UPPER THERMAL LIMITS AND ACCLIMATION POTENTIAL OF ARCTIC COD (*BOREOGADUS SAIDA*): A KEY FOOD WEB SPECIES IN THE ARCTIC OCEAN

by

Helen E. Drost

M.Sc., The University of British Columbia, 2007

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES

(Zoology)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

April, 2017

Abstract

The recent rapid and unprecedented changes to the physical and biogeochemical properties of the Arctic Ocean have gained worldwide attention. The greater than 50% reduction in sea ice volume below average is of great concern. My thesis investigates the potential effects of a warmer Arctic Ocean upon the indigenous Arctic cod, *Boreogadus saida*. This fish make up a significant proportion of the lower trophic energy reserve available in Arctic marine systems. Predators rely upon Arctic cod to provide them bite sized access to those critical energy reserves. Yet despite their key role in the Arctic food web, their upper thermal limits are not well studied. Thus, my three objectives were: a) quantify, for the first time in this species, their upper thermal limits b) determine the acclimation potential of the species at three acclimation temperatures and c) contrast the results generated by different methods that quantify thermal limits for declining physiological performance (rate transition temperatures) that I determined.

Boreogadus saida upper thermal limits were tested under acute warming conditions using three different methods: loss of equilibrium (T_{cmax}), absolute aerobic scope (AAS) derived from oxygen uptake rates and the cardiac method, which uses maximum heart rate (f_{Hmax}) to detect change in whole animal performance. In conducting the thermal acclimation studies, I discovered foremost that 6.5°C-acclimated fish grew at that temperature but, to date, have only produced eggs at 3.5°C water temperatures. The T_{cmax} significantly increased with acclimation temperature (0.5, 3.5 and 6.5°C) from 14.4, 15.5, up to 17.1°C respectively, while the more ecologically relevant AAS transition temperature limits were found at lower temperatures from 1.0 to 5.5°C. The temperature for peak f_{Hmax} (T_{max}) occurred between 11.0 to 12.0°C and the performance of f_{Hmax} for larval *B. saida* during acute warming was not significantly different from the adults until T_{max} was reached.

This novel study of the thermal physiology of this key Arctic marine food web species revealed a greater than expected thermal tolerance and a significant acclimation potential up to 6.5°C, suggesting that this species may be more resilient to rapid climate change than previously thought.

Preface

A Science publication in 2008 by Pörtner and Farrell on animal physiology and climate change was the inspiration for the following study. Dr. Eddy Carmack read this paper a year after publication and asked me, a research assistant at the time, to “look into it”. These are the results of that assignment.

I contributed to the identification and design of the research program that resulted in this thesis document. I collected the fish, helped rear a 2nd generation, analyzed and presented the research data to the public and wrote, with significant editorial assistance from T. Farrell, the 4 thesis related, peer reviewed journal publications. Below, as required, is the publication list with details on co-author contributions.

Publications:

Chapter 2
Drost H. E., Lo, M., Carmack, E., Farrell, A. P. (2016). Acclimation potential of *Boreogadus saida* Lepechin in the rapidly warming Arctic Ocean – Advanced publication: Journal of Experimental Biology, DOI: 10.1242/jeb.140194

- This publication is based on work conducted at the Vancouver Aquarium (VA: Vancouver, B.C and in Cambridge Bay, Nunavut at two improvised field laboratories). I designed the study, conducted the research, analysed the data and wrote the paper.
- All the co-authors of the publication contributed in one or other of the following: field work (T. Farrell & E. Carmack), study design (M. Lo & T. Farrell), data collection (M. Lo & E. Carmack), editing (all authors particularly T. Farrell) and with publication submission (T. Farrell &, M. Lo)

Chapter 3
Drost, H. E., Carmack, E. C., Farrell, A. P. (2014). Upper thermal limits of cardiac function for *Boreogadus saida*, a key food web fish species in the Arctic Ocean. J Fish Biol. 84 1781-1792, DOI: 10.1111/jfb.12397

- This publication is based on work conducted at the Vancouver Aquarium (VA: Vancouver, B.C and in Cambridge Bay, Nunavut (at two improvised field laboratories). I designed the study, conducted the research, analysed the data and wrote the paper.
- All the co-authors of the publication contributed in one or other of the following: field work (T. Farrell & E. Carmack), study design (T. Farrell & E. Carmack), editing (all authors particularly T. Farrell) and with publication submission replies and proofing (T. Farrell & E. Carmack)

Chapter 4
Drost, H. E., Fisher, J. Randall, F., Kent, D. Carmack, E. C., Farrell. A. P. (2016). Upper thermal limits of the hearts of *Boreogadus saida:* Adults compared with larvae – J Fish Biol. 88, 718-726, DOI:10.1111/jfb.12807

- This publication is based on work conducted at the Vancouver Aquarium (VA: Vancouver, B.C) from 2011 to 2015 and in Cambridge Bay (CB: Nunavut) at two

improvised field laboratories from 2011 to 2012. I designed the study, conducted the research, analysed the data and wrote the paper.

- All the co-authors of the publication contributed in one or other of the following: rearing larvae (D. Kent, J. Fisher. & VA staff), study design (T. Farrell & E. Carmack), Data Analysis (F. Randall & T. Farrell) editing (all authors particularly T. Farrell) and with publication submission (T. Farrell)

Appendix B

D. Kent, H. E. Drost, J. Fisher, T. Oyama and A. P. Farrell, Journal of Fish Biology 88 (3), 1241-1248 (2016). I analysed the data and wrote the paper.

- This publication is based on work conducted at the Vancouver Aquarium (VA: Vancouver, B.C from 2011 to 2015.
- The first author and the co-authors of the publication contributed in one or other of the following: rearing larvae (D. Kent, J. Fisher. T. Oyama), study design (T. Farrell), Data Analysis (F. Randall & T. Farrell) editing (all authors particularly T. Farrell) and with publication submissions (T. Farrell)

Permits:

Fish were collected, held and tested in accordance with permits issued by:

- The Kitikmeot Hunters and Trappers Association – Nunavut (letter of approval),
- The University of British Columbia Animal Care Committee (A11-0267),
- The Freshwater Institute Science Laboratories Animal Care Committee – Arctic Aquatic Research (FWI-ACC-2012-050),
- Vancouver Aquarium Animal Care Committee (2011–04).

Table of contents

List of tables

List of figures

Glossary and abbreviations

AAS	Absolute aerobic scope (MMR – RMR) defines the absolute aerobic capacity to perform activities (such as movement, feeding, growth and reproduction)
bpm	Beats per minute
CB	Cambridge Bay, Nunavut
EKG	Electrocardiogram recording of electricity generated by the heart
EPOC	Excess post oxygen consumption
FAS	Factorial aerobic scope (MMR/RMR)
f_H	Routine heart rate
f_{Hmax}	Maximum heart rate
Lf	Fork length
h	Hour
hp	Horse power
K	Kelvin
Kg	Kilogram
kJ	Kilojoule - a unit of energy
l	Litre
m	Meter
M_b	Body mass
MMR	Maximum metabolic rate
MO_2	Rate of oxygen uptake (measured in mg O_2 kg^{-1} h^{-1})
MS-222	Tricaine methanesulfonate anaesthetic
OCLTT	The oxygen and capacity-limited thermal tolerance hypothesis suggests that a fish's capacity to supply oxygen to tissues becomes limited at temperature extremes
ppm	Unit - Parts per million
ppt	Ocean Salinity unit - kg salt per kg water in parts per thousand
psu	Practical Salinity scale (PSS-78) to replace ppt for consistency
P-wave	EKG recording that represents atrial contraction
Q_{10}	The Q_{10} temperature coefficient represents the factor by which the rate of a reaction increases for every 10°C rise in the temperature
QRS-complex	EKG recording that represents ventricular contraction
RMR	Routine metabolic rate

R-R interval	Time between beats - used to calculate heart rate
s.e.m.	Standard error of the mean
SST	Sea surface temperature
T_{AB}	The first Arrhenius breakpoint temperature, when f_{Hmax} first fails to keep up with acute thermal warming (see Farrell, 2016)
T_{AR}	The temperature when f_{Hmax} becomes arrhythmic
T_{cmax}	Critical temperature when fish first roll over due to acute warming (2°C/h)
T_{crit}	Critical temperature when AAS = 0extrapolated from AAS regression curve. Beyond this temperature a fish is forced into an anaerobic and time-limited lifestyle
T_{FS}	The temperature when FAS drops permanently below 2
T_{lpej}	Lower pejus temperature when the aerobic scope drops below 90% of $T_{opt(AAS)}$
T_{max}	When f_{Hmax} first reaches maximum bpm
$T_{opt(AAS)}$	The optimal temperature under which an animal has the greatest capacity to perform a certain activity
T_{upej}	Upper pejus temperature when the aerobic scope drops below 90% of $T_{opt(AAS)}$
T_{pej}	Pejus temperature when performance begins to decline
T_{QB}	The temperature when incremental Q_{10} drops permanently below 2
T_{QR}	The temperature when the EKG recording of the QRS peak height (measured from Q to R) starts to permanently decline
T_{upej}	Upper pejus temperature when the aerobic scope drops below 90% of $T_{opt(AAS)}$
VA	Vancouver Aquarium
y	Year

Acknowledgements

This thesis is a result of collaboration with and input from the co-authors, Cambridge Bay resident experts in fishing and coastal ice dynamics, my thesis committee and anonymous peer reviewers of the publications. This study would not have happened without the help from local fishers J. Puglik and M. Townsend and the generous support from F. Buchan- Director of the Arctic College and college staff. I received invaluable support and fish collecting, holding and shipping advice from D. Kent, J. Fisher and T. Oyama. I am indebted to all the staff and volunteers from the Vancouver Aquarium. I also relied upon the support from staff at University of British Columbia and other students, in particular M. Lo and F. Randall for their invaluable help running experiments and analyzing data. Any positive contributions that this thesis provides are due to the many persons involved with the study. Any errors or oversights are mine alone.

Funding

A.P.F. and E.C.C. were funded by the Natural Sciences and Engineering Research Council of Canada. A.P.F. holds a Canada Research Chair. The field study was financed in part by grants from the Northern Students Training Programme (2011 and 2012) and the Arctic Research Foundation (2012).

Volunteers

The authors would like to thank those from the Cambridge Bay community including F. Buchan-Corey, B. Sitatak and J. Pzanioyak and to all those who helped with field work including M. Townsend, J. Puglik, M. Havioyak, S. Dockerill, A. Townsend, T. Rutherford and D. Varella. The captain and crew of the Canadian Coast Guard ice breaker Sir Wilfrid Laurier helped tremendously with equipment logistics during the Arctic summers of 2011 and 2012 . I am also indebted to Vancouver Aquarium volunteers for looking after the fish during the long term acclimation study including: M. Larrea, D. Richards, S. Vitkauskaite, J. Yan and E. Chan.

Government

The access to data and equipment provided by the Canadian Hydrography Department and the Institute of Ocean Sciences, Pat Bay, including M. Dempsy, S. Vagel and S. Zimmerman was greatly appreciated. I also thank those from the Cambridge Bay including B. Sitatak and J. Pzanioyak

NGO

I am very appreciative of the support provided by the Vancouver Aquarium staff, in particular J. Fisher, D. Kent, J. Nightingale, D. Thoney, E. Solomon, T. Myers and M. Haulena.

Academic institutes

The thesis supervisory committee, the Farrell lab - especially M. Casselman for devising a field friendly apparatus template for heart rate measurements in fish and M. Lo who, as an undergraduate student, courageously withstood the cold conditions of a temperature controlled laboratory in the basement of biosciences to measure *B. saida* respiration and critical temperature limits.

Family and Friends
I am grateful for all the help and support from family and friends.
To all my relations. Thank you!

Dedication
To Neil K. Benson
(MCMLVII - MMXIV)

Chapter 1: Thesis introduction

1.1 Arctic oceanography and climate change

The Arctic marine system is changing physically (e.g. increased temperatures, loss of sea ice, increased stratification, altered light climate) and chemically (e.g. reduced pH), altered terrestrial inputs. Consequently biological changes are occurring (e.g. altered productivity, rates of respiration, poleward migration of non-native species). The physiological limits of key endemic species however, remain relatively unknown. The goal of this work, therefore, is to quantify the thermal limits and acclimation potential of an important Arctic forage fish species *Boreogadus saida* Lepechin, 1774. This fish species plays a vital role moving energy stores, such as essential fatty acids, from algae and zooplankton up to the higher trophic levels in the Arctic.

Physical conditions in the Arctic Ocean are changing at unprecedented rates, at least twice the rate of warming than the global average (Gaston et al., 2003; Polyakov et al., 2005; Steele et al., 2011; Barber et al., 2015; Berge et al., 2015; Carmack et al., 2015, 2016). Alarmingly, both the quality (Krishfield et al., 2014) and quantity (Vaughan et al., 2013; Perovich et al., 2014) of summer sea ice (from July to September) has decreased appreciably. The lowest Arctic sea ice extent on record occurred in the fall of 2016 (NOAA - Arctic sea ice data, November, 2016). Sea surface temperature (SST) in the summer of 2007 was 7°C greater than the previous 30-y average (Steele et al., 2008; Timmermans and Proshutinsky, 2014). Water temperatures at depths from 60 to 800 m below the surface layer are also increasing due to warmer inflows from the subarctic Atlantic and Pacific (Shimada et al., 2006; Polyakov et al., 2010).

The world oceans have absorbed an estimated 90% of the Earth's extra heat since 1955 (IPCC, 2013; Levitus et al., 2012). However, there is a limit to how much heat the ocean can absorb before other climate system thresholds are reached. For example Carson et al. (2016) suggest continued global warming may trigger a cascade of catastrophic events such as the collapse of Greenland and Antarctic land based ice sheets and methane hydrate destabilization (Carson et al., 2016; Peterson and Rocha, 2016).

These extreme deviations from normal ice coverage, low pH and anomalous Arctic sea and air temperatures such as the recent spikes of greater than 20°C above normal, are now being directly attributed to climate change with positive feedback loops that further accelerate global warming (Carson et al., 2016; Feely, 2016). For instance, ordinarily Arctic sea ice prevents ~80% of solar radiation from being absorbed into the surface layer of the ocean (Carson et al., 2016) yet as warming melts the ice, the open sea absorbs more heat which increases surface warming and leads to even more sea ice loss. There are also negative feedback loops in the global system. Greater open ocean allows for increased air-sea gas exchange, which allows increased ocean absorption of excess, anthropogenic CO_2 from the atmosphere. It is estimated that world oceans have already absorbed ~ 1/3rd excess CO_2 (Fabry et al., 2008). However, the chemical composition of seawater becomes more acidic as it absorbs CO_2.

Ocean acidification has now reached critical levels in parts of the Arctic Ocean (Yamamoto-Kawai et al., 2011). Since the industrial revolution an average of 30% more [H^+] ions have been measured in seawater (Fabry et al., 2008). Increased [H^+] ions increase acidity and thus lower the pH value. Ocean acidification of the Arctic Ocean is twice as high as the global average not only because cold water absorbs more CO_2 than warm water but also because fresh water, from sea ice melt and riverine input, is naturally more acidic.

Respiration of organic matter and upwelling events also contribute to reduced seawater pH (Yamamoto-Kawai et al., 2011; Mathis et al., 2015). The Arctic Ocean is particularly vulnerable to ocean acidification as it is naturally quite low in carbonate ions (CO_3^{2-}). These ions react with calcium (Ca) in seawater to form calcium carbonate minerals - a critical component of marine animal shells and diatom tests (Mathis et al., 2015). The Arctic pelagic pteropod species *Limacina helicina* is particularly vulnerable to shell dissolution (Comeau et al., 2009: Feely et al., 2016).

It is predicted, and recent observations are confirming, that the rapid changes in both the physical and chemical nature of the Arctic Ocean will directly and significantly impact resident marine biota. Climate change will also cause secondary, cascading effects that also have the potential to alter the entire structure of existing Arctic marine food webs (Carmack and McLaughlin, 2001; Gaston et al., 2003; Perry et al., 2005; Grebmeier et al., 2006; Wassmann, 2011; Yamamoto-Kawai et al., 2011; Hutchings et al., 2012; Barber et al., 2015; Mathis et al., 2015; Steiner et al., 2015, Carson et al., 2016). The multiple stressors of climate change, including temperature, ocean acidification, hypoxia and species migration are potentially acting synergistically upon indigenous marine species (Pörtner et al., 2005; Pörtner and Farrell, 2008). Using idealized curves, Pörtner and Farrell, 2008 explained how acute warming alters the aerobic performance of an aquatic animal. The curves quantify thermal optimum windows based on peak values of aerobic scope that are species and even, in some instances, population specific (Fry, 1947; Eliason et al., 2011). Thermal windows are predicted to narrow both in height and width when an ectotherm species is exposed to additional stressors (Pörtner and Farrell, 2008) but temperature as a single variable is still a relevant measure as it is a main driver of biogeography and has a direct impact on an ectotherm's physiology (Angel, 1991; Farrell, 1997; Pörtner, 2001; Payne et al., 2016).

Pörtner and Farrell (2008) also predicted that the performance curves would shift to the right with warm acclimation. Acclimation potential can be quantified by comparing the temperatures when fish lose equilibrium after exposure to at least 3 different acclimation temperatures. Metabolic performance (e.g. cardio-respiratory) of a species can also be compared at different acclimation test temperatures for a specified duration of time (10 days to 6 months are used in the chapter studies below). Another way to quantify acclimation potential is by contrasting the Q_{10} values of a species metabolic rate at two different acclimation temperatures. The Q_{10} represents the factor by which the rate of a reaction increases over a specified rise in temperature (traditionally 10°C). When Q_{10} is 1 the rate of reaction has plateaued i.e. the reaction rate no longer changes with an increase in temperature. Therefore, when contrasting the heart rate of fish from 2 acclimation temperatures, a Q_{10} value of 1 would indicate complete acclimation. This application of Q_{10} was recently coined the Acclimation Potential Index - API (Seebacher et al., 2015). The API for *B. saida* is calculated in the conclusion of this thesis.

Climate change in the Arctic threatens *B. saida* with extirpation due to ocean warming and the loss of ice-associated niches if they cannot acclimate to warmer conditions (Wyllie-Echeverria et al., 1997; Carmack and McLaughlin, 2001; Cheung et al., 2008; Thorsteinson and Love, 2016). It is estimated, from model simulations, that a 2°C increase in bottom water temperatures could reduce *B. saida* distribution in the eastern Bering Sea by 80% and their relative abundance by as much as 90% percent (Thorsteinson and Love, 2016). Such a reduction in numbers of *B. saida* and available habitat would have a significant, cascading impact throughout the Arctic marine ecosystem and would directly impact subsistence and commercial fisheries (Thorsteinson and Love, 2016). There are empirical observations of a northward retreat of *B. saida* from their southern-most distributions, e.g., waters off Disko

Bay, Greenland, Iceland-East Greenland waters and the Barents Sea (Hansen et al., 2012; Farrell et al., 2013; Astthorsson, 2015). These observations add evidence to the dire predictions for the future of *B. saida,* yet only a limited number of field and even fewer laboratory-based studies of thermal physiology exist for this key Arctic marine species.

1.2 Arctic Ocean food web

The importance of *B. saida* in the marine Arctic food web (Figure 1.1) was summed up by Welch et al. (1992) and Bradstreet et al. (1986) with the observation that few alternative food sources to *B. saida* existed.

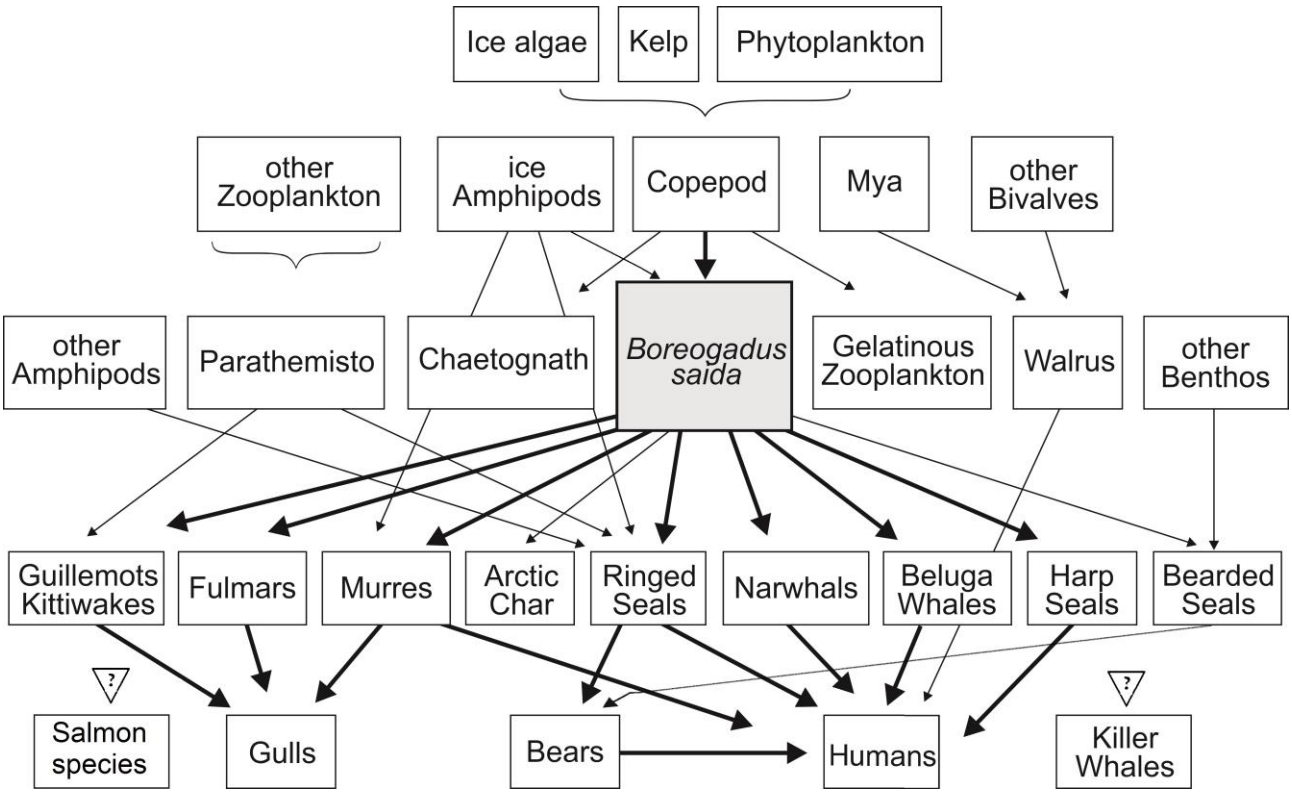

Figure 1.1 Arctic marine food web

This diagram was adapted from Welch et al. (1992). The heavy arrows highlight the primary role of *B. saida* in the transfer of energy (amount ingested calculated in the original food web as kJ m^{-2} y^{-1}) from zooplankton to Arctic mega fauna. Other zooplankton species energy contribution via *B. saida* is not as significant as amphipods and copepods. Welch et al. (1992) estimated that the ringed seal ingests more than 2x's the amount of *B. saida* than Narwhals and Beluga and

more than 3x's the amount than any other predator. Light thin arrows originating from *B. saida* indicate species that opportunistically consume them (pers. obs.) but are not usually listed as one of their predators. The energy transfer of migrating species (e.g. killer whales and various salmon species) recently observed in the Canadian Arctic are unknown. These example groups - potential disruptors of present day Arctic marine ecosystems - are assigned question mark symbols inside open downward pointing triangles.

In fact *B. saida* was recently referred to as a keystone species, which implies that the removal of this fish from the Arctic food web would destabilize the entire Arctic marine ecosystem and lead to further loss of other species (Nahrgang et al., 2016; Thorsteinson and Love, 2016). The designation of keystone however, also implies that the species has a larger role than their abundance or biomass would suggest and thus keystone is not an appropriate term for *B. saida* because they are the key food web fish species due to their total numbers. For instance, it is estimated that 150 000 t of *B. saida* are consumed annually by predators in Lancaster Sound, Nunavut (Welch et al., 1992; Crawford and Jorgenson, 1996) and a recent estimate of under ice larval fish abundance was in the billions (David et al., 2015).

1.3 Arctic cod (*Boreogadus saida* Lepichin)

Boreogadus saida survive in ice-covered, sub-zero waters due to the presence of anti-freeze glycoproteins, specialized kidney function (Osuga and Feeney, 1978; Christiansen et al.,1996) and the ability to digest food at -1.4°C water temperatures (Hop and Tonn, 1998). Within the literature, common names used for *Boreogadus saida* include both Arctic cod and Polar cod. The sister taxa, ice cod (*Arctogadus glacialis)* are also referred to as Polar cod and, to add to the confusion, Atlantic cod (*Gadus morhua*) from the Barents Sea are often referred to as Northeast Arctic cod. Consequently there is potential overlap and miss-representation of the various cod species when using common names. The American Fisheries Society have updated their naming conventions in 2014 and now recommend that the common name Polar cod be used when referring to *B. saida*. However, as Polar and

Arctic cod have other associations I will use *B. saida* throughout this document. I identified *B. saida* morphologically and, having inadvertently caught two *A. glacialis*, was able to directly compare differences between the sister taxa to ensure correct species identification.

Boreogadus saida are a diminutive forage fish that grow to ~40cm total length, live to the age of 7 years and spawn during winter months under the sea ice (Craig et al., 1982; Hop and Graham,1995) starting at age 3 years (Lear,1983). It is thought that they can reproduce more than once but with great expenditure of energy and limited survivorship (Sakurai et al., 1998; H. Drost, pers. obs.). The eggs take a minimum of 70 days to hatch in water temperatures below 0°C (Sakurai et al.,1998; Kent et al., 2016) and it is thought that larvae and juveniles prefer to live beneath the sea ice and most are hatched under ice (Falardeau et al., 2014). However, *B. saida* eggs, larvae and juveniles have been reared in ice free conditions and have been observed to exist in ice free zones, which suggests they may be associated with, rather than dependent on, sea ice (Bouchard and Fortier, 2011; Kent et al., 2016; Thorsteinson and Love, 2016). Whether potential future ice free conditions enhance arctic cod larvae survival (Bouchard and Fortier, 2011) or negatively impact them (Geoffroy et al., 2016; Kohlbach et al., 2017) is still contested. Dependent or not, a recent estimate of under ice larval fish abundance is in the billions. Thus, sea ice is a significant habitat for this most northerly distributed of all fish species (David et al., 2015).

1.3.1 *B. saida* distribution and migration

Boreogadus saida are pan-Arctic in distribution. Genetic analysis confirms that there is insufficient variation to differentiate *B. saida* to the population level (Nelson et al., 2013). Catch data for the large schools seen during ice break-up in summer (Moulton and Tarbox, 1987; Crawford et al., 2012; H. Bain and A. D. Sekerak, unpubl. data) show *B. saida* congregating at depths of 100 - 250 m, ranging from the surface to 1383 m (Coad and Reist,

2004). During the summer months in coastal waters, they most often occur in water with temperatures ranging from 0 to 4°C (Bain and Sekerak, unpubl. data), but in the Lancaster Sound region, acoustic data from 1989 and 1990 suggest *B. saida* sought > 2°C near the surface when a strong thermocline existed, but were found to be evenly distributed in a well-mixed water column (Crawford and Jorgenson, 1996). In shelf-break and slope waters in the Beaufort Sea, Crawford et al. (2012) showed a distinct preference for either near-surface waters or the deeper (0 - 2°C) Atlantic water as opposed to the intermediate-depth Pacific water where water temperatures are below 0°C. Moulton and Tarbox (1987) suggest that *B. saida* in the Beaufort Sea occupy the boundary of colder, underlying saline water and the warmer fresh surface waters, when they congregate nearshore in the summer months. They found that the peak in *B. saida* density corresponded to increasing salinity and preceded local upwellings. They suggest that the behavioural preference for 2 - 9°C may be due to food abundance. Thus, occupying this warmer water could help maintain a faster metabolism to consume and convert as much food as possible during times of bounty (Moulton and Tarbox,1987). Before this thesis was undertaken, however, there was no consensus regarding optimum temperatures based on existing observations.

1.4 Known temperature limits of *B. saida* from laboratory studies

One central question regarding the future distribution and survival of this fish species is ultimately based on whether *B. saida* have the ability to acclimate to the rapidly changing thermal conditions in the Arctic.

Another concern is the development and adaptation of eggs. I found that Arctic cod can develop from egg to reproductive adult in constant 3.5°C water temperatures (Kent et al., 2016 - see Appendix B). Previous research had shown normal development occurs from -1.0 up to 3°C but no study has reported normal development temperatures ≥ 5°C (Aronovich et

al.,1975; Sakurai et al.,1998; Kent et al., 2016). Graham and Hop (1995) found that developing eggs and newly hatched larvae will die or exhibit severe deformities when exposed to 9°C water temperatures for 24 h.

The temperature preferendum of adult *B. saida* was found to be between 2.8 and 4.4°C (over a 0 to 8°C range) depending on the time of day (Schurmann and Christiansen, 1994). Also distribution analysis of larval *B. saida* catch data from the Barents Sea Ecosystem Survey (1986 - 2008) indicate that 85.5% of the 0 to 1 year age group are found in water temperatures between 1 - 5°C, with a peak abundance between 2 - 4°C depending on average summer temperatures (Rajasakaren, 2013). A study that appeared during the course of my Ph.D. research showed daily growth rates of juvenile *B. saida* were similar at 5 and 9°C and in these relatively warm conditions growth rates were faster than at 0°C water temperature (Laurel et al., 2015).

