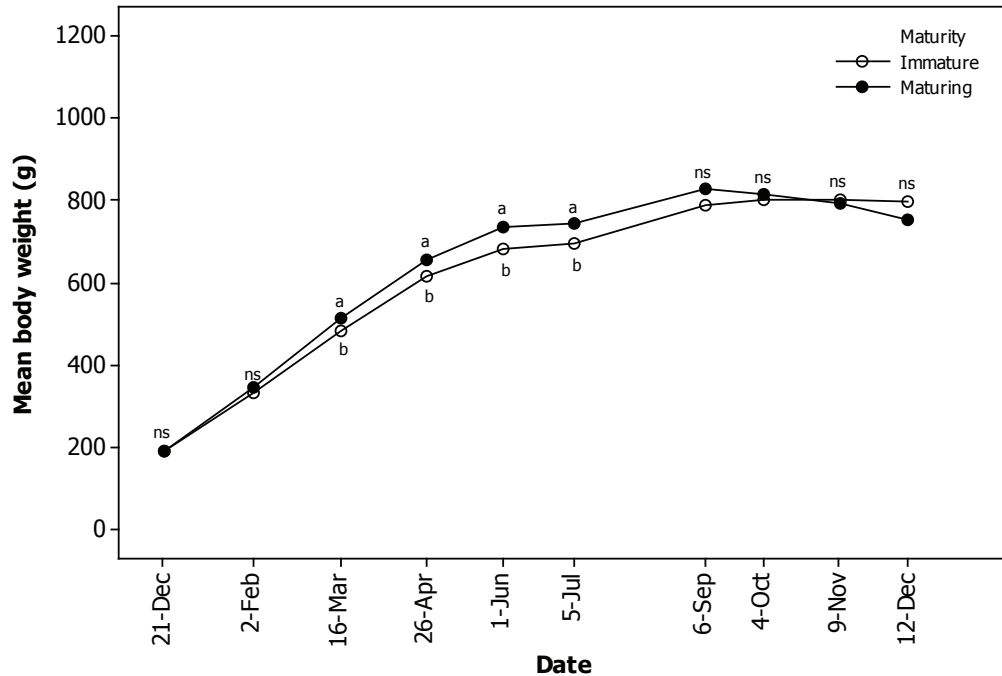


Fig. 8. Interaction plot of mean body weight (g) of sexually immature and maturing Arctic charr from December 21, 2005 to December 12, 2006. Within each date, means sharing the same letter are not significantly different at the 5 % level. ns = not significant.

5.4.2 Incidence of Mortality

The incidence of mortality throughout the 12 month trial (December 2005 to 2006) was significantly affected by treatment path ($p = 0.0351$). Paths 1, 3 and 10 had the lowest percent mortality at 0.1 %, and Path 2 had the highest percentage at 15.6 % (Fig. 9). With the exception of Path 2, mortality was less than 10 % among the treatment paths. An abrupt change in temperature from 10 to 5 °C may have affected the incidence of mortality, however, Paths 2 and 10 both experienced this change and their mortalities were significantly different (Fig. 9). Dempson (1993) reported that Arctic charr undergoing a seawater challenge test (30 ppt) in warm water (above 7 °C) survived for a longer period of time than those in cold water (less than 4 °C). However, those Arctic charr were quite small, less than 105 mm in fork length (approximately 50 g in body weight), much smaller than the Arctic charr in the current study. The relatively low incidence of mortality may have been attributed to the large body size of the Arctic charr in this experiment.

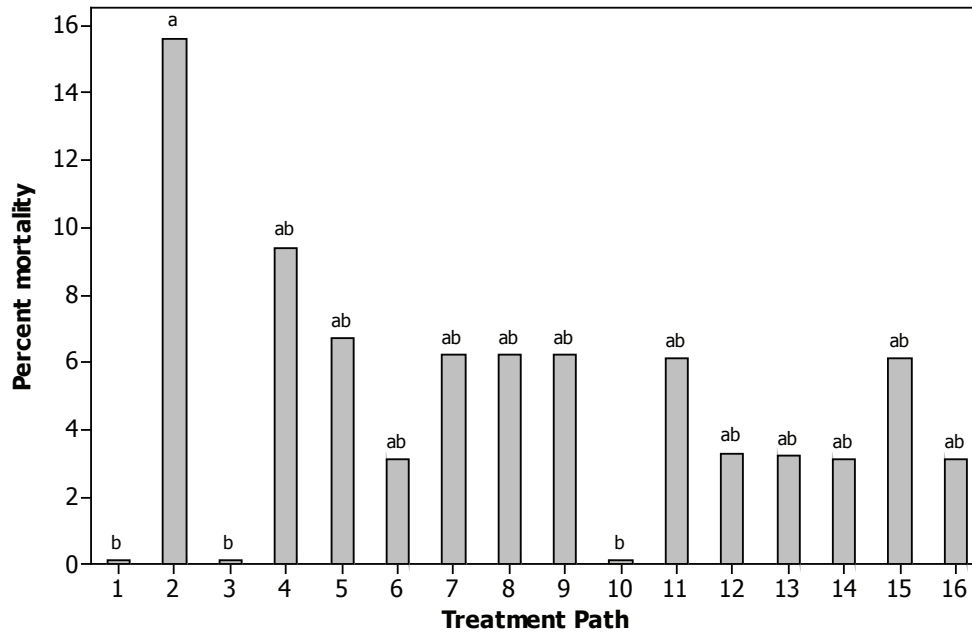


Fig. 9. Bar graph of the incidence of mortality among Arctic charr reared in 16 treatment paths, from December 21, 2005 to December 12, 2006. Treatment paths correspond to a combination of four photoperiod treatments (*LDN*; and *Early*, *Mid*, and *Late* timing of *LD*18:6 from December 21 to April 26), two freshwater temperatures (10 and 5 °C) in May and the transfer from freshwater to 20 ppt seawater at 10 or 5 °C in June. Proportions sharing the same letter are not significantly different at the 5 % level.

5.4.3 Incidence of Sexual Maturation

The effect of photoperiod on the incidence of sexual maturation in December was significant ($p = 0.0007$) but the effect of sex was not significant ($p = 0.0845$; Table 8). Both the *Early* and *Mid* photoperiods were the most effective at reducing the incidence of sexual maturation, with only 43 and 57 % of the population maturing, compared to *Late* (68 %) and *LDN* (77 %; Fig. 10).

Table 8

P values of the main and interaction effects of photoperiod and sex on the incidence of sexual maturation of Arctic charr.

Source of variation	<i>P</i> values
Photoperiod	0.0007
Sex	0.0845
Photoperiod x Sex	0.7824

In contrast to the results of the present experiment, Duston et al. (2003) found a significant effect of sex on the incidence of sexual maturation. A reduction to 66 % in males and 32 % in females, compared to controls under *LDN* (83 and 50 %, respectively) was achieved after subjecting Arctic charr to the *Long-to-Ambient* regime starting February 3. The percentage of controls that matured in Duston et al. (2003), > 50 %, was similar to the *Late* (68 %) and *LDN* (77 %) treatments in this experiment, and in males (47 %) and females (74 %) in Experiment 2. Percent maturation in both of these experiments was much higher than those found in Duston et al. (2007): 5 % (females) and 22 % (males).

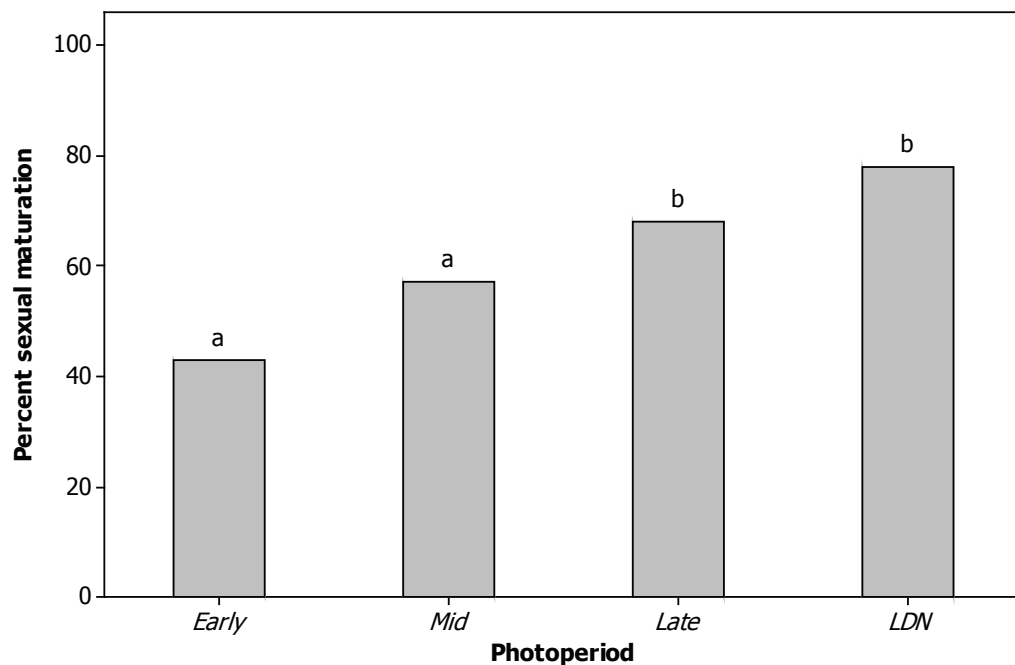


Fig. 10. Bar graph of the incidence of sexual maturity among Arctic charr reared in 5 °C on December 12, 2006. The fish were exposed to four photoperiod treatments (*LDN*; and *Early*, *Mid*, and *Late* timing of *LD18:6* from December 21, 2005 to April 26, 2006). Sexes have been pooled. Proportions sharing the same letter are not significantly different at the 5 % level.

A significant reduction in sexual maturation among the *Early* and *Mid* photoperiods, compared to *Late* and *LDN*, confirms the hypothesis that a larger reduction occurs earlier in the winter, during the proposed ‘critical decision’ to mature (Rowe and Thorpe 1990). Duston et al. (2003) suggested that the long photoperiod in winter was arresting a process of maturation that was already underway, and that the timing of the switch from long to short (or ambient, in this experiment) was critical. This is in

agreement with the results of this experiment. The switch from long to ambient photoperiod on April 26 (*Late* treatment) would only result in minor differences in actual daylength compared to *LDN*, as opposed to the switch from long to ambient on February 1 (*Early* treatment), where the natural daylength is quite short (Duston et al. 2003).

Differences in body size by September, one year prior to sexual maturation, had an effect on the incidence of maturity in June among chinook salmon (*Oncorhynchus tshawytscha*; Silverstein et al. 1998). As the fish in the present study did not arrive in the lab until November, it was not possible to determine the weight of individual fish during this critical time. Variation in growth rate during the Atlantic salmon parr stage has been postulated to be of vital importance to the fish's decision to mature the following year (Rowe and Thorpe 1990). A recommendation would be to acquire Arctic charr earlier in the year, during the winter/early spring prior to experimentation, to be able to track their growth for a longer period of time.

The chinook salmon in Silverstein et al. (1998) and the Arctic charr in Imsland and Gunnarsson (2011) were less than 21 g in the autumn prior to their trial, whereas the Arctic charr in the present study were approximately 200 g in December. This was also established in another trial involving chinook salmon, where the initiation of sexual maturation was predicted to be in fish less than 7 g (Shearer et al. 2006). Fish reproduce as soon as they are able to do so 'developmentally', meaning at the earliest opportunity (Thorpe 1995). The large size of the fish in December in the present study may have contributed to the high incidence of sexual maturity the following autumn, as gonadal development may have already been in progress by this time (Rowe and Thorpe 1990). The Arctic charr in Duston et al. (2003) were also quite large, 300 g in February, which may explain why the incidence of sexual maturation in the *Long-to-Ambient* regime was still quite high at 32 % (females) and 66 % (males). Although the *Long-to-Ambient* regime was effective at reducing the incidence of sexual maturation compared to controls, the high percentage of fish (> 43 %) that matured in the present study still needs to be addressed.

5.4.4 Final Body Weight and Marketable Product

There was a large variation in body weight among immature and maturing Arctic charr on December 12, with means ranging from 100 to over 1600 g (Fig. 11). At the end of the trial, there was 34.2 kg of high-value marketable product and a total of 151 kg of non-marketable product.

The *Early* (13.3 kg) and *Mid* (13.3 kg) photoperiod treatments generated the largest amount of marketable biomass compared to the *Late* (5.1 kg) and *LDN* (2.5 kg) treatments. However, only 10 out of 60 (*Early*) and 11 out of 56 (*Mid*) fish were marketable. Across all photoperiod treatments, only 11.5 % (27 of 234) fish were marketable. This is much lower than the 20.0 % reported in Experiment 1, where no treatments were used to control for sexual maturation.