Overall, both field observations (catch and acoustic) and laboratory studies report several different adult *B. saida* optimum temperatures ranging from 0 to 9°C (Aronovich et al.,1975; Moulton and Tarbox, 1987; Coad and Reist, 2004; Graham and Hop,1995; ; Crawford and Jorgenson, 1996; Sakurai et al.,1998; Walkusz et al., 2011 & 2013; Crawford et al., 2012; Laurel et al., 2015). A 9°C temperature optimum range is unusual for a cold water, stenothermal fish (Pörtner and Farrell, 2008; Ferreira et al., 2014). Clearly, our understanding of the thermal physiology of *B. saida* was far from complete.

1.5 Methods to quantify thermal tolerance acclimation potential of *B. saida*

My research combined three measurements to characterize thermal tolerance and acclimation potential including: 1) T_{cmax} (a replacement for upper incipient lethal temperature; Fry, 1947); 2) absolute aerobic scope (AAS; Fry, 1947); and 3) routine heart rate (f_H) and maximally stimulated heart rate (f_{Hmax}) (Fry, 1947; Casselman et al., 2012).

Critical temperature (T_{cmax}) was defined in this study as the temperature when a fish first began to roll over (loss of equilibrium) during acute warming at a rate of 3°C h^{-1}. No fish mortality occurred at this highly replicable end point.

Aerobic scope (the difference between standard and maximum metabolic rate; Fry, 1947) is a useful metric of thermal performance in fishes because it represents the amount of oxygen available to perform fundamental activities of life such as reproduction, feeding and locomotion. AAS is a valuable index of the capacity of a fish to remove oxygen from water. If a fish has less aerobic capacity, it cannot fuel metabolic activities and thus will not perform as well. The oxygen and capacity-limited thermal tolerance (OCLTT) hypothesis, suggests that a fish's capacity to supply oxygen to tissues becomes limited at temperature extremes (Pörtner, 2001). Survival of fish at high temperatures was shown experimentally to be directly related to oxygen availability (Alabaster and Welcome, 1962). Absolute aerobic scope has an optimal temperature (T_{opt}) where it is maximal and a critical temperature (T_{crit}) where it is zero (Fry, 1947; Pörtner, 2001; Pörtner and Farrell, 2008; Farrell, 2016).The T_{opt} and T_{crit} indices describe individual capacity and provide the foundation upon which the observed limitations of an ectotherms activities, distribution and survival can be interpreted for a wide and diverse number of geographic applications (Forster et al., 1987; Hinds et al., 1993; Claireaux et al., 2000; Claireaux and Lefrancois, 2005; Bremer et al., 2007; Pörtner and Knust, 2007; Farrell, 2009; Eliason et al., 2011).

Difficult field conditions, however, may make it impractical to accurately measure aerobic scope in fishes in remote locations such as the Arctic. Hence, Casselman et al. (2012) introduced methodology for measuring maximum heart rate (f_{Hmax}) during acute warming,

first performed on Coho salmon (*Oncorhynchus kisutch*), as a means of providing indirect measures of T_{opt} and T_{crit} indices.

When considering the replacement of the AAS method for the field friendly f_{Hmax} method, the assumption is that when oxygen uptake (MO_2) increases with temperature, the principal variable that changes is heart rate (f_H). Given the Fick equation [$MO_2 = f_H \times V_S \times (Ca_{O_2} - Cv_{O_2})$] and that when MO_2 increases with temperature neither stroke volume (V_S) nor the amount of O_2 extracted from tissue ($Ca_{O_2} - Cv_{O_2}$) change appreciably until near critical temperature maximum (T_{cmax}) (Heath and Hughes, 1973; Sartoris et al., 2003; Franklin et al., 2007, Steinhausen et al., 2008, Clark et al., 2008; Farrell 2009, Eliason et al., 2011, Gamperl, 2011), it seems reasonable to assume that the change in MO_2 is indeed directly proportional to f_H.

One advantage of the f_{Hmax} technique is that it permits a high-throughput of assessments under field conditions. This technique was subsequently used for laboratory studies by Anttila et al. (2013) for juvenile rainbow trout *Oncorhynchus mykiss*, by Verhille et al. (2013) to identify subtle differences in temperature tolerance between diploid and triploid *O. mykiss*, and by Chen et al. (2013) to examine the effect of different rearing temperatures on upper temperature tolerance of juvenile sockeye salmon *Oncorhynchus nerka.* Therefore, this study represents the first use of this technique for any polar marine fish species and the first attempt to compare data derived before and after the transport of an Arctic species to test them in a laboratory at lower latitudes.

1.6 *B. saida* respiration physiology

Prior to this thesis, nothing was known about the absolute aerobic scope for *B. saida*. Studies of oxygen uptake have only reported measurements of *B. saida's* routine metabolic

rate (RMR) between -1.5 and 8.0°C (Holeton, 1974; Steffensen et al., 1994; Hop and Graham, 1995; Kunz et al., 2016). When these studies are combined, based on temperature and holding duration for *B. saida,* they produce remarkably similar values of oxygen uptake (see Figure 1.2.) considering the different holding conditions and duration, life history, and location of study.

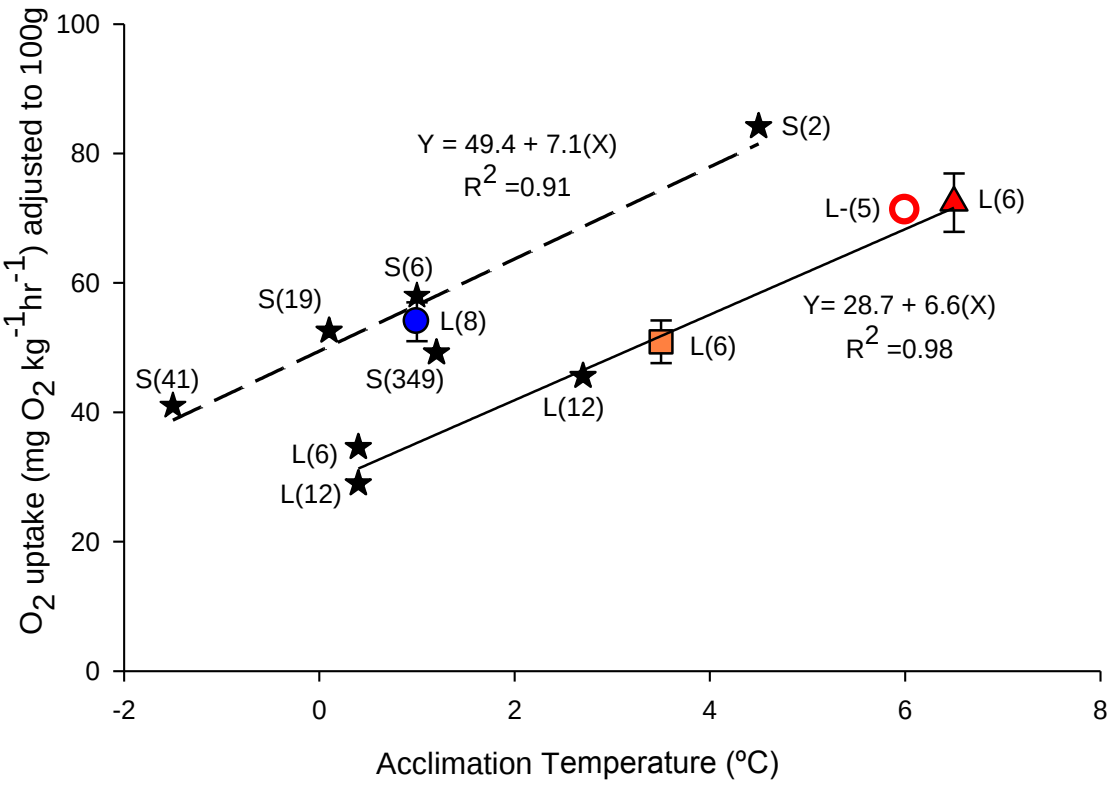

Figure 1.2 Linear regression analysis of routine metabolic rate (RMR) of *B. saida* from past research (black stars) including: Holton (1974), Steffensen et al. (1994), Hop and Graham (1995) and most recently, Kunz et al. (2016) (open red circle). These published data were compared with the present study of RMR values at 3 acclimations: 1°C (blue circle), 3.5°C (orange square) and 6.5°C (red triangle). Sample ID includes: short- (S) or long-term (L) acclimation and sample size in brackets. The regression analysis did not include data collected at the 1°C acclimation temperature (blue circle) for this study. All data were standardized to 100 g body weight (M_b) based on the equation from Steffensen *et al.,* 1994: $MO_{2(100\ g)} = MO_{2(l)} * (M_b / 100)^{(1-A)}$, where A is the scaling factor of 0.80.

In Figure 1.2., single regression lines fit closely to both short- and long-term acclimation data (R^2 = 0.91 and 0.98, respectively). However, Figure 1.2. shows one long-term acclimation data point (1 month holding at 1°C of fish that were reared at 3.5°C) that associates with short term holding values of oxygen uptake. Perhaps this anomalous result is due to the finding, published over 60 years ago, that fish take longer to acclimate to cold rather than warmer water (Fry, 1947). Assuming that cold acclimation takes longer than warm, this data set was omitted from the long term regression analysis.

The compilation of data in Figure 1.2. highlights the ability of *B. saida* to acclimate to different temperatures depending on the duration of exposure. This finding is not unexpected as it has been shown that even true stenothermal Antarctic fish species are able to acclimate to warmer temperatures (Pörtner et al., 2000; Lanning et al., 2005; Seebacher et al., 2005; Franklin et al., 2007; Robinson and Davidson, 2008; Peck et al., 2014). The challenge is quantifying acclimation potential as well as the cost and benefits of acclimation on ectotherms, both the individual and populations. Results from this study and past research suggests that the cost of acclimation may, in some cases, outweigh the benefits (Woods and Harrison, 2001; Seebacher et al., 2005; Deutsch et al., 2015; Pershing et al., 2015; Drost et al., 2016).

1.7 Study goals

Quantifying the thermal limits and the acclimation potential of *B. saida* were the primary goals of this thesis (Chapter 2.) and the methods used included heart rate, which was a potential candidate to replace the more arduous and time consuming AAS method. Thus a key goal was to compare the AAS and the f_{Hmax} methods. I wanted to see if the AAS method could be replaced, particularly in remote field settings, by the f_{Hmax} method, which is more practical and can generate more data in a given time period.

Another goal (Chapter 3.) was to test the field capability of measuring f_{Hmax} and to compare the data to those duplicated in the laboratory to ensure cardiac physiology is not significantly altered after transporting fish to different laboratory facilities. Data applicability is a common concern of physiologists when using laboratory results to forecast real-life situations (Larsson et al., 1985; Pörtner et al., 2007; Marentette et al., 2012). The ability to transport animals to long term holding facilities requires that the animals in question are able to display a similar thermal dependence of metabolic rates, in this case f_{Hmax}.

The aquarists at the Vancouver Aquarium successfully reared the eggs and larvae up to reproductive adults - a first in North America. This provided me with the opportunity to achieve, at least in part, the goal to study younger life stages of *B. saida* (Chapter 4.).

1.8 Study location: Cambridge Bay (69°06N, 105°04W), Nunavut

Cambridge Bay is a very sheltered 8 km^2 fjord type estuary (Figure 1.3) with two entrance sills, both less than 20 m in depth (Gade et al., 1974). An inner sill (~50 m depth) cuts the bay into two sections along a west-east orientation (The western section, near the entrance of Freshwater Creek is deeper (~90 m) than the eastern side. There is only a small diurnal tide less than a meter in height that, when combined with the bathymetry, limits circulation within the bay. In fact, it was the calm nature of this Bay that attracted the early researchers.

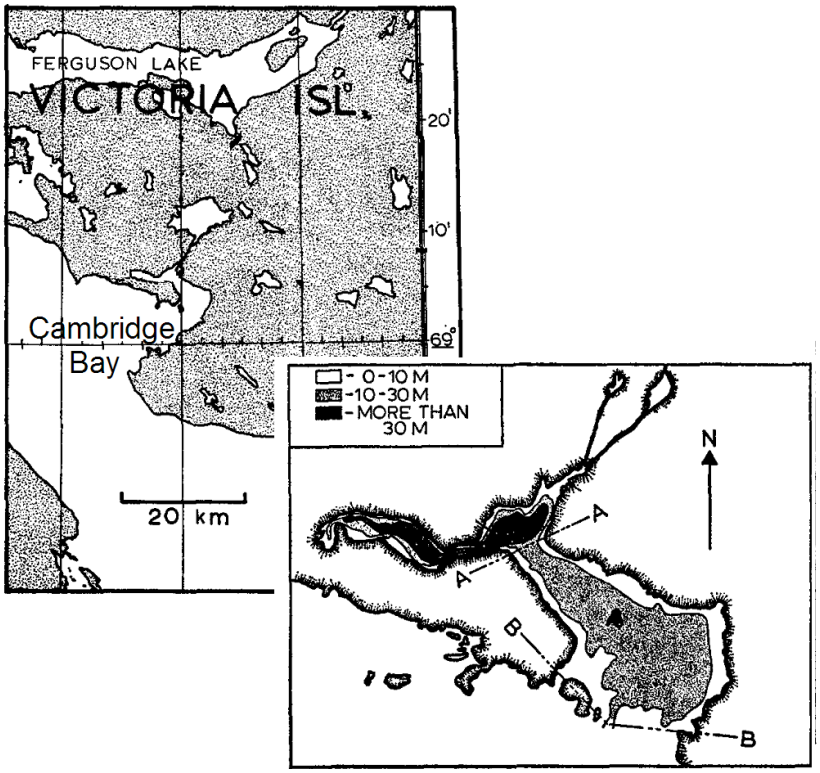

Figure 1.3 Map of Cambridge Bay (69°06N, 105°04W), on Victoria Is., Nunavut
Map adapted from Gade et al., 1974. The top map shows Cambridge Bay position south of Ferguson Lake. The length of the bay is less than 20 km. The lower inset highlights the 2 main entrance sills (B) and the inner sill (A). The deeper western arm of the bay (black rather than grey shading) is > 30 m with a maximum depth of 90 m measured by Gade et al., in 1974 and in this study in 2011.

On August the 25th, 2011 up to 10°C water temperature was recorded in the top 5 m of the water column (Figures 1.4A) but below 20 m, across the entire bay, water temperatures less than 0°C dominate.

Wind mixing primarily dictates the characteristics of the surface layer (Gade et al., 1974). On the day this CTD transect was conducted there was a ~15 knot wind blowing from the southeast. Perhaps the prevailing SE wind and reduced mixing can account for the deeper lens of fresh water at the end of the fjord (Figure 1.4B)

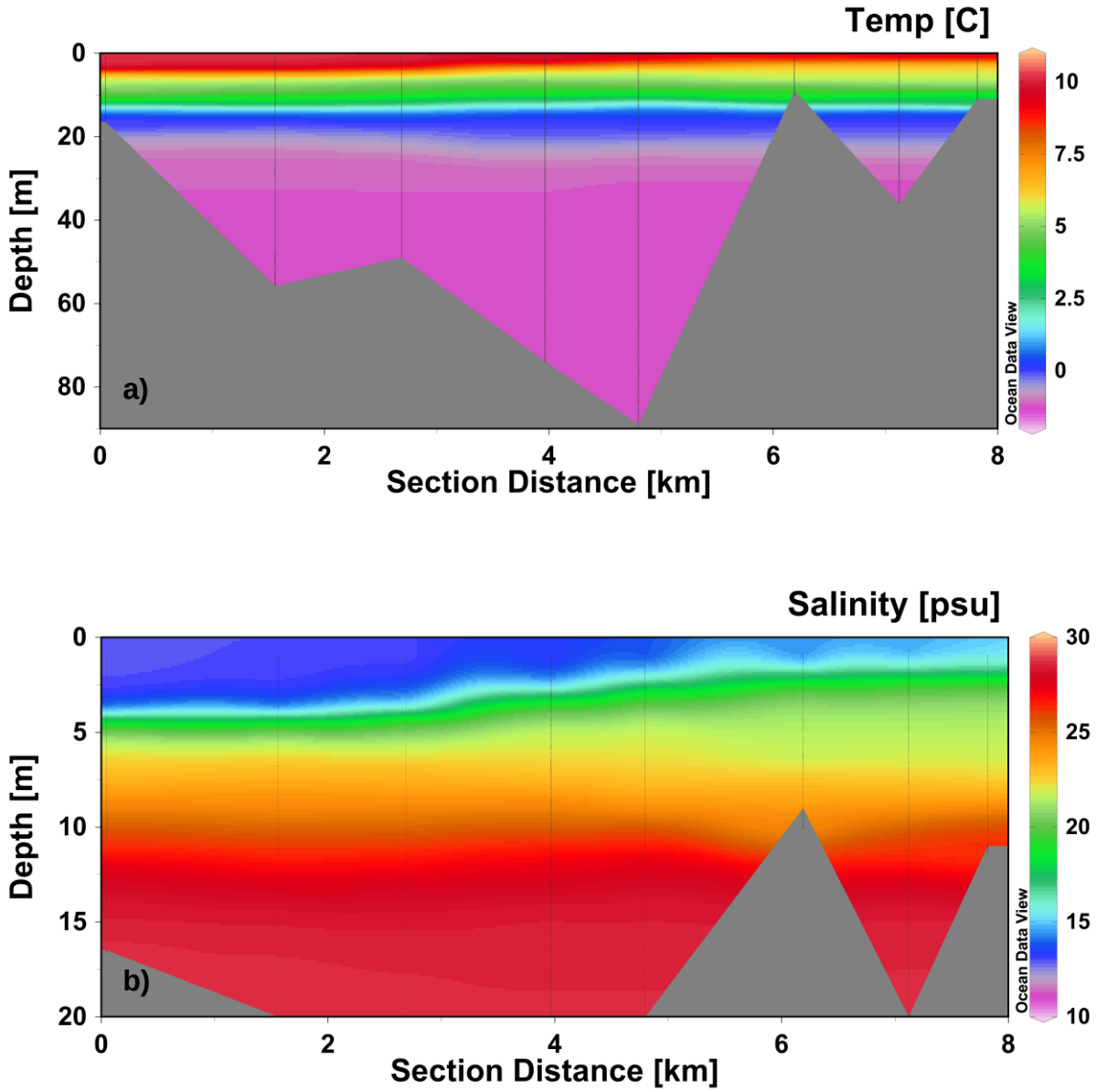

Figure 1.4 Temperature & Salinity in Iqaluktuuttiaq / ᐃᖃᓗᒃᑑᑎᐊᖅ / Cambridge Bay
The traditional Inuinnaqtun language name for Cambridge Bay (CB) is translated as “good fishing place” and was the main reason for selecting the study site. **a)** The temperature profile was taken on August 25th, 2011. It is focused on the western portion of CB, starting at SW end of the Inlet and ends at the mouth of Freshwater Creek. **b)** Summer salinity profile was taken on August 25th, 2011. It starts at western arm and ends at the mouth of Freshwater Creek. The depth profile was limited to 20 m to increase resolution in the surface layer.

The temperature/salinity correlation (TS) plot (Figure 1.5) is a comparison of profiles from three stations: from the end of the Fjord (station F-0), the deepest mid section (CB-1) and

between the two sills at the mouth of Freshwater Creek (R-2). These TS plots show a distinct two-layer structure as described by both Lewis and Walker (1970) and Gade et al. (1974). Warm and fresh on the surface layer and cold saline waters at depth.

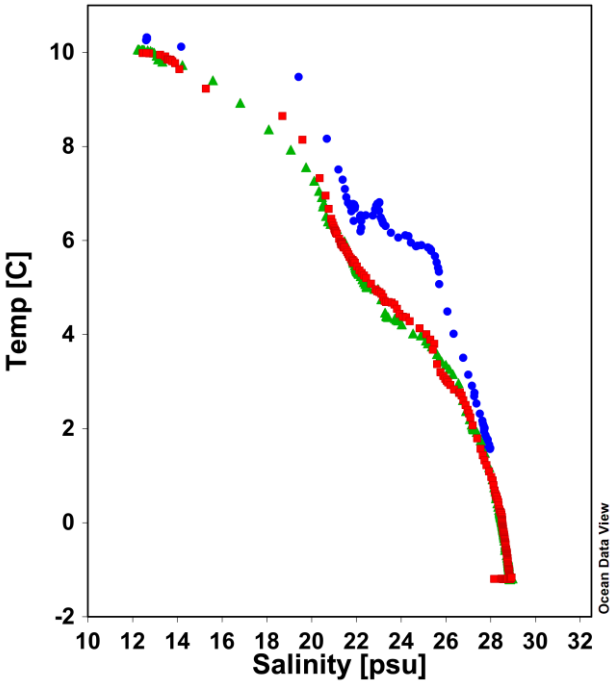

Figure 1.5 Temperature and salinity (TS) plots at three stations in Cambridge Bay station at the west end of the fjord (●); at the deepest part in the middle of the bay (▲) and east side between 2 sills at the mouth of Freshwater Creek (■).

As expected, due to distance from the main freshwater source, station F0 is more saline below the surface fresh water lens than the other two stations. The F0 station also differs from the other two stations at ~6°C. At this temperature the F0 profile becomes relatively stable yet salinity continues to increase. Perhaps the 3rd sill, that isolates the western arm of the fjord, has an influence on mixing at mid-depths. Overall the salinity profile has changed little from data collected on the 15th of September 1965. In fact, since the 1970's little has changed regarding the general oceanography of Cambridge Bay except for one very important feature - water temperature.

Gade et al. (1974) reported that summer temperatures reach a maximum of 5°C. The data I collected in 2011 and, with help from volunteers, in 2012 (data not shown) show maximum temperatures of ~10°C in August. In 2012 Ocean Network Canada deployed a permanent near-shore observatory with a continuous recording CTD situated at ~6m depth, which is just shallow enough, based on my 2011 data, to record temperatures close to maximum in the surface layer (Figure 1.6).

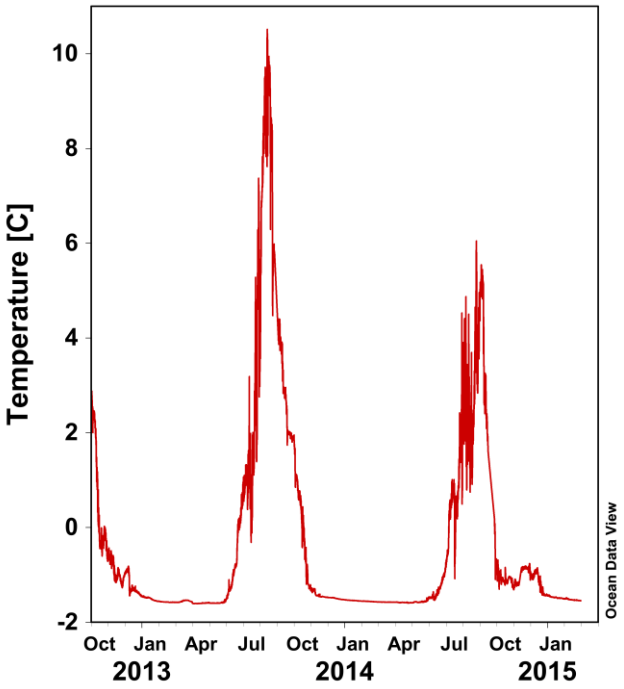

Figure 1.6 Continuous temperature profile in Cambridge Bay
Data was provided by Ocean Network Canada (ONC) from October 2013 to March 2015.

The ONC Arctic node continuously records water temperature every hour and this recording shows that on July 29th water temperature rose above 5°C. On the 14th of August at 13:30 the temperature went above 10°C and reached a maximum of 10.5°C that same day at 19:30 local time. Twenty four hours later the temperature dropped by 1°C and by August 25th it was back down to 5°C and remained below 5°C until the following year. The lower maximum temperatures in 2014 suggests that 10°C water temperatures are episodic and

either the 1970's researchers missed these surface warming events or they are associated with Arctic warming.

Lewis and Walker (1970) mention that on the 10th of July water temperature reached ~2.5°C at roughly the same depth as the present day ONC node. In comparison, it was not until July 23rd in 2013 that the water temperature rose above 2°C and in 2014 this warming did not occur until the end of July. In 2014, the maximum temperature of 6°C peaked just past midnight on the 26th of August, 12 days later than 2013.

Cambridge Bay, a sheltered fjord type estuary, has a distinct 2-layer structure in summer months that has essentially not changed since it was first described in the 1970's. The main difference may be maximum water temperatures and thickness of the fresh water lens in the top 5 m. How these changes will impact *B. saida* is unknown. If the water temperatures historically did not exceed 5°C then these fish could access the entire water column without exceeding their temperature optimum window. However, water temperatures of 10°C are getting close to *B.saida* thermal tolerance limits (see Chapter 2.), which may inhibit their access to the surface. It is not known if the surface layer of the water column during ice free summer conditions is important habitat for *B. saida.*

1.9 Study hypotheses

My main hypothesis was that the cardio-respiratory system of *B. saida* would thermally acclimate to water temperatures of 0.5, 3.5 and 6.5°C. I also hypothesized that 1°C-acclimated fish breathing rates would recover faster after being chased than 6.5°C-acclimated fish.

Past research on thermal limits in fish have shown that fish hearts become arrhythmic when upper temperature limits are reached (Farrell, 2009; Eliason et al., 2013; Chen et al., 2013; Ferreira et al., 2014). Thus arrhythmia can act as a clear indicator that the upper

temperature tolerance of an individual fish has been surpassed. The physiological basis of heart arrhythmia is currently being explored (Badr et al., 2016; Vornanen, 2016). For this study, I hypothesized that i) regardless of acclimation temperature, the temperature at which cardiac arrhythmias develop (T_{AR}) would be lower than T_{cmax} and ii) that the temperature for peak AAS (T_{opt}) would be similar to when heart rate first fails to keep up with acute thermal warming (T_{AB}). I anticipated that the suite of physiological rate transition temperatures would show a predictable order, as seen previously in goldfish (Ferreira et al., 2014; Farrell, 2016).

I also tested if transporting fish to different laboratories impacted cardiac performance by comparing the heart rate of 0.5°C field-acclimated *B. saida* (10+ days after capture) with fish transported to the Vancouver Aquarium and held at the same temperature for 6 months.

A final key hypothesis to test was whether *B. saida* larvae were more sensitive than adults to warm temperatures, as has been found with other fish larvae (Burgess et al., 2006; Burggren and Reyna, 2011; Dionisio et al., 2012). I was concerned that only quantifying physiological limits of adult populations may underestimate the vulnerability of younger life stages.

Chapter 2: Acclimation potential of *Boreogadus saida*[1]

2.1 Introduction

The physics and biogeochemistry of the Arctic Ocean have changed significantly in the past century and are predicted to continue changing as rapidly (Carmack and McLaughlin, 2001; Overpeck et al., 2005; Polyakov et al., 2005; Grebmeier et al., 2006; Wassmann, 2011; Yamamoto-Kawai et al., 2011). Missing, however, is a general knowledge on the thermal tolerance ranges for Arctic ectotherms and their acclimation potential, which are minimum knowledge requirements to accurately forecast the distributional patterns of marine species and their chances of survival in a changing environment (Pörtner and Farrell, 2008; Farrell, 2009; Somero, 2010; Niehaus et al., 2012). Thus, it is imperative to understand thermal optima, tolerance and acclimation potential of Arctic fishes to predict food web consequences with continued warming of the Arctic Ocean.

Boreogadis saida are able to acclimate to different temperatures over time as are (despite the differences in evolution) Antarctic species, which experience true stenothermal conditions year round (Pörtner et al., 2000; Lanning et al., 2005; Seebacher et al., 2005; Franklin et al., 2007). I quantified acclimation potential using 3 methods including: the critical thermal maximum of 3 acclimation groups (0.5, 3.5, 6.5°C); the aerobic scope of similarly acclimated *B. saida*; and the heart rate of long term (6 month) acclimated fish. Both the aerobic scope (e.g., Brett, 1962; Ultsch et al., 1980; McKenzie et al., 2012; Eliason et al., 2013; Killen et al., 2014; Del Raye and Weng, 2015) and f_H or f_{Hmax} (Stillman, 2002; Blank et al., 2004; Braby

[1] A version of this chapter was published as: Drost, H. E., Lo, M. Carmack, E.C. and Farrell, A.P. (2016). Acclimation potential of Arctic cod (*Boreogadus saida*) from the rapidly warming Arctic Ocean. *Journal of Experimental Biology* 219: 3114-3125.

and Somero, 2006; Franklin et al., 2007; Chen et al., 2013; Verhille et al., 2013; Anttila et al., 2014; Ferreira et al., 2014; Sidhu et al., 2014) measurements have been re-employed to investigate the thermal niches of fishes in this era of rapid climate change. All three methods employed testing fish with an acute temperature challenge that can be applied without harm or mortality, even at T_{cmax} temperature limits.

2.2 Materials and methods

Animal care

Fish were collected, held and tested in accordance with the Canadian Council on Animal Care regulations and permits were issued by the Kitikmeot Hunters and Trappers Association – Nunavut, the University of British Columbia Animal Care Committee (A11-0267), the Freshwater Institute Science Laboratories Animal Care Committee – Arctic Aquatic Research (FWI-ACC-2012-050) and the Vancouver Aquarium Animal Care Committee (2011–04).