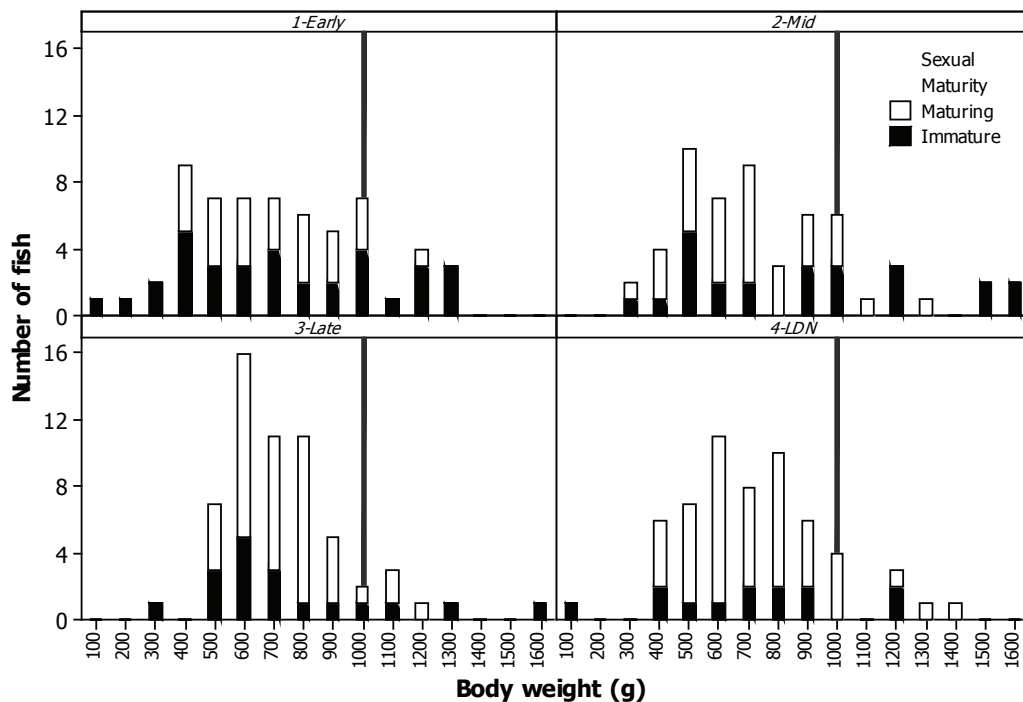


Fig. 11. Bar graph of final body weight distribution of Arctic charr reared in 5 °C on December 12, 2006, sub-divided by photoperiod (*LDN*, and *Early*, *Mid*, and *Late* timing of *LD18:6* from December 21, 2005 to April 26, 2006) and sexual maturity status (immature and maturing). Sexes have been pooled. Immature fish greater than 1 kg, to the right of the vertical line, are of marketable size.

One recommendation would be to harvest the fish at a smaller pan-size (< 400 g), rather than market size (1 – 3 kg), to maximize yield (Jobling et al. 1998, Smith et al.

1992). Pan-sized coho salmon (*Oncorhynchus kisutch*) do not require high flesh carotenoid values to achieve consumer acceptance (Christiansen and Wallace 1988, Smith et al. 1992). By recalculating the biomass to include immature Arctic charr from 200 to over 1000 g, there would be 37 % (87 of 234) and 73.3 kg of marketable product, double the amount described previously. Further research should be conducted to determine if small (< 400 g) immature Fraser River Arctic charr could have a desirable flesh coloration to meet market demands. Norwegian Arctic charr > 200 g fed astaxanthin have deposited enough pigment to attain desirable flesh coloration (Hatlen et al. 1995). Another recommendation would be to harvest the fish at a larger body size than 400 g, but before secondary sexual characteristics appear, i.e., before the carotenoids are mobilized from the flesh into the developing ova of females and the skin of males (Hatlen et al. 1996, Leclercq et al. 2010).

Chapter 6.0 Combined Effect of Temperature, a *Long-to-Ambient* Photoperiod, and Restricted Food Intake in Winter on the Incidence of Sexual Maturation of Arctic Charr

6.1 Introduction

Arctic charr aquaculture in Atlantic Canada is under-developed due to the high incidence of early sexual maturation that occurs prior to harvesting (Duston et al. 2003). In Experiment 2, a *Long-to-Ambient* photoperiod applied either December 21 or February 1 was the most effective at reducing the incidence of sexual maturation among Arctic charr (43 and 57 %, respectfully) compared to 77 % in controls under simulated natural photoperiod. However, this was only a modest reduction, and the incidence of sexual maturation is still too high. Therefore, the current study investigated the combined effects of the *Long-to-Ambient* photoperiod (applied December 21), a restricted food intake December 21 through March 15, and a cold water temperature (5 °C) from December 21 onwards. A restricted food intake (Rowe and Thorpe 1990) and rearing in cold water (Saunders et al. 1983) during the winter months both act to slow somatic growth of the fish and divert energy from gonadal growth. Sexual maturation may be arrested due to insufficient acquisition of energy reserves during the winter months, which are required for the development of gonads the following autumn (Saunders et al. 1983, Rowe and Thorpe 1990).

6.2 Objectives and Hypotheses

The objectives of this experiment were to (i) investigate the growth performance and survival among Arctic charr reared in two temperatures (5 and 10 °C), in combination with a *Long-to-Ambient* photoperiod and a restricted feeding regime (December 21 through March 15), and (ii) investigate how this treatment combination affected the incidence of sexual maturation among Arctic charr.

It was hypothesized that a treatment combination with a *Long-to-Ambient* photoperiod (December 21 through February 1), 5 °C water temperature, and a restricted feeding regime (December 21 through March 15) would further reduce the incidence of sexual maturation among Arctic charr.

Lastly, it was hypothesized that the number of sexually maturing fish would decrease and the number of immature fish of marketable quality and size would increase with each succession of treatments throughout the three experiments.

6.3 Materials and Methods

6.3.1 Experimental Design

The experiment was a 2x2x2 factorial design with 2 replicate tanks. The treatments were temperature (5 and 10 °C), photoperiod (*LDN* and *Long-to-Ambient*), and ration (*Satiation*: fed to apparent satiation throughout the experiment; and *Restricted*: restricted food intake for 12 weeks). Although not a part of the true experimental design, the fish in 5 and 10 °C freshwater were transferred to 20 ppt seawater (5 and 10 °C; respectfully) on June 5.

6.3.2 Experimental Procedures

Arctic charr (Fraser River, Labrador stock, 0+ year-old, ~ 197 g) from 12 full-sib families (from the F4 generation of class 2004) of a pedigreed breeding program by the CZRI were used in the study. F4 means the first family from the 4th generation. In November 2006, 600 fish were transferred by truck from Shippagan to the NSAC Aquaculture Centre and were held in four tanks within a freshwater recirculation system supplied with well water (10 °C). On November 27, the fish were anesthetized with *TMS*, PIT-tagged by injection into the dorsal musculature, and graded by body weight (± 1 g); fish weighing less than 120 g were not used in the experiment.

On December 20, fish were anesthetized, measured for body weight (± 1 g) and fork length (± 1 mm), PIT-tag number recorded, and then randomly allocated in groups of 10 to 16 tanks within two independent freshwater recirculation systems (8 tanks per system) supplied with well water (10 °C). In total there were $n = 280$ fish per recirculation system and $n = 35$ per tank (~ 7.5 kg/m³).

The experiment commenced on December 21, 2006 and ended on November 16, 2007. At this time, all fish were anesthetized, PIT-tag recorded, and measured for body size at 6-week intervals. The experiment was conducted in two phases: freshwater phase from December 21 to June 5 and seawater phase from June 5 to November 16. At the

start of the freshwater phase, the temperature in one of the recirculation systems was gradually decreased from 10 to 5 °C over a period of 3 days. In addition, two photoperiod treatments commenced December 21 within the two recirculation systems. The treatments were *LDN* and *Long-to-Ambient* until February 1 (4 tanks each within both systems). Also, two ration treatments commenced December 21: fish in two of the tanks within each photoperiod treatment were fed to apparent satiation (*Satiation*), while fish in the other two tanks were not fed until March 15 (restricted ration for 12 weeks; *Restricted*). The fish within the *Restricted* ration treatment were weighed at 2-week intervals to closely monitor for significant weight loss. From March 15 onwards, the photoperiod was *LDN* in all tanks and all fish were fed to apparent satiation until the end of the experiment.

On June 5, the freshwater fish (5 and 10 °C) were relocated to two seawater recirculation systems (20 ppt, 5 and 10 °C, respectively). The systems were maintained at 20 ppt until July 18, and then the salinity was gradually increased to 30 ppt over a period of two weeks using a combination of natural and artificial seawater (Instant Ocean® Sea Salt, Dynamic Aqua-Supply Ltd., Surrey, BC). The systems were maintained at 30 ppt until the end of the experiment. On September 14, 57 % (155 of 273) fish from the 5 °C seawater treatment died (for the incident report, see Appendix 1.3). Dead fish were removed from the system, PIT-tag recorded, measured for body size, and dissected to determine sex and sexual maturity status. The decision was made to continue the experiment, as the surviving fish recovered their appetite and appeared normal, and data could still be collected from the survivors and the 10 °C treatment. The surviving fish in 5 °C were routinely monitored for any health problems and were maintained in the recirculation system until the end of the experiment. The surviving fish were not weighed in October to prevent any additional stress from netting and anesthetization. The surviving fish were weighed in November, and their growth, sex, and sexual maturation data were used in the data analysis. The experiment was terminated November 16 when all fish were euthanized by *TMS* overdose. PIT-tag numbers were recorded and each fish was measured for body size and dissected to determine sex and sexual maturity status.

6.3.3 Statistical Analysis

The effect of temperature, photoperiod, ration, and sexual maturation on body weight was analyzed as repeated measures using the MIXED procedure of SAS as a five-factor factorial, with individual fish ($n = 515$) as the experimental unit (SAS Institute 2008). The factors were temperature (5 and 10 °C), photoperiod (*LDN* and *Long-to-Ambient*), ration (*Satiation* and *Restricted*), sexual maturation (immature and maturing), and measurement date (December 21, February 1, March 15, April 24, June 5, July 5, August 22, September 14, October 2, and November 16). The most appropriate covariance structure was determined to be compound symmetry, and mean body weight required a square root transformation to satisfy the normality and constant variance assumptions (Littell et al. 1998). The statistical model for the five-factor factorial in this analysis was expressed as

$$Y_{ijklmn} = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + \delta_k + (\tau\delta)_{ik} + (\beta\delta)_{jk} + (\tau\beta\delta)_{ijk} + \gamma_l + (\tau\gamma)_{il} + (\beta\gamma)_{jl} + (\tau\beta\gamma)_{ijl} + (\delta\gamma)_{kl} + (\tau\delta\gamma)_{ikl} + (\beta\delta\gamma)_{jkl} + (\tau\beta\delta\gamma)_{ijkl} + \eta_m + (\tau\eta)_{im} + (\beta\eta)_{jm} + (\tau\beta\eta)_{ijm} + (\delta\eta)_{km} + (\tau\delta\eta)_{ikm} + (\beta\delta\eta)_{jkm} + (\tau\beta\delta\eta)_{ijkm} + (\gamma\eta)_{lm} + (\tau\gamma\eta)_{ilm} + (\beta\gamma\eta)_{jlm} + (\tau\beta\gamma\eta)_{ijlm} + (\delta\gamma\eta)_{klm} + (\tau\delta\gamma\eta)_{iklm} + (\beta\delta\gamma\eta)_{jklm} + (\tau\beta\delta\gamma\eta)_{ijklm} + \epsilon_{ijklmn}$$

where Y_{ijklmn} was the response variable (body weight), μ was the overall mean, τ_i was the effect of date ($i = 1, 2, \dots, 10$), β_j was the effect of temperature ($j = 1, 2$), δ_k was the effect of photoperiod ($k = 1, 2$), γ_l was the effect of ration ($l = 1, 2$), η_m was the effect of sexual maturation ($m = 1, 2$), the number of replicates was denoted by n ($n = 1, 2, \dots, n_{ijklmn}$), and ϵ_{ijklmn} was the error term.

Food conversion ratio and specific growth rate from December 21 to July 5 were both analyzed as a four-factor factorial using the MIXED procedure of SAS, with tank ($n = 2$) as the experimental unit (SAS Institute 2008). The factors in both analyses were temperature (5 and 10 °C), photoperiod (*LDN* and *Long-to-Ambient*), ration (*Satiation* and *Restricted*), and measurement date (December 21, February 1, March 15, April 24, June 5, and July 5). The analysis was only conducted until July 5 because a change in personnel occurred from July 9 to mid-August, and the data was highly variable. For both analyses, the most appropriate covariance structure was determined to be compound symmetry. FCR required a reciprocal transformation to satisfy the normality and

constant variance assumptions. SGR did not require a transformation (Littell et al. 1998). The statistical models for both of the four-factor factorials were expressed as

$$Y_{ijklm} = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + \delta_k + (\tau\delta)_{ik} + (\beta\delta)_{jk} + (\tau\beta\delta)_{ijk} + \gamma_l + (\tau\gamma)_{il} + (\beta\gamma)_{jl} + (\tau\beta\gamma)_{ijl} + (\delta\gamma)_{kl} + (\tau\delta\gamma)_{ikl} + (\beta\delta\gamma)_{jkl} + (\tau\beta\delta\gamma)_{ijkl} + \varepsilon_{ijklm}$$

where Y_{ijklm} is the response variable (blood plasma osmolality), μ is the overall mean, τ_i is the effect of date ($i = 1, 2, \dots, 6$), β_j is the effect of temperature ($j = 1, 2$), δ_k is the effect of photoperiod ($k = 1, 2$), γ_l is the effect of ration ($l = 1, 2$), the number of replicates is denoted by m ($m = 1, 2, \dots, n_{ijklm}$), and ε_{ijklm} is the error term.

The p -values in all factorial tests were estimated using the Restricted Maximum Likelihood method. Least squares means of the treatment combinations from the highest order significant interaction effects were computed, and their letter groupings were generated to identify the means that were significantly different at either a 5 % (within each date) or a 1 % (within each treatment over time) level of significance.