Adult *B. saida* were caught in August 2011 and July 2012 near CB on Victoria Island, Nunavut, Canada (69·12°N; 105·05°W). A trap was set at 17 m to collect fish in 2011 and a dip net was used down to 2 m to collect fish in 2012 during ice break-up. The water temperature in both cases ranged between −1 and 0°C. Fish were transported in buckets to the field laboratory (CB) and were held in Coleman coolers (86 cm×40 cm×45 cm; length, width and height) filled with local aerated sea water and maintained at the acclimation temperatures (0.0 and 3.5°C) using chilling units (Fisher Isotemp 3016d; precision ± 0.1°C, Fisher Scientific Company; www.fishersci.com). Fish were held for up to 4 weeks to ensure good health before air transport to the VA. Fish were starved for 48 h prior to transport. Pairs of fish were placed in double plastic bags containing 5 l of 0°C sea water and pure oxygen before creating an airtight seal and positioning, with bubble wrap, in an ice-filled cooler for air

travel. Thirty-six hours of flight and transport were required to transport fish to the VA, water temperature remained at 0°C throughout.

At the VA laboratory, *B. saida* were held in a closed-system 450 l tank with a daily 50% replacement of sump water as well as cleaning. Fish were fed to satiation with frozen krill usually every 1-2 days and were exposed to a fluorescent light and dark cycle that represented Vancouver (49°N) daylight conditions. Food was withheld for a minimum of 36 h before any experimentation. The order of experiments minimized the risk of fish mortality, testing f_{Hmax} first and T_{cmax} last.

Thermal acclimation

Fish were maintained at acclimation temperatures of 1.0, 3.5 and 6.5°C (± 0.5°C) for a minimum of 1 month before T_{cmax} and AAS measurements were performed. A 6-month acclimation period was used prior to f_{Hmax} measurements. Fish were introduced to the new acclimation temperatures within a few days by raising or lowering the temperature 1°C/day. Limited fish numbers required some, but not all fish being used for more than one test (after at least 7 days recovery) and at more than one acclimation temperature. Previous studies have shown a significant reduction in routine metabolic rate after > 5 months in captivity (Hop and Graham, 1995; Donelson et al., 2011) , but the present fish were in captivity much longer before testing, some more than one year. Also, the response of f_{Hmax} to acute warming was similar when measured in CB just 10 days after capture and acclimation to 0.5°C and 3.5°C when compared with measurements at VA more than 6 months after capture.

Critical thermal maximum (T_{cmax})

Each T_{cmax} measurement used 10 fish that were progeny from the 2011 wild fish that had bred at the VA (4 years old) and 3 fish that were wild-caught (estimated at 6 years old) collected either in 2011 or 2012 near Cambridge Bay. The range in mass was from 32.9 to

101.8 g, with the combined average mass of 65.8 ± 5.4 g (see supplementary Table S1 for individual fish mass). For each test, fish were not fed for 48 h before transfer into an individual insulated cooler with aerated and temperature-controlled InstantOcean® seawater (volume = 27 l; salinity = 30 ppt) where they were held overnight to recover from handling stress at their acclimation temperature. Water temperature was regulated with a refrigerant coil attached to a programmable chiller (Fisher Isotemp 3016d) and 2 thermometers (Fisher Scientific Type K digital thermometer probe; FireSting Y, with ± 0.1°C precision) that were calibrated to 0°C in ice-water during the trials using a Fisher Scientific Type K digital thermometer. Black netting was placed over the top of the cooler to maintain low light conditions and ensure fish containment. Water was acutely warmed until the fish first lost equilibrium rather than waiting for a full 10 s of disequilibrium (Chen et al., 2015). This endpoint and a quick transfer into a recovery tank at 4°C prior to return to their holding tank resulted in no fish mortality. No fish was retested without at least a 7-day recovery period.

Absolute aerobic scope (AAS)

Routine oxygen uptake (RMR) was measured from the decline in dissolved oxygen saturation of water within two custom-made, intermittent-flow, airtight and lightproof respirometers 8.0 x 15.5 x 22.5 cm. Gut evacuation from repletion in *B. saida* took 36 - 70 h at -1.5 to -0.5°C, with an average of 51 h (Hop and Tonn, 1998). Thus, after a 48-h fasting period, a fish was transferred to each of the two respirometers, which were connected to a 32-l, closed-circuit sump that contained two refrigerant coils attached to two programmable chillers (Fisher Isotemp 3016 d; www.fisherssci.com) filled with 60% propylene glycol antifreeze. The seawater sump was continuously aerated and also held a magnetic drive pump. Water temperature was controlled by the recirculating chillers and measured to a precision of ± 0.1°C (Fisher Scientific Type K digital thermometer probe). A pilot experiment

that measured oxygen uptake over 47.5 h while wild-caught *B. saida* became accustomed to the respirometer found that RMR stabilized between 12 h and 22 h (see Fig. 2Ai). Consequently, all RMR measurements began following an overnight acclimation of minimally 12 h. Water temperature was adjusted at a rate of 3°C h^{-1} to the desired acute test temperature for that experiment: 0.5°C, 2.0°C, 3.5°C, 5.0°C and 7.5°C for the 3.5°C-acclimation group, and 0.5°C, 2.5°C, 4.5°C, 6.5°C and 8.5°C for the 1.0°C- and 6.5°C-acclimation groups. At the test temperature, fish were held for 1 h before measuring RMR using closed respirometry that recorded the depletion of oxygen from the water with a fibre optic oxygen meter for up to 30 min (Firesting O_2, PyroScience GmbH, Aachen, Germany). This procedure was repeated 2 - 3 times and the lowest value was reported as RMR. Then the fish was removed from the respirometer and placed in a ~12 l circular tank containing aerated water at the test temperature for exhaustive exercise. Chasing involved a 5-min period of hand chasing, gentle tail pinches and lifting until unresponsive to touch, followed by brief air exposure (Norin and Clark, 2016). The fish was returned to the respirometer and oxygen uptake measurement resumed within 30 s and continued over a 5 to 30-min period, depending on the test temperature. The maximum oxygen uptake (MMR) was calculated from the steepest 2-5% decrease in percent water saturation, which occurred consistently at the start of recording. Water oxygen saturation never decreased below 75% saturation for any measurement. After the MMR measurement, fish were weighed, pit tagged (if newly tested) and returned to their acclimation tank. AAS was calculated as (MMR − RMR) and factorial aerobic scope (FAS) as (MMR x RMR^{-1}). Excess post-exercise oxygen consumption (EPOC) was measured at 0.5 and 1 h after MMR to compare the % return to initial RMR values for the 1.0°C- and 6.5°C-acclimated fish. Tests with the 1.0°C-acclimation group used 20 fish bred at VA with an average mass of 59.8 ± 2.7 g. Tests with the 3.5°C acclimation

group used 19 wild fish caught in 2011 with an average mass of 111.5 ± 6.1 g. Tests with the 6.5°C-acclimation group used 11 fish bred at VA and 6 wild fish caught in 2012 with an average mass of 74.1 ± 7.6 g.

Maximum heart rate (f_{Hmax})

The response of f_{Hmax} to acute warming used a technique and apparatus detailed previously (Casselman et al., 2012) and modified for *B. saida* (Drost et al., 2014). Briefly, two fish were anaesthetized in 75 mg l^{-1} tricaine methanesulphonate (MS-222, Sigma-Aldrich Products; www.sigmaaldrich.com) until they were unresponsive to a tail pinch before being transferred to individual 30 cm by 10 cm Plexiglas water-bath chambers (water volume = 2 l) where the anaesthetized state was maintained with gill irrigation using seawater containing 50 mg l^{-1} MS-222. Due to the possible effect of anaesthesia on unstimulated hearts, I tested the efficacy of the initial atropine injection during test trials and was satisfied that maximum heart rate was maintained throughout the trial period.

The chambers were connected to a 15 l closed-circuit, continuously aerated seawater sump, which contained a magnetic drive pump and two refrigerant coils attached to programmable chillers (Fisher Isotemp 3016d; www.fisherssci.com) filled with 60% propylene glycol antifreeze. Water temperature was controlled by the recirculating chiller and measured to a precision of ± 0.1°C (Fisher Scientific Type K digital thermometer probe). The fish were positioned dorsal side down on a fine mesh screen to enable placement of two custom-made chromel-A electrodes on the skin near the heart to record an ECG. The acute warming increased water temperature in 0.5°C increments every 15 min (2°C h^{-1}), which allowed both water temperature and f_{Hmax} to stabilize. For the 0.5°C- and 3.5°C-acclimation groups, the experiment was terminated when water temperature reached 9.5°C or earlier if the QRS wave amplitude began to decline so that there was a greater chance of reviving the fish. For

the 6.5°C-acclimation group warming continued until cardiac arrhythmia first developed. Thus, T_{max} and T_{AR} were not measured for the 0.5°C- and 3.5°C-acclimation groups.

Data analysis and statistical testing

The equality of data variance was tested using Levene's method on normally distributed data. T_{cmax} was calculated as the sample mean ± s.e.m. for 13 fish at each acclimation temperature. The results were compared across acclimation temperatures using one-way ANOVA with Tukey post-hoc test. Wild versus reared were treated as random factors in the analysis. A linear regression was applied to water saturation measurements to determine oxygen uptake (MO_2, mg O_2 kg^{-1} h^{-1}) as:

Eqn 1. $MO_2 = ([O_2]_{t1} - [O_2]_{t2}) \times V (t_2-t_1)^{-1}$,

where $[O_2]_{t1}$ is oxygen concentration (mg O_2 l^{-1}) at time t_1 (h); $[O_2]_{t2}$ is oxygen concentration at time t_2; V is the respirometer volume minus the volume (l) of the fish, using saltwater correction to relate volume (l) to body mass (M_b; kg). All oxygen uptake data are presented as mean ± s.e.m. To account for differences in body mass, which ranged from 32.5 g to 163.8 g (mean = 78.1 ± 3.0 g) all individual oxygen uptake measurements were adjusted to a 100 g fish using the equation:

Eqn 2. $MO_{2(100\ g)} = MO_2 * (M_b 100^{-1})^{(1-A)}$,

where $MO_{2(100\ g)}$ is the oxygen uptake for a 100 g fish, M_b is the fish body mass and A is the mass exponent (0.80) describing the relationship between metabolic rate and body mass for *B. saida* (Steffensen et al., 1994). A one-way ANOVA confirmed RMR and MMR data, for wild and reared fish from the 6.5°C-acclimation group, were not significantly different and so wild and reared fish were combined.

The AAS data for different acute temperatures were subjected to regression analysis. A log normal, 3-parameter regression was a good fit (R^2 = .99) for the skewed 1.0°C-

acclimation data. Whereas Weibull 4 parameter regressions (used for parametric survival analysis - see Ricklefs and Scheuerlein, 2001) were applied to the 3.5°C- and 6.5°C-acclimation data, which resulted in an R^2 of 0.96 and 0.65, respectively, and a realistic T_{crit} extrapolation, where AAS approaches zero. The log normal regression fitted a curve to the 1.0°C-acclimation data to estimate the temperature for peak AAS (T_{opt}) and the lower and the upper pejus temperatures (T_{pej}; Pörtner and Farrell, 2008), equal to 90% of peak AAS, was calculated. The Weibull regressions also estimated peak and pejus temperatures for 3.5°C- and 6.5°C-acclimation data, which allowed calculation of a T_{opt} window (T_{lpej} - T_{upej}; Eliason et al., 2013). Statistical differences among acclimation groups and among acute test temperatures were tested using a one-way ANOVA and a Tukey post-hoc test.

The 1.0°C- and 6.5°C-acclimation fish excess post-exercise oxygen consumption (EPOC) was measured at 0.5 and 1 h after MMR. Significant differences ($P < 0.05$) were identified at the two acclimation temperatures (1.0°C and 6.5°C) and at the 5 acute test temperatures (0.5°C, 2.5°C, 4.5°C, 6.5°C, 8.5°C), which directed additional Tukey Pairwise Comparison post-hoc testing using the transformed proportional data.

f_{Hmax} was calculated at each test temperature for individual fish using top of 1st peak to the top of the 2nd peak, the R-R interval, which averaged over 30 consecutive heartbeats from an EKG recording with a rhythmic heartbeat. The mass average for the 3 acclimation temperatures (0.5, 3.5 and 6.5°C) was 31.8 ± 2.4 g, 80.4 ± 5.7 g and 117.5 ± 7.6 g, respectively. Rate transition temperatures for f_{Hmax} were calculated for individual fish (as described in Casselman et al., 2012; Anttila et al., 2013). The first Arrhenius breakpoint temperature (T_{AB}) (Yeager and Ultsch, 1989) was determined by plotting the natural log of the heart rate ($\ln f_{Hmax}$) of individual fish against the inverse of temperature (1000 K^{-1}) and running best-fit linear regressions (SigmaPlot 11.0, Systat Software; www.sigmaplot.com) to

determine the lowest temperature when the slope of the Arrhenius line decreased. The incremental Q_{10} transition temperature for f_{Hmax} (T_{QB}) was determined by calculating the Q_{10} for each 1°C change in temperature using:

$Q_{10} = (f_{Hmax2}\,(f_{Hmax1})^{-1})\text{^}[10(T_2 - T_1)^{-1}]$.

T_{QB} was assigned when the incremental Q_{10} decreased and remained below 2.0 because a $Q_{10} > 2$ is considered a normal rate of change of routine fish metabolism with temperature (Fry and Hochachka, 1970; Miller and Mann, 1973; Holeton, 1974). The transition temperature at which the heartbeat first reached the peak f_{Hmax} was recorded as T_{max} and the temperature at which the heart first started an arrhythmic heartbeat was recorded as T_{AR}. In addition, the amplitude (mV) of the QR-wave was calculated, when possible, from each individual EKG trace at each test temperature and was used to determine the temperature when the QR-wave reached a peak value (T_{QR}). QR-wave amplitudes were then expressed relative to the largest value for each individual.

2.3 Results

Critical thermal maximum (T_{cmax})

T_{cmax} was recorded when fish lost equilibrium (LOE). Their ability to remain upright increased significantly by 2.2°C (from 14.9°C to 17.1°C) with acclimation from 1°C to 6.5°C, which represented a 0.43°C change in T_{cmax} per °C in acclimation temperature (Fig. 2.1.B).

Respiratory performance (AAS)

RMR increased exponentially with acute warming at all acclimation temperatures (Fig. 2.2. A). However, RMR measured at a test temperature of 0.5°C for 6.5°C-acclimation was significantly lower than for 1.0°C-acclimation, a response consistent with thermal compensation.

For 1.0°C-acclimated fish, MMR did not increase significantly with test temperature (Fig. 2.2.B) and AAS and FAS decreased with increasing testing temperature (Fig. 2.2.C and 2.2.D, respectively). The T_{opt} window extended from 0.2°C to 3.4°C (Table 2.1) for AAS, with the peak (Fig. 2.3.A) occurring at 0.5°C. At a test temperature of 8.5°C, AAS was 73% of that measured at 0.5°C.

The 3.5°C- and 6.5°C-acclimated fish both increased AAS with acute warming, reaching their peak AAS near their acclimation temperature (Fig. 2.2.B). For 3.5°C-acclimated fish the highest measured FAS value was at 3.5°C (Fig. 2.2.D), the T_{opt} window for AAS was from 1.6 to 5.4°C (Table 2.1), peak AAS (T_{opt}) occurred at 3.5°C (Fig. 2.3.B), and AAS at a test temperature of 7.5°C was 60% of the peak AAS measured at 3.5°C. For 6.5°C-acclimated fish, the T_{opt} window was from 2.4 to 8.1°C (Table 2.1), T_{opt} was 5.4°C with the peak AAS at 6.5°C (Fig. 2.3.C). AAS at a test temperature of 8.5°C was 80% of the peak AAS measured at 6.5°C. Thus, both the T_{opt} and the T_{opt} window increased with acclimation temperature (Table 2.1) and FAS never decreased below 2 provided the test temperature was < 6.5°C, independent of acclimation temperature (Fig 2.2.D).

Peak AAS was similar for 1.0°C- and 3.5°C-acclimated fish, but peak AAS and FAS were significantly higher for 6.5°C-acclimated fish. Even so, some delayed mortality unexpectedly followed the MMR measurement for the 6.5°C-acclimated fish tested at 8.5°C (50% of fish) and the 3.5°C-acclimated fish tested at 7.5°C (6% of fish). Extrapolation of the AAS curves produced upper T_{crit} values of 15.1°C and 18.2°C, respectively, for 3.5°C- and 6.5°C-acclimation groups, which were similar to measured T_{cmax} values (15.5°C and 17.1°C, respectively).

As expected, recovery from exhaustion (as measured by the % of AAS available) was more complete after 1.0 h than after 0.5 h ($P < 0.000$) for 1.0°C- and 6.5°C-acclimated fish

(Fig. 2.4). In fact, within a 1.0 h at least 79% of AAS was restored independent of test or acclimation temperatures. For 1.0°C-acclimated fish, post-hoc testing revealed a significantly slower recovery after 0.5 h, at test temperatures of 6.5 and 8.5°C (P = 0.0001) and after 1.0 h recovery was significantly slower at the 6.5°C test temperature (P = 0.008). For the 6.5°C-acclimated fish, recovery was independent of the acute test temperature (P = 0.072 after 0.5 h and P = 0.061 after 1.0 h). When comparing the difference in recovery between 1°C- and 6.5°C-acclimated fish over all test temperatures, the 6.5°C-acclimated fish recovered significantly faster at 0.5 h (P = 0.027). However, there was no statistical difference in recovery between temperature acclimations after 1.0 h (P = 0.342).

f_{Hmax}

As expected, f_{Hmax} increased with acute warming for each individual and acclimation group (Fig. 2.5). Indeed, warming accelerated f_{Hmax} by a consistent amount between 0.5 and 1.5°C (Q_{10} ~ 3) independent of acclimation temperature. Neither T_{AB}, T_{QB} nor peak f_{Hmax} varied significantly with acclimation temperature, with the exception of the 6.5°C-acclimation group having a significantly higher T_{QB} (P = 0.024) (Table 2.1).

T_{AR} and T_{max} for 6.5°C-acclimated fish were compared with published field data for T_{max} and T_{AR} using 0.5°C- and 3.5°C-acclimated fish (Table 2.1) in the absence of T_{AR} and T_{max} measurements here. T_{AR} and T_{max} did not differ significantly among acclimation temperatures. However, individual variation in T_{AR} was considerable, ranging from 7.6 to 15.2°C.

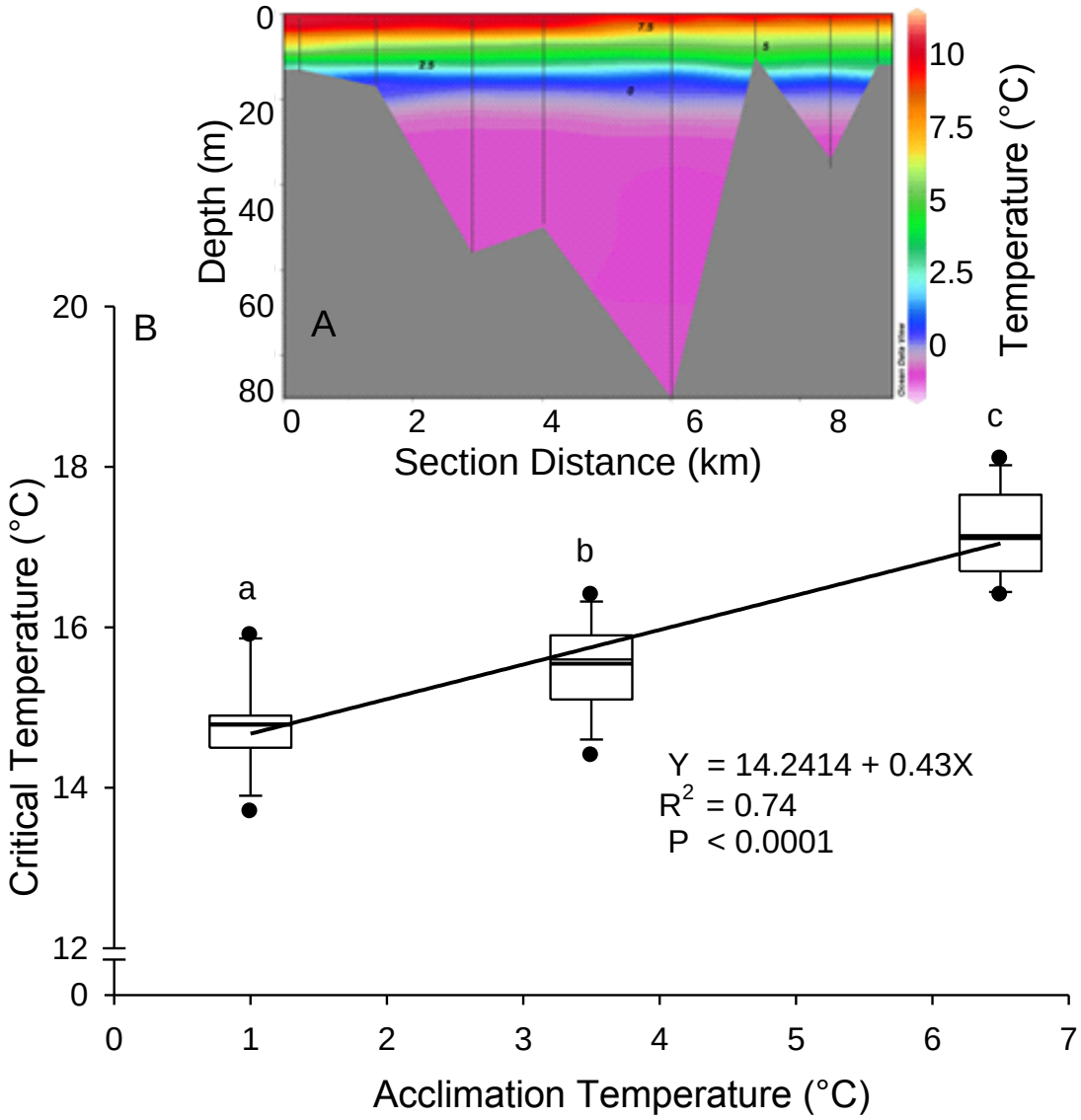

Figure 2.1 Slope of T_{cmax} acclimation potential compared with SST maximum
A) In Cambridge Bay, Nunavut, water temperatures ranged from -1.5°C at >30m depth to ~ 10°C at the surface on August 11th, 2011, as shown above from a 9 km transect measuring temperature and depth at 8 stations. **B)** Box plots of the critical thermal maximum (T_{cmax} mean ± s.e.m.) of individual *Boreogadus saida* (n=13) acclimated for 1 month at 1.0, 3.5 and 6.5°C. The boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Error bars above and below the box indicate the 90th and 10th percentiles and black circle symbols are the outlying points. Different letters at each acclimation temperature denote statistical difference for mean values using one-way ANOVA (P<0.05) and slope=0.43, derived from the linear regression line equation. *B. saida* were collected near Cambridge Bay (69°N, 105°W), Nunavut, Canada.

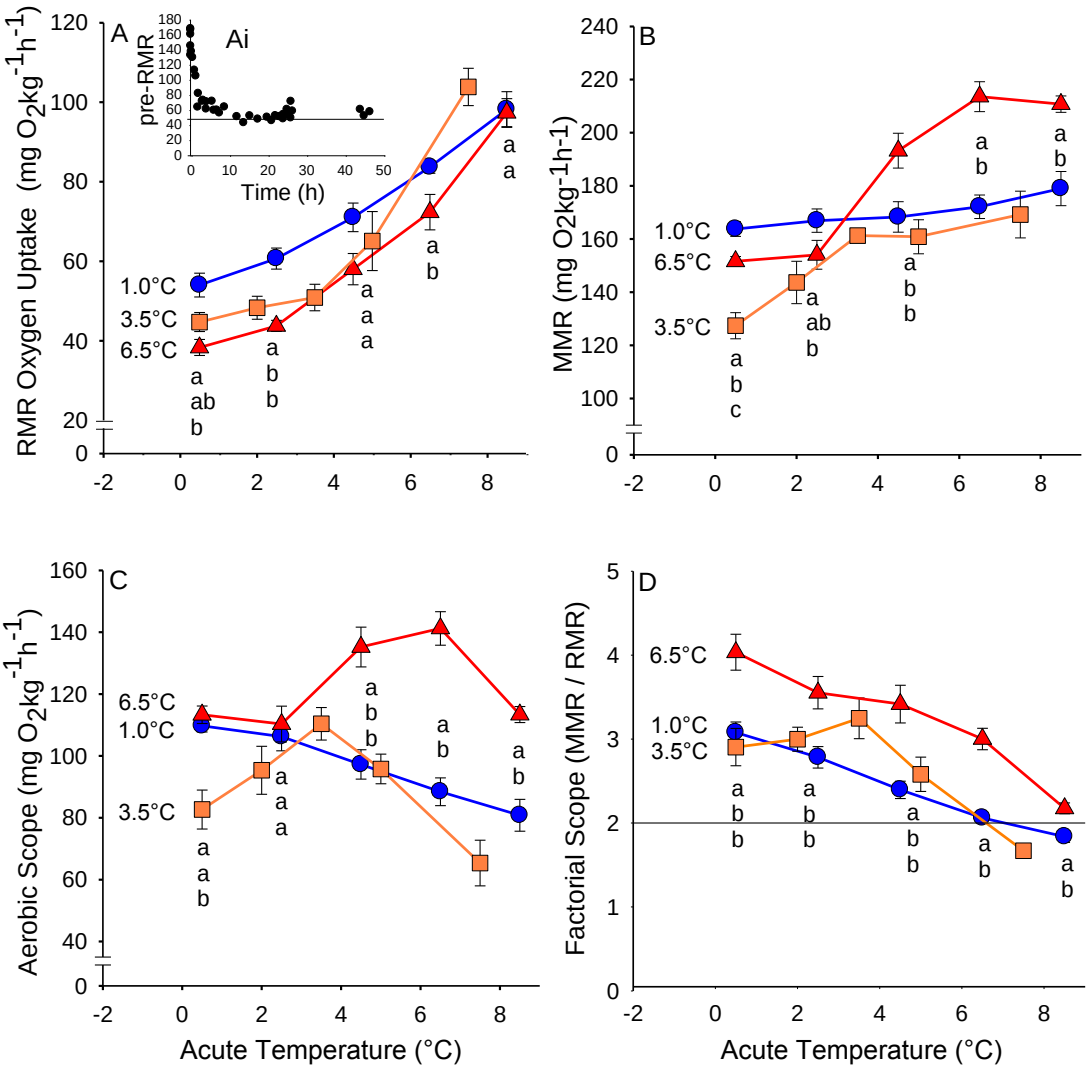

Figure 2.2 Oxygen uptake (mean ± s.e.m.) of *B. saida*

Boreogadis saida acclimated to three temperatures: 1.0°C, blue circle, n=8; 3.5°C, orange square, n=6; 6.5°C, red triangle, n=8 (except at test temperature 8.5°C when n=6). Means that do not share a letter are significantly different. **A)** Individual routine metabolic rate (RMR) and Ai) Oxygen uptake by wild-caught *B. saida* from 0 to 47.5 h after introduction to the respirometer **B)** Maximum metabolic rate (MMR) **C)** Aerobic scope (AAS), the absolute difference between MMR and RMR and **D)** Factorial scope (FS), the fraction of MMR and RMR. Stacked letters are positioned near the data tested at each acute test temperature. Mean values that do not share a letter are significantly different. Data for 3.5°C-acclimated fish at test temperatures 3.5 and 7.5°C are not included in the statistical analysis.

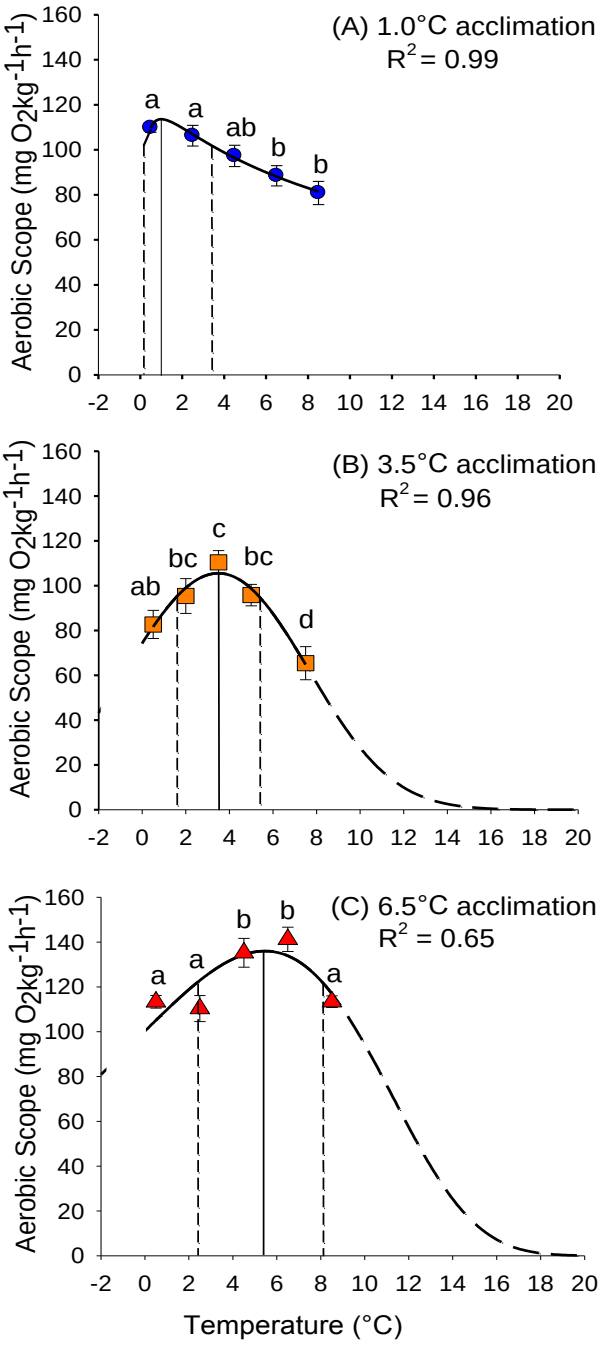

Figure 2.3 ***B. saida*** **absolute aerobic scope (AAS) and** T_{opt}

Optimum temperature (T_{opt}, solid vertical line) and upper and lower pejus estimates (where 90% AS at T_{opt}, dashed lines) for *B. saida;* **A)** 1.0°C, blue circle, n=8; **B)** 3.5°C, orange square, n=6; **C)** 6.5°C, red triangle, n=8 (except at test temperature 8.5°C when n=6). Regression equations calculated using a log normal, 3-parameter regression for the 1.0°C data and Weibull 4 parameter regressions for the 3.5 and 6.5°C data. Means that do not share a letter are significantly different.

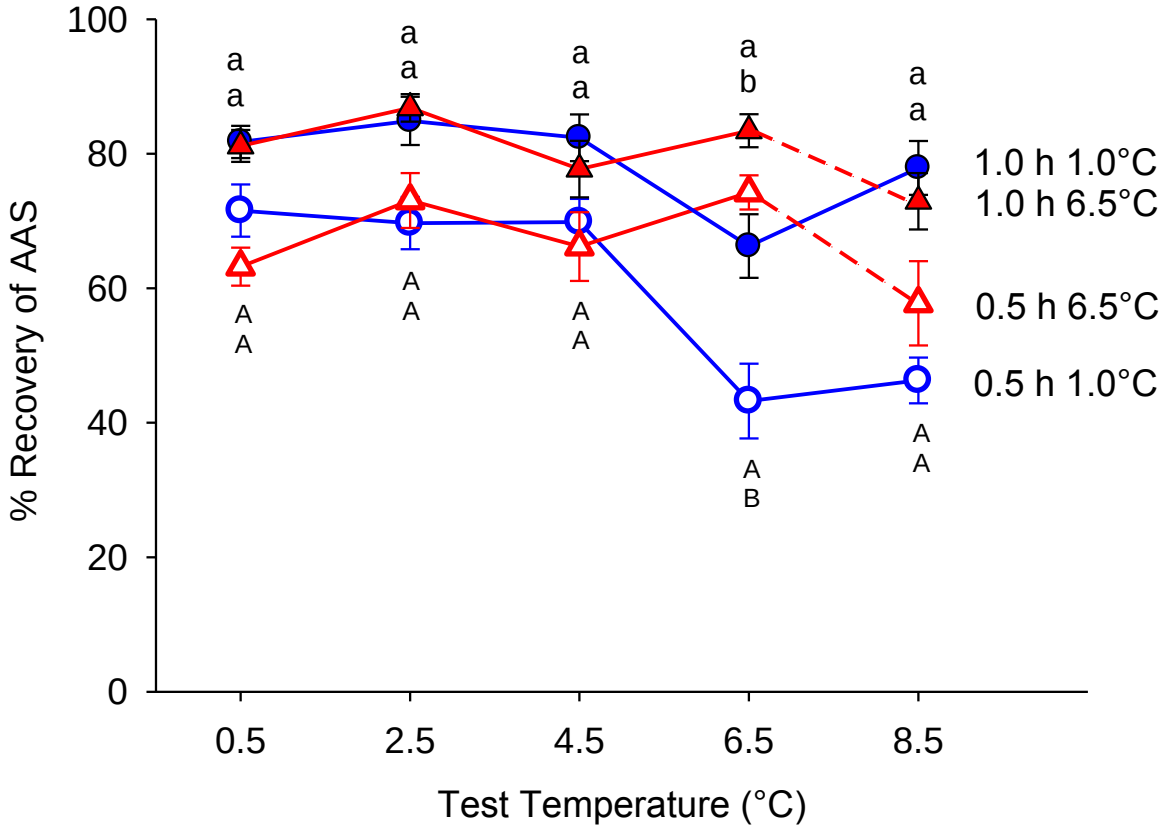

Figure 2.4 ***B. saida*** **excess post-oxygen consumption (EPOC)**
A comparison of the percent recovery to previously tested RMR (mean values ± s.e.m.) of oxygen uptake between acclimation temperatures 1°C (blue circle); 6.5°C (red triangle) after 0.5 h (half-filled symbol) and 1 h (filled symbol) at each test temperature. The solid lines connect data with the same sample size (n=8) and dashed lines highlight the change in sample size (n=6) for 6.5°C-acclimated fish at 8.5°C test temperature. Different capital letters denote statistical significance for the 0.5 h data and different small letters denote statistical significance for the 1.0 h data.

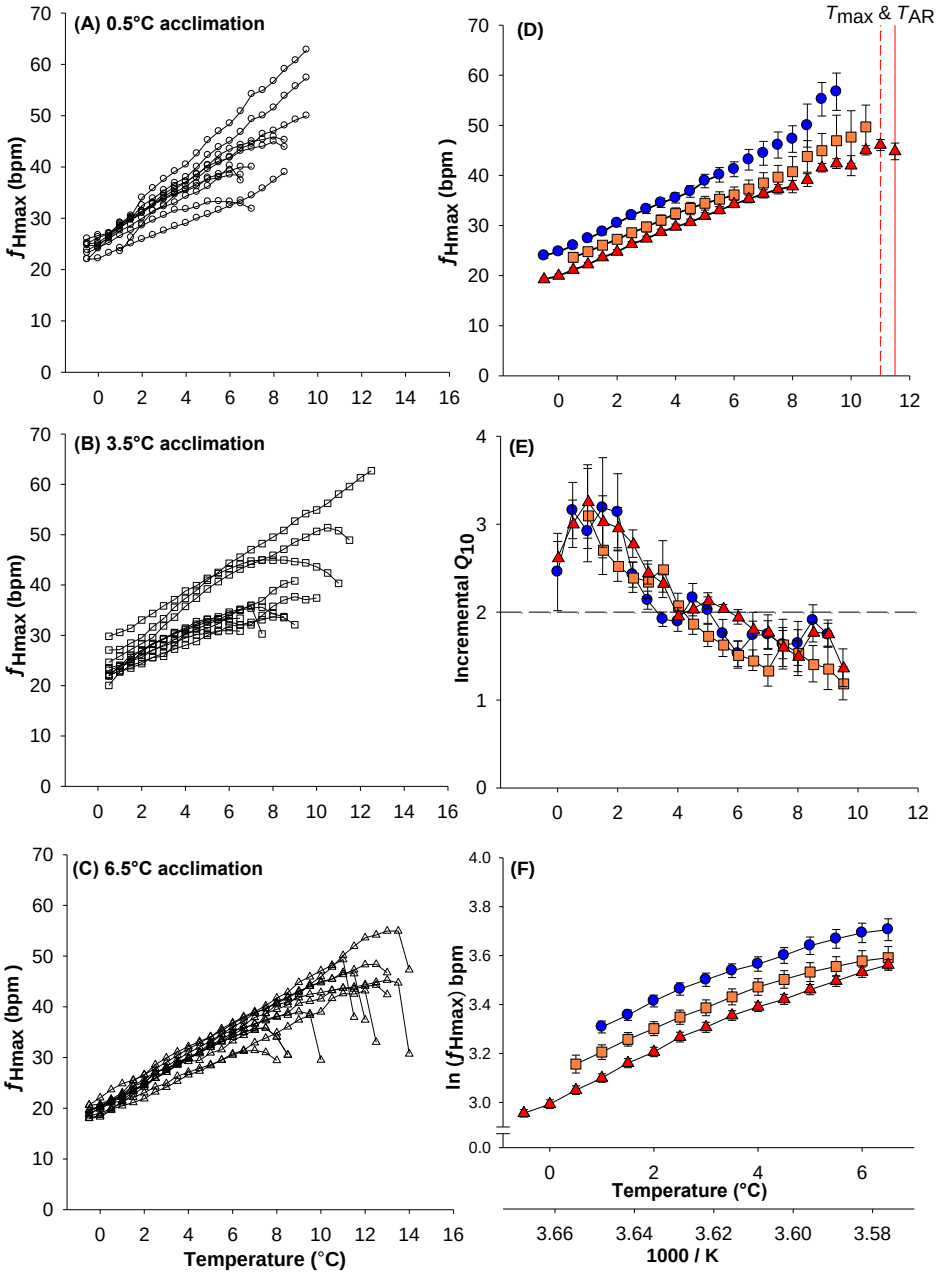

Figure 2.5 ***B. saida*** **heart rate (*f_{Hmax}*)**

Responses of maximum heart rate (f_{Hmax}) to test temperatures during incremental warming of individual anesthetized *B. saida* (n=10) acclimated for 6 month intervals to **A)** 0.5°C, blue circle; **B)** 3.5°C, orange square; **C)** 6.5°C, red triangle; **D)** The change in maximum heart rate (f_{Hmax}) (mean ± s.e.m.) of *B. saida* in response to temperature increase. The red dashed and solid lines depict the T_{max} and T_{AR} data that were measured for the 6.5°C- acclimated group held at the Vancouver Aquarium. **E)** Incremental Q_{10} for f_{Hmax} was derived from the individual fish responses summarized in A) at each 1°C change in temperature.

The temperature at which the Q_{10} irrevocably remained below 2 (black horizontal reference line) was assigned as the second breakpoint temperature (T_{QB}) ± s.e.m. **F)** The first significant rate decrease in heart rate as water temperature increases (T_{AB}) was determined for individual fish at each acclimation temperature using best-fit regression analysis of data arranged in Arrhenius plots. The mean (n=10) natural log of heart rates ($\ln f_{Hmax}$) at three acclimation temperatures ± s.e.m. (0.5°C, blue circle; 3.5°C, orange square; 6.5°C, red triangle) are depicted. Data points are connected by lines indicating that the sample size remains unchanged.

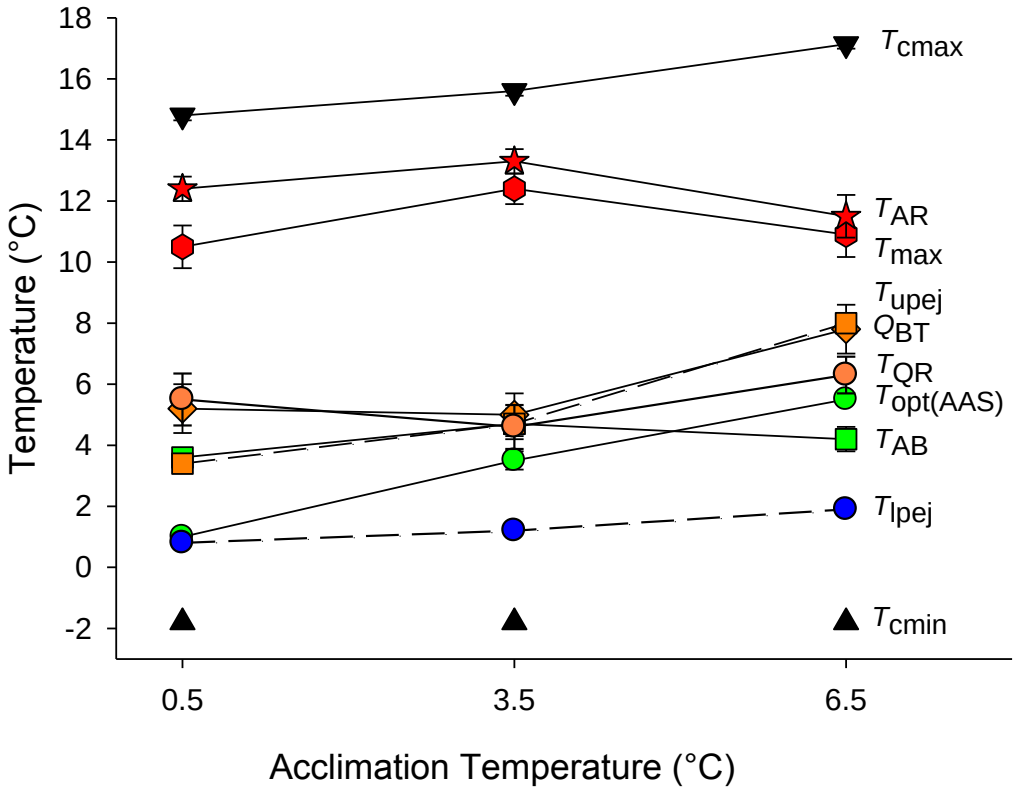

Figure 2.6 Fry thermal polygon for *B. saida*

The minimum lethal temperature ($T_{cmin;}$ black upward triangle) for *B. saida* is assumed, across all acclimation groups, to be -1.8°C (the approximate freezing temperature of surface seawater). The transition temperatures depicted include: Lower temperature pejus ($T_{lpej;}$ blue circle), Arrhenius break point temperature ($T_{AB;}$ green square), AAS derived temperature optimum ($T_{opt(AAS);}$ green circle), Incremental Q_{10} (< 2) break point (T_{QB}; orange diamond), Upper temperature pejus (T_{upej}; orange square), QRS peak height (T_{QR}; orange circle), Peak heart rate (T_{max}; red circle); Arrhythmic f_{Hmax} (T_{AR}; red star) and Loss of equilibrium (T_{cmax}; black downward triangle). Dashed lines indicate values based on 90% T_{opt} and approximate value (-1.8°C) of seawater freezing (not directly measured). All data are presented with error ± s.e.m. except for $T_{cmin,}$ T_{opt}, T_{lpej} and T_{upej}.

Statistical difference in key transition temperatures between 0.5, 3.5 and 6.5°C- acclimation groups is presented below (Table 2.1).

Table 2.1 Key transition temperatures

Group	T_{AB}	T_{lpej}	T_{opt}	T_{upej}	T_{QR}	T_{FS}	T_{QB}	T_{max}*	T_{AR}*	T_{cmax}
0.5°C	3.6[a]	0.2	1.0	3.4	5.5[a]	8.5	5.2[ab]	10.8[a]	12.3[a]	14.9[a]
3.5°C	4.7[a]	1.6	3.5	5.4	4.6[a]	7.5	5.0[a]	12.3[a]	13.4[a]	15.5[b]
6.5°C	4.3[a]	2.4	5.4	8.1	6.3[a]	~9.0	7.8[b]	10.9[a]	11.1[a]	17.1[c]
	P = 0.209	n/av	n/av	n/av	P = 0.244	n/av	***P = 0.024***	P = 0.224	P = 0.066	***P = 0.000***

***** The data for 0.5°C- and 3.5°C-acclimated fish are from field measurements previously published.

2.4 Discussion

Fish energetics rely on oxygen being extracted (respiration) from the water and delivered (cardiac) to tissues (Campbell et al., 2009). Both of these vital processes are temperature dependent (Crozier, 1924). Here I identified thermal limits and rate transition temperatures for cardio-respiratory performance that can potentially dictate migration and limit survival (Fry, 1947; Pörtner, 2001; Farrell, 2002; Somero 2005; Pörtner and Farrell, 2008; Farrell et al., 2009; Iftikar and Hickey, 2013; Deutsch et al., 2015). Even though the thermal performance of biochemical reactions, cells, tissues, organs and organ systems may be quite disparate (Schulte, 2015), the thermal niche of a whole animal must be bounded by its critical thermal limits (T_{cmax} and T_{cmin} - the latter taken here as the freezing point of seawater at -1.8°C in the absence of experimental data). Yet, Antarctic stenotherms, even with a narrow window of thermal tolerance, do acclimate to warmer temperatures to some degree (Pörtner et al., 2000; Lannig et al., 2005; Seebacher et al., 2005; Pörtner et al., 2007), despite the fact that their biogeographic and thermal isolation is more extreme than that of Arctic fishes and has

been this way for around 30,000 years. For example, Seebacher et al. (2005) acclimated the Antarctic notothenioid *Pagothenia borchgrevinki* to 4°C, a temperature likely 3.5°C greater than they experience in the wild. I proposed and provided support for the hypothesis that the cardio-respiratory system of *B. saida*, have some capacity for thermal acclimation, which likely translates into a capacity for *B. saida* to exploit the thermally stratified Arctic Ocean in the summer (see Fig. 2.1.A).

The maximum cardio-respiratory capacity of *B. saida* did well over a range of temperatures that it is likely to experience both under ice and even in Arctic surface water during peak summer temperatures (see Fig.2.1.B). This discovery poses a challenge to whether or not this species should be considered a true polar stenotherm, unlike, for example, burbot, a freshwater cold stenothermal fish that loses cardiac pumping capacity beyond 1°C despite a steadily increasing heart rate (Tiitu and Vornanen, 2002). We also observed a variety of compensatory responses to thermal acclimation that would benefit *B. saida* in a warmer environment. These compensations include an increase in T_{cmax}, increases in peak AAS and FAS, a > 2°C increase in the T_{opt} window for AAS, a faster recovery of AAS after exhaustion and a significant down regulation of f_{Hmax}.

Boreogadus saida, when acclimated to 6.5°C, could maintain their vertical orientation up to 17.3°C and had a T_{cmax} that was 2.2°C higher than 1.0°C-acclimated fish. Fry (1971) defined thermal acclimation as at least a 1.0°C increase in T_{cmax} when acclimation temperature is increased by 3°C (ratio = 0.33). Thus, *B. saida* met the standard criterion for thermal acclimation. Similarly, stenothermal Antarctic fish species were able to significantly increase T_{cmax} by > 2°C, with a range of 15 to 18°C, when tested at ambient -1.5°C and then acclimated to 4°C water temperatures (Somero and DeVries, 1967; Podrabsky, and Somero, 2006; Bilyk and DeVries 2011). From an evolutionary perspective, there is a remarkable

similarity in the ratio of T_{cmax} to acclimation temperature for different fish species, which is 0.43 for *B. saida*, 0.41 (range 0.27 - 0.50) for 20 species of North American freshwater fishes (Beitinger et al., 2000) and 0.44 (range 0.24 – 0.65) for 8 Antarctic species (Bilyk and DeVries, 2011).

From an environmental perspective it is interesting that the measured T_{cmax} for both *B. saida* and Antarctic species, lies well beyond present day surface water temperatures in both polar environments (Fig. 2.1A). T_{cmax} is a thermal tolerance limit and one probably not normally experienced in their thermal niche (Kerr, 1976). For instance, 16°C-acclimated *B. saida* died in a laboratory feeding study (Laurel et al., 2015). The same study found maximum growth rate for *B. saida* occurred at acclimation temperatures between 5 and 9°C (Laurel et al., 2015), a result that is consistent with the observation here that 6.5°C-acclimated fish had the highest AAS and FAS.

A link between increased AAS (and FAS) and increased growth (and condition factor) was also found in a warm acclimation study of spine cheek anemone fish (Donelson, 2015). Cardio-respiratory links to performance were also demonstrated in 14°-acclimated rainbow trout (*Oncorhynchus mykiss*), which had a T_{upej} between 19 and 20°C (Chen et al., 2015), decreased food consumption rate at 19°C and starved at 22°C (Myrick and Cech, 2000). The apparent link between cardio-respiratory transition temperatures and performance has been further illustrated in the eurythermic goldfish (*Carassius auratus*). A T_{opt} of 20°C was calculated for goldfish acclimated to 12°C water temperature (Ferreira et al., 2014). The maximum swimming rate of goldfish, acclimated to 15°C water temperatures, declined at 20°C (Fry and Hart, 1948; Johnston and Temple, 2002). Furthermore, T_{max} of goldfish acclimated to 12°C water was 27°C (Ferreira et al., 2014), which is the same temperature

that goldfish acclimated to 10°C lost their ability to escape predators (Johnson and Temple, 2002).

Measurements of EPOC provided additional evidence of acclimation to 6.5°C in *B. saida* because recovery was fastest at 6.5°C, particularly when compared with 1°C-acclimated fish at the same test temperature, which was contrary to our hypothesis that the colder acclimated group would perform better. Improved performance with acclimation is species specific. Each species has different capacities to perform life-sustaining activities, with individual variation. However, for all species it is predicted that temperature acclimation changes the shape of their aerobic scope curve and the values for T_{opt} and the T_{opt} window for AAS (Pörtner and Farrell, 2008; Schulte, 2015), as seen recently for the eurythermal goldfish (12 to 28°C; Ferreira et al., 2014). We found that *B. saida* aerobic scope curve, unlike the eurythermal goldfish, broadened with warmer acclimation and their T_{opt} and their T_{opt} window for AAS increased by ~2°C. Yet despite the clear evidence of an increase in performance capacity of *B. saida* at warmer temperatures, thermal acclimation may exact a cost to whole animal performance (Woods and Harrison, 2002; Seebacher et al., 2005; Deutsch et al., 2015; Pershing et al., 2015). For instance, acute exposure to a temperature higher than 6.5°C presented severe problems with post-exhaustion mortality at test temperatures higher than the acclimation temperature, e.g., an unexpected 50% of 6.5°C-acclimated fish tested at 8.5°C, something I never observed at test temperatures of 6.5°C or lower. Thus, the high growth rate seen for *B. saida* at 9°C in a protected laboratory with ample food (Laurel et al., 2015) may not be possible in the natural environment.

Depression of biological rates (*i.e.* compensation) is another sign of warm acclimation in ectotherm species including arthropods, molluscs, fish, amphibians and reptiles (Lillywhite et al., 1999; Aho and Vornanen, 2001). This was evident in *B. saida* with the significant

reduction in f_{Hmax} (6 ± 0.1 bpm) at 6.5°C-acclimation when compared with 0.5°C-acclimation over the test temperature range from 0.5 to 8°C, when sample size remained the same. A reduction of f_H is predicted with an increase in tolerance to warmer water (Farrell, 1997; 2016) and, at least in rainbow trout, appears to be caused by modification of the pacemaker action potential (Haverinen and Vornanen, 2007). A Q_{10} effect has been used to describe acclimation potential (Du et al., 2010; Seebacher et al., 2015). For *B. saida,* f_{Hmax} shows, on average, a moderate acclimation response ($Q_{10\ (6)}$ = 1.7) when acclimated from 0.5 to 6.5°C.

One area of concern is that anaesthetics, particularly MS-222 is known to have membrane destabilizing properties, which could alter the thermal response of f_{Hmax} and perhaps help trigger cardiac arrhythmias. Regarding cardiac arrhythmias, their physiological basis is being explored (Badr et al., 2016; Vornanen, 2016), but more important to the present concern, they are observed with acute warming of perfused working heart preparations (pers. comm.) where no anaesthetic is present. Cardiac arrhythmias are also observed in unanaesthetized fish during acute warming (Clark et al., 2008; Eliason et al., 2013), but the presence of intact control mechanisms hampers results interpretation. In terms of the accuracy of the rate transition temperatures for f_{Hmax}, a comparison of two anaesthetics with different mechanisms of action (15 ppm clove oil and 50 ppm MS222) produced similar results for f_{Hmax} in coho salmon, with MS222 having the lower individual variability for T_{AB} (Casselman et al., 2012). Furthermore, the same study measured T_{opt} for aerobic scope for unanaesthetized coho salmon and it was not significantly different from the T_{AB} estimated from f_{Hmax} for any of the anaesthetic treatments tested. Thus, while any effect of anaesthetics on f_{Hmax} with this technique seem minor at best, care is still needed in choosing the best type and dose of anaesthetic when other fish species are tested.

A ‘Fry thermal polygon’ can be used to distinguish various zones of thermal tolerance for reproduction, activity, tolerance and lethality with respect to acclimation temperature (Fry, 1974). In our study, the cardio-respiratory transition temperatures (*i.e.* performance limits) were incorporated into a modified Fry temperature polygon to graphically represent *B. saida* windows of thermal tolerance (Farrell, 2016). Previously, the rate transition temperatures for f_{Hmax} and AAS have been placed in a hierarchy within a Fry thermal polygon for goldfish (Ferreira et al., 2014) and rainbow trout (Chen et al., 2015). We do likewise in Fig. 2.6. Both previous studies found that T_{AR} was 1 to 3°C below T_{cmax}. Similarly, T_{AR} for *B. saida* was at least a 2°C below T_{cmax}. However, the relationship between T_{opt} and T_{AB} varied according to acclimation temperature because, while T_{opt} approximated the acclimation temperature in all three fish species, T_{AB} was independent of acclimation temperature in *B. saida*. Thus, with 0.5°C- and 3.5°C-acclimation, T_{opt} was slightly lower than T_{AB} and closer to the T_{upej} values, whereas with 6.5°C-acclimated fish the T_{AB} was almost 1°C lower than T_{opt}. Even so, the absolute differences between T_{AB} and T_{opt} were never large and T_{AB} was always within the T_{opt} window (Fig. 2.6).

Abrupt changes in respiration and heart rate, due to increasing temperature, highlight ecologically relevant physiological limitations. The results of this study demonstrate the potential rewards of combining whole animal cardio-respiratory performance with ecosystem observations. Transition temperatures, when added to Fry temperature polygon graphs, estimate a hierarchy of temperature limits to fundamental activities that could also include other physiological functions such as reproduction and growth.

In summary, when considering the full life history of *B. saida*, it appears that egg development is the critical life stage with respect to temperature, a limit of 3 to 3.5°C (Sakurai

et al., 1998; Kent et al., 2016). Similarly 3.5°C-acclimated larvae T_{AB} is 3.3°C, when hearts first fail to keep up with steadily increasing water temperature as described in Chapter 4.

As researchers have known for decades, the *B. saida* life history is inextricably linked with Arctic sea ice. Sakshaug and Skjoldal (1989) coined the term “ice-edge effect” to describe the physical and biological activities that occur around the marginal ice zone, which are vital feeding grounds for *B. saida* larvae and juveniles (Bradstreet et al., 1986; Arrigo, 2014). Food, safety and also the results from this study suggest that ice-induced water temperature suppression help to explain *B. saida* abundance, estimated to be in the billions, under ice (David et al., 2015). Such abundance is required to maintain existing marine Arctic food webs (Hop and Gjøsæter, 2013).

One concern regarding this study was whether there was any change in *B. saida* physiology after transporting them ~2,500 km from where they were collected. Key differences included holding them in different sea water, feeding them on a constant fixed diet of primarily Antarctic krill and exposing them to 49°latitude light conditions. Thus It was necessary to test the hypothesis that transport and holding *B. saida* in different conditions, except temperature, did not significantly alter their cardiac performance. I measured f_{Hmax} in wild fish held for 10 days in local 0.5°C seawater and compared it with the data from fish that were transported to the Vancouver Aquarium and kept at the same temperature for 6 months. Potential changes in cardiac performance after relocating fish are discussed in the following chapter.

Chapter 3: Cardiac function of *Boreogadus saida*: laboratory vs. field[2]

3.1 Introduction

While the physiology of polar fishes is well studied for a few species from the Antarctic Ocean, this is not the case for Arctic fishes (Farrell and Steffensen, 2005; Farrell et al., 2013). Here, the focus is on the impact of an acute increase in water temperature on the cardiac performance of *Boreogadus saida* (Lepechin, 1774), a key food web fish species in the Arctic Ocean (Bradstreet et al., 1986; Coad and Reist, 2004). The role of B. saida in the marine Arctic food web is crucial as predation upon them efficiently moves a large proportion of energy and nutrients from Arctic algae and invertebrates to higher trophic levels (Welch et al., 1993; Crawford and Jorgenson, 1996). The impact to Arctic marine food webs, if water temperatures exceed the limits of cardiac performance, could potentially be circumpolar in scope. This species distribution is typically north of the Arctic Circle in pan Arctic water that range in temperature from freezing in winter to 6 - 10°C in summer (Bradstreet et al., 1986; DeVries and Steffensen, 2005; Bain and Sekerak, unpubl. data; Drost et al., 2016).

Thermal acclimation of *B. saida* is an important factor in oxygen consumption (MO_2) estimates (Hop and Graham, 1995). When two acclimation temperatures were compared (0.4 and 2.7°C), MO_2 was ~ 30% higher in the warmer water and a linear relationship existed between oxygen consumption and acclimation temperature. The Q_{10} temperature coefficient is a standardized measure of the rate of change of a chemical or biological system (oxygen consumption in this case) due to increasing water temperature. Despite the differences in methodology of three studies, which measured the routine oxygen consumption of *B. saida*,

[2] A version of this chapter was published as: Drost, H. E., Carmack, E. C., Farrell, A. P. (2014). Upper thermal limits of cardiac function for Arctic cod *Boreogadus saida*, a key food web fish species in the Arctic Ocean. J Fish Biol. DOI: 10.1111/jfb.12397

the Q_{10} values were similar over the temperature range between −1·5 and 1.0°C (Holeton, 1974; Steffensen et al., 1994; Hop and Graham, 1995). The duration of acclimation was also a significant factor in these studies. When fish were held at 0·4°C for 14 days before testing, the MO_2 consumption rate was 86.0 $O_2\,kg^{-1}\,h^{-1}$. After holding the fish at 0.4°C for 5 months, the MO_2 consumption rate had decreased appreciably to 46.5 mg $O_2\,kg^{-1}\,h^{-1}$ (Hop and Graham, 1995). These limited data, however, did not reveal the upper temperature limit for the most basal values of MO_2 recorded in *B. saida* nor has maximum MO_2 been measured.