The incidence of mortality and sexual maturity were both analyzed using the CATMOD procedure of SAS with the contrast statement (SAS Institute 2008). Mortality throughout the experiment was analyzed as a three-factor factorial: temperature (5 and 10 °C), photoperiod (*LDN* and *Long-to-Ambient*), and ration (*Satiation* and *Restricted*). Sexual maturity in November was analyzed as a four-factor factorial with temperature (5 and 10 °C), photoperiod (*LDN* and *Long-to-Ambient*), ration (*Satiation* and *Restricted*), and sex (male and female) as factors. The response variables % mortality and % sexual maturation were calculated, the proportions were further compared using the analysis of contrasts, and their letter groupings were generated at the 5 % level of significance.

Immature fish with a round weight > 1 kg were categorized as marketable. The biomass of marketable fish was determined as the sum of the body weight (kg) of the all of the immature fish that were over 1 kg round weight on November 16. Maturing fish with a round weight > 1 kg were categorized as non-marketable, and their biomass was determined in the same manner as the immature fish. In addition, all other fish (both immature and maturing) with round weights less than 999 g were included in the total biomass of non-marketable fish. The biomass of marketable and non-marketable fish

was also determined for each treatment combination of (1) temperature and (2) temperature and sex. Photoperiod was not included, mainly to simplify the calculations, and also due to the ineffectiveness of the treatment with respect to females in 5 °C.

6.4 Results and Discussion

6.4.1 Somatic Growth

Mean body weight from December 21, 2006 to November 16, 2007 was not significantly affected by photoperiod ($p = 0.0932$), but was affected by the interaction effect of temperature*ration*date ($p < 0.0001$) and temperature*maturation*date ($p < 0.0001$; Table 9).

Table 9

P values of the main and interaction effects of temperature, photoperiod, ration, and sexual maturation from Repeated Measures Analysis on mean body weight of Arctic charr from December 21, 2006 to November 16, 2007.

Source of variation	<i>P</i> values
Date	<0.0001
Temperature	<0.0001
Date x Temperature	<0.0001
Photoperiod	0.0932
Date x Photoperiod	0.3282
Temperature x Photoperiod	0.8228
Date x Temperature x Photoperiod	0.3712
Ration	<0.0001
Date x Ration	<0.0001
Temperature x Ration	<0.0001
Date x Temperature x Ration	<0.0001
Photoperiod x Ration	0.6091
Date x Photoperiod x Ration	0.1036
Temperature x Photoperiod x Ration	0.1147
Date x Temperature x Photoperiod x Ration	0.2160
Maturation	0.1263
Date x Maturation	<0.0001
Temperature x Maturation	0.0053
Date x Temperature x Maturation	<0.0001
Photoperiod x Maturation	0.8568
Date x Photoperiod x Maturation	0.5493
Temperature x Photoperiod x Maturation	0.3774
Date x Temperature x Photoperiod x Maturation	0.5210
Ration x Maturation	0.0948
Date x Ration x Maturation	0.2708
Temperature x Ration x Maturation	0.9185
Date x Temperature x Ration x Maturation	0.6783
Photoperiod x Ration x Maturation	0.2279
Date x Photoperiod x Ration x Maturation	0.8676
Temperature x Photoperiod x Ration x Maturation	0.3150
Date x Temperature x Photoperiod x Ration x Maturation	0.7396

Fish in 10 °C *Satiation* had the highest mean body weight compared to the other treatments. They attained a mean weight of 998 g by November, whereas the fish in 10 °C *Restricted* were only 812 g (Fig. 12, right panel). On April 24, one month after the 12 week period of restricted food intake had ended, the 5 and 10 °C *Restricted* fish had the same mean body weight (300 and 339 g, respectively), but were significantly different

from June 5 onwards (Fig. 12). Mean body weight of 10 °C *Restricted* fish surpassed the 5 °C *Satiation* fish by July 5 (Fig. 12). Fish in 5 °C *Restricted* were the slowest growing fish throughout the trial, although their mean body weight was similar to the 5 °C *Satiation* fish by November (564 and 586 g, respectively; Fig. 12, left panel). The *Restricted* fish in both 5 and 10 °C increased in mean body weight after the period of restricted food intake ended. Long-term fasting in Arctic charr is natural, and wild Arctic charr rely on endogenous energy reserves throughout the winter months (Aas-Hansen et al. 2003, Tveiten et al. 1996). If a restricted food intake for a short period during the winter reduces the incidence of sexual maturation the following autumn, this may be a good technique for the fish farmer to use as the somatic growth of the fish throughout the remainder of the spring and summer will not be sacrificed.

Mean body weight of the fish within each treatment combination (temperature and ration) from December 21 through November 16 is shown in Figure 12. Mean body weight increased significantly from December 21 to August 22 in 5 °C (with the exception of the period of restricted food intake). By October 2, there was no significant increase in mean body weight, and weight decreased in the 5 °C *Satiation* fish by November 16. Mean body weight of the 5 °C *Restricted* fish leveled off from August 22 to November 16 (Fig. 12, left panel). Mean body weight of the 10 °C *Restricted* fish increased significantly from December 21 through October 2 (with the exception of the period of restricted food intake). For the fish in 10 °C *Satiation*, mean body weight increased from December 21 through July 5, leveled off in August, and increased significantly October 2 (Fig. 12, right panel).

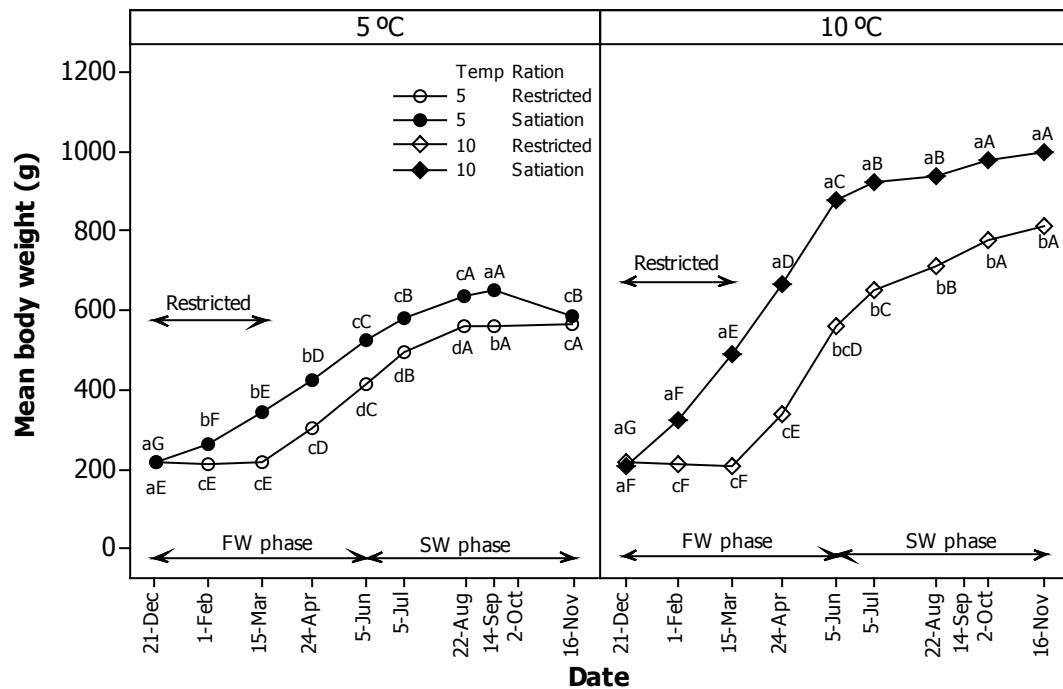


Fig. 12. Interaction plot of mean body weight (g) of Arctic charr from December 21, 2006 to November 16, 2007. The fish were reared in a combination of two temperatures (5 and 10 °C) and two rations (*Satiation*: fed to apparent satiation throughout the experiment; and *Restricted*: restricted food intake for 12 weeks, from December 21, 2006 to March 15, 2007). Panels are subdivided by temperature. Within each date (among both panels), means sharing the same letter (lower case) are not significantly different at the 5 % level. Within each treatment over time, means sharing the same letter (upper case) are not significantly different at the 1 % level.

The 10 °C immature and maturing fish had the same growth trajectory from December through August, however, mean body weight differed significantly in October (932 and 818 g, respectively) and November (1013 and 799 g, respectively; Fig. 13, right panel). A similar trend was observed in Experiment 1 and Duston et al. (2007), where significant differences in body weight were only found in late autumn. This is in contrast to Experiment 2, where there were no differences observed between immature and maturing fish from September through December. The fish in Experiment 2 did not gain a significant amount of body weight due to the cold rearing temperature (5 °C) June through December, and therefore the fish could not express their potential to diverge in growth with respect to sexual maturity. By contrast, the 5 °C maturing fish in this experiment were consistently larger in mean body weight compared to immatures, from December 21 through November 16 (Fig. 13, left panel). This may be attributed to the

fact that the fish in this experiment were grown in 5 °C starting in December, compared to the fish in Experiment 2 that were grown in 5 °C starting May/June. Overall, the 10 °C *Satiation* fish and 10 °C immature fish were the only ones that achieved a mean body weight of 1 kg. In terms of aquaculture production, growing Arctic charr in 5 °C would not be recommended, as the fish grew poorly compared to those in 10 °C and only achieved a final mean weight of approximately 600 g.

Mean body weight of the fish within each treatment combination (temperature and maturation) from December 21 through November 16 is shown in Figure 13. The maturing fish in 5 °C increased in mean body weight from December 21 through August 22, then growth leveled off from August through November. Among the immature fish in 5 °C, mean body weight increased until September 14, then decreased in November (Fig. 13, left panel). In 10 °C, both the immature and maturing fish had the same growth trajectory from December 21 through July 5. By August 22, the mean body weight of the immature fish leveled off, and then increased significantly in both October and November (Fig. 13, right panel). By contrast, the mean body weight of the maturing fish increased until August 22, leveled off in October, and decreased significantly by November. The growth trajectory of the maturing fish in the current study was comparable to the growth of the maturing fish in Duston et al. (2007), which appeared to be leveling off in November. The growth of both the 5 and 10 °C fish in 30 ppt in the current study was significantly better than the growth of the fish that were directly transferred 30 ppt (10 °C) in Duston et al. (2007). The fish in the current study were transferred from freshwater to 20 ppt in June, and then acclimated to 30 ppt in July. This appears to be a safe protocol for rearing Arctic charr in full-strength seawater, even in 5 °C.

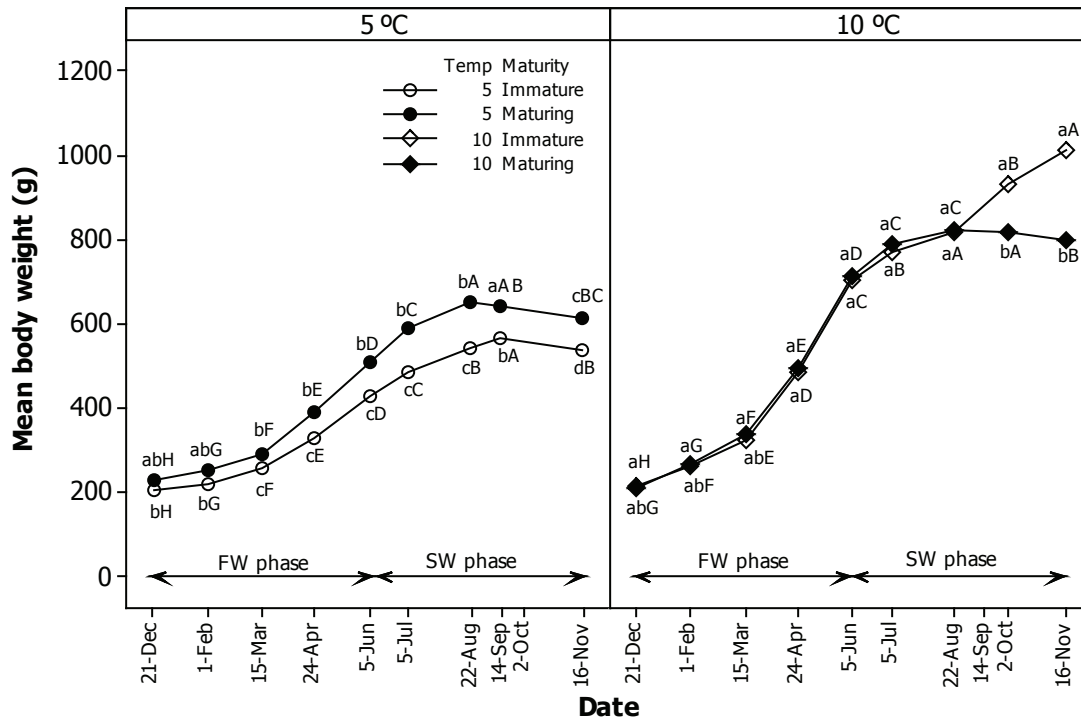


Fig. 13. Interaction plot of mean body weight (g) of immature and maturing Arctic charr reared in 5 and 10 °C from December 21, 2006 to November 16, 2007. Panels are sub-divided by temperature. Within each date (among both panels), means sharing the same letter (lower case) are not significantly different at the 5 % level. Within each treatment over time, means sharing the same letter (upper case) are not significantly different at the 1 % level.

6.4.2 Food Conversion Ratio and Specific Growth Rate

Mean FCR of the fish from February 1 to July 5, 2006 was independent of photoperiod ($p = 0.5320$), however, mean FCR was affected by the interaction effect of date*temperature*ration ($p = 0.0122$; Table 10). Food consumption being independent of photoperiod is in agreement with Sæther et al. (1996), who found that Norwegian Arctic charr displayed distinct seasonal cycles in feeding, even when reared under a long photoperiod (LD12:12) and in constant temperature (4 °C).

Table 10

P values of the main and interaction effects of temperature, photoperiod, and ration from Repeated Measures Analysis on mean food conversion ratio of Arctic charr from December 21, 2006 to July 5, 2007.

Source of variation	<i>P</i> values
Date	<0.0001
Temperature	0.0179
Date x Temperature	0.0067
Photoperiod	0.5320
Date x Photoperiod	0.2487
Temperature x Photoperiod	0.0767
Date x Temperature x Photoperiod	0.7191
Ration	<0.0001
Date x Ration	0.0011
Temperature x Ration	0.0219
Date x Temperature x Ration	0.0122
Photoperiod x Ration	0.6367
Date x Photoperiod x Ration	0.9697
Temperature x Photoperiod x Ration	0.4487
Date x Temperature x Photoperiod x Ration	0.6644

From February to June (freshwater phase), mean FCR was good at less than 1.2 for both the fish in 5 and 10 °C *Satiation* (Fig. 14). By July (seawater phase; 20 ppt), the mean FCR of the 10 °C fish deteriorated to 2.0.

In April, the FCR of the *Restricted* fish was excellent, with a mean of 1.02 in 10 °C and 0.85 in 5 °C, although by June and July there was no significant difference between the temperatures (Fig. 14). The FCR of the *Restricted* fish was good with a mean of 1.2 in July. This was significantly better (i.e. lower) than the mean FCR of fish in *Satiation*, which ranged from 1.4 to 2.0 (Fig. 14). The *Restricted* fish may have exhibited compensatory growth after the period of restricted food intake ended (Imslund and Gunnarsson 2011).

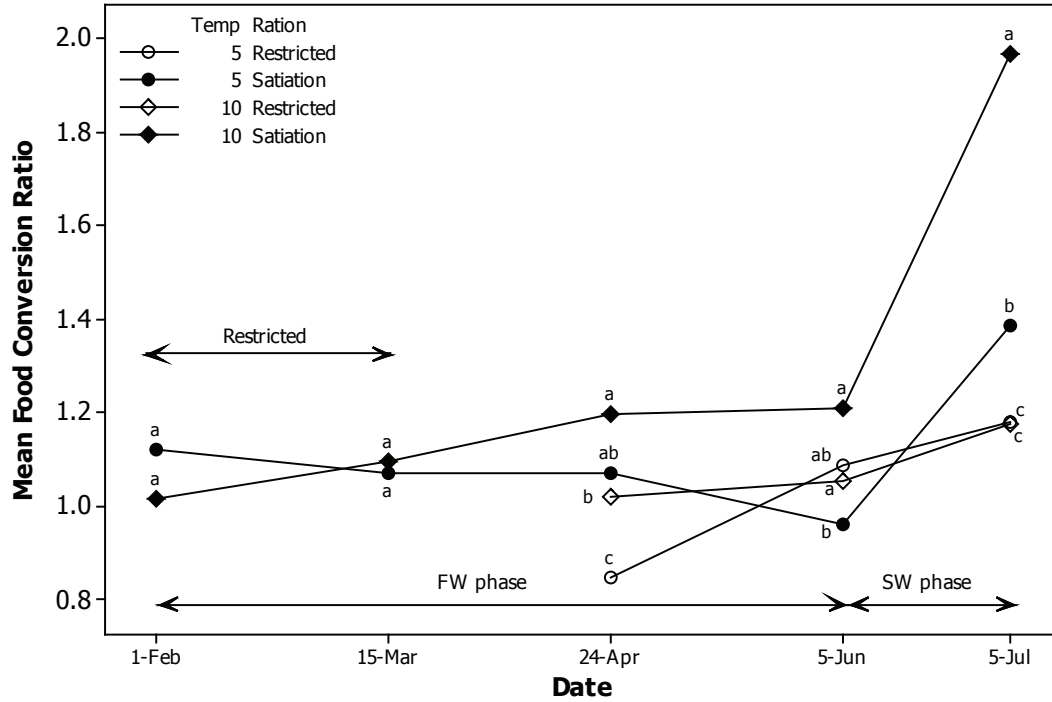


Fig. 14. Interaction plot of mean food conversion ratio (FCR) of Arctic charr from December 21, 2006 to July 5, 2007. The fish were reared in a combination of two temperatures (5 and 10 °C), two photoperiods (*LDN* and *Long-to-Ambient, LD18:6*, from December 21, 2006 to February 1, 2007), and two rations (*Satiation*: fed to apparent satiation throughout the experiment; and *Restricted*: restricted food intake for 12 weeks, from December 21, 2006 to March 15, 2007). The x-axis shows the final date of each data sub-set (~ 6 weeks). Within each date, means sharing the same letter are not significantly different at the 5 % level. ns = not significant.

Mean SGR from February 1 to July 5 was also independent of photoperiod ($p = 0.8695$), but was affected by the interaction effect of date*temperature*ration ($p < 0.0001$; Table 11).

Table 11

P values of the main and interaction effects of temperature, photoperiod, and ration from Repeated Measures Analysis on mean specific growth rate of Arctic charr from December 21, 2006 to July 5, 2007.

Source of variation	P values
Date	<0.0001
Temperature	<0.0001
Date x Temperature	<0.0001
Photoperiod	0.8695
Date x Photoperiod	0.0998
Temperature x Photoperiod	0.8695
Date x Temperature x Photoperiod	0.6443
Ration	<0.0001
Date x Ration	<0.0001
Temperature x Ration	<0.0001
Date x Temperature x Ration	<0.0001
Photoperiod x Ration	0.7425
Date x Photoperiod x Ration	0.0790
Temperature x Photoperiod x Ration	0.0853
Date x Temperature x Photoperiod x Ration	0.6443

Mean SGR for 5 °C *Satiation* remained between 0.4 and 0.6 % d⁻¹ from February to July. Mean SGR for 10 °C *Satiation* started at 1.08 % d⁻¹ in February and decreased progressively to 0.17 % d⁻¹ by July (Fig. 15). By contrast, the mean SGR of the *Restricted* fish increased dramatically from 0 in March to 0.87 % d⁻¹ (5 °C) and 1.21 % d⁻¹ (10 °C) by April. It has been suggested that after a period of restricted food intake, the fish feed more rapidly than if they had been fed to satiation throughout (Talbot et al. 1984). This is in agreement with the results of the current study. Mean SGR remained the same for 10 °C *Restricted* in June and then decreased to 0.54 % d⁻¹ by July. Mean SGR for 5 °C *Restricted* decreased slightly from 0.87 to 0.76 % d⁻¹ by June and to 0.57 % d⁻¹ by July (Fig. 15). By July, the *Restricted* group had a significantly higher mean SGR than the *Satiation* group.

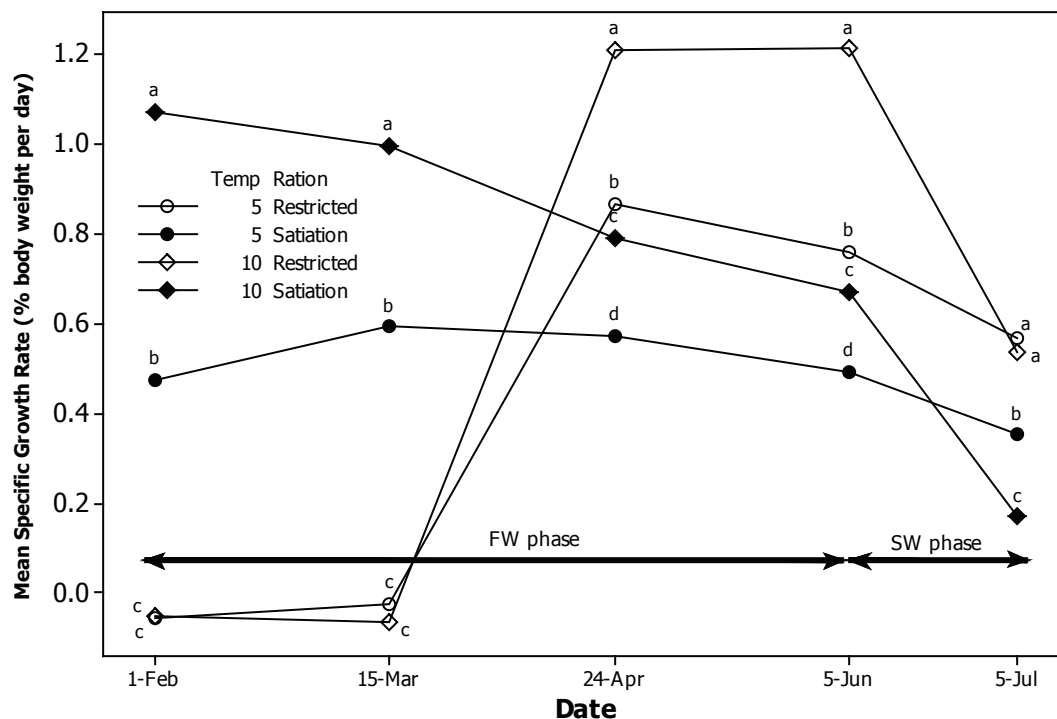


Fig. 15. Interaction plot of mean specific growth rate (SGR, % body weight per day) of Arctic charr from December 21, 2006 to July 5, 2007. The fish were reared in a combination of two temperatures (5 and 10 °C), two photoperiods (*LDN* and *Long-to-Ambient*, *LD18:6*, from December 21, 2006 to February 1, 2007), and two rations (*Satiation*: fed to apparent satiation throughout the experiment; and *Restricted*: restricted food intake for 12 weeks, from December 21, 2006 to March 15, 2007). The x-axis shows the final date of each data sub-set (~ 6 weeks). Within each date, means sharing the same letter are not significantly different at the 5 % level.

6.4.3 Incidence of Mortality

The incidence of mortality throughout the 11 month trial (December 2006 to November 2007) was not significantly affected by temperature ($p = 0.1037$, Table 12).

Table 12

P values of the main and interaction effects of temperature, photoperiod, and ration on the incidence of mortality of Arctic charr.

Source of variation	<i>P</i> values
Temperature	0.1037
Photoperiod	0.3659
Temperature x Photoperiod	0.8565
Ration	0.5875
Temperature x Ration	0.3659
Photoperiod x Ration	0.0467
Temperature x Photoperiod x Ration	0.8565

Most of the fish were simply recorded as being moribund. A few fish were euthanized for being moribund for too long (several days). Upon dissection, one fish had a distended abdomen. Four fish had caudal fin erosion, one fish had scoliosis, and one had jumped out of the tank.

A study on Atlantic salmon parr (< 22 g) revealed that mortality increased (7 and 22 %) in fish reared under two temperature treatments (3-7 °C and 7-14 °C, respectively) and were fed a reduced ration (Herbinger and Friars 1992). The large body size of the Arctic charr in the current study may have contributed to the negative effects of temperature on the incidence of mortality.

The incidence of mortality was significantly affected by the interaction effect of photoperiod*ration ($p = 0.0467$; Table 12). The effect of photoperiod on the incidence of mortality depended on the ration treatment. The fish in the *Long-to-Ambient* photoperiod that were fed to *Satiation* had a lower incidence of mortality than the fish in *LDN* that were fed to *Satiation* (3.6 and 9.3 %, respectfully), however, there was no significant difference between the *Long-to-Ambient* and *LDN* photoperiods for those fish on the *Restricted* ration (6.4 and 4.3 %, respectfully; Fig. 16). Overall, the incidence of mortality in this experiment was less than 10 % in each treatment combination, similar to the results in Experiments 1 and 2.

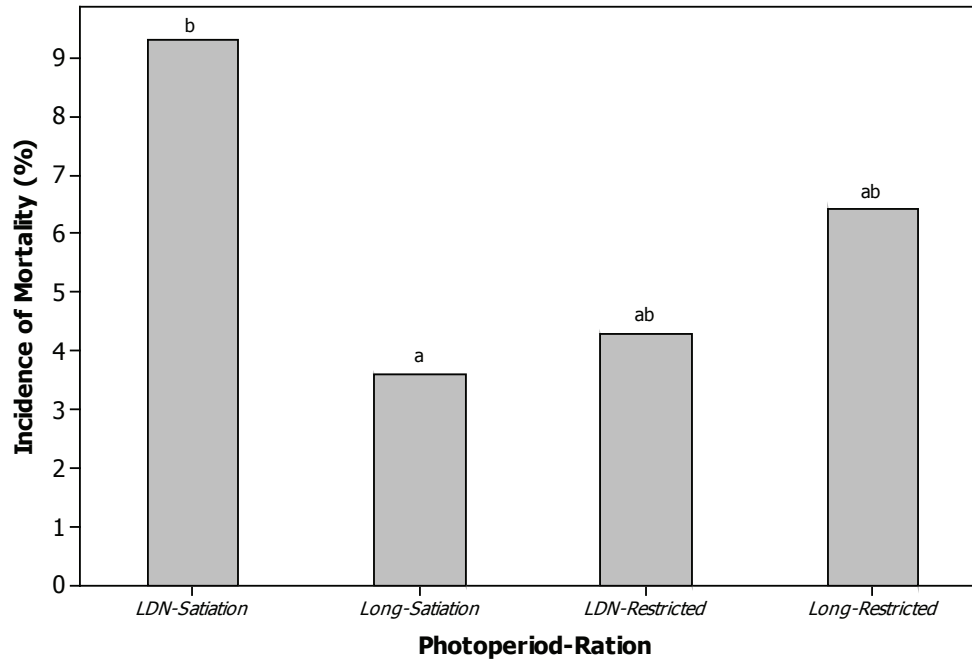


Fig. 16. Incidence of mortality among Arctic charr from December 21, 2006 to November 16, 2007. The fish were reared in a combination of photoperiod (*LDN* and *Long-to-Ambient*, *LD18:6*, from December 21, 2006 to February 1, 2007) and ration (*Satiation*: fed to apparent satiation throughout the experiment; and *Restricted*: restricted food intake for 12 weeks, from December 21, 2006 to March 15, 2007). Proportions sharing the same letter are not significantly different at the 5 % level.