I decided to use cardiac function instead of MO_2 and aerobic scope to test if there were differences in field vs. laboratory fish physiology because it is a simpler method to use in the field and, in general, cardiac function decreases precipitously upon reaching acute thermal limits, thereby implicating it as “an obvious candidate for a cause of acute thermal death” (Somero, 2010). Indeed, an increasing number of studies on a variety of fish species have used maximum heart rate (f_{Hmax}) to provide valuable and reliable information on the rate transition temperatures associated with upper thermal performance of the fish heart (Steinhausen et al., 2008; Casselman et al., 2012; Anttila et al., 2013; Chen et al., 2013; Ferreira et al., 2014; Munoz et al., 2014; Sidhu et al., 2014).

3.2 Materials and methods

3.2.1 Fish collection

The fish were collected, held and tested in accordance with the Canadian Council on Animal Care regulations and permits were issued by the University of British Columbia Animal Care Committee (A11-0267), the Freshwater Institute Science Laboratories Animal Care Committee – Arctic Aquatic Research (FWI-ACC-2012-050) and the Vancouver Aquarium Animal Care Committee (2011–04).

Adult *Boreogadus saida* (Lepechin 1774) were caught in August 2011 and in July 2012 near Cambridge Bay (CB) on Victoria Island, Nunavut, Canada (69°12′N; 105°05′W), as detailed previously in Chapter 2. In brief, adult *B. saida* were held in 0°C water temperatures for up to 4 weeks at CB to ensure good health before being transported by air at 0°C to the Vancouver Aquarium (VA), British Columbia, Canada. Thirty-six hours of flight and transport were required to transport fish to the VA, water temperature remained at 0°C throughout.

At the VA laboratory, *B. saida* were held in a closed-system 450 l tank with a daily 50% replacement of sump water as well as cleaning. Fish were fed to satiation with frozen krill usually every 1-2 days and were exposed to a fluorescent light and dark cycle that represented Vancouver (49°N) daylight conditions. Food was withheld for a minimum of 36 h before any experimentation. The order of experiments minimized the risk of fish mortality, testing f_{Hmax} first and T_{cmax} last.

In 2012, the fish were held at 0°C for 10 days before testing in situ in Nunavut. The 0°C acclimation results from 2011 at VA are compared with those of the 2012 in CB. Two of the fish that had been tested in the CB laboratory were also transported to VA. The summer photoperiod, from July to September, was between18 and 24 h natural daylight. The fish were fed and accepted 2·0 mm BioTrout pellets (www.136 bio-oregon.com) for at least 10 days before the CB tests were conducted. At the VA laboratory, fish were held in a 450 l tank at 0°C and fed to satiation within 48 h of arrival with frozen krill *Euphausia pacifica* and *Euphausia superba*, and occasionally cut herring *Clupea pallasii* Valenciennes 1847, caplin *Mallotus villosus* (Müller 1776) and squid *Loligo* sp.

Fish caught in 2011 were maintained at 0°C for 6 months under a light and dark cycle with fluorescent lighting that represented Vancouver daylight conditions (49°N) until tests were performed. The fish tested in CB (n=12) weighed 39.7 ± 2.0 g (mean ± s.e.m.) and were 17.0

± 0.3 cm in total length (LT), while the fish when tested at VA (n=10) were 31.8 ± 2.4 g and 16.5 ± 0.4 cm, respectively.

3.2.2 Measuring maximum heart rate during acute warming

f_{Hmax} was measured in *B. saida* after a 24 h fast using the technique and apparatus detailed in Casselman et al. (2012). There were four different temperature endpoints used in these experiments. In the CB laboratory, warming continued until electrocardiogram (ECG) became arrhythmic (T_{AR}), where upon the experiment was terminated and each pair of test fish were quickly returned to fresh sea water at the acclimation temperature (0°C) to promote recovery. Two of the revived fish were passive integrated transponder (PIT) tagged and transported to the VA laboratory and retested along with the other fish held at the VA laboratory. Given a concern over satisfactory post-test recovery for the costly to catch fish, a different endpoint was adopted for VA tests during acute warming: reaching the characteristic plateau (or a decline) for f_{Hmax}, a decreased amplitude of the QRS wave [see Fig. 1(b)], or simply reaching a maximum warming temperature of 9.5°C. By warming fish no higher than 9.5°C at VA, all test fish were revived rapidly. Only three of the fish tested at VA did not reach the characteristic plateau in f_{Hmax} before 9.5°C, which indicates that the mean transition temperature T_{max} was slightly underestimated.

The method used to measure the response of f_{Hmax} to acute warming was to simultaneously anaesthetize two fish in 75 mg l^{-1} tricaine methanesulphonate (MS222, Sigma-Aldrich Products; www.sigmaaldrich.com) until they were unresponsive to a tail pinch. Fish were then transferred to individual 30 cm×10 cm Plexiglas water-bath chambers (water volume = 2 l) where the anaesthetized state was maintained with gill irrigation (c. 150 ml min^{-1} with seawater containing 50 mg l^{-1}MS-222). The chambers were connected to a 15 l closed-circuit sump, which contained two refrigerant coils attached to programmable chillers

(Fisher Isotemp 3016d; www.fisherssci.com) filled with 60% propylene glycol antifreeze. Water temperature, and hence fish temperature, was controlled by the recirculating chiller and measured to a precision of ± 0.1°C (Fisher Scientific Type K digital thermometer probe). The seawater sump was continuously aerated and also held a magnetic drive pump. The fish were positioned dorsal side down on a fine mesh screen to enable placement of two custom-made chromel-A electrodes on the skin near the heart to record an ECG. The electrodes were connected via amplifiers (×1000; Grass P55 AC – Astro-Med Inc.; www.astro-med.ca) and filters (low-pass 30 Hz with a 3 Hz transition width) to a PowerLab ML870 data acquisition unit and Labchart 7 Pro software (ADinstruments; www.adinstruments.com). The initial water temperature was set at acclimation temperature. After 1 h of recording to ensure a stable $f_{H,}$ intraperitoneal injections of 10 mg kg^{-1} atropine sulphate and 8 μg kg^{-1} isoproterenol (Sigma-Aldrich Products) were administered to block any vagal inhibition of the f_H and maximize any β-adrenergic stimulation of f_H, respectively. Typically, the injections resulted in ~5 bpm increase after ~15 min, mostly in response to atropine. Pilot experiments with additional drug injections either at the start or near the end of the warming period had no further effect on f_{Hmax}, which suggested that the selected drug treatments were effective in both generating a f_{Hmax} and maintaining this state during the experiment. The acute warming trial consisted of incrementally increasing temperature by 0.5°C every 15 min and waiting at each increment until water temperature and f_{Hmax} stabilized.

3.2.3 Data analysis and statistical testing

The f_{Hmax} was calculated using the R-R interval (time between beats of an ECG recording) from 30 heartbeats of a continuous ECG recording with a rhythmic heartbeat. Calculation of the highest absolute value for f_{Hmax} and the temperature at which this occurred were based on individual data. The warmest measurement reported was 0.5°C below the temperature

that triggered cardiac arrhythmia (T_{AR}) (CB) or a plateau (VA) (Fig. 3.1.), and the proportion of fish reaching this trigger is presented in Fig. 3.1.a. and was used to calculate the temperature for arrhythmic heartbeat (T_{AR}) in fish tested at the CB laboratory. The two fish tested in CB then transported to VA were not included in statistical tests.

The T_{AR} was considered by Casselman et al. (2012) to be an index of the T_{crit}. An Arrhenius breakpoint temperature (T_{AB}) analysis (Yeager and Ultsch, 1989) was performed by plotting the natural log of the heart rate ($\ln f_{Hmax}$) of individual fish against the inverse of temperature (1000 K−1) and running best-fit regressions (SigmaPlot 11.0, Systat Software; www.sigmaplot.com) to determine where the slope of the line in the Arrhenius plot decreased; i.e. the breakpoint (Casselman et al., 2012; Anttila et al., 2013). The T_{AB}, based on individual determinations [and not the averaged transformed data that are shown in Fig. 1(c)], was considered by Casselman et al. (2012) to be an index of T_{opt} . Again for individual fish, an incremental Q_{10} for f_{Hmax} was determined for each 1°C change in temperature. The temperature at which the incremental Q_{10} for a fish abruptly decreased below 2.0 and remained below 2.0 was assigned as a second breakpoint temperature T_{QB}. A Q_{10} value of 2 is regarded as a normal rate of change of routine metabolism with temperature (Fry and Hochachka, 1970; Miller and Mann, 1973; Holeton 1974).

All data are presented as a mean ± s.e.m. unless otherwise stated. Mean values derived from f_{Hmax} data for CB tests were compared with the VA tests using at-test and $P<0.05$ as the fiducial limit. Power analysis was tested using Minitab 16 (www.minitab.com).

3.3 Results

At 0.5°C, f_{Hmax} was 26.4 ± 0.8 bpm for tests at the CB field laboratory and 26.0 ± 0.4 bpm for tests at the VA laboratory. As expected, f_{Hmax} increased with temperature and with similar slopes for individuals between 0.5 and 5.5°C for the CB and VA tests [Fig. 3.1.a]. In fact, the

average response of f_{Hmax} to acute warming was indistinguishable for CB and VA tests [two-sample t-test, d.f. =20; P>0.05; n=12 and n=10, respectively; Fig. 3.1.b]. Also, T_{AB} was not significantly different for CB (3.2 ± 1.1°C) and VA (3.6 ± 0.9°C) tests [two-sample t-test; d.f. = 20; P > 0.05; Fig. 3.1.c) and Table 3.1]. The incremental Q_{10} for f_{Hmax} remained > 2.0 between 0.5 and 5.5°C for CB and VA tests [Fig. 3.1.d] and their Q_{10} (ΔT=1°C) values over this temperature range were not significantly different (2.3 ± 0.1°C and 2.4 ± 0.2°C, respectively, two sample t-test d.f. = 20, P > 0·05). Inspection of Fig. 3.1.c and Fig.3.1.d suggests that T_{AB} coincided with the temperature where the incremental Q_{10} decreased to around 2.0. Thus, T_{QB} was always greater than T_{AB}. Although the T_{AB} comparison introduced the possibility of a type II statistical error (power = 0.3), a power analysis revealed that 50 fish would have been needed to sufficiently raise the statistical power (0.8) and confidence (P=0.05) that a type II error was not being made had T_{AB} differed by just 0.5°C. Thus, in the range of water temperatures in which wild *B. saida* are most typically found year round, an excellent agreement existed for the performance of f_{Hmax} between the CB and VA tests.

With acute warming beyond 5.5°C, f_{Hmax} began to diverge for fish tested at CB and at VA and for the two fish re-tested at VA. For CB tests, T_{QB} was 8.0°C compared to 5.5°C at the VA [Fig. 3.1.d]. Thus, the temperature range over which an incremental Q_{10} of ~2 was maintained was greater for the CB than the VA. Also, for fish tested at CB, the highest absolute value for f_{Hmax} was 54.5 ± 2.9 bpm at a T_{max} of 10.5 ± 0·8°C, whereas VA fish only achieved 45.2 ± 2.9 bpm at a T_{max} of 7.8 ± 0·5°C [Fig. 3.1.a]. Two fish re-tested at the VA after being warmed to T_{AR} in CB had a lower T_{AB}, f_{Hmax} and T_{AR} but were within the minimum range reported for both CB and VA tested fish (Table 3.I). The fact that the T_{AR} was 12.4±0.4°C in CB tests (Table 3.I) suggests that the upper temperature tolerance for *B. saida*

acclimated to 0°C lies well above the T_{AB}. Similarly, in the VA tests, fish were warmed to 9.5°C, again well above the T_{AB}.

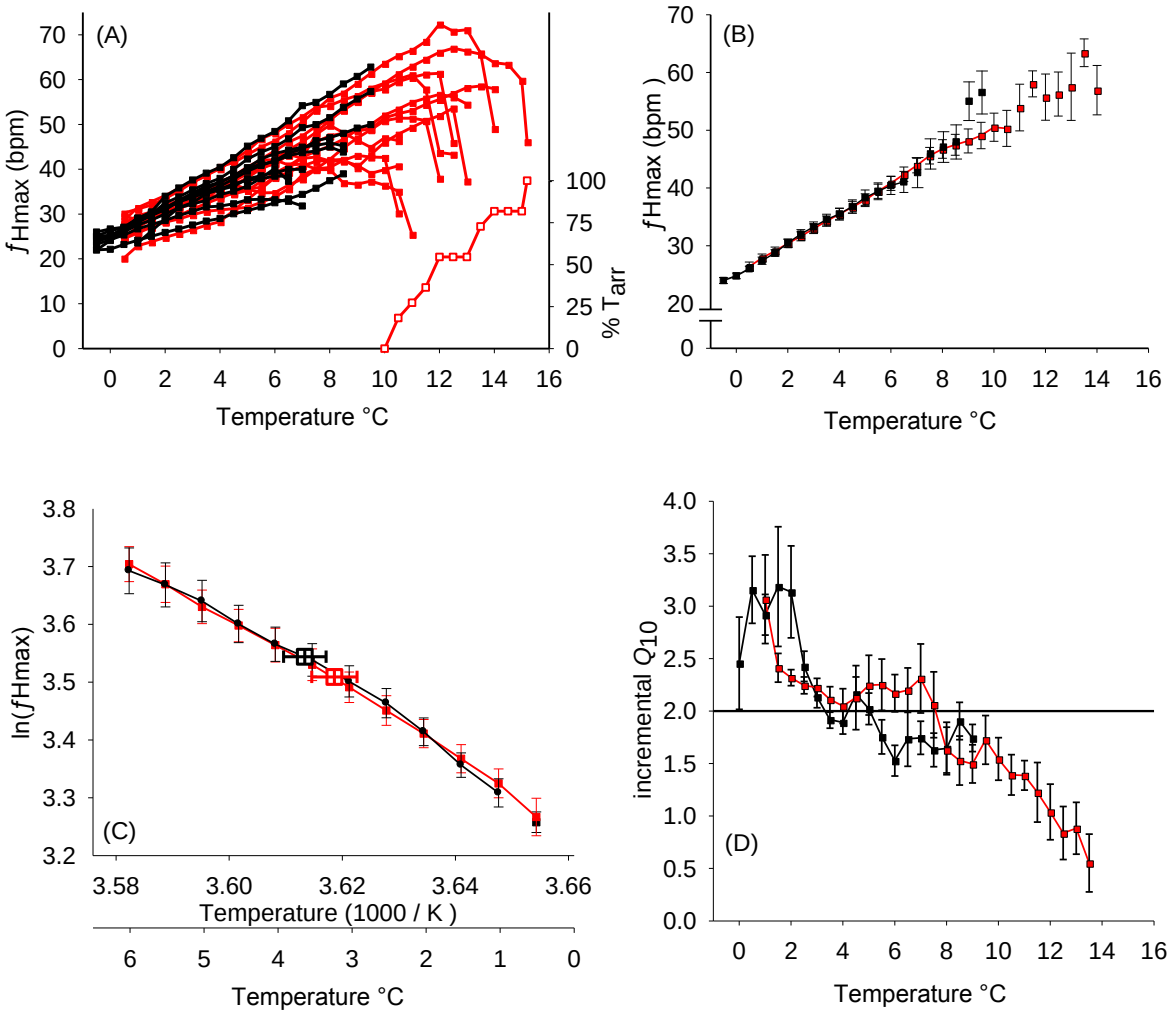

Figure 3.1 Effects of acute warming on maximum heart rate

The effects of acute warming on the maximum heart rate (f_{Hmax}) of anaesthetized *Boreogadus saida* acclimated to 0°C. Tests were conducted in Cambridge Bay (CB), Nunavut (n=12;■) and Vancouver Aquarium (VA), British Columbia (n=10;■). **A)** Individual responses of f_{Hmax} acutely warmed in 0.5°C increments for fish tested at the CB field laboratory and the VA laboratory. Lines connect the data for each fish. Also shown is the per cent of individuals developing cardiac arrhythmia and the temperature when it occurred (T_{AR}) from the CB field laboratory (n=11). **B)** Mean ± s.e.m. f_{Hmax} derived from the individual data presented in (a). Data points for f_{Hmax} are connected by lines when CB and VA sample size remains unchanged (n=12 and n=10, respectively). Two fish were

not tested below 0.5°C at VA (n=8). **C)** The Arrhenius plot of f_{Hmax} based on the mean values presented in (b). The first Arrhenius breakpoint temperature (T_{AB}) was determined from individual Arrhenius plots and the mean ± s.e.m. T_{AB} is indicated for CB and VA; horizontal error bars indicate ± s.e.m. using the Arrhenius convention y-axis is the natural log of heart rate [ln(f_{Hmax})]. Data points are connected by lines only when sample size remains unchanged. **D)** The incrementalQ_{10} analysis of f_{Hmax} for 0.5°C temperature increments (ΔT=1°C bars). Lines connect all points to highlight the abrupt decrease when $Q_{10} < 2$, which is called the incremental Q_{10} breakpoint temperature (T_{QB}).

Table 3.1 A comparison of transition temperatures

Boreogadus saida heart rate (f_{Hmax}) indices (mean ± s.e.m.).

Parameter (°C)	CB 0°C *n* = 12	VA 0°C *n* = 10	CB 0°C *n* = 2	VA 0°C *n* = 2 retested
T_{AB}	3.2 ± 0.3	3.6 ± 0.3	2.5, 4.7	1.3, 1.5
Q_{10} (0.5 - 5.5)	2.3 ± 0.1	2.4 ± 0.2	3.1, 2.5	3.0, 2.3
T_{QB}	8.0	5.5	4.0, 5.0	4.0, 3.0
(0.5) f_{Hmax} bpm	26.4 ± 0.8	26.0 ± 0.4	20.2, 28.6	21.9, 28.2
Highest f_{Hmax} bpm	54.5 ± 2.9	45.2 ± 2.9	53.7, 71.1	39.4, 46.2
T_{max}	10.5 ± 0.8	7.8 ± 0.5	12.5, 12.0	10.0, 9.5
T_{AR}	12.4 ± 0.4 *	Not available	13.3, 13.6	10.1, 10.0

*****n =11; T_{AB} (first Arrhenius break point temperature); T_{QB} (incremental Q_{10} break point temperature); T_{max} (temperature for the highest f_{Hmax} recorded); T_{AR} (temperature when cardiac arrhythmia first developed)

3.4 Discussion

This study reports cardiac function measurements that were made before and after transport to examine the potential for further study of *B. saida* in a more amenable laboratory facility. The data from the CB laboratory, which is the first application of this technique in a remote location, characterized several transition temperatures that may have biological importance. The f_{Hmax} increased from 26·4 bpm at 0.5°C to the highest rate of 54·5 bpm at a T_{max} of 10·4∘C, before Cardiac arrhythmias developed at a T_{AR} of 12.4°C and in between

these endpoints there were two more transition temperatures: T_{AB} (3·2∘C) and T_{QB} (8∘C). Given these high transition temperatures for f_{Hmax}, a concern is that the anaesthetic exerted a protective effect on the heart and so extended apparent temperature tolerance. While there are no *in vivo* measurements of f_{Hmax} in *B. saida*, there are reasons to propose that the anaesthetic did not protect the heart. Comparisons of aerobic scope and critical thermal maxima (T_{cmax}) with heart rate transition temperatures have demonstrated that T_{AR} is lower than T_{crit} and T_{cmax} (Casselman et al., 2012; Anttila et al., 2013). Indeed, this is an element of the reasoning behind the proposition that the heart starts to collapse before the whole animal during acute warming of fishes. Of course, T_{AR} could be lower and closer to T_{opt}. This possibility should be tested with *in vivo* studies.

Over the range from 0.5 to 5.5°C, the results for tests performed in CB and at VA were indistinguishable. All the same, T_{QB}, the highest f_{Hmax} and the temperature at which that occurred (T_{max}) differed. While all tests were performed on fish acclimated to 0°C, it is possible that these upper temperature differences were related to the shorter acclimation period at CB, preceded by more variable temperatures experienced in the wild by *B. saida* just before CB tests (e.g. seasonal changes and vertical migration to warmer surface waters) when compared with 6-month acclimation to constant temperature at VA. In a laboratory study, Schurmann and Christiansen (1994) showed that *B. saida* preferred 3 – 6°C within a thermal gradient from 0 to 8°C. This temperature preference corresponds with the CB and VA tests, which show that the heart rate steadily increases until T_{AB} (3.2 and 3.6°C) and that the fish maintain a Q_{10} of ~2·0 until T_{QB} (5.5 and 8.0°C).

Casselman et al. (2012), who introduced the f_{Hmax} index, showed that the T_{AB} was very similar to measured values of T_{opt} in the stenothermal salmonid *O. kisutch*. They suggested that the T_{AB} was an index of T_{opt} while T_{AR} was an index of upper T_{crit}. Subsequently, Anttila

et al. (2013) suggested that, rather than T_{opt}, T_{AB} may be a proxy for a transition temperature coined by Frederich and Pörtner (2000) as the lower pejus temperature (T_{pej}; getting worse) below T_{opt}. While the interpretation of these transition temperatures for f_{Hmax} is still under debate, the temperature difference between T_{AB} and T_{AR} is much smaller (4 – 6°C) for the stenothermal salmonids compared with *B. saida* (9.2°C), which was a surprise given the restricted Arctic distribution of the latter. T_{AR} associated with 0°C acclimation was substantially higher than the acclimation temperature itself and the frigid temperatures *B. saida* experience most of the year. During summer, however, the fish have been trapped in fyke nets in Simpson Lagoon in Prudhoe Bay on the Alaskan North Slope, at temperatures up to 13.5°C (Craig et al., 1982).

The high T_{AR} and field observations raise considerable interest in whether or not *B. saida* should be classed as stenothermal and also what factors other than temperature might restrict its geographic distribution in the Arctic. That they could routinely recover from being acutely warmed up to 9.5°C suggests a high temperature tolerance and maintaining a Q_{10} for f_{Hmax} of ≥ 2.0 up to and perhaps beyond 5.5°C represents an unexpected finding in terms of the heart's ability to maintain activity. It is also clear that in summer, *B. saida* often prefer temperatures warmer than the near-freezing ones where they were captured for this study. Why is *B. saida's* biogeographic distribution above the Arctic Circle when they can apparently tolerate much warmer water? The upper limit temperatures they can tolerate in the laboratory do not seem to be realized in nature, at least for any significant length of time. Sea temperatures off the west coast of Greenland have progressively increased by ~ 2°C since 1997, with a present range in temperature between - 0.5°C in winter and up to 5°C in summer (Hansen et al., 2012; NOAA, 2016). Although this is within *B. saida's* T_{AB} - T_{QB} range, they are no longer caught near the Danish Polar Marine Station situated on Disko

Island, just north of the Arctic Circle (Farrell et al., 2013). This suggests that the temperature dependence of cardiac performance per se is not the primary driver of the realized *B. saida* distribution. Species interactions also help define distribution (Holt, 2003). Diet could be an important consideration in the competitive interactions between *B. saida*, Atlantic cod *Gadus morhua* L. 1758, haddock *Melanogrammus aeglefinus* (L. 1758), capelin *Mallotus villosus* (Müller 1776) and pacific sand lance (*Ammodytes hexapterus*) (Renaudet al., 2012; Hop and Gjøsæter, 2013; Suzuki et al., 2015), but strong competitive interactions have yet to be documented.

Antarctic fishes have been successfully relocated for physiological studies for two decades (Axelsson et al., 1992), and here, the potential to relocate *B. s*aida to a southern laboratory location without a major disruption to their cardiac physiology is shown. The subtle differences observed here for relocated fish could have been related to the more variable water temperatures that the fish experience in the wild preceding the field tests. The relatively high temperature tolerance was a surprising discovery for *B. saida*, a species that inhabits cold, near-freezing waters.

In summary, based on the physiological evidence presented in this study, *B. saida* may be among the survivors of the anthropogenic climate change affecting the Arctic Ocean. Due to the higher than expected tolerance of Adult *B. saida*, the next question to investigate is whether there may be lower temperature limits when considering the full life history of *B. saida*. The water temperature tolerance of eggs is estimated to be ~3°C (Sakurai et al., 1998; Kent et al., 2016) and may limit their ability to adapt to the loss of sea ice. Fortunately, the fish collected in 2011 were fecund that winter and reproduced at the Vancouver Aquarium. This allowed testing of egg development at 3.5°C (Kent et al., 2016) and larval cardiac system temperature tolerance (Chapter 4).

Chapter 4: Cardiac performance of adult and larvae *Boreogadus saida*[3]

4.1 Introduction

The temperature limits of developing larvae of Boreogadus saida had not been determined before this study. Given the central role of *Boreogadus saida* (Lepechin 1774) in Arctic food webs, knowledge of their upper thermal performance limits is critical for resource management and conservation in this era of rapid climate change in the Arctic. Therefore, this study compared the upper thermal limit of cardiac performance of adult and larval *B. saida* by acutely warming (2°C h^{-1} and 4°C h^{-1}, respectively) individuals that had been acclimated to 3.5°C. This type of study was performed with adult *B. saida* acclimated to 0.5°C (Chapter 3) and it was discovered that the first Arrhenius breakpoint temperature for f_{Hmax} (T_{AB}) occurred at 3.2°C. This was a surprising result for a fish with a restricted polar habitat because T_{AB} is a rate transition temperature that is thought to be near the thermal optimum for aerobic scope in fishes (Casselman et al., 2012; Anttila et al., 2013; Ferreira et al., 2014). Moreover, f_{Hmax} reached its absolute maximum (T_{max}, a rate transition temperature thought to be just below the critical thermal maximum) at an impressive 10.5°C.

Despite this unexpectedly high upper thermal tolerance, populations of *B. saida* are reported to have declined off Disko Bay, Greenland near the Arctic Circle, where maximum summer water temperatures have increased but still do not exceed 5°C (Hansen et al., 2012). A decline in *B. saida* populations was also reported during the 2013 Barents Sea fish stock assessments conducted by both the Alfred-Wegener Institute and the Norwegian Institute of Marine Research (Astthorsson, 2015). This decline was attributed to secondary

[3] A version of this chapter was published as: Drost, H. E., Fisher, J. Randall, F., Kent, D. Carmack, E. C., Farrell. A. P. (2015). Upper thermal limits of the hearts of Arctic cod *Boreogadus saida*: Adults compared with larvae – J Fish Biol. 10.1111/jfb.12807

consequences of rapidly warming Arctic waters, which includes a de-coupling of predator/prey synchronicity and changing competitor and predator relationships due to in-migration of other fish species from the south. In the present study, however, the idea that water temperature can be a primary determinant of habitat range was explored with the hypothesis that larvae are more sensitive to acute warming than adults. This prediction is based on three main assumptions. First, growth and survival of larval fish is typically more sensitive to warm temperature than adult fish (Burgess et al., 2006; Burggren and Reyna, 2011; Dionisio et al., 2012). Second, an accidental 24-h exposure to 9°C of *B. saida* larvae may have caused spinal scoliosis and jaw deformities (Graham and Hop, 1995). Third, *B. saida* eggs are produced during winter months from November to February (Graham and Hop, 1995) and the larvae emerge and grow under the winter ice at a stenothermal sub-zero water temperature. It is not until after ice break-up, usually in late July, when they are exposed to summer water temperatures.

4.2 Materials and methods

4.2.1 Collection and care

To test the upper thermal performance of the heart, cardiac frequency was monitored from an electrocardiogram recording in adult fish and was visually counted in translucent larvae. The adult fish were collected, held and tested in accordance with Canadian Council on Animal Care regulations and permits were issued by the University of British Columbia Animal Care Committee (A11-0267), the Freshwater Institute Science Laboratories Animal Care Committee – Arctic Aquatic Research (FWI-ACC-2012-050) and the Vancouver Aquarium Animal Care Committee (2011-04). Adult *B. saida* were caught with a dip net from depths up to 2 m in July 2012 near Cambridge Bay on Victoria Island, Nunavut, Canada during ice break up. The ambient surface water temperature at capture was 0 - 4°C

(depending on ice position) and 0°C at 2 m depth. The adult fish tested at a field laboratory in Cambridge Bay (*n* = 9) weighed 35.7 ± 2.3 g (mean ± s.e.m.) and had a fork length of 17.7 ± 0.4 cm (mean ± s.e.m.). Food was withheld for 24 h before testing. Eggs from gravid adult *B. saida* were fertilized artificially at Vancouver Aquarium on September 26, 2011. The parentage cross was a female caught in Resolute Bay by VA staff in 2008 and a male fish I caught in Cambridge Bay (see Chapter 2 for capture details). Vancouver Aquarium staff kept fertilized eggs at a constant 3.5°C water temperature in a 300 l (80 cm diameter x 60 cm deep) circular holding tank with re-circulating water and constant air provided. Earlier breeding at Vancouver Aquarium had shown that fertilized eggs could withstand temperatures to > 4°C and that larval transition from yolk absorption to live food was enhanced at 3.5°C because of a greater survivability of their food. Eggs hatched in 29-34 days following fertilization on October 25th. Larvae depleted their yolk sacs over 7 to13 days and were first fed Selco-soaked (Easy DHA Selco, Inve Aquaculture) gut-loaded rotifers *Brachionus spp's*, Leeuwenhoek 1676. Gut-loading the rotifers consisted of feeding them a solution of frozen copepods (Cyclopeeze brand, Argent) emulsified with fish flakes and water. Rotifers were also gut-loaded with algae (Innovative Aquaculture, Starter Formula). Selco-soaked, gut-loaded *Artemia,* Schlösser 1756) were introduced once the larvae had grown large enough to eat them. Frozen finely shaved krill *Euphausiid pacifica,* Hansen 1911 and Otohime pellets (Marubeni Nisshin Feed) were fed thereafter to the stabilized population. Testing began at 86 days post-fertilization (dpf) when the free-swimming larvae (6.9 ± 0.4 mg) were still translucent and about 2 months post-yolk absorption. Larvae were not fed for 24 h before testing.