6.4.4 Incidence of Sexual Maturation

The incidence of sexual maturation in November was independent of ration ($p = 0.7657$), however, there was a significant interaction effect of temperature*photo-period*sex ($p = 0.0482$; Table 13).

Table 13

P values of the main and interaction effects of temperature, photoperiod, ration, and sex on the incidence of sexual maturation of Arctic charr.

Source of variation	<i>P</i> values
Temperature	<0.0001
Photoperiod	0.0261
Temperature x Photoperiod	0.8746
Ration	0.7657
Temperature x Ration	0.1937
Photoperiod x Ration	0.7114
Temperature x Photoperiod x Ration	0.8226
Sex	<0.0001
Temperature x Sex	<0.0001
Photoperiod x Sex	0.3007
Temperature x Photoperiod x Sex	0.0482
Ration x Sex	0.8750
Temperature x Ration x Sex	0.0754
Photoperiod x Ration x Sex	0.6941
Temperature x Photoperiod x Ration x Sex	0.1130

Perhaps the long-term fasting that occurs during over-wintering in wild Arctic charr reduces the effectiveness of a restricted ration in large farmed Arctic charr (Tveiten et al. 1996). Contrasting results were found in Atlantic salmon (450 g; Thorpe et al. 1990) of similar body size to the Arctic charr in this trial (> 200 g). Thorpe et al. (1990) reported that restricted food intake during February-March was effective at reducing sexual maturation, a period when appetite was increasing with the seasonal increase in photoperiod and temperature. One recommendation would be to apply the restricted ration treatment in the spring/summer (early March through late June) rather than early winter, to determine if the later timing of the restricted ration would be more effective in Arctic charr. This would correspond with the natural migratory patterns of wild anadromous Arctic charr (Klemetsen et al. 2003a). The fish in the current study were quite large, and perhaps they had sufficient energy reserves to proceed with sexual maturation, regardless of a restricted ration treatment.

Silverstein et al. (1998) conducted an experiment using a ration treatment with chinook salmon. The ration treatment consisted of two groups: fish fed to apparent satiation, and fish fed half of the amount that were fed to the apparent satiation group. The treatments commenced in January, approximately 18 months prior to the onset of

sexual maturation. By contrast, the ration treatment in the current study commenced in December, less than one year prior to sexual maturation. The ration treatment in Silverstein et al. (1998) had a significant effect on the incidence of sexual maturation, in contrast to the results of the current study. Perhaps the treatment in the current study occurred too late in the season, after the critical period where the fish have already made the decision to mature and gonadal development was already in progress (Rowe and Thorpe 1990). A second recommendation would be to start a restricted ration treatment at least 12-18 months prior to the onset of sexual maturation.

The incidence of sexual maturation in November ranged from 15 to 98 %. The highest incidence of sexual maturation was 98 %, which occurred in female fish reared in 10 °C and *LDN* (Fig. 17). This is much higher than the results reported in Duston et al. (2003), and in Experiments 1 and 2.

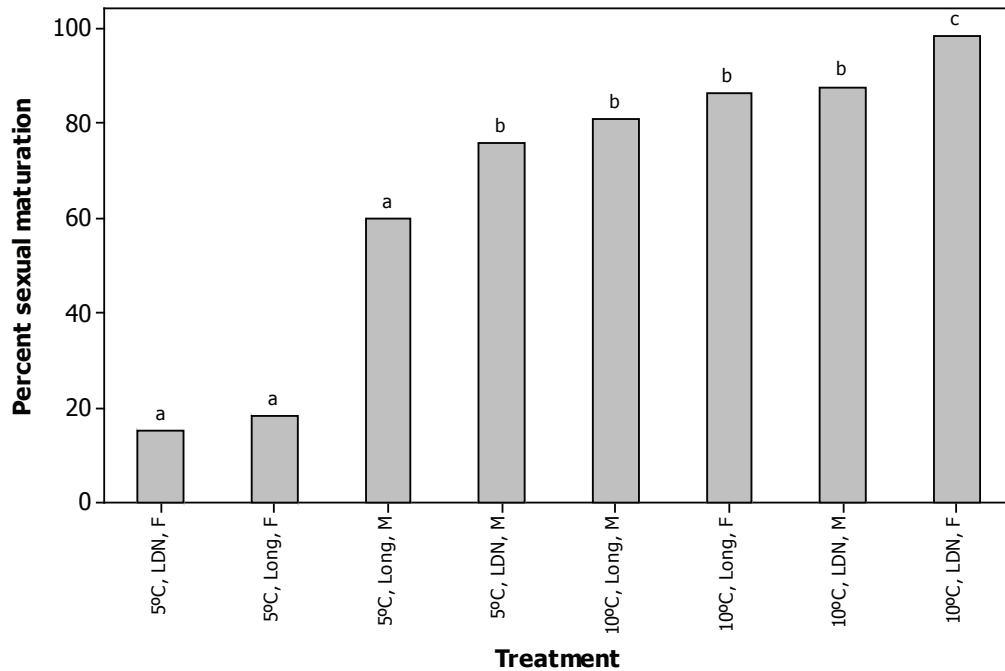


Fig. 17. Incidence of sexual maturity (immature and maturing) in November 2007 among male and female (M = male, F = female) Arctic charr, reared in a combination of two temperatures (5 and 10 °C) and two photoperiods (*LDN* and *Long-to-Ambient*, *LD18:6*, from December 21, 2006 to February 1, 2007). Proportions sharing the same letter are not significantly different at the 5 % level.

Photoperiod was effective at reducing sexual maturation among females reared in 10 °C; under *LDN*, 98 % matured, compared to *Long-to-Ambient*, in which 86 % matured (Fig. 17). The lowest incidence of sexual maturation occurred in 5 °C females: 15 % in *LDN* and 18 % in *Long-to-Ambient*, with no significant difference among the photoperiods (Fig. 17). This was comparable to the incidence of sexual maturation found in Duston et al. (2007), however, the authors believed that the low incidence of sexual maturation in their study (males 22 %, females 5 %) was an anomaly. Due to the higher energy requirements required for the development of oocytes compared to sperm, perhaps the effects of a cold rearing temperature and thus a lower growth rate was more pronounced in females compared to males. The high incidence of sexual maturation in 10 °C compared to 5 °C in the current study is in agreement with Silverstein et al. (1998), who found that the larger chinook salmon in their trial were fatter and matured at a higher rate. If sexual maturation could easily be inhibited in female Arctic charr by rearing in low water temperature, the production of all-female stocks may be a viable option for Arctic charr farming (Jobling et al. 1998).

The incidence of sexual maturation of males in 10 °C was independent of photoperiod: 88 % under *LDN*, and 81 % under *Long-to-Ambient* (Fig. 17). Among males in 5 °C, the *Long-to-Ambient* regime was effective, reducing sexual maturation to 60 % compared to 76 % in *LDN* (Fig. 17). Rearing males in 5 °C under *Long-to-Ambient* would also be recommended, as the incidence of sexual maturation (60 %) was comparable to the females in 5 °C (15 and 18 %; Fig. 17). Similar results were found in Atlantic salmon reared in temperatures less than 5 °C (Saunders et al. 1983). Saunders et al. (1983) postulated that exposure to low water temperatures resulted in reduced feeding and the depletion of energy reserves, and as such Atlantic salmon exposed to these low water temperatures during the winter months may make the physiological “decision” not to mature that year.

6.4.5 Final Body Weight and Marketable Product

There was a large variation in body weight among immature and maturing Arctic charr on November 16, with means ranging from 100 to over 1700 g (Fig. 18).

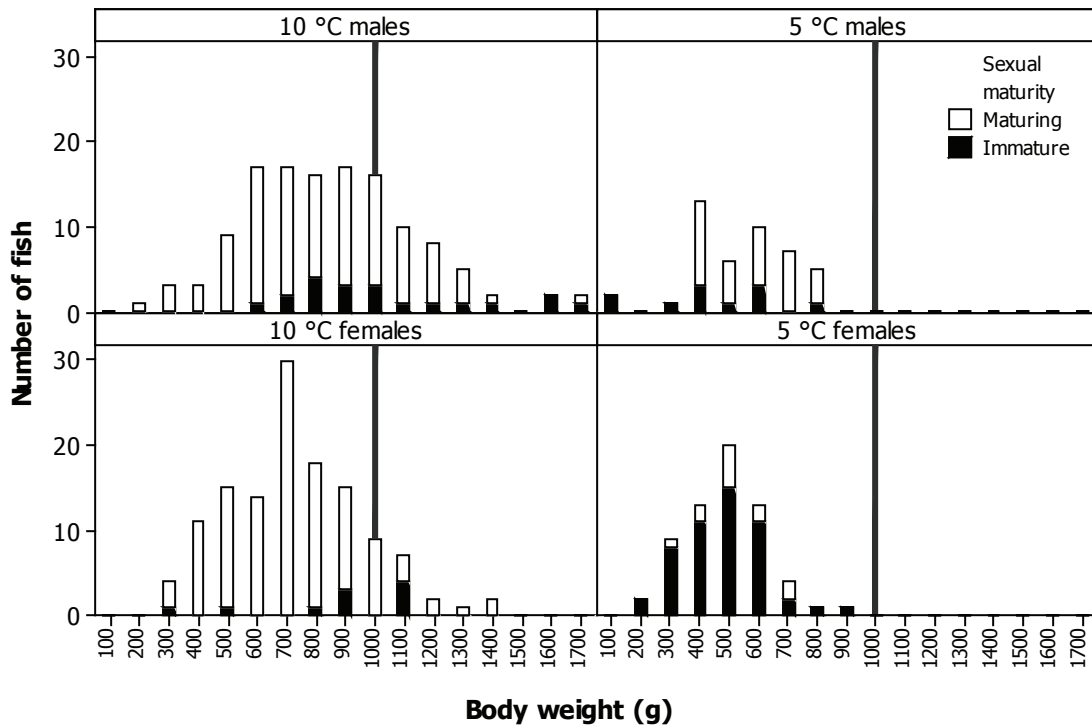


Fig. 18. Bar graph of final body weight distribution of Arctic charr on November 16, 2007, subdivided by temperature (5 and 10 °C), sex (male and female), and sexual maturity status (immature and maturing). Immature fish greater than 1 kg, to the right of the vertical line, are of marketable size.

The large variation in body weight has been a problem, and has been observed in the three experiments described here, and in experiments performed using other Arctic charr in the NSAC lab (Duston et al. 2003, 2007). One exception is the group of 5 °C females in this experiment, which displayed less variation in final body weight (Fig. 18). At the end of the trial, there was only 18.3 kg of high-value biomass (immature fish greater than 1000 g) and a total of 256.9 kg of non-marketable biomass. This amounted to a mere 3.9 % (14 of 363) of marketable product.

Following the example set in Experiment 2, the biomass was recalculated for both the 5 and 10 °C fish to include all immature fish greater than 200 g. This was particularly important for fish in 5 °C, as there were no immatures that attained a body weight over 1 kg. In 5 °C, 56.1 % of the population (60 of 107) would be marketable. In contrast, only 11.7 % (30 of 256) would be marketable in 10 °C.

With respect to temperature and sex, 81.0 % (51 of 63) of females in 5 °C were marketable, much higher than the 7.8 % (10 of 128) of females in 10 °C. In males, 21.4 % (9 of 42) in 5 °C were marketable, compared to 15.6 % (20 of 128) in 10 °C.

In terms of aquaculture production, growing Arctic charr in 5 °C would not be recommended, as the fish grew poorly compared to those in 10 °C and only achieved a final mean weight of approximately 600 g. However, if the fish grown in 5 °C were all females, and were marketed at pan-size (Smith et al. 1992) or before the onset of secondary sexual characteristics (Hatlen et al. 1996), the farmer would achieve a higher yield.

It was hypothesized that the number of maturing fish would decrease and the number immature fish of marketable quality and size would increase with each succession of treatments across the three experiments. This did not happen. The percent marketable fish decreased from 20.0, to 11.5, to 3.9 %. This may be attributable to the favorable environmental conditions in these experiments, as farmed Arctic charr often reach a body size in one year that would take many years in nature (Jobling et al. 1998). Future research on the interactions between body size, photoperiod, temperature, and the nutritional status of 0+ Arctic charr may enable farmers to produce fish of consistent body size and of marketable quality (Bromage et al. 2001).

Chapter 7.0 General Discussion and Conclusions

7.1 General Discussion

The direct transfer of Arctic charr from freshwater to 30 ppt resulted in reduced growth rate compared to those transferred from freshwater to 20 ppt. This indicated that even though the fish in Experiment 1 were large, over 600 g, they cannot be safely transferred from freshwater to 30 ppt. Fraser River Arctic charr may have a lower degree of anadromy compared to Norwegian Arctic charr, due to the difference in their geographic locations (56 ° and 70 °N, respectively). Although *CLP* was not effective during the autumn, the use of *CLP* from December 21 onwards should be evaluated, as it may improve the growth performance of the fish during the winter months.

The *Long-to-Ambient* photoperiod regime applied on both December (*Early*) and February (*Mid*) was effective at reducing the incidence of sexual maturation compared to controls reared under *LDN*. The large body size of the fish at the start of Experiment 2 may have contributed to the high incidence of sexual maturation. In terms of somatic growth, acclimating Arctic charr from 10 to 5 °C in freshwater before the transfer to seawater does not appear to be beneficial; however, the body size of the fish was quite large at the time of transfer.

A restricted feeding regime significantly improved the growth and food conversion ratio of the *Restricted* fish compared to the *Satiation* fish during the spring. It was expected that the *Restricted* ration would lead to a reduction in the incidence of sexual maturation by altering the growth and energy reserves of the fish during the critical time when the decision to mature was thought to be made, however, the effect of the *Restricted* ration on the incidence of sexual maturation was not significant. In terms of aquaculture production, growing Fraser River Arctic charr in 5 °C would not be recommended, as the fish grew poorly compared to those in 10 °C. However, the incidence of sexual maturation was high in 10 °C. As a result, only 3.9 % of the population was of marketable size and quality in Experiment 3, much less than what was reported in the previous experiments: 20.0 % (Experiment 1) and 11.5 % (Experiment 2). Interestingly, female fish in 5 °C displayed less variability in final body weight and had a low incidence of sexual maturation, regardless of photoperiod regime (< 18 %).

7.2 Conclusions

Fraser River Arctic charr can be successfully acclimated from 20 to 30 ppt seawater, at 10 °C (Experiment 1). In Experiment 2, the *Long-to-Ambient* photoperiod regime applied on both December (*Early*) and February (*Mid*) was effective at reducing the incidence of sexual maturation compared to controls, however, there is still a high percentage of fish that matured (> 43 %). In Experiment 3, a restricted feeding regime significantly improved the growth and food conversion ratio of the *Restricted* fish compared to the *Satiation* fish during the spring, however, the incidence of sexual maturation was independent of the ration treatment. Growing Fraser River Arctic charr in 5 °C would not be recommended, as the fish grew poorly compared to those in 10 °C (Experiment 3). However, growing female fish in 5 °C resulted in less variability in final body weight and they had a low incidence of sexual maturation, regardless of photoperiod regime.

7.3 Future Recommendations

Future laboratory experiments should assess the rate of acclimation from brackish to full-strength seawater, as the acclimation period was over two weeks. This may not be feasible on a fish farm. The acclimation from freshwater or 10 ppt to full-strength seawater should also be assessed, as 20 ppt seawater may not be accessible on a fish farm. The acclimation of small Fraser River Arctic charr (< 400 g) to full-strength seawater should be assessed to determine the appropriate body size for safe transfer to seawater. In addition, the hypo-osmoregulatory ability and growth performance of Arctic charr should be evaluated, when the fish are grown in 20 ppt and 30 ppt at different temperatures throughout the winter months.

Future laboratory experiments should evaluate the effectiveness of the *Long-to-Ambient* photoperiod regime, using small sized (~ 20 g) 0+ Arctic charr. In addition, the timing of the application of the *Restricted* regime should be investigated, as significant differences in the incidence of sexual maturation may be observed if the *Restricted* regime occurs earlier in the year when the fish are small (0+ in age), or later in the spring/summer (as 1+ fish) to correspond with the natural migratory pattern of anadromous Arctic charr. If sexual maturation could be inhibited in female Arctic charr

by rearing in low water temperature, the production of all-female stocks may be a viable option for Arctic charr farming.

Before Fraser River Arctic charr can be successfully grown in sea-cages in Atlantic Canada, more research needs to be conducted in the laboratory to establish safe protocols for rearing in seawater, and to determine the most effective methods to reduce the high incidence of sexual maturation.

Bibliography

- Aas, G.H., Bjerkgeng, B., Hatlen, B., Storebakken, T. 1997. Idoxanthin, a major carotenoid in the flesh of Arctic charr (*Salvelinus alpinus*) fed diets containing astaxanthin. *Aquaculture* 150, 135-142.
- Aas-Hansen, Ø., Johnsen, H.K., Vijayan, M.M., Jørgensen, E.H. 2003. Development of seawater tolerance and concurrent hormonal changes in fed and fasted Arctic charr at two temperature regimes. *Aquaculture* 222, 135-148.
- Adams, C.E., Huntingford, F.A. 1997. Growth, maturation and reproductive investment in Arctic charr. *Journal of Fish Biology* 51, 750-759.
- Aksnes, A., Gjerde, B., Roald, S.O. 1986. Biological, chemical and organoleptic changes during maturation of farmed Atlantic salmon, *Salmo salar*. *Aquaculture* 53, 7-20.
- Amano, M., Iigo, M., Ikuta, K., Kitamura, S., Yamada, H., Yamamori, K. 2000. Roles of melatonin in gonadal maturation of underyearling precocious male masu salmon. *General and Comparative Endocrinology* 120, 190-197.
- Arnesen, A.M., Johnsen, H.K., Mortensen, A., Jobling, M. 1998. Acclimation of Atlantic salmon (*Salmo salar* L.) smolts to 'cold' sea water following direct transfer from fresh water. *Aquaculture* 168, 351-367.
- Arnesen, A.M., Jørgensen, E.H., Jobling, M. 1993a. Feed intake, growth and osmoregulation in Arctic charr, *Salvelinus alpinus* (L.), transferred from freshwater to saltwater at 8 °C during summer and winter. *Fish Physiology and Biochemistry* 12(4), 281-292.
- Arnesen, A.M., Jørgensen, E.H., Jobling, M. 1993b. Feed intake, growth and osmoregulation in Arctic charr, *Salvelinus alpinus* (L.), following abrupt transfer from freshwater to more saline water. *Aquaculture* 114, 327-338.
- Belkovskiy, N.M., Lega, Y.V., Chernitskiy, A.G. 1991. Disruption of water-salt metabolism in rainbow trout, *Salmo gairdneri*, in seawater at low temperatures. *Voprosy ikhtiologii* 31(3), 511-515.
- Bjerknes, V., Duston, J., Knox, D., Harmon, P. 1992. Importance of body size for acclimation of underyearling Atlantic salmon parr (*Salmo salar* L.) to seawater. *Aquaculture* 104, 357-366.
- Boghen, A.D. (Ed.) 1995. Cold-water Aquaculture in Atlantic Canada (2nd edition). The Canadian Institute for Research on Regional Development, Moncton, NB.

- Bottengård, L., Jørgensen, E.H. 2008. Elevated spring temperature stimulates growth, but not smolt development, in anadromous Arctic charr. *Comparative Biochemistry and Physiology Part A* 151, 596-601.
- Bourlier, A., Billard, R. 1984. Delayed gametogenesis and spawning in rainbow trout (*Salmo gairdneri*) kept under permanent light during the first and second reproductive cycles. *Aquaculture* 43, 259-268.
- Brännäs, E., Linnér, J., Eriksson, L.-O. 2002. Aggression and growth as an effect of size composition in groups of Arctic charr. *Journal of Fish Biology* 60, 1331-1334.
- Brännäs, E., Wiklund, B.-S. 1992. Low temperature growth potential of Arctic charr and rainbow trout. *Nordic Journal of Freshwater Research* 67, 77-81.
- Bromage, N., Duston, J. 1986. The control of spawning in the rainbow trout (*Salmo gairdneri* Richardson) using photoperiod techniques. Report from the Institute of Freshwater Research, Drottningholm 63, 26-35.
- Bromage, N.R., Elliott, J.A.K., Springate, J.R.C., Whitehead, C. 1984. The effects of constant photoperiods on the timing of spawning in the rainbow trout. *Aquaculture* 43, 213-223.
- Bromage, N., Porter, M., Randall, C. 2001. The environmental regulation of maturation in farmed finfish with special reference to the role of photoperiod and melatonin. *Aquaculture* 197, 63-98.
- Canadian Council on Animal Care (CCAC) (2005). CCAC Guidelines on the Care and Use of Fish in Research, Teaching and Testing, 87 pp. CCAC, Ottawa ON. www.ccac.ca/en/CCAC_Programs/Guidelines_Policies/GDLINES/Fish/Fish_Guidelines_English.pdf.
- Christiansen, J.S., Wallace, J.C. 1988. Deposition of canthaxanthin and muscle lipid in two size groups of Arctic charr, *Salvelinus alpinus* (L.). *Aquaculture* 69, 69-78.
- CZRI. 2002. Commercialization of high pedigreed Arctic charr products, Shippagan, New Brunswick; November 2002.
- Damsgård, B., Arnesen, A.M., Jobling, M. 1999. Seasonal patterns of feed intake and growth of Hammerfest and Svalbard Arctic charr maturing at different ages. *Aquaculture* 171, 149-160.
- Davie, A., Porter, M.J.R., Bromage, N.R., Migaud, H. 2007. The role of seasonally altering photoperiod in regulating physiology in Atlantic cod (*Gadus morhua*). Part I. Sexual maturation. *Canadian Journal of Fisheries and Aquatic Sciences* 64, 84-97.

- Davies, B., Bromage, N., Swanson, P. 1999. The brain-pituitary-gonadal axis of female rainbow trout *Oncorhynchus mykiss*: effects of photoperiod manipulation. *General and Comparative Endocrinology* 115, 155-166.
- Delabbio, J. 1995. Arctic charr culture in Atlantic Canada. pp. 85-106 In A.D. Boghen, ed. 1995. *Cold-water Aquaculture in Atlantic Canada* (2nd ed). The Canadian Institute for Research on Regional Development, Moncton, NB. 672 pp.
- Delabbio, J.L., Glebe, B.D., Sreedharan, A. 1990a. Variation in growth and survival between two anadromous strains of Canadian Arctic charr (*Salvelinus alpinus*) during long-term saltwater rearing. *Aquaculture* 85, 259-270.
- Delabbio, J.L., Sreedharan, A., Glebe, B.D. 1990b. Important considerations in the mariculture of Arctic charr (*Salvelinus alpinus* L.). Pages 119-124 In R.L. Saunders (Ed.) *Proceedings of Canada-Norway finfish aquaculture workshop*, September 11-14, 1989. Canadian Technical Report of Fisheries and Aquatic Sciences 1761.
- Dempson, J.B. 1993. Salinity tolerance of freshwater acclimated, small-sized Arctic charr *Salvelinus alpinus* from northern Labrador. *Journal of Fish Biology* 43, 451-462.
- Dempson, J.B. 2001. North Labrador Arctic charr. DFO Stock Status Report D2-07. 7 pp.
- Dempson, J., Green, J. 1985. Life history of anadromous Arctic charr, *Salvelinus alpinus*, in the Fraser River, northern Labrador. *Canadian Journal of Zoology* 63, 315-324.
- Duston, J., Astatkie, T., MacIsaac, P.F. 2003. Long-to-short photoperiod in winter halves the incidence of sexual maturity among Arctic charr. *Aquaculture* 221, 567-580.
- Duston, J., Astatkie, T., Murray, S.B. 2007. Effect of salinity at constant 10 °C on grow-out of anadromous Arctic charr from Labrador. *Aquaculture* 273, 679-686.
- Duston, J., Bromage, N. 1986. Photoperiodic mechanisms and rhythms of reproduction in the female rainbow trout. *Fish Physiology and Biochemistry* 2, 35-51.
- Duston, J., Bromage, N. 1987. Constant photoperiod regimes and the entrainment of the annual cycle of reproduction in the female rainbow trout (*Salmo gairdneri*). *General and Comparative Endocrinology* 65, 373-384.
- Duston, J., Bromage, N. 1988. The entrainment and gating of the endogenous circannual rhythm of reproduction in the female rainbow trout (*Salmo gairdneri*). *Journal of Comparative Physiology Part A* 164, 259-268.