4.2.2 Measurements

The methods employed to measure the rate transition temperatures for f_{Hmax} in 3.5°C-acclimated adult *B. saida* (*n* = 9) during acute warming were the same as those detailed in Drost *et al.* (2014), as adapted from Casselman *et al*. (2012). Briefly, two fish were simultaneously anesthetized in 75 mg l^{-1} tricaine methanesulfonate (MS-222; Sigma-Aldrich Products; www.sigmaaldrich.com) until they were unresponsive to a tail pinch. They were then transferred to water bath chambers where the anaesthetized state was maintained with gill irrigation using a closed seawater loop than contained MS-222. Two custom-made chromel-A electrodes were placed on the skin near the heart to record an electrocardiogram (ECG). The electrodes were connected via amplifiers (x1000; Grass P55 AC – Astro-Med Inc.; www.astro-med.ca) and filters (low pass 30 Hz with a 3 Hz transition width) to a PowerLab ML870 data acquisition unit and Labchart 7 Pro software (AD instruments; www.adinstruments.com). Water temperature, and hence fish temperature, was controlled by the chiller. The initial water temperature was set at acclimation temperature. After 1 h of recording to ensure a stable f_H, intraperitoneal injections of 10 mg kg^{-1} atropine sulfate and 8 µg kg^{-1} isoproterenol (Sigma-Aldrich Products) were administered to block any vagal inhibition of the heartbeat and maximize any β-adrenergic stimulation, respectively. Temperature was then raised incrementally by 0·5°C every 15 min., when water temperature and f_{Hmax} had stabilized. For the translucent larvae (*n* = 12), heartbeats were visually counted after an individual was transferred into and submerged 3 cm below the surface of a 250 ml water-jacketed glass chamber that contained 50 ppm MS-222 dissolved in the seawater. The seawater was kept thermally homogenized by constant mixing and aeration. The temperature was raised in increments of 1°C and, after 15 min to stabilize the heartbeats were recorded using a digital SLR Nikon DL-700 with the 12.5 mm macro lens attached to a

Zeiss dissection microscope. Preliminary experiments explored the potential to use atropine sulphate dissolved in water to block any vagal inhibition of the larval heartbeat, as it is not possible to easily inject cardioactive drugs into small larvae to produce f_{Hmax} (Randall, 1966; Miller et al., 2011).

Concentration-response tests were conducted (0.5 – 6.0 ppm; n = 4) and produced no effect on f_H, except for two concentrations (≥ 4 mg kg^{-1}) that reduced f_H, which suggested a non-specific effect. In view of these findings, no further efforts were made to pharmacologically manipulate the heartbeat as was done with the adults. Therefore, larval heart rate (f_H) is reported with the expectation that f_{Hmax} was approached, if not present, given the lack of a stimulatory effect of atropine. Larvae body mass was measured after the trial.

At each temperature increment, f_{Hmax} of adults was calculated from the R-R interval for 30 rhythmic heartbeats. For larvae, f_H was calculated from the timed interval for 30 consecutive heartbeats. As in previous studies (Casselman et al., 2012; Anttila et al., 2013), the following rate transition temperatures were calculated for each individual to provide indices of how f_H changed during acute warming. The first Arrhenius breakpoint temperature (the T_{AB}) (Yeager and Ultsch,1989) was determined by plotting the natural log of the heart rate ($\ln f_{Hmax}$) of individual fish against the inverse of temperature (1000 / K) and running best-fit regressions (SigmaPlot 11.0, Systat Software; www.sigmaplot.com) to determine the lowest temperature when the slope of the Arrhenius line decreased. The T_{AB} is considered an index of the optimum temperature (T_{opt}) for aerobic scope (Casselman et al., 2012; Ferreira et al., 2014). The transition temperature at which the incremental Q_{10} for f_H abruptly decreased and remained below 2.0 (the T_{QB}) was determined for individual fish by calculating an incremental Q_{10} for each 1°C change in temperature using:

Q_{10} = (f_H2/f_H1)^(10/(T2-T1)). A Q_{10} value of 2 is regarded as a normal rate of change of routine fish metabolism with temperature (Fry and Hochachka, 1970; Miller and Mann, 1973; Holeton, 1974). The temperature at which the heartbeat first reached its absolute maximum rate was recorded as T_{max} and the temperature at which the heart started an arrhythmic heartbeat was recorded as T_{AR}. No larval hearts became arrhythmic before warming was terminated at 8.0°C.

4.2.3 Data and statistical analysis

Rate transition temperatures were determined for each individual yet mean values were used in figures for illustration purposes only. Each rate transition index derived for adult fish was compared with larvae using one way ANOVA and $P < 0.05$ as the test for statistical significance.

The initial f_{Hmax} of anaesthetized, 3.5°C-acclimated adults at 0.5°C was 25.9 ± 0.9 bpm (Table 4.I). The f_{Hmax} increased with acute warming (Fig. 4.1.a) with T_{AB} occurring at 4.4 ± 0.4°C (Fig. 4.2.c) and T_{QB} occurring at 8.4 ± 0.9°C (Fig. 4.2.a). f_{Hmax} reached its highest absolute value with individual variability in the T_{max} (11 to 14.5°C) and averaged 12.3 ± 0.4°C. The absolute f_{Hmax} ranged from 50 to 71 bpm, with an average of 58.6 ± 2.6 bpm (Fig. 4.1.a and 4.2.a). Beyond T_{max}, f_{Hmax} decreased and then became arrhythmic with T_{AR} averaging 13.3 ± 0.4°C (Fig. 4.2.a) and more than 60% of the individual fish beginning arrhythmic heartbeats between 13 and 14°C.

4.3 Results

In anaesthetized, 3.5°C-acclimated larvae f_H was initially 36.3 ± 0.4 bpm at -1°C. At all comparable test temperatures, larval f_H was statistically different ($P < 0.05$) when compared with that for adults (1-way ANOVA). For larvae, the highest absolute value f_H was 91.3 ± 2.1 bpm and T_{max} was 7.6 ± 0.2°C (Fig. 4.2.). T_{max} was significantly lower ($P = 0.01$) when

compared with the adults (Table 4.I). The plateau in f_H (Fig. 4.2.a) was less discernable in larvae than adults and cardiac arrhythmia was not observed for any larvae.

The T_{QB} occurred at 7.4 ± 0.1°C (Fig. 4.2.b) and f_H increased exponentially with warming reaching T_{AB} at 3.3 ± 0.3°C (Fig. 4.2.c). Neither of these transition temperatures, T_{QB} or T_{AB} were significantly different (P = 0.07; P = 0.3) to those for adults (Table 4.I).

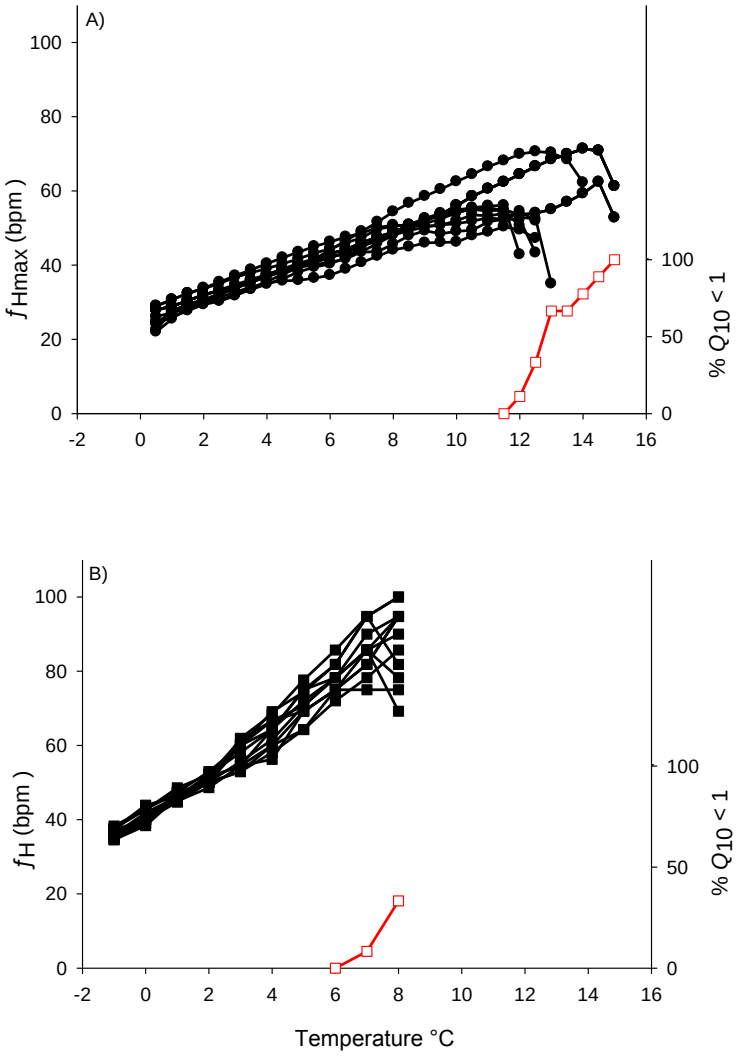

Figure 4.1 Acute warming on f_{Hmax} of Individual adults and f_H of larvae
Effect of acute warming on maximum heart rate (f_{Hmax}) of and **A)** Individual routine f_{Hmax} of *B. saida* adults (n = 9; ●) that had been acclimated to 3.5°C water temperature for 10 days. **B)** Individual routine f_H of larvae (n = 12; ■) also acclimated at 3.5°C since inception. The open red symbols show the percent of

B. saida that decreased f_{Hmax} at the highest test temperature, when Q_{10} values are less than 1.

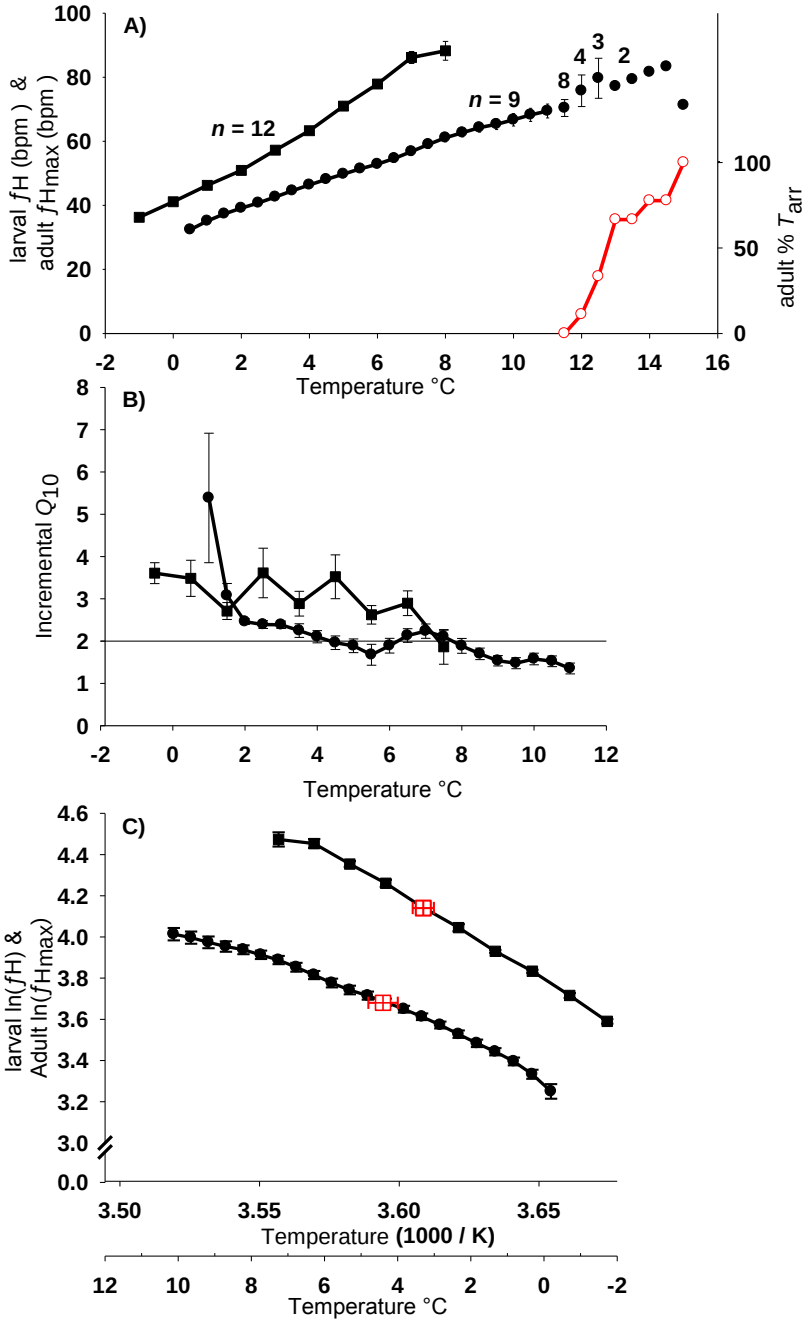

Figure 4.2 Adult and larval heart rate transition temperatures
Mean values for rate transition temperatures are used here for illustration purposes although values were determined for each individual: **A)** Adult (●) and larvae (■) mean (± s.e.m.) f_{Hmax} derived from Fig. 4.1. A solid line connects

temperature data when the sample size remains the same. The change in sample number is indicated on graph. Larvae sample size did not change (n = 12). Only the *B. saida* adult hearts went arrhythmic (T_{AR}) and thereafter removed from analysis. Also indicated on this graph is the percent occurrence of T_{AR} as water temperature increased for adults (o). **B)** The incremental Q_{10} of f_{Hmax} derived from individual data. The T_{QB} is 8.4 ± 1.0°C when break point is set at Q_{10} = 2. **C)** The Arrhenius plot of heart rate (f_{Hmax} ± s.e.m.) derived from the individual data. The first Arrhenius break point temperature (T_{AB} = 4.4 ± 0.4°C) for *B. saida* adults and (T_{AB} = 3.3 ± 0.3°C.) for the larvae overlay the data (red rectangle with middle horizontal line).

Table 4.1 Adult and larvae heart rate transition temperatures

Mean ± s.e.m. transition temperatures of *B. saida* routine heart rate f_{Hmax} for Cambridge Bay (CB) adults acclimated for 10 days to 3.5°C and 0.5°C water temperatures and (f_H) for Vancouver Aquarium laboratory (VA) larvae reared at 3.5°C water temperature.

Transition Temperature Indices	CB Adults 3.5°C n = 9	CB Adults 0.5°C n = 12	VA Larvae 3.5°C n = 12
T_{AB} (^{o}C)	4.4 ± 0.4[a]	3.2 ± 0.3[a]	3.3 ± 0.3[a]
T_{QB} (°C)	8.4 ± 0.9[a]	7.8 ± 0.6[a]	7.4 ± 0.1[a]*
T_{max} (°C)	12.3 ± 0.4[a]	10.5 ± 0.8[a]	7.6 ± 0.2[b]**
T_{AR} (°C)	13.3 ± 0.4[a]	12.4 ± 0.4[a]	Not available
0.5°C f_{Hmax} (bpm)	25.9 ± 0.9[a]	26.4 ± 0.8[a]	41.1 ± 0.5[b]***
Highest f_{Hmax} (bpm)	58.6 ± 2.6[a]	54·5 ± 2·9[a]	91.3 ± 2.1[b]**

T_{AB}, first Arrhenius breakpoint temperature; T_{QB}, incremental Q_{10} breakpoint temperature; f_{Hmax}, maximum heart rate; T_{max}, temperature for the highest f_{Hmax} recorded; T_{AR}, temperature when cardiac arrhythmia first developed. Heart rate values within an age class that are not statistically different (P > 0.05) are indicated by the same letter; *n = 8; **could be underestimated; *** 0°C water temperature for larvae f_H (bpm)

4.4 Discussion

If one accepts the idea that T_{AB} is a reasonable approximation of T_{opt} for aerobic scope, as suggested by others (see Casselman et al., 2011; Ferreira et al., 2014), the present findings indicate that 3.5°C-acclimated adult and larval *B. saida* have a T_{opt} close (4.4°C and 3.3°C) to their acclimation temperature of 3.5°C. For 0.5°C-acclimated *B. saida* adults, T_{AB} was similarly 3.2°C (Chapter 3). It is postulated that tropical ectotherms live much closer to their thermal optimum for aerobic scope than do temperate and polar species (Pörtner and Farrell, 2008). The present results support this suggestion.

I expected the heart rate of larvae would be greater than adults due to the allometric relationship between heart rate and body mass. Indeed this study supports the assumption that resting heart rate is higher in smaller fish as it is in mammals (Farrell and Jones 1992). Important differences were observed between adults and larvae even though T_{AB} and T_{QB} were similar (Table 4.I). Notably, T_{max} was 3 to 4°C warmer for adults than larvae. This difference in upper thermal tolerance supports the working hypothesis that larvae are less tolerant of warming than adults. Also, warming of adult *B. saida* to T_{AR} and larvae to T_{max} was invariably harmful, but adult *B. saida* fully recovered if warmed to just T_{max} (Chapter 3). Why adults typically developed cardiac arrhythmias as they approached their upper thermal limit but larvae did not is unclear. Individual variability in T_{max} was considerable for adults, a range of 3.5°C. This individual variability may prove important in a warming Arctic, especially if the variability has a genetic basis, as was found for land-locked Atlantic cod *Gadus morhua*, L 1758 in the stenothermal Ogac Lake (Bradbury et al., 2010).

Fertilized *B. saida* eggs develop slowly below sea ice at as low as -1.8°C (Aronovich et al., 1974; Melnikov and Chernova, 2013). At such low temperatures, hatching would not normally occur until May-June and yolk absorption would last another month. Independent feeding

would then coincide with the initial bloom of algae and the availability of copepod eggs and nauplii (Graham and Hop, 1995; Walkusz et al., 2011). This is when surface seawater temperatures begin to rise to a maximum of 11°C after ice melt (unpublished observation, 2012; Ocean Networks Canada, 2015). The present study, which is the first report of successful laboratory rearing a full life cycle of *B. saida* from eggs to adults whom in turn became reproductive, shortened the period between fertilization and depletion of egg yolk to 2 months with a 3.5°C acclimation temperature. Nonetheless, the translucent, free-swimming larvae used here may be exposed to warmer summer temperatures, making the present experiment relevant to excursions of larvae into warm surface seawater near Cambridge Bay.

In summary, the lower T_{max} of larvae compared with adults, along with the peak summer temperature, could be important determinants of the southern extent of the *B. saida* habitat range. Furthermore, unless the nearly 3-month old larvae can thermally acclimate, they may not live at their T_{opt} (near 3.5°C) until after the polar ice breaks up and the surface water warms. The potential for thermal acclimation of larvae was not tested here due to limited resources and difficulty of laboratory rearing. The comparison of results for adult *B. saida* acclimated to 0.5°C (Chapter 3) and 3.5°C reveals a remarkable similarity for both T_{AB}, T_{QB} and T_{AR} (Table I). Yet Fry (1971) stated that fish that can acclimate to 3°C warmer water typically have an increase of ~1°C in upper temperature tolerance. A similar trend was noted between the 0.5 and 3.5°C acclimated groups of fish studied in Cambridge Bay (CB - see Table 4.1).

Chapter 5: Conclusions

In response to the growing concern about the rapid warming of the Arctic Ocean, this study quantified the thermal limits and acclimation responses of *Boreogadus saida*, a key Arctic food web fish. Physiological rates for cardio-respiratory functions MO_2 (the rate of oxygen uptake measured in mg) and f_{Hmax} (maximum heart rate) were compared with T_{cmax} (critical temperature maximum for loss of equilibrium) at 3 acclimation temperatures. The transition temperatures for these functions during acute warming were used to gauge phenotypic plasticity after thermal acclimation between 0.5°C and 6.5°C for 1 month (T_{cmax} and MO_2 measurements) and 6 months (f_{Hmax} measurements).

As hypothesized, adult *B. saida* exhibit the potential to acclimate to warmer temperatures. This acclimation potential was evident by the 2.3°C increase in T_{cmax} from 14.9°C to 17.1°C, a 4.5°C increase in AAS, and a down regulation of 6 ± 0.1 bpm in f_{Hmax} (from 0.5 to 8°C test temperatures) in 6.5°C-acclimated fish compared with 0.5°C-acclimated fish. A slope value (0.42) derived from regression analysis of the 3 acclimation T_{cmax} measurements was similar to that found for 20 species of North American freshwater fish and 8 species of Antarctic fish (Beitinger et al., 2000; Bilyk and DeVries, 2011; Chapter 2). Perhaps this slope of acclimation to critical temperatures is specific to teleosts.

Another way to assess the vulnerability to climate warming is by calculating the Acclimation Potential Index (API) to estimate a species phenotypic plasticity (Seebacher et al., 2015). The calculated index as depicted in Figure 5.1 is based on the rate equation to determine Q_{10} (see Chapter 2 for details) wherein total acclimation is defined as $Q_{10} = 1$. The results of this study suggests that the cardiac system of *B. saida* can acclimate up to water temperatures of 6.5°C.

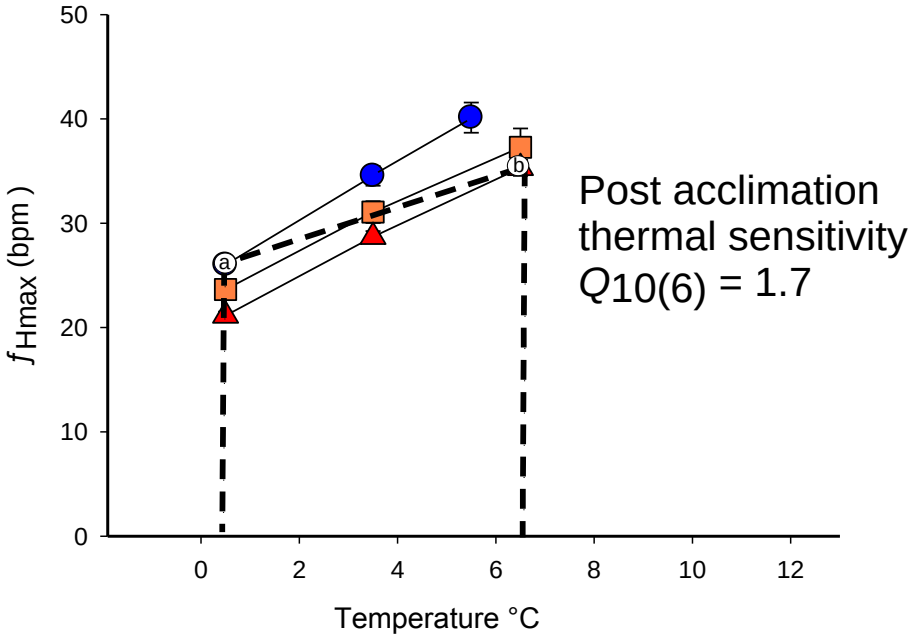

Figure 5.1 Acclimation Potential Index (API)
The acclimation group 1.0°C (point a: blue circles), 3.5°C (orange squares) and 6.5°C (point b: red triangles). The points a & b are the values used in the calculation. API equation $Q_{10} = (R_{2,2}/R_{1,1})^{(10/(T2- T1))}$, where $R_{2,2}$ is the heart rate of warm acclimated (6.5°C) fish at 6.5°C acute test temperature (T_2); $R_{1,1}$ is the heart rate of cold acclimated (0.5°C) fish at 0.5°C acute test temperature (T_1)

Another indicator of acclimation potential was the finding that, after one month acclimation, there was no difference in AAS between the 0.5 and 3.5°C-acclimated fish (Fry Curve - Figure 5.2). This equality in AAS values represents perfect compensation to a 3°C change in water temperature (i.e. when Q_{10} = 1) but when acclimated to 6.5°C, the capacity for aerobic scope increased by ~20%. Despite this increase in aerobic capacity at 6.5°C water temperatures and despite the statistically lower excess post-exercise oxygen consumption (EPOC) values these fish had after 30 mins. post exercise, over 50% of the 6.5°C acclimated fish died after being chased at the highest test temperature (8°C). Could acclimation to warmer temperatures exact a cost to the whole animal? Further investigation is required. However, no cost was noted in larvae reared at 3.5°C (see Appendix B). Larvae grew to

reproductive adulthood without any apparent detrimental effects. If it is assumed they develop at -1.8°C water temperatures in the wild, rearing at 3.5°C represents ~5°C acclimation.

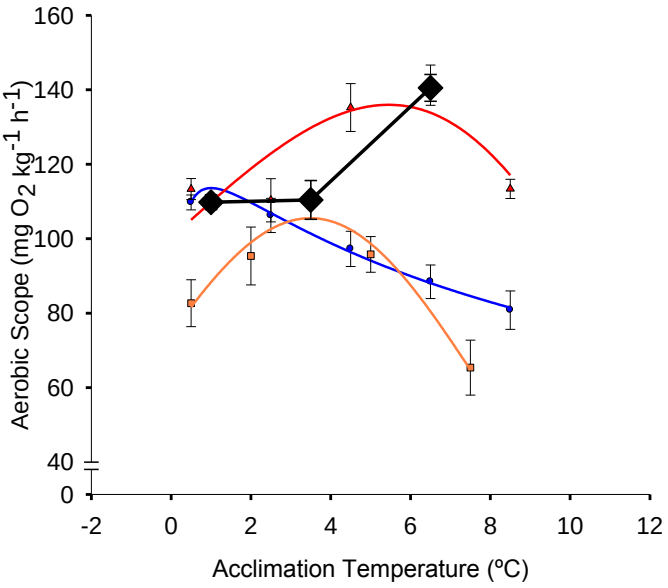

Figure 5.2 *Boreogadus saida* AAS Fry curves.
The blue, orange and red curves reflect the average (± s.e.m.) of 1 month acclimation groups (1°C, blue circle; 3.5°C, orange square; 6.5°C, red upward triangle). The black diamonds connected by the black line are the AAS values of fish measured at their acclimation temperatures.

While testing the hypothesis that larvae were less tolerant than adults to warm temperatures, I found that, while they indeed had a lower T_{max} than adults, they were generally more resilient to acute changes in water temperature than expected. Having a T_{max} of ~8°C (possibly underestimated) was a surprising discovery considering they usually remain, for at least the first year of development, beneath sea ice in -1.8°C water temperatures (Lønne and Gulliksen, 1989; Melnikov and Chernova, 2013; David et al., 2015).

This thesis also demonstrated the practicality of using a relatively fast, field-friendly technique to measure maximally stimulated heart rates of fish (Casselman et al., 2012) for the first time in cold Arctic marine water. I also have shown, in Chapter 3, the potential to

relocate *B. saida* to a southern laboratory location without a major disruption to their cardiac physiology.

A main goal of this thesis was to compare the AAS and the f_{Hmax} methods. I wanted to see if the AAS method could be replaced, particularly in remote and logistically expensive field settings, by the f_{Hmax} method. This method is more practical and can generate more data in a given time period. I found that the respiratory and cardiac performance levels plateau within similar, well defined, thermal windows (Figure 5.3). Yet within the optimum window and certainly in the upper thermal limits the two methods were complimentary but not identical.