- Duston, J., Bromage, N. 1991. Circannual rhythms of gonadal maturation in female rainbow trout (*Oncorhynchus mykiss*). *Journal of Biological Rhythms* 6(1), 49-53.
- Duston, J., Knox, J.D.E. 1992. Acclimation of Atlantic salmon (*Salmo salar*) parr to seawater in autumn: stimulatory effect of a long photoperiod. *Aquaculture* 103, 341-358.
- Duston, J., Saunders, R.L., Knox, D.E. 1991. Effects of increases in freshwater temperature on loss of smolt characteristics in Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences* 48, 164-169.
- Duston, J., Saunders, R.L. 1995. Increased winter temperature did not affect completion of smolting in Atlantic salmon. *Aquaculture International* 3, 196-204.
- Duston, J., Saunders, R.L. 1999. Effect of winter food deprivation on growth and sexual maturity of Atlantic salmon (*Salmo salar*) in seawater. *Canadian Journal of Fisheries and Aquatic Sciences* 56, 201-207.
- Dutil, J.-D. 1986. Energetic constraints and spawning interval in the anadromous Arctic charr (*Salvelinus alpinus*). *Copeia* 4, 945-955.
- Eddy, F.B. 1981. Effects of stress on osmotic and ionic regulation in fish. pp. 77-102 In A.D. Pickering (Ed.) *Stress in fish*. Academic Press, London.
- Finstad, B., Nilssen, K.J., Gulseth, O.A. 1989. Sea-water tolerance in freshwater-resident Arctic charr (*Salvelinus alpinus*). *Comparative Biochemistry and Physiology Part A* 92, 599-600.
- Finstad, B., Staurnes, M., Reite, O.B. 1988. Effect of low temperature on sea-water tolerance in rainbow trout, *Salmo gairdneri*. *Aquaculture* 72, 319-328.
- Fitzgerald, R., Stefansson, S.O., Garforth, D., Irwin, S. 2002. Production II: From egg to market size: onrearing in freshwater and marine environments. pp. 65-104 In S.M. Stead and L. Laird (Eds.) 2002. *Handbook of salmon farming*. Praxis Publishing Ltd., Chichester, UK. 502 pp.
- Fletcher, G.L., Kao, M.H., Dempson, J.B. 1988. Lethal freezing temperatures of Arctic char and other salmonids in the presence of ice. *Aquaculture* 71, 369-378.
- Frantzen, M., Arnesen, A.M., Damsgård, B., Tveiten, H., Johnsen, H.K. 2004a. Effects of photoperiod on sex steroids and gonad maturation in Arctic charr. *Aquaculture* 240, 561-574.
- Frantzen, M., Damsgård, B., Tveiten, H., Moriyama, S., Iwata, M., Johnsen, H.K. 2004b. Effects of fasting on temporal changes in plasma concentrations of sex steroids, growth hormone and insulin-like growth factor I, and reproductive investment in Arctic charr. *Journal of Fish Biology* 65, 1526-1542.

- Frantzen, M., Johnsen, H.K., Mayer, I. 1997. Gonadal development and sex steroid in a female Arctic charr broodstock. *Journal of Fish Biology* 51, 697-709.
- Gulseth, O.A., Nilssen, K.J., Iversen, M., Finstad, B. 2001a. Seawater tolerance in first-time migrants of anadromous Arctic charr (*Salvelinus alpinus*). *Polar Biology* 24, 270-275.
- Gulseth, O.A., Steen, K., Nilssen, K.J. 2001b. Seawater tolerance in captive high-Arctic Svalbard charr (*Salvelinus alpinus*): effect of photoperiod and body size. *Polar Biology* 24, 276-281.
- Halvorsen, M., Arnesen, A.M., Nilssen, K.J., Jobling, M. 1993. Osmoregulatory ability of anadromous Arctic char, *Salvelinus alpinus* (L.), migrating towards the sea. *Aquaculture and Fisheries Management* 24, 199-211.
- Handeland, S.O., Stefansson, S.O. 2001. Photoperiod control and influence of body size on off-season parr-smolt transformation and post-smolt growth. *Aquaculture* 192, 291-307.
- Hatlen, B., Aas, G.H., Jørgensen, E.H., Storebakken, T., Goswami, U.C. 1995. Pigmentation of 1, 2, and 3 year old Arctic charr (*Salvelinus alpinus*) fed two different dietary astaxanthin concentrations. *Aquaculture* 138, 303-312.
- Hatlen, B., Arnesen, A.M., Jobling, M. 1996. Muscle carotenoid concentrations in sexually maturing and immature Arctic charr, *Salvelinus alpinus* (L.). *Aquaculture Nutrition* 2, 207-212.
- Heasman, M. S., Black, K. D. 1998. Mini review: The potential of Arctic charr, *Salvelinus alpinus* (L.) for mariculture. *Aquaculture Research* 29, 67-76.
- Heen, K., Thorpe, J., Ridler, N., Monahan, R.L., Mahnken, C., Lindbergh, J. 1993. The distribution of salmon aquaculture. pp.10–58. In K. Heen, R.L. Monahan, and F. Utter (Eds.). *Salmon aquaculture*. Halsted Press: an Imprint of John Wiley and Sons, Inc., Toronto, Ontario.
- Herbinger, C.M., Friars, G.W. 1992. Effects of winter temperature and feeding regime on the rate of early maturation in Atlantic salmon (*Salmo salar*) male parr. *Aquaculture* 101, 147– 162.
- Hoar, W.S. 1988. The physiology of smolting salmonids. pp. 275-343. In W.S. Hoar and D.J. Randall (Eds.) *Fish Physiology*, Volume 6B, Academic Press, New York. 436 pp.
- Hoar, W.S. 1976. Smolt transformation: evolution, behavior, and physiology. *Journal of the Fisheries Research Board of Canada* 33, 1234-1252.

- Imslund, A.K, Gunnarsson, S. 2011. Growth and maturation in Arctic charr (*Salvelinus alpinus*) in response to different feed rations. *Aquaculture* 318, 407-411.
- Jensen, J. L. A., Rikardsen, A. H. 2008. Do Northern riverine anadromous Arctic charr *Salvelinus alpinus* and sea trout *Salmo trutta* overwinter in estuarine and marine waters? *Journal of Fish Biology* 73, 1810-1818.
- Jobling, M. (Ed.) 1995. *Environmental Biology of Fishes*. Fish and Fisheries series 16. Chapman and Hall, London. 455 pp.
- Jobling, M. 1983. Influence of body weight and temperature on growth rates of Arctic charr, *Salvelinus alpinus* (L.). *Journal of Fish Biology* 22, 471-475.
- Jobling, M., Baardvik, B.M. 1991. Patterns of growth of maturing and immature Arctic charr, *Salvelinus alpinus*, in a hatchery population. *Aquaculture* 94, 343-354.
- Jobling, M., Jørgensen, E., Arnesen, A., Ringø, E. 1993. Feeding, growth and environmental requirements of Arctic charr: a review of aquaculture potential. *Aquaculture International* 1, 20-46.
- Jobling, M., Tveiten, H., Hatlen, B. 1998. Cultivation of Arctic charr: an update. *Aquaculture International* 6, 181-196.
- Johnson, L. 1980. The Arctic charr, *Salvelinus alpinus*. In E.K. Balon (Ed.) *Charrs, Salmonid Fishes of the Genus Salvelinus*. Dr. W. Junk, The Hague, pp. 15-98.
- Johnston, G. (Ed.) 2002. *Arctic charr aquaculture*. Blackwell Publishing, Oxford, UK. 272 pp.
- Jørgensen, E.H., Arnesen, A.M. 2002. Seasonal changes in osmotic and ionic regulation in Arctic charr, *Salvelinus alpinus*, from a high- and a sub-arctic anadromous population. *Environmental Biology of Fishes* 64, 185-193.
- Jørgensen, E.H., Aas-Hansen, Ø., Moriyama, S., Iwata, M., Strand, J.E.T. 2007. The parr-smolt transformation of Arctic charr is comparable to that of Atlantic salmon. *Aquaculture* 273, 227-234.
- Kadri, S., Thorpe, J.E., Metcalfe, N.B. 1997. Anorexia in one-sea-winter Atlantic salmon (*Salmo salar*) during summer, associated with sexual maturation. *Aquaculture* 151, 405-409.
- Klemetsen, A., Amundsen, P.-A., Dempson, J.B., Jonsson, B., Jonsson, N., O'Connell, M.F., Mortensen, E. 2003a. Atlantic salmon *Salmo salar* L., brown trout *Salmo trutta* L. and Arctic charr *Salvelinus alpinus* (L.): a review of aspects of their life-histories. *Ecology of Freshwater Fish* 12, 1-59.

- Klemetsen, A., Knudsen, R., Staldvik, F.J., Amundsen, P.-A. 2003b. Habitat, diet and food assimilation of Arctic charr under the winter ice in two subarctic lakes. *Journal of Fish Biology* 62, 1082-1098.
- Leclercq, E., Taylor, J.F., Hunter, D., Miguad, H. 2010. Body size dimorphism of sea-reared Atlantic salmon (*Salmo salar* L.): Implications for the management of sexual maturation and harvest quality. *Aquaculture* 301, 47-56.
- Le François, N.R., Blier, P., Adambounou, L.T., Lacroix, M. 1997. Alteration of gonadal development of brook charr (*Salvelinus fontinalis*): Impact on salinity tolerance following transfer in estuarine conditions. *Journal of Experimental Zoology* 279, 273-283.
- Le François, N.R., Blier, P. 2000. Branchial N^+K^+ ATPase activity in brook charr (*Salvelinus fontinalis*): effect of gonadal development in hypo- and hyperosmotic environments. *Journal Experimental Biology* 286, 647-655.
- Le François, N.R., Lemieux, H., Blier, P.U. 2002. Biological and technical evaluation of the potential of marine and anadromous fish species for cold-water mariculture. *Aquaculture Research* 33, 95-108.
- Lega, Y.V., Chernitsky, A.G., Belkovsky, N.M. 1992. Effect of low sea water temperature on water balance in the Atlantic salmon, (*Salmo salar* L.). *Fish Physiology and Biochemistry* 10(2), 145-148.
- Littell, R.C., Henry, P.R., Ammerman, C.B. 1998. Statistical analysis of repeated measures data using SAS procedures. *Journal of Animal Science* 76, 1216-1231.
- Mason, E. 1993. Prince Edward Island char production, p. 8-9. In B. Glebe and J. Delabbio (Eds.) Proceedings of the Canadian Arctic charr conference, St. Andrews, New Brunswick, November 1992. Bulletin of the Aquaculture Association of Canada 93-2.
- McClure, C.A., Hammell, K.L., Moore, M., Dohoo, I.R., Burnley, H. 2007. Risk factors for early sexual maturation in Atlantic salmon in seawater farms in New Brunswick and Nova Scotia, Canada. *Aquaculture* 272, 370-379.
- McCormick, S.D., Björnsson, B.Th., Sheridan, M., Eilertson, C., Carey, J.B., O'Dea, M. 1995. Increased daylength stimulates plasma growth hormone and gill Na^+ , K^+ -ATPase in Atlantic salmon (*Salmo salar*). *Journal of Comparative Physiology Part B* 165, 245-254.
- McCormick, S.D., Saunders, R.L. 1987. Preparatory physiological adaptations for marine life of salmonids: osmoregulation, growth, and metabolism. *American Fisheries Society Symposium* 1, 211-229.

- McGeachy, S. 1993. Arctic charr production in New Brunswick, p. 6-7. In B. Glebe and J. Delabbio (Eds.) Proceedings of the Canadian Arctic charr conference, St. Andrews, New Brunswick, November 1992. Bulletin of the Aquaculture Association of Canada 93-2.
- Murphy, J. 1993. An overview of the Arctic char industry in Nova Scotia, p. 7. In B. Glebe and J. Delabbio (Eds.) Proceedings of the Canadian Arctic charr conference, St. Andrews, New Brunswick, November 1992. Bulletin of the Aquaculture Association of Canada 93-2.
- Nordeng, H. 1983. Solution to the "Char Problem" based on Arctic char (*Salvelinus alpinus*) in Norway. Canadian Journal of Fisheries and Aquatic Sciences 40, 1372-1387.
- Pankhurst, N.W. 1998. Reproduction. pp. 1-26 In K.D. Black and A.D. Pickering (Eds.) Biology of Farmed Fish. Sheffield Academic Press Ltd., Sheffield, England. 415 pp.
- Peterson, R.H., Harmon, P.R. 2005. Changes in condition factor and gonadosomatic index in maturing and non-maturing Atlantic salmon (*Salmo salar* L.) in Bay of Fundy sea cages, and the effectiveness of photoperiod manipulation in reducing early maturation. Aquaculture Research, 1-8.
- Power, M., Dempson, J.B., Power, G., Reist, J.D. 2000. Environmental influences on an exploited anadromous Arctic charr stock in Labrador. Journal of Fish Biology 57, 82-98.
- Power, M., Power, G., Caron, F., Doucett, R., Guiguer, K. 2002. Growth and dietary niche in *Salvelinus alpinus* and *Salvelinus fontinalis* as revealed by stable isotope analysis. Environmental Biology of Fishes 64, 75-85.
- Randall, C.F., Bromage, N.R., Thorpe, J.E., Miles, M.S., Muir, J.S. 1995. Melatonin rhythms in Atlantic salmon (*Salmo salar*) maintained under natural and out-of-phase photoperiods. General and Comparative Endocrinology 98(1), 73-86.
- Rikardsen, A.H., Amundsen, P.-A. 2005. Pelagic marine feeding of Arctic charr and sea trout. Journal of Fish Biology 66, 1163-1166.
- Rikardsen, A.H., Amundsen, P.-A., Bodin, P.J. 2003. Growth and diet of anadromous Arctic charr after their return to freshwater. Ecology of Freshwater Fish 12, 74-80.
- Rogers, B., Davidson, W. 2001. Arctic charr aquaculture review. Prince Edward Island Aquaculture Alliance and the Aquaculture Association of Nova Scotia. 23 pp.
- Rounsefell, G.A. 1958. Anadromy in North American Salmonidae. Fishery Bulletin of the Fish and Wildlife Service, 58 (131), 171-185.