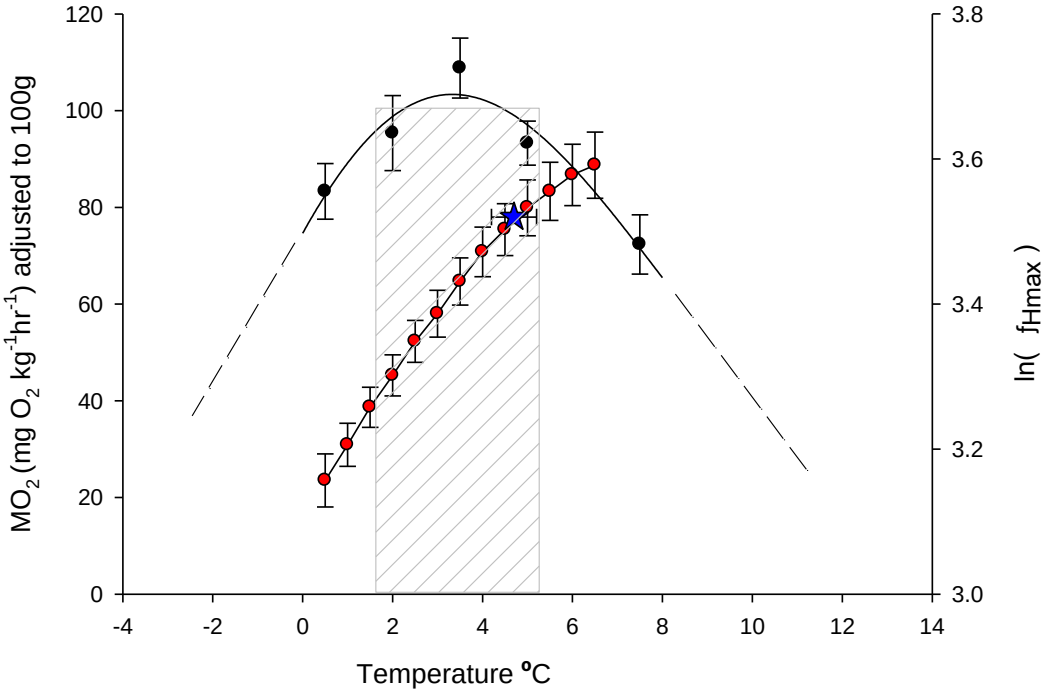

Figure 5.3 Comparing 3.5°C-acclimated *B. saida* T_{opt} with T_{AB}
Heart rate ($f_{Hmax;}$ red circle) and Absolute Aerobic Scope (AAS; black circle) mean values ± s.e.m. The blue star highlights the Arrhenius break point temperature (T_{AB}) which situates within the T_{opt} window (grey shaded area)

In fact, there appears to be a fixed hierarchy of transition temperatures and these transition temperature limits tend not to overlap - apart from 2 exceptions (see the Fry

thermal polygon for *B. saida* - Figure 2.6). The first exception was the inverted relationship between T_{AB} and T_{opt} for 6.5°C-acclimated fish compared to the 1.0 and 3.5°C-acclimated fish. T_{AB} occurred at higher water temperatures than T_{opt} for the latter two acclimations but the 6.5°C fish heart rate began to decrease, despite increasing water temperatures, ~1°C before T_{opt} temperatures were reached.

Munoz et al., 2014 found that the T_{AB} index changes with acclimation for pacific salmon species. This was not the case for adult *B. saida* acclimated for 6 months before testing the change in heart rate with acute warming. Their T_{AB}, unlike T_{opt}, was independent of acclimation temperature (Chapter 2) yet it was always situated within the T_{opt} window. Furthermore, the larval T_{AB} (3.3 ± 0.3°C) was not significantly different from adults (Chapter 4). The T_{AB} values of B. saida matched the observations of preferred temperature in the laboratory and in the wild, whereas T_{opt} reflected the temperature that the fish were acclimated to and the T_{cmax} values exceeded historical and present day maximum summer sea surface water temperatures (SST). Rather than critical maximum temperatures it is the lower transition temperature limits (T_{opt} and T_{AB}) of *B. saida* that may dictate their future migration and survival.

For over a century water temperature has been regarded as one of the main drivers of fish distribution (Davenport and Castle, 1895). Of all the transition temperatures calculated in this study, it is the T_{opt} window range and specifically the T_{AB} index (which situates within the T_{opt} window) that can explain recent observations of the northward migration of *B. saida*. They are moving away from regions where SST is rapidly increasing. Temperature anomalies as high as 7°C have been verified (Steele et al., 2008; Timmermans and Proshutinsky, 2014). Present day water temperatures could be directly influencing *B. saida* distribution in addition to the reduction in summer sea ice extent and potential changes in prey and predator

composition and distribution as well as other variables associated with climate change (e.g. declining pH and O_2 concentrations).

The second exception to the fixed hierarchy of the transition temperatures was found with the new T_{QR} index I introduced in this study. This index measures the decline in EKG peak height at elevated water temperatures. T_{QR} was similar in value to the theoretical T_{upej} (arbitrarily set at 90% of T_{opt}) and the incremental $Q_{10,}$ which I defined as the point when Q_{10} values drop, irrevocably, below 2. These three indices overlapped each other, particularly for the 3.5°C-acclimated fish. Perhaps T_{QR} provides a mechanistic explanation for this significant tipping point in the deterioration in cardiac performance.

Given that it is reasonable to assume that the change in MO_2 is indeed directly proportional to f_H, I actually measured f_{Hmax} because I wanted to determine the limits of the heartbeat. When I compare the changes in MMR and f_{Hmax} what I discovered was there are complex relationships (Figure 5.4) that are worthy of further study. Thorarensen et al. (1996) concluded that the relationship between oxygen uptake and heart rate in chinook salmon was

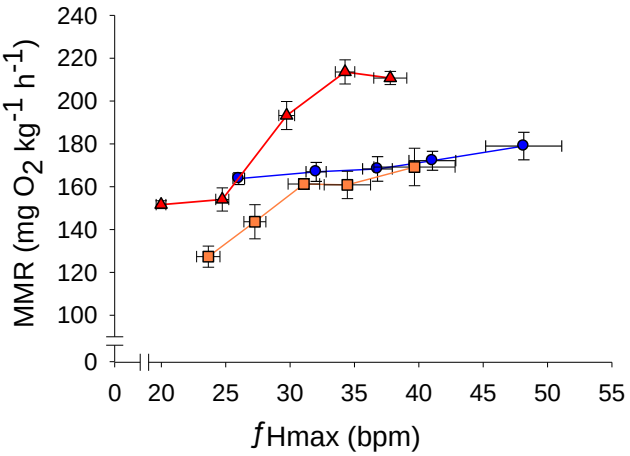

Figure 5.4 Respiratory (MO2) and cardiac (f_{Hmax}) comparison
Comparison of MMR and f_{Hmax} at 5 acute test temperatures recorded after chasing fish that were acclimated for 1 month intervals to water temperatures (1°C: ●, 3.5°C: ■, 6.5°C:▲).

too variable to be considered interchangeable whereas Butler et al. (2002) considered the relationship between heart rate and MO_2 (for air breathing iguanas) was sufficiently described by the simplified Fick equation as more than 50% of the change in MO_2 was accounted for by heart rate. I concluded that both methods provide data that are uniquely informative and not necessarily interchangeable especially when spanning different acclimation temperatures.

I have demonstrated that the 3 relatively simple, non-lethal methods (T_{cmax}, AAS and f_{Hmax}) provide a detailed description of the upper thermal limits of *B. saida*. These experiments can be conducted in laboratories with limited resources and could be modified slightly to include various life stages, multi-stressors and multi-generations. In this era of rapid climate change, it is important to quantify key food web species range in thermal tolerance and their overall acclimation potential to improve our understanding of realized niches.

Quantifying transition temperatures will also help improve model predictions of ectotherm species future migration and survival potential. Knowing the temperature limits of key food web species adds to our understanding of overall food web dynamics. This information could help inform the public, particularly subsistence hunters and resource managers, about food security in a rapidly changing climate.

References

Aho, E. and Vornanen, M. (2001). Cold acclimation increases basal heart rate but decreases its thermal tolerance in rainbow trout (*Oncorhynchus mykiss*). *Journal of Comparative Physiology B* **171**, 173-179.

Alabaster, J. S. and Welcome, R. L. (1962). Effect of concentration of dissolved oxygen on survival of trout and roach in lethal temperatures. *Nature*, London **194**, 107

Angel, M.V. (1991). Variations in time and space: is biogeography relevant to studies of long-time scale change? *Journal of the Marine Biological Association of the United Kingdom* **71**:191-206.

Anttila, K., Casselman, M. T., Schulte, P. M. and Farrell, A. P. (2013). Optimum temperature in juvenile salmonids: connecting subcellular indicators to tissue function and whole-organism thermal optimum. *Physiological and Biochemical Zoology* **86**, 245-256.

Anttila, K., Couturier, C. S., Overli, O., Johnsen, A., Marthinsen, G., Nilsson, G. E. and Farrell, A. P. (2014). Atlantic salmon show capability for cardiac acclimation to warm temperatures. *Nature Communications* **5**, 4252

Aronovich, T. M., Doroshev, S. I., Spectorova, L. V. and Makhotin, V. M. (1975). Egg incubation and larval rearing of navaga (*Eleginus navaga* Pall.), polar cod (*Boreogadus saida* lepechin) and Arctic flounder (*Liopsetta glacialis* Pall.) in the laboratory. *Aquaculture* **6**, 233-242.

Arrigo, K. R. (2014). Sea Ice Ecosystems. *Annual Review of Marine Science,* **6**, 439-467.

Astthorsson, O. S. (2015). Distribution, abundance and biology of polar cod, *Boreogadus saida*, in Iceland–East Greenland waters. *Polar Biology*, **39**, 995-1003.

Axelsson, M., Davison, W., Forster, M. E., Farrell, A. P. (1992). Cardiovascular responses of the red-blooded Antarctic fishes *Pagothenia bernacchii* and *P. borchgrevinki*. *Journal of Experimental Biology* **167**, 179-201.

Badr, A., El-Sayed, M. F. and Vornanen, M. (2016). Effects of seasonal acclimatization on temperature dependence of cardiac excitability in the roach, *Rutilus rutilus*. *Journal of Experimental Biology* **219**, 1495-1504.

Bain, H. and Sekerak, A. D. (1978). Aspects of the biology of Arctic cod, *Boreogadus saida*, in the central Canadian Arctic. Unpubl. Rep. by LGL Ltd., Toronto, for Polar Gas Project, Toronto, 104.

Barber, D. G., Hop, H., Mundy, C. J., Else, B., Dmitrenko, I. A., Tremblay, J. E., Ehn, J. K., Assmy, P., Daase, M., Candlish, L. M. et al. (2015). Selected physical, biological and biogeochemical implications of a rapidly changing Arctic Marginal Ice Zone. *Progress in*

Oceanography **139**, 122-150.

Beitinger, T. L., Bennett, W. A. and McCauley, R. W. (2000). Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. *Environmental Biology of Fishes* **58**, 237-275.

Berge, J., Heggland, K., Lonne, O. J., Cottier, F., Hop, H., Gabrielsen, G. W., Nottestad, L. and Misund, O. A. (2015). First records of Atlantic Mackerel (*Scomber scombrus*) from the Svalbard Archipelago, Norway, with possible explanations for the extension of its distribution. *Arctic* **68**, 54-61.

Bilyk, K. T. and DeVries, A. L. (2011). Heat tolerance and its plasticity in Antarctic fishes. *Comparative Biochemistry and Physiology a- Molecular & Integrative Physiology* **158**, 382-390.

Blank, J. M., Morrissette, J. M., Landeira-Fernandez, A. M., Blackwell, S. B., Williams, T. D. and Block, B. A. (2004). In situ cardiac performance of Pacific bluefin tuna hearts in response to acute temperature change. *Journal of Experimental Biology* **207**, 881-890.

Bouchard, C. & Fortier, L. (2011). Circum-arctic comparison of the hatching season of polar cod *Boreogadus saida*: A test of the freshwater winter refuge hypothesis. *Progress in Oceanography* **90**, 105-116.

Braby, C. E. and Somero, G. N. (2006). Following the heart: temperature and salinity effects on heart rate in native and invasive species of blue mussels (genus *Mytilus*). *Journal of Experimental Biology* **209**, 2554-2566.

Bradbury, I. R., Hubert, S., Higgins, B., Borza, T., Bowman, S., Paterson, I. G., Snelgrove, P. V. R., Morris, C. J., Gregory, R. S., Hardie, D. C. et al. (2010). Parallel adaptive evolution of Atlantic cod on both sides of the Atlantic Ocean in response to temperature. *Proceedings of the Royal Society B-Biological Sciences* **277**, 3725-3734.

Bradstreet, M. S., Finley, K. J., Sekerak, A. D., Griffiths, W. B., Evans, C. R., Fabijan, M. F. and Stallard, H. E. (1986). Aspects of the biology of *Boreogadus saida* and its importance in Arctic marine food chains, pp. 1-192. Winnipeg: Central and Arctic Region, Department of Fisheries and Oceans.

Bremer, K., Melzner, F., Lucassen, M. and Pörtner, H. (2007). Thermal acclimation of aerobic scope in a southern North Sea cod population. *Comparative Biochemistry and Physiology a- Molecular & Integrative Physiology* **146**, S207-S208.

Brett, J. R. (1962). Some considerations in the study of respiratory metabolism in fish, particularly salmon. *Journal of the Fisheries Research Board of Canada* **19**, 1025-1038.

Burgess, E.A., Booth, D.T. and Lanyon, J.M. (2006) Swimming performance of hatchling green turtles is affected by incubation temperature. *Coral Reefs* **25**:341-349.

Burggren W. W., and Reyna K. S. (2011). Developmental trajectories, critical windows and phenotypic alteration during cardio-respiratory development. *Respiratory Physiology & Neurobiology* **178**:13–21.

Butler, P. J., Frappell, P. B., Wang, T. & Wikelski, M. (2002). The relationship between heart rate and rate of oxygen consumption in Galapagos marine iguanas (*Amblyrhynchus cristatus*) at two different temperatures. *Journal of Experimental Biology* **205**, 1917-1924.

Campbell, H., Davison, W., Fraser, K. P. P., Peck, L. S. and Egginton, S. (2009). Heart rate and ventilation in Antarctic fishes are largely determined by ecotype. *Journal of Fish Biology* **74**, 535-552.

Carmack, E. and McLaughlin, F. A. (2001). Arctic Ocean change and consequences to biodiversity: A perspective on linkage and scale. *Memoirs of National Institute of Polar Research* **54**, 365-375.

Carmack, E., I. Polyakov, L. Padman, I. Fer, E. Hunke, J. Hutchings, J. Jackson, D. Kelley, R. Kwok, C. Layton, H. Melling, D. Perovich, O. Persson, B. Ruddick, M.L. Timmermans, J. Toole, T. Ross, S. Vavrus, and P. Winsor. (2015). Towards quantifying the increasing role of oceanic heat in sea ice loss in the new Arctic. *Bulletin of the American Meteorological Society* **96**, 2079–2105.

Carmack, E. C., Yamamoto-Kawai, M., Haine, T. W. N., Bacon, S., Bluhm, B. A., Lique, C., Melling, H., Polyakov, I. V., Straneo, F., Timmermans, M. L. and Williams, W. J. (2016). Freshwater and its role in the Arctic Marine System: Sources, disposition, storage, export, and physical and biogeochemical consequences in the Arctic and global oceans. *Journal of Geophysical Research: Biogeosciences* **121**, 675-717.

Carson, M., M. Sommerkorn, C. Behe, S. Cornell, J. Gamble, T. Mustonen, G. Peterson, T. Vlasova, and F.S. Chapin. (2016). Chp. 1. An Arctic resilience assessment. In Arctic resilience report: Arctic council. Eds. M. Carson, and G. Peterson, Stockholm Environment Institute and Stockholm Resilience Centre, Stockholm.

Casselman, M. T., Anttila, K. and Farrell, A. P. (2012). Using maximum heart rate as a rapid screening tool to determine optimum temperature for aerobic scope in Pacific salmon *Oncorhynchus* spp. *Journal of Fish Biology* **80**, 358-377.

Chen, Z., Anttila, K., Wu, J., Whitney, C. K., Hinch, S. G. and Farrell, A. P. (2013). Optimum and maximum temperatures of sockeye salmon (*Oncorhynchus nerka*) populations hatched at different temperatures. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* **91**, 265-274.

Chen, Z. Q., Snow, M., Lawrence, C. S., Church, A. R., Narum, S. R., Devlin, R. H. and Farrell, A. P. (2015). Selection for upper thermal tolerance in rainbow trout (*Oncorhynchus mykiss* Walbaum). *Journal of Experimental Biology* **218**, 803-812.

Cheung, W. L., Lam, V. W. and Pauly, D. (2008). Modelling present and climate shifted distribution of marine fishes and invertebrates. *Fisheries Centre Research Reports* **16**, 72.

Christiansen, J. S., Dalmo, R. A. and Ingebrigtsen, K. (1996). Xenobiotic excretion in fish with aglomerular kidneys. *Marine Ecology Progress Series* **136**, 303-304.

Claireaux, G. and Lefrancois, C. (2005). Ecological implications of environmental influences on the aerobic metabolic scope of fish. *Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology* **141**:S165-S165.

Claireaux, G., Webber, D. M., Lagardere, J. P. and Kerr, S. R. (2000). Influence of water temperature and oxygenation on the aerobic metabolic scope of Atlantic cod (*Gadus morhua*). *Journal of Sea Research* **44**, 257-265.

Clark, T. D., Sandblom, E., Cox, G. K., Hinch, S. G. and Farrell, A. P. (2008). Circulatory limits to oxygen supply during an acute temperature increase in the Chinook salmon (*Oncorhynchus tshawytscha*). *American Journal of Physiology-Regulatory Integrative and Comparative Physiology* **295**, R1631-R1639.

Coad, B. W. and Reist, J. D. (2004). Annotated list of the Arctic marine fishes of Canada. *Canadian Manuscript Reports Fish and Aquatic Science* **2674**, 112.

Comeau, S., G. Gorsky, R. Jeffree, J.L. Teyssié, and J.P. Gattuso. (2009). Impact of ocean acidification on a key Arctic pelagic mollusc (*Limacina helicina*). *Biogeosciences* **6**,1877-1882.

Craig, P. C., Griffiths, W. B., Haldorson, L. and McElderry, H. (1982). Ecological studies of *Boreogadus saida* in Beaufort Sea coastal waters, Alaska. *Canadian Journal of Fisheries and Aquatic Sciences* **39**, 395-406.

Crawford, R. E. and Jorgenson, J. K. (1996). Quantitative studies of *Boreogadus saida* schools: Important energy stores in the Arctic food web. *Arctic* **49**, 181-193.

Crawford, R. E., Vagle, S. and Carmack, E. C. (2012). Water mass and bathymetric characteristics of polar cod habitat along the continental shelf and slope of the Beaufort and Chukchi seas. *Polar Biology* **35**, 179-190.

Crozier, W. J. (1924). On biological oxidations as function of temperature. *The Journal of General Physiology* **7**, 189-216.

Davenport, C. B. and Castle, W. E. (1895). On the acclimatization of organisms to high temperatures. *Arch Entwick - lungsmech*. Org. **2**, 227-249.

David, C., Lange, B., Krumpen, T., Schaafsma, F., van Franeker, J. and Flores, H. (2015). Under-ice distribution of polar cod *Boreogadus saida* in the central Arctic Ocean and their association with sea-ice habitat properties. *Polar Biology* **39**, 981-994.

Davison, W., Franklin, C. E., McKenzie, J. C. (1994). Haematological changes in an Antarctic teleost, *Trematomus bernacchi* following stress. *Polar Biology* **14**, 463-466.

Davison, W., Axelsson, M., Forster, M., Nilsson, S. (1995). Cardiovascular responses to acute handling stress in the Antarctic fish *Trematomus bernacchii* are not mediated by circulatory catecholamines. *Fish Physiology and Biochemistry* **14**, 253-257.

Del Raye, G. and Weng, K. C. (2015). An aerobic scope-based habitat suitability index for predicting the effects of multi-dimensional climate change stressors on marine teleosts. *Deep Sea Research Part II: Topical Studies in Oceanography* **113**, 280-290.

Deutsch, C., Ferrel, A., Seibel, B., Pörtner, H.-O. and Huey, R. B. (2015). Climate change tightens a metabolic constraint on marine habitats. *Science* **348**, 1132-1135.

DeVries, A. L. and Steffensen, J. F. (2005). The Arctic and Antarctic polar marine environments. In *The physiology of polar fishes*, vol. 22 eds. A.P. Farrell and J. F. Steffensen), pp. 1- 22. New York and London: Academic Press.

Dionisio, G., Campos, C., Valente, L. M. P., Conceicao, L. E. C., M. L. Cancela, M. L. and P. J. Gavaia, P. J. (2012). Effect of egg incubation temperature on the occurrence of skeletal deformities in *Solea senegalensis*. *Journal of Applied Ichthyoloy* **28**, 471-4766.

Donelson, J. M., Munday, P. L., McCormick, M. I. and Nilsson, G. E. (2011). Acclimation to predicted ocean warming through developmental plasticity in a tropical reef fish. *Global Change Biology* **17**, 1712-1719.

Donelson, J. M. (2015). Development in a warm future ocean may enhance performance in some species. *Journal of Experimental Marine Biology and Ecology* **472**, 119-125.

Drost, H. E., Carmack, E. C. and Farrell, A. P. (2014). Upper thermal limits of cardiac function for *Boreogadus saida*, a key food web fish species in the Arctic Ocean. *Journal of Fish Biology* **84**, 1781-1792.

Drost, H. E., Fisher, J., Randall, F., Kent, D., Carmack, E. C. and Farrell, A. P. (2015). Upper thermal limits of the hearts of *Boreogadus saida*: adults compared with larvae. *Journal of Fish Biology* **88**, 718-726.

Drost, H.E., M. Lo, E.C. Carmack, and A.P. Farrell. (2016). Acclimation potential of Arctic cod (*Boreogadus saida*) from the rapidly warming Arctic Ocean. *Journal of Experimental Biology* **219,** 3114-3125.

Du, W.-G., Ye, H., Zhao, B., Warner, D. A. and Shine, R. (2010). Thermal acclimation of heart rates in reptilian embryos. *Plos One* **5**, e15308.

Eliason, E. J., Clark, T. D., Hague, M. J., Hanson, L. M., Gallagher, Z. S., Jeffries, K. M., Gale, M. K., Patterson, D. A., Hinch, S. G. and Farrell, A. P. (2011). Differences in thermal tolerance among sockeye salmon populations. *Science* **332**, 109-112.

Eliason, E. J., Wilson, S. M., Farrell, A. P., Cooke, S. J. and Hinch, S. G. (2013). Low cardiac and aerobic scope in a coastal population of sockeye salmon *Oncorhynchus nerka* with a short upriver migration. *Journal of Fish Biology* **82**, 2104-2112.

Fabry, V.J., B.A. Seibel, R.A. Feely, and J.C. Orr. (2008). Impacts of ocean acidification on marine fauna and ecosystem processes. *ICES Journal of Marine Science* **65**, 414–432.

Falardeau, M., Robert, D. & Fortier, L. (2014). Could the planktonic stages of polar cod and Pacific sand lance compete for food in the warming Beaufort Sea? *ICES Journal of Marine Science* **71**, 1956-1965.

Farrell, A. P. (1997). Effects of temperature on cardiovascular performance. In *Global warming: implications for freshwater and marine fish*, Eds. C. M. Wood and D. G. McDonald, pp. 135-158. Cambridge, U.K.: Cambridge Univ. Press.

Farrell, A. P. (2002). Cardiorespiratory performance in salmonids during exercise at high temperature: insights into cardiovascular design limitations in fishes. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **132**, 797-810.

Farrell, A. P. and Steffensen, J. F. (2005). The Physiology of Polar Fishes: Gulf Professional Publishing. **22**, xi-xii

Farrell, A. P. (2009). Environment, antecedents and climate change: lessons from the study of temperature physiology and river migration of salmonids. *Journal of Experimental Biology* **212**, 3771-3780.

Farrell, A. P., Altimiras, J., Franklin, C. E., Axelsson, M. (2013). Niche expansion of the shorthorn sculpin to Arctic waters is supported by a thermal independence of cardiac performance at low temperature *Canadian Journal of Zoology* **91**, 1-8.

Farrell, A. P. (2016). Pragmatic perspective on aerobic scope: peaking, plummeting, pejus and apportioning. *Journal of Fish Biology* **88**, 322-343.

Farrell, A. P., Eliason, E. J., Sandblom, E. and Clark, T. D. (2009). Fish cardiorespiratory physiology in an era of climate change. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* **87**, 835-851.

Farrell, A.P. and Jones, D.R. (1992). The heart. In: Fish Physiology, Vol. XIIA., Eds. W.S. Hoar, D.J. Randall and A.P. Farrell. Academic Press, San Diego. pp. 1-88.

Feely, R.A., S.R. Alin, B. Carter, N. Bednaršek, B. Hales, F. Chan, T.M. Hill, B. Gaylord, E. Sanford, R.H. Byrne, C.L. Sabine, D. Greeley, and L. Juranek. (2016). Chemical and biological impacts of ocean acidification along the west coast of North America. *Estuarine, Coastal and Shelf Science* **183**, Part A:260-270.

Ferreira, E. O., Anttila, K. and Farrell, A. P. (2014). Thermal optima and tolerance in the

eurythermic goldfish (*Carassius auratus*): Relationships between whole-animal aerobic capacity and maximum heart rate. *Physiological and Biochemical Zoology* **87**, 599-611.

Forster, M. E., Franklin, C. E., Taylor, H. H. and Davison, W. (1987). The aerobic scope of an Antarctic fish, *Pagothenia borchgrevinki* and its significance for metabolic cold adaptation. *Polar Biology* **8**, 155-159.

Franklin, C. E., Davison, W. and Seebacher, F. (2007). Antarctic fish can compensate for rising temperatures: thermal acclimation of cardiac performance in *Pagothenia borchgrevinki*. *Journal of Experimental Biology* **210**, 3068-3074.

Frederich, M. and Pörtner, H. O. (2000). Oxygen limitation of thermal tolerance defined by cardiac and ventilatory performance in spider crab, *Maja squinado*. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* **279**, 1531-1538.

Fry, F. E. J. (1947). Effects of the environment on animal activity. *University of Toronto Studies Biological Series* **No. 55**, 1-62.

Fry, F. E. J. (1971). The effect of environmental factors on the physiology of fish. In *Fish Physiology*, vol. 6, Eds. W. S. Hoar and D. J. Randall), pp. 1-98. New York, NY: Academic Press.

Fry, F. E. J. and Hart, J. S. (1948). The relation of temperature to oxygen consumption in the goldfish. Biological Bulletin Wood's Hole **94**, 66-77.

Fry, F. E. J. and Hochachka, P. W. (1970). Fish. *Comparative physiology of thermoregulation Vol. 1. Invertebrates and nonmammalian vertebrates* 79 -134.

Gade, H.G., R.A. Lake, E.L. Lewis, and E.R. Walker. (1974). Oceanography of an Arctic bay. *Deep Sea Research and Oceanographic Abstracts* **21,** 547-571.

Gamperl, A.K. (2011). Integrated control and response of the circulatory system: Integrated responses of the circulatory system to hypoxia In *Encyclopedia of Fish Physiology*. A.P. Farrell, editor Academic Press, San Diego. 1221-1228

Gaston, A. J., Woo, K. and Hipfner, J. M. (2003). Trends in forage fish populations in northern Hudson Bay since 1981, as determined from the diet of nestling thick-billed murres *Uria lomvia*. *Arctic* **56**, 227-233.

Geoffroy, M., Majewski, A., LeBlanc, M., Gauthier, S., Walkusz, W., Reist, J. D. & Fortier, L. (2016). Vertical segregation of age-0 and age-1+ polar cod (*Boreogadus saida*) over the annual cycle in the Canadian Beaufort Sea. *Polar Biology* **39**, 1023-1037.
Graham, M. and Hop, H. (1995). Aspects of reproduction and larval biology of Arctic cod (*Boreogadus saida*). *Arctic* **48**, 130-135.

Grebmeier, J. M., Overland, J. E., Moore, S. E., Farley, E. V., E. C. Carmack, L. W. Cooper, K. E. Frey, J. H. Helle, F. A. McLaughlin, S. L. McNutt. (2006). A major

ecosystem shift in the Northern Bering Sea. *Science* **311**, 1461-1464.

Hansen, M. O., Nielsen, T. G., Stedmon, C. A. and Munk, P. (2012). Oceanographic regime shift during 1997 in Disko Bay, Western Greenland. *Limnology and Oceanography* **57**, 634-644.

Haverinen, J. and Vornanen, M. (2007). Temperature acclimation modifies sinoatrial pacemaker mechanism of the rainbow trout heart. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* **292**, R1023-R1032.

Heath, A. G. and Hughes, G. M. (1973). Cardiovascular and respiratory changes during heat stress in rainbow trout. *Journal of Experimental Biology* **59**, 323-338.

Hinds, D. S., Baudinette, R. V., Macmillen, R. E. and Halpern, E. A. (1993). Maximum metabolism and the aerobic factorial scope of endotherms. *Journal of Experimental Biology* **182**, 41-56.

Holeton, G. F. (1974). Metabolic cold adaptation of polar fish - fact or artifact. *Physiological Zoology* **47**, 137-152.

Holt, R. D. (2003). On the evolutionary ecology of species' ranges. *Evolutionary Ecology Research* **5**, 159-178.

Hop, H. and Gjøsæter, H. (2013). Polar cod (*Boreogadus saida*) and capelin (*Mallotus villosus*) as key species in marine food webs of the Arctic and the Barents Sea. *Marine Biology Research* **9**, 878-894.

Hop, H. and Graham, M. (1995). Respiration of juvenile Arctic cod (*Boreogadus saida*) - effects of acclimation, temperature and food intake. *Polar Biology* **15**, 359-367.

Hop, H. & Tonn, W. M. (1998). Gastric evacuation rates and daily rations of Arctic cod (*Boreogadus saida*) at low temperatures. *Polar Biology* **19**, 293-301.

Hop, H., Tonn, W. M. and Welch, H. E. (1997). Bioenergetics of Arctic cod (*Boreogadus saida*) at low temperatures. *Canadian Journal of Fisheries and Aquatic Sciences* **54**, 1772-1784.