- Rowe, D.K., Thorpe, J.E. 1990. Suppression of maturation in male Atlantic salmon (*Salmo salar* L.) parr by reduction in feeding and growth during spring months. *Aquaculture* 86, 291-313.
- Sæther, B.-S., Johnsen, H.K., Jobling, M. 1996. Seasonal changes in food consumption and growth of Arctic charr exposed to either simulated natural or a 12:12 LD photoperiod at constant water temperature. *Journal of Fish Biology* 48, 1113-1122.
- SAS Institute Inc. 2008. SAS/STAT® 9.2 User's Guide, SAS Institute Inc., Cary, NC.
- Saunders, R.L. 1995. Salmon aquaculture: present status and prospects for the future. pp. 37-81 In A.D. Boghen (Ed.) 1995. *Cold-water Aquaculture in Atlantic Canada* (2nd edition). The Canadian Institute for Research on Regional Development, Moncton, NB. 672 pp.
- Saunders, R.L., Henderson, E.B., Glebe, B.D. and Loudenslager, E.J. 1983. Evidence of a major environmental component in determination of the grilse : larger salmon ratio in Atlantic salmon (*Salmo salar*). *Aquaculture* 33, 107-118.
- Silverstein, J.T., Shearer, K.D., Dickhoff, W.W., Plisetskaya, E.M. 1998. Effects of growth and fatness on sexual development of chinook salmon (*Oncorhynchus tshawytscha*) parr. *Canadian Journal of Fisheries and Aquatic Sciences* 55, 2376-2382.
- Sinnott, R. 2002. Fish farming and the feed companies. pp. 105-185 In S.M. Stead and L. Laird (Eds.) 2002. *Handbook of salmon farming*. Praxis Publishing Ltd., Chichester, UK. 502 pp.
- Shearer, K., Parkins, P., Gadberry, B., Beckham, B., Swanson, P. 2006. Effects of growth rate/body size and a low lipid diet on the incidence of sexual maturation in juvenile male spring chinook salmon (*Oncorhynchus tshawytscha*). *Aquaculture* 252, 545-556.
- Skuladottir, G.V., Schiöth, H.B., Gudmundsdottir, E., Richards, B., Gardarsson, F., Jonsson, L. 1990. Fatty acid composition of muscle, heart and liver lipids in Atlantic salmon, *Salmo salar*, at extremely low environmental temperature. *Aquaculture* 84, 71-80.
- Smith, B.E., Hardy, R.W., Torrissen, O.J. 1992. Synthetic astaxanthin deposition in pan-size coho salmon (*Oncorhynchus kisutch*). *Aquaculture* 104, 105-119.
- Stacey, N.E. 1984. Control of the timing of ovulation by exogenous and endogenous factors. In G.W. Potts and R.J. Wootton (Eds.) *Fish Reproduction: Strategies and Tactics*. Academic Press, London, pp. 207-222.

- Stagg, R.M., Talbot, C., Eddy, F.B., Williams, M. 1989. Seasonal variations in osmoregulatory and respiratory responses to seawater exposure of juvenile Atlantic salmon (*Salmo salar*) maintained in freshwater. *Aquaculture* 82, 219-228.
- Staurnes, M. 1993. Difference between summer and winter in gill Na-K-ATPase activity and hypoosmoregulatory ability of seafarmed anadromous Arctic char (*Salvelinus alpinus*). *Comparative Biochemistry and Physiology Part A* 105, 475-477.
- Strand, J.E.T., Johnsen, H.K., Arnesen, A.M. 2007. Comparison of parr-smolt transformation in hatchery reared offspring of one domesticated and two wild populations of Atlantic salmon (*Salmo salar* L.). *Aquaculture* 273, 250-256.
- Strand, J.E.T., Aarseth, J.J., Hanebrette, T.L., Jørgensen, E.H. 2008. Keeping track of time under ice and snow in a sub-arctic lake: plasma melatonin rhythms in Arctic charr overwintering under natural conditions. *Journal of Pineal Research* 44, 227-233.
- Sumpter, J.P., Scott, A.P., Baynes, S.M., Witthames, P.R. 1984. Early stages of the reproductive cycle in virgin female rainbow trout (*Salmo gairdneri* Richardson). *Aquaculture* 43, 235-242.
- Swanson, H.K., Kidd, K.A., Babaluk, J.A., Wastle, R.J., Yang, P.P., Halden, N.M., Reist, J.D. 2010. Anadromy in Arctic populations of lake trout (*Salvelinus namaycush*): otolith microchemistry, stable isotopes, and comparisons with Arctic charr (*Salvelinus alpinus*). *Canadian Journal of Fisheries and Aquatic Sciences* 67, 842-853.
- Takashima, F., Yamada, Y. 1984. Control of maturation in masu salmon by manipulation of photoperiod. *Aquaculture* 43, 243-257.
- Talbot, C., Higgins, P.J., Shanks, A.M. 1984. Effects of pre- and post-prandial starvation on meal size and evacuation rate of juvenile Atlantic salmon, *Salmo salar* L. *Journal of Fish Biology* 25, 551-560.
- Taranger, G.L., Haux, C., Hansen, T., Stefansson, S.O., Björnsson, B.Th., Walther, B.T., Kryvi, H. 1999. Mechanisms underlying photoperiodic effects on age at sexual maturity in Atlantic salmon, *Salmo salar*. *Aquaculture* 177, 47-60.
- Taranger, G.L., Haux, C., Stefansson, S.O., Björnsson, B.Th., Walther, B.T., Hansen, T. 1998. Abrupt changes in photoperiod affect age at maturity, timing of ovulation and plasma testosterone and oestradiol-17 β profiles in Atlantic salmon, *Salmo salar*. *Aquaculture* 162, 85-98.
- Thomsen, D.S., Koed, A., Nielsen, C., Madsen, S.S. 2007. Overwintering of sea trout (*Salmo trutta*) in freshwater: escaping salt and low temperature or an alternate life strategy? *Canadian Journal of Fisheries and Aquatic Sciences* 64, 793-802.

- Thorpe, J. 1995. Present and future charr research: reactions of a salmon biologist. *Nordic Journal of Freshwater Research* 71, 438-444.
- Thorpe, J.E., Talbot, C., Miles, M.S., Keay, D.S. 1990. Control of maturation in cultured Atlantic salmon, *Salmo salar*, in pumped seawater tanks, by restricting food intake. *Aquaculture* 86, 315-326.
- Tveiten, H., Johnsen, H.K., Jobling, M. 1996. Influence of maturity status on the annual cycles of feeding and growth in Arctic charr reared at constant temperature. *Journal of Fish Biology* 48, 910-924.
- Usher, M.L., Talbot, C., Eddy, F.B. 1991. Effects of transfer to seawater on growth and feeding in Atlantic salmon smolts (*Salmo salar* L.). *Aquaculture* 94, 309-326.
- Wandsvik, A., Jobling, M. 1982a. Overwintering mortality of migratory Arctic charr, *Salvelinus alpinus* (L.), reared in salt water. *Journal of Fish Biology* 20, 701-706.
- Wandsvik, A., Jobling, M. 1982b. Observations on growth rates of Arctic charr, *Salvelinus alpinus* (L.), reared at low temperature. *Journal of Fish Biology* 20, 689-699.

Appendix 1: Incident Reports

A1.1 Experiment 1

On Saturday August 6, 2005 (9am) all of the fish (n = 153) in Biosystem 1 (30 ppt salinity) were found dead. There were no alarms sent to the NSAC Aquaculture Centre pager nor were there any trend logs showing dramatic changes. An Aquaculture staff member assessed the situation in the Biosystem. Water was passing through each tank, through the biological filter and the oxygen exchanger. The temperature was within normal range, and ammonia and nitrite levels were below 1 ppm. A portion of the fish had flared gills, a sign of hypoxia, however, hypoxia was not suspected as the major cause of the die-off. There were no obvious signs of malfunction within the Biosystem. At the end of the work day on Friday August 5, 2005, the fish were in good health. On Friday afternoon, sodium bicarbonate had been added to the Biosystem to raise the pH above 7.6, and salt was also added to raise the salinity above 30 ppt. This was repeated in the other two Biosystems used in the trial, however, there was no mortality in these systems. The source of the problem was not determined.

A1.2 Experiment 2

On June 1, 2006 the fish were transferred from a freshwater Biosystem to two seawater Biosystems (20 ppt). Biosystem 2 was at 5 °C, and Biosystem 4 was at 10 °C. During the first week in seawater, the fish in Biosystem 4 had a good feeding response, but the fish in Biosystem 2 were less interested in food. The first fish to exhibit a loss of equilibrium was from Biosystem 2 on June 5. A few tanks of fish in this Biosystem had a low food response compared to Biosystem 4. The Total Ammonia Nitrogen (TAN) level in Biosystem 2 had been over-range for a few days, and nitrite (NO₂) in Biosystem 4 was 2.978 mg/L on June 7. TAN and nitrite assays were performed in the lab in the NSAC Aquaculture Centre. Total Ammonia Nitrogen is the combination of unionized ammonia (NH₃) and ionized ammonia (ammonium, NH₄⁺) that are dissolved in water, and NH₃ is the principal form that is toxic to aquatic animals. Toxicity increases as both temperature and pH decrease.

On Thursday June 8, the fish were only fed once in the morning, and a series of water samples were taken on the hour from 9:40am to 2:40pm to monitor TAN and nitrite

levels. TAN levels were converted to unionized ammonia by multiplying TAN mg/L by 0.0053 for Biosystem 2 (5 °C) and by 0.0073 for Biosystem 4 (10 °C). During the day, unionized ammonia was less than 0.0189 mg/L and 0.0125 mg/L for Biosystems 2 and 4, respectively. From June 9-12, the fish in both Biosystems were not fed to reduce the TAN and nitrite levels. On June 13 and 14, the fish were fed one meal, and then were not fed June 15 through June 27. One meal was fed June 28. TAN slowly decreased during this time. The 5 °C were eating well, and the 10 °C fish were very lively and hungry.

On July 5, 2006 the fish were all weighed. July 7, the fish were fed two meals. July 8, the water in Biosystem 4 was discoloured, most likely due to increased feeding. July 17, the freshwater make-up was turned off in both Biosystems to allow salinity to gradually increase to 30 ppt. July 20 (2:30pm), an Aquaculture staff member inoculated both Biosystems with cow manure. Two handfuls of dry cow manure were hydrated with 2 to 3 L of tap water. The manure mixture was blended to create a slurry. The slurry was filtered through two aquarium dipnets (no cheesecloth available) and collected into a 2 L container. The volume (1.5 L) was split between the two Biosystems. The biofilters were inoculated from the top of the towers to broadcast the fluid through the system.

At 3pm on July 20, there was one moribund fish and one dead fish in Biosystem 4. Oxygen saturation was 9.88 mg/L and a water sample was taken at 3:30pm. Biosystem 2 (TAN = 0.633 mg/L, nitrite = 3.155 mg/L). Biosystem 4 (TAN = 0.201 mg/L, nitrite = 1.897 mg/L). By 5pm, the fish in Biosystem 4 (10 °C) had a poor appetite. By 6pm, the moribund fish had died. 8:30pm, two more fish were moribund. 10pm, 5 fish moribund. At 10pm a water sample was taken. Biosystem 4 (TAN = 0.146 mg/L, nitrite = 1.307 mg/L). 10:15pm, one fish died and 6 were moribund. July 21 8:15am a major die-off had occurred in Biosystem 4. No mortalities were observed in Biosystem 2. All fish that were alive or moribund were netted and moved to a freshwater Biosystem that was held at 10 °C.

A veterinarian came at 1:30pm and took three dead fish and sacrificed four moribund fish for pathology. During the day, the fish in freshwater were slowly dying and were usually moribund and dead within one hour. Dead fish were removed every half an hour until 9pm. All dead fish were measured for weight and length, PIT-tag recorded, and dissected to determine sex and sexual maturity status. Biosystem 4 was

drained, flushed and refilled at 20 ppt. At 4:45pm, ten “lively” fish were added to a tank to monitor their health and survival overnight. Mortalities occurred for a few days afterwards. July 22 (n = 16), July 23 (n = 7), July 24 (n = 3). In total, there were 177 mortalities.

A1.3 Experiment 3

In January 2007, Biosystem 2 and 4 were filled with seawater (5 and 10 °C, respectively). The aim was to pre-condition the biofilters before introducing the fish in June. Starting in January, 150 g of ammonium chloride was added to each biosystem every 3 to 4 days, and TAN (mg/L) and nitrite (mg/L) were measured on a regular basis. The temperature, oxygen, salinity, and pH were also recorded. On May 29, the seawater was drained, the tanks were cleaned, and tanks refilled with seawater (5 and 10 °C) in preparation for the transfer of fish on June 5.

September 13, 2007 fish in Biosystem 2 (5 °C, 30 ppt) did not have an appetite. The next morning, there were a lot of fish that were dying. Oxygen, pH, salinity, and temperature were all within normal range. TAN was 0.279 mg/L and nitrite was 0.546 mg/L. Mortalities occurred for almost a week afterwards. September 14 (n = 46), September 15 (n = 38), September 16 (n = 40), September 17 (n = 19), September 18 (n = 9), September 19 (n = 2), and September 20 (n = 1). In total, 155 fish died.

The veterinarian took 4 moribund fish for pathology and took water samples. Water samples were sent to Maxxam Analytical lab in Bedford, Nova Scotia. The necropsy revealed no pathogenic agent. Histo-pathology (Dr. Groman AVC, UPEI) revealed clear evidence of gill damage and red cell hemolysis and erythrophagia. These changes are consistent with exposure to a water-born toxin or oxidizing agent. These changes have been seen most often when oxidizing agents such as chlorine or iodine have found their way into the water system. Toxins such as nitrite, hydrogen sulphate, and high unionized ammonia can be ruled out, also pathogenic microbes and hypoxia can be ruled out. Water quality results from Maxxam, confirmed NSAC data that ammonia and nitrite were normal. Chlorine was undetectable. However, there was an air-gap in the water sample bottle and several weeks elapsed before the sample was analyzed. It is possible that any chlorine present could have dissipated.