Hutchings, J. A., Côté, I. M., Dodson, J. J., Fleming, I. A., Jennings, S., Mantua, N. J., Peterman, R. M., Riddell, B. E. and Weaver, A. J. (2012). Climate change, fisheries, and aquaculture: trends and consequences for Canadian marine biodiversity. *Environmental Reviews* **20**, 220-311.

IPCC, (2013). *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 1535 pp, doi:10.1017/CBO9781107415324.

Iftikar, F. I. and Hickey, A. J. R. (2013). Do mitochondria limit hot fish hearts? Understanding the role of mitochondrial function with heat stress in *Notolabrus celidotus*. *Plos One* **8**, e64120

Johnston, I. A. and Temple, G. K. (2002). Thermal plasticity of skeletal muscle phenotype in ectothermic vertebrates and its significance for locomotory behaviour. *Journal of Experimental Biology* **205**, 2305-2322.

Kent, D., Drost, H. E., Fisher, J., Oyama, T. and Farrell, A. P. (2016). Laboratory rearing of wild *Boreogadus saida* from egg to adulthood. *Journal of Fish Biology,* **88**:1241-1248.

Kerr, S. R. (1976). Ecological analysis and the Fry paradigm. *Journal of the Fisheries Research Board of Canada* **33**, 329-335.

Killen, S. S., Mitchell, M. D., Rummer, J. L., Chivers, D. P., Ferrari, M. C. O., Meekan, M. G. and McCormick, M. I. (2014). Aerobic scope predicts dominance during early life in a tropical damselfish. *Functional Ecology* **28**, 1367-1376.

Krishfield, R. A., Proshutinsky, A., Tateyama, K., Williams, W. J., Carmack, E. C., McLaughlin, F. A. and Timmermans, M. L. (2014). Deterioration of perennial sea ice in the Beaufort Gyre from 2003 to 2012 and its impact on the oceanic freshwater cycle. *Journal of Geophysical Research-Oceans* **119**, 1271-1305.

Kunz, K. L., Frickenhaus, S., Hardenberg, S., Johansen, T., Leo, E., Pörtner, H.-O., Schmidt, M., Windisch, H. S., Knust, R. and Mark, F. C. (2016). New encounters in Arctic waters: a comparison of metabolism and performance of polar cod (*Boreogadus saida*) and Atlantic cod (*Gadus morhua*) under ocean acidification and warming. *Polar Biology* **39**, 1137-1153.

Lannig, G., Storch, D. and Pörtner, H. O. (2005). Aerobic mitochondrial capacities in Antarctic and temperate eelpout (*Zoarcidae*) subjected to warm versus cold acclimation. *Polar Biology* **28**, 575-584.

Larsson, A., C. Haux, and M.L. Sjobeck. (1985). Fish physiology and metal pollution: results and experiences from laboratory and field studies. *Ecotoxicology and Environmental Saftey* **9**:250-281.

Laurel, B., Spencer, M., Iseri, P. and Copeman, L. (2015). Temperature-dependent growth and behavior of juvenile *Boreogadus saida* and co-occurring North Pacific gadids. *Polar Biology*, 1-9.

Lear, W. H. (1983). Distribution, size and sexual maturity of *Boreogadus saida* in the northwest Atlantic during 1959–1978. *Canadian Atlantic Fisheries Science Advisory Commission Research* **79**, 40.

Levitus, S., J.I. Antonov, T.P. Boyer, O.K. Baranova, H.E. Garcia, R.A. Locarnini, A.V.

Mishonov, J.R. Reagan, D. Seidov, E.S. Yarosh, and M.M. Zweng. (2012). World ocean heat content and thermosteric sea level change (0–2000 m), 1955–2010. *Geophysical Research Letters* **39:** L10603

Lewis, E. L. and Walker, E. R. (1970). The water structure under a growing sea ice sheet. *Journal of Geophysical Research* **75**, 6836-6845.

Lillywhite, H. B., Zippel, K. C. and Farrell, A. P. (1999). Resting and maximal heart rates in ectothermic vertebrates. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **124**, 369-382.

Lønne, O. J. and Gulliksen, B. (1989). Size, age and diet of polar cod, *Boreogadus saida* (Lepechin 1773), in ice covered waters. *Polar Biology* **9**, 187-191.

Marentette, J.R., S. Tong, G. Wang, N.M. Sopinka, M.D. Taves, M.A. Koops, and S. Balshine. (2012). Behavior as biomarker? Laboratory versus field movement in round goby (*Neogobius melanostomus*) from highly contaminated habitats. *Ecotoxicology* **21**:1003-1012.

Mathis, J.T., J.N. Cross, W. Evans, and S.C. Doney. (2015). Ocean acidification in the surface waters of the Pacific-Arctic boundary regions. *Oceanography* **28,**122-135.

McKenzie, D. J., Steffensen, J. F., Taylor, E. W. and Abe, A. S. (2012). The contribution of air breathing to aerobic scope and exercise performance in the banded knife fish *Gymnotus carapo* L. *Journal of Experimental Biology* **215**, 1323-1330.

Melnikov, I. A. and Chernova, N. V. (2013). Characteristics of under-ice swarming of polar cod *Boreogadus saida* (Gadidae) in the Central Arctic Ocean. *Journal of Ichthyology* **53**, 7-15.

Miller, R. J. and Mann, K. H. (1973). Ecological energetics of seaweed zone in a marine bay on Atlantic coast of Canada. 3. Energy transformations by sea-urchins. *Marine Biology* **18**, 99-114.

Miller, S. C., Gillis, T. E. and Wright, P. A. (2011). The ontogeny of regulatory control of the rainbow trout (*Oncorhynchus mykiss*) heart and how this is influenced by chronic hypoxia exposure. *Journal of Experimental Biology* **214**, 2065-2072.

Moulton, L. L. and Tarbox, K. E. (1987). Analysis of *Boreogadus saida* movements in the Beaufort Sea nearshore region, 1978-79. *Arctic* **40**, 43-49.

Munoz, N. J., Anttila, K., Chen, Z. Q., Heath, J. W., Farrell, A. P. and Neff, B. D. (2014). Indirect genetic effects underlie oxygen-limited thermal tolerance within a coastal population of chinook salmon. *Proceedings of the Royal Society B-Biological Sciences* **281,** 20141082

Myrick, C. A. and Cech, J. J. (2000). Temperature influences on California rainbow trout physiological performance. *Fish Physiology and Biochemistry* **22**, 245-254.

Nahrgang, J., P. Dubourg, M. Frantzen, D. Storch, F. Dahlke, and J.P. Meador. (2016). Early life stages of an Arctic keystone species (*Boreogadus saida*) show high sensitivity to a water-soluble fraction of crude oil. *Environmental Pollution* **218,** 605-614.

Nelson, R. J., Bouchard, C., Madsen, M., Praebel, K., Rondeau, E., von Schalburg, K., Leong, J. S., Jantzen, S., Sandwith, Z., Puckett, S., Messmer, A., Fevolden, S. E. and Koop, B. F. (2013). Microsatellite loci for genetic analysis of the Arctic gadids *Boreogadus saida* and *Arctogadus glacialis*. *Conservation Genetics Resources* **5**, 445-448.

Niehaus, A. C., Angilletta, M. J., Sears, M. W., Franklin, C. E. and Wilson, R. S. (2012). Predicting the physiological performance of ectotherms in fluctuating thermal environments. *Journal of Experimental Biology* **215**, 694-701.

NOAA - Arctic Sea ice data. (2016). Available at: http://nsidc.org/arcticseaicenews/

Norin, T. and Clark, T. D. (2016). Measurement and relevance of maximum metabolic rate in fishes. *Journal of Fish Biology* **88**, 122-151.

Osuga, D. T. and Feeney, R. E. (1978). Antifreeze glycoproteins from Arctic fish. *Journal of Biological Chemistry* **253**, 5338-5343.

Overpeck, J. T., M. Strum, J.A. Francis, D.K. Perovich, M.C. Serreze and 18 others. (2005). Arctic system on trajectory to new, seasonally ice-free state. *EOS* **86**, 313.

Ocean Networks Canada Data Archive, http://www.oceannetworks.ca, Oceans Networks Canada, University of Victoria, Canada. Downloaded on (March, 2015).

Payne, N.L., J.A. Smith, D.E. van der Meulen, M.D. Taylor, Y.Y. Watanabe, A. Takahashi, T.A. Marzullo, C.A. Gray, G. Cadiou, and I.M. Suthers. (2016). Temperature dependence of fish performance in the wild: links with species biogeography and physiological thermal tolerance. *Functional Ecololgy.* 30, 903-912.

Peck, L.S., S.A. Morley, J. Richard, and M.S. Clark. (2014). Acclimation and thermal tolerance in Antarctic marine ectotherms. *The Journal of Experimental Biology* 217,16-22.

Penney, C. M., Nash, G. W. and Gamperl, A. K. (2014). Cardiorespiratory responses of seawater-acclimated adult Arctic char (*Salvelinus alpinus*) and Atlantic salmon (*Salmo salar*) to an acute temperature increase. *Canadian Journal of Fisheries and Aquatic Sciences* **71**, 1096-1105.

Perovich, D., Gerland, S., Hendricks, S., Meier, W., Nikolaus, M. and Tschudi, M. (2014). Sea Ice. *Arctic report card*. Available at http://arctic.noaa.gov/Report-Card

Perry, A. L., Low, P. J., Ellis, J. R. and Reynolds, J. D. (2005). Climate change and distribution shifts in marine fishes. *Science* **308**, 1912-1915.

Pershing, A. J., Alexander, M. A., Hernandez, C. M., Kerr, L. A., Le Bris, A., Mills, K. E.,

Nye, J. A., Record, N. R., Scannell, H. A., Scott, J. D. et al. (2015). Slow adaptation in the face of rapid warming leads to collapse of the Gulf of Maine cod fishery. *Science*. **350**, 809-812

Peterson, G., and J.C. Rocha. (2016). Chp. 3. Arctic regime shifts and resilience. In *Arctic resilience report: Arctic council*. M. Carson, and G. Peterson, Eds. Stockholm Environment Institute and Stockholm Resilience Centre, Stockholm.

Podrabsky, J. E. and Somero, G. N. (2006). Inducible heat tolerance in Antarctic notothenioid fishes. *Polar Biology* **30**, 39-43.

Polyakov, I. V., Beszczynska, A., Carmack, E. C., Dmitrenko, I. A., Fahrbach, E., Frolov, I. E., Gerdes, R., Hansen, E., Holfort, J., Ivanov, V. V. et al. (2005). One more step toward a warmer Arctic. *Geophysical Research Letters* **32**, L17605

Polyakov, I. V., Timokhov, L. A., Alexeev, V. A., Bacon, S., Dmitrenko, I. A., Fortier, L., Frolov, I. E., Gascard, J.-C., Hansen, E., Ivanov, V. V. et al. (2010). Arctic Ocean warming contributes to reduced polar ice cap. *Journal of Physical Oceanography* **40**, 2743-2756.

Pörtner, H. O. (2001). Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. *Naturwissenschaften* **88**, 137-146.

Pörtner, H. O. and Farrell, A. P. (2008). Ecology, physiology and climate change. *Science* **322**, 690-692.

Pörtner, H.O., and R. Knust. (2007). Constraints and trade-offs in climate dependent adaptation: Energy budgets and growth in a latitudinal cline. *Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology* **146,** S205-S206.

Pörtner, H.O., M. Langenbuch, and B. Michaelidis. (2005). Synergistic effects of temperature extremes, hypoxia, and increases in CO_2 on marine animals: From Earth history to global change. *Journal of Geophysical Research: Oceans* **110**,1-15.

Pörtner, H. O., Peck, L. and Somero, G. (2007). Thermal limits and adaptation in marine Antarctic ectotherms: An integrative view. *Philosophical Transactions of the Royal Society B - Biological Sciences* **362**, 2233-2258.

Pörtner, H. O., Van Dijk, P. L. M., Hardeqig, I. and Sommer, A. (2000). Levels of metabolic cold adaptation: Tradeoffs in eurythermal and stenothermal ectotherms. In *Antarctic Ecosystems: Models for Wider Ecological Understanding.* Eds. W. Davison C. Howard-Williams and P. Broady), pp. 109-122. Christchurch, New Zealand: Caxton Press.

Rajasakaren, B. (2013). Distribution of polar cod (*Boreogadus saida*) in the Barents Sea – A useful indicator of climate change? Thesis: Master of Science, pp. 220: University of Bergen.

Randall, D. J. (1966). Nervous control of cardiac activity in Tench (*Tinca tinca*) and goldfish (*Carassius auratus*). *Physiological Zoology* **39**, 185-191.

Renaud, P. E., Berge, J., Varpe, O., Lonne, O. J., Nahrgang, J., Ottesen, C. and Hallanger, I. (2012). Is the poleward expansion by Atlantic cod and haddock threatening native polar cod, *Boreogadus saida*? *Polar Biology* **35**, 401-412.

Ricklefs, R. E. and Scheuerlein, A. (2001). Comparison of aging-related mortality among birds and mammals. *Experimental gerontology* **36**, 845-857.

Robinson, E. and Davison, W. (2008). Antarctic fish can survive prolonged exposure to elevated temperatures. *Journal of Fish Biology* **73**, 1676-1689.

Sakshaug, E. and Skjoldal, H. R. (1989). Life at the ice edge. *Ambio* **18**, 60-67.

Sakurai, Y., Ishii, K., Nakatani, R., Yamaguchi, H., Anma, G. and Jin, M. (1998). Reproductive characteristics and effects of temperature and salinity on the development and survival of eggs and larvae of *Boreogadus saida*. *Memoires of Faculty Fisheries, Hokkaido University* **45**, 77-89.

Sartoris, F. J., Bock, C., Serendero, I., Lannig, G. and Pörtner, H. O. (2003). Temperature-dependent changes in energy metabolism, intracellular pH and blood oxygen tension in the Atlantic cod. *Journal of Fish Biology* **62**, 1239-1253.

Schulte, P. M. (2015). The effects of temperature on aerobic metabolism: towards a mechanistic understanding of the responses of ectotherms to a changing environment. *Journal of Experimental Biology* **218**, 1856-1866.

Schurmann, H. and Christiansen, J. S. (1994). Behavioral thermoregulation and swimming activity of two Arctic teleosts (subfamily Gadinae)-the polar cod (*Boreogadus saida*) and the navaga (*Eleginus navaga*). *Journal of Thermal Biology* **19**, 207-212.

Seebacher, F., Davison, W., Lowe, C. J. and Franklin, C. E. (2005). A falsification of the thermal specialization paradigm: compensation for elevated temperatures in Antarctic fishes. *Biology letters* **1**, 151-154.

Seebacher, F., White, C. R. and Franklin, C. E. (2015). Physiological plasticity increases resilience of ectothermic animals to climate change. *Nature Climate Change* **5**, 61-66.

Shimada, K., Kamoshida, T., Nishino, S., Itoh, M., McLaughlin, F. A., Carmack, E. C., Zimmerman, S. and Proshutinsky, A. (2006). Pacific Ocean Inflow: influence on catastrophic reduction of sea ice cover in the Arctic Ocean. *Geophysical Research Letters* **33**, L08605.

Sidhu, R., Anttila, K. and Farrell, A. P. (2014). Upper thermal tolerance of closely related Danio species. *Journal of Fish Biology* **84**, 982-995.

Somero, G. N. (2005). Linking biogeography to physiology: Evolutionary and acclimatory adjustments of thermal limits. *Frontiers in Zoology* **2**, 1-9.

Somero, G. N. (2010). The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. *Journal of Experimental Biology* **213**, 912-920.

Somero, G. N. and Devries, A. L. (1967). Temperature tolerance of some Antarctic fishes. *Science* **156**, 257-258.

Steele, M., Ermold, W. and Zhang, J. (2011). Modeling the formation and fate of the near-surface temperature maximum in the Canadian Basin of the Arctic Ocean. *Journal of geophysical research. C, Oceans* **116**, C11015 doi:10.1029/2010JC006803

Steele, M., Ermold, W. and Zhang, J. L. (2008). Arctic Ocean surface warming trends over the past 100 years. *Geophysical Research letters* **35**, L02614 doi:10.1029/2007GL031651

Steffensen, J. F., Schurmann, H. and Bushnell, P. G. (1994). Oxygen consumption in 4 species of teleosts from Greenland - no evidence of metabolic cold adaptation. *Polar Biology* **14**, 49-54.

Steiner, N., Azetsu-Scott, K., Hamilton, J., Hedges, K., Hu, X., Janjua, M. Y., Lavoie, D., Loder, J., Melling, H., Merzouk, A. et al. (2015). Observed trends and climate projections affecting marine ecosystems in the Canadian Arctic. *Environmental Reviews* **23**, 191-239.

Steinhausen, M. F., Sandblom, E., Eliason, E. J., Verhille, C. and Farrell, A. P. (2008). The effect of acute temperature increases on the cardiorespiratory performance of resting and swimming sockeye salmon (*Oncorhynchus nerka*). *Journal of Experimental Biology* **211**, 3915-3926.

Stillman, J. H. (2002). Causes and consequences of thermal tolerance limits in rocky intertidal porcelain crabs, genus *Petrolisthes*. *Integrative and Comparative Biology* **42**, 790-796.

Suzuki, K. W., Bouchard, C., Robert, D. and Fortier, L. (2015). Spatiotemporal occurrence of summer ichthyoplankton in the southeast Beaufort Sea. *Polar Biology* **38**, 1379-1389.

Thorarensen, H., Gallaugher, P. E. and Farrell, A. P. (1996). The limitations of heart rate as a predictor of metabolic rate in fish. *Journal of Fish Biology* **49**, 226-236.

Thorsteinson, L.K., and Love, M.S., Eds, (2016). Alaska Arctic marine fish ecology catalog: U.S. Geological Survey Scientific Investigations Report 2016-5038 (OCS Study, BOEM 2016-048), 768 p., http://dx.doi.org/10.3133/sir20165038

Tiitu, V. and Vornanen, M. (2002). Regulation of cardiac contractility in a cold stenothermal fish, the burbot *Lota lota* L. *Journal of Experimental Biology* **205**, 1597-1606.

Timmermans, M. L. and Proshutinsky, A. (2014). Arctic Ocean sea surface temperature. In *Arctic report card: Update for 2014*: NOAA Reports. Available at

http://arctic.noaa.gov/Report-Card

Ultsch, G. R., Ott, M. E. and Heisler, N. (1980). Standard metabolic rate, critical oxygen tension, and aerobic scope for spontaneous activity of trout (*Salmo gairdneri*) and carp (*Cyprins carpio*) in acidified water. *Comparative Biochemistry and Physiology a-Physiology* **67**, 329-335.

Use of Fishes in Research Committee - joint committee of the American Fisheries Society, the American Institute of Fishery Research Biologists, and the American Society of Ichthyologists and Herpetologists. (2014). Guidelines for the use of fishes in research. American Fisheries Society, Bethesda, Maryland.

Vaughan, D. G., Comiso, J. C., Allison, I., Carrasco, J., Kaser, G., Kwok, R., Mote, P., Murray, T., Paul, F., Ren, J. et al. (2013). Observations: Cryosphere. In *Climate Change 2013: The physical science basis. Contribution of Working Group 1 to the fish assessment report of the intergovernmental panel of climate change*, Eds. T. F. Stocker D. Qin G.-K. Plattner M. Tignor S. K. Allen J. Boschung A. Nauels Y. Xia V. Bex and P. M. Midgley). Cambridge, UK and New York, USA.

Verhille, C., Anttila, K. and Farrell, A. P. (2013). A heart to heart on temperature: Impaired temperature tolerance of triploid rainbow trout (*Oncorhynchus mykiss*) due to early onset of cardiac arrhythmia. *Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology* **164**, 653-657.

Vornanen, M. (2016). The temperature dependence of electrical excitability in fish hearts. *Journal of Experimental Biology* **219**, 1941-1952.

Walkusz, W., Majewski, A. and Reist, J. D. (2013). Distribution and diet of the bottom dwelling Arctic cod in the Canadian Beaufort Sea. *Journal of Marine Systems* **127**, 65-75.

Walkusz, W., Paulic, J. E., Williams, W. J., Kwasniewski, S. and Papst, M. H. (2011). Distribution and diet of larval and juvenile *Boreogadus saida* in the shallow Canadian Beaufort Sea. *Journal of Marine Systems* **84**, 78-84.

Wassmann, P. (2011). Arctic marine ecosystems in an era of rapid climate change. *Progress in Oceanography* **90**, 1-17.

Welch, H.E., M.A. Bergmann, T.D. Siferd, K.A. Martin, M.F. Curtis, R.E. Crawford, R.J. Conover, and H. Hop. (1992). Energy flow through the marine ecosystem of the Lancaster Sound region, Arctic Canada. *Arctic* **45,** 343-357.

Welch, H. E., Crawford, R. E. and Hop, H. (1993). Occurrence of *Boreogadus saida* schools and their vulnerability to predation in the Canadian High Arctic. *Arctic* **46**, 331-339.

Woods, H. A. and Harrison, J. F. (2001). The beneficial acclimation hypothesis versus acclimation of specific traits: physiological change in water-stressed *Manduca sexta* caterpillars. *Physiological and Biochemical Zoology* **74**, 32-44.

Wyllie-Echeverria, T., Barber, W. E. and Wyllie-Echeverria, S. (1997). Water masses and transport of age-0 *Boreogadus saida* and age-0 Bering flounder into the northeastern Chukchi Sea. In *American Fisheries Society Symposium*, vol. **19** (ed. J. B. Reynolds), pp. 60-80.

Yamamoto-Kawai, M., McLaughlin, F. A. and Carmack, E. C. (2011). Effects of ocean acidification, warming and melting of sea ice on aragonite saturation of the Canada Basin surface water. *Geophysical Research Letters* **38**, 5.

Yeager, D. P. and Ultsch, G. R. (1989). Physiological regulation and conformation: A BASIC program for the determination of critical points. *Physiological Zoology* **62**, 888-907.

Appendices

Appendix A *Boreogadus saida* mass and total length (TL)

	T_{cmax}						MO_2		
	1°C		3.5°C		6.5°C		1.0°C	3.5°C	6.5°C
Location	UBC		UBC		UBC		UBC	VA	UBC
Fish #	Mass (g)	TL(cm)	Mass (g)	TL(cm)	Mass (g)	TL(cm)	Mass (g)	Mass (g)	Mass (g)
1	41.6	n/av	46.4	17.4	47.2	17.6	49.5	82.4	54.8
2	69.2	17.5	32.9	14.8	55.1	18.2	56.3	108.5	47.1
3	49.1	17.5	61.8	17.3	74.5	18.9	42.3	131.0	89.6
4	69.3	18.9	72.5	19.6	72.8	19.5	56.7	77.1	42.9
5	52.4	19.0	47.3	17.5	41.6	17.4	72.1	132.2	43.6
6	63.8	19.6	60.8	18.2	78.9	19.6	52.4	87.0	110.3
7	51.8	17.7	67.9	19.5	51.4	17.7	87.5	84.8	32.5
8	46.9	17.4	42.5	17.4	35.6	14.8	61.2	120.4	64.0
9	54.0	17.3	57.4	19.0	92.4	20.8	61.5	89.5	93.7
10	56.6	17.6	74.8	17.5	76.3	18.8	56.2	110.5	44.6
11	85.4	21.5	94.2	23.8	96.1	24.2	70.2	98.8	73.8
12	101.8	23.8	94.5	24.2	96.6	23.8	57.8	150.5	68.6
13	101.1	24.2	70.3	21.5	82.2	21.5	41.4	115.0	104.0
14							48.2	94.3	80.0
15							56.8	140.8	146.9
16							61.3	163.8	110.4
17							61.9	89.4	52.2
18							59.8	90.1	
19							87.9	152.0	
20							54.0		

Table A.1 Mass and total length of *B. saida* from T_{cmax} and MO_2 experiments

	f_{Hmax}									
	0.5°C		0.5°C		3.5°C		3.5°C		6.5°C	
Location	VA		CB		VA		CB		VA	
Fish #	Mass (g)	TL (cm)	Mass (g)	TL (cm)	Mass (g)	TL (cm)	Mass (g)	TL (cm)	Mass (g)	TL (cm)
1	32.5	16.5	30.0	15.8	67.0	21.5	37.0	16.7	146.4	n/av
2	30.0	16.4	34.0	16.0	76.0	22.0	23.5	14.8	136.3	n/av
3	26.5	15.7	49.0	18.5	102.0	n/av	50.0	18.2	162.0	30.0
4	50.0	19.5	33.5	16.2	84.0	23.0	34.5	16.8	116.9	26.0
5	31.5	16.7	45.0	17.6	81.0	21.0	35.0	17.0	118.1	26.0
6	27.0	15.8	46.0	17.8	98.0	24.0	39.0	16.6	105.6	25.5
7	24.5	15.6	35.0	16.5	68.0	22.5	33.0	15.7	84.9	24.0
8	26.0	16.1	51.5	18.4	85.0	23.4	37.0	17.1	95.1	24.5
9	30.5	15.5	40.0	16.0	43.0	18.4	32.5	17.1	106.8	23.0
10	39.5	17.5	35.0	16.1	100.0	23.0			103.3	25.5
11			41.0	18.1						
12			36.0	16.6						

Table A.2 Mass and total length of *B. saida* from f_{Hmax} experiments

Appendix B Laboratory rearing of wild Arctic cod *B. saida* from egg to adulthood

Brief Communication:
Journal of Fish Biology (2016) 88, 1241–1248
doi:10.1111/jfb.12893, available online at wileyonlinelibrary.com

D. Kent*, H. E. Drost†‡, J. Fisher*, T. Oyama* and A. P. Farrell†§
*Vancouver Aquarium, 845 Avison Way, Vancouver, BC, V6G 3E2, Canada,
†Zoology Department, University of British Columbia, 6270 University Boulevard, Vancouver, BC, V6T1Z4, Canada and
§Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC, V6T 1Z4, Canada

The techniques and protocols used to successfully capture, transport and breed Arctic cod *Boreogadus saida*, as well as to rear their larvae through to adulthood are summarized. Breeding *B. saida* will increase the opportunity to study this fish species, which is a critical part of the Arctic food web.

Key words: climate change; full life cycle rearing; larval feeding regimen; rearing temperature.

The threat to the Arctic ecosystem from climate change cannot be overstated. Indeed, the early predictions of the rate of ice melting in the Arctic were understated (Stroeve et al., 2007). What remains unclear is how Arctic fishes will respond: will fishes migrate, be extirpated, acclimate or adapt? Some of this uncertainty stems from a dearth of information on relevant Arctic fish species under relevant conditions, forcing extrapolations from knowledge on better-studied temperate fish species. Ongoing challenges include the high cost of Arctic field research and access to experimental fishes, both of which are hampered by severe winter weather conditions and limited field stations. Clearly, a partial solution to experimental fish availability is to transport wild fishes from remote polar locations to permanent southern laboratories where experimental work can be performed year round Christiansen and George, 1995; Hop et al., 1997; Sakurai et al., 1998; Pörtner et al., 2000; Lannig et al., 2005; Drost et al., 2014). Similarly, Antarctic fishes have been successfully transported to explore their physiology (Davison et al., 1994, 1995; Seebacher et al., 2005). If polar fishes can be successfully bred under laboratory conditions, then full life-history studies become a possibility. Here, details of the techniques and protocols that were used to successfully capture and transport wild Arctic cod *Boreogadus saida* (Lepechin 1774) and then breed and rear them are presented.

The developmental pattern of *B. saida*, based on pigment patterns and meristic characteristics, is found in a broader comparative developmental study of northern gadid larvae (Dunn and Vinter, 1984). Also, captured adult *B. saida* were first bred at the Vancouver Aquarium Marine Science Centre (VAMSC), which allowed a study of larval growth for ~ 4 weeks (Graham and Hop, 1995). Successful rearing of larval *B. saida* to adulthood is a more recent achievement and the focus of this report. In fact, some of the adults and progeny described in this *B. saida* breeding programme have been used already for physiological experiments, as permitted under the Canadian Council on Animal Care (A10-0236), the University of British Columbia Animal Care Committee (A11-0267), the Freshwater Institute Science Laboratories Animal Care Committee – Arctic Aquatic Research (FWI-ACC-2012-050) and the Vancouver Aquarium Animal Care Committee (2011-04).

Boreogadus saida can be captured with minimal trauma using a trap, seine or dip-net. Trapping 1–2 year-old *B. saida* was successful at 20 m depths in late July using tinned sardines as bait suspended in mesh bags. Other researchers report catching *B. saida*

individually using hand-nets when diving, and collecting *B. saida* from bottom trawls and trap nets (Aronovich et al., 1975; Graham and Hop, 1995; Hop et al., 1997; Christiansen et al., 2012; VAMSC, pers. obs.). By far, the most successful, most cost-effective and least harmful capture method takes advantage of a natural behaviour of *B. saida*. During ice break-up (July to August), they swarm in huge numbers [Fig. B.1(a)] near the water surface for a short time period. Thus, large numbers of *B. saida* were quickly and easily dip-netted near Cambridge Bay, Nunavut, Canada (69.12°N; 105.05°W) during the swarming. *Boreogadus saida* were then quickly transferred to the temporary holding facility prior to transport (Graham and Hop, 1995; Drost et al., 2014). The holding facility kept the water temperature below 4°C and ~25% of the aerated seawater was changed daily. *Boreogadus saida* were fed daily to satiation with frozen invertebrate species such as krill *Euphausia pacifica* and *Euphausia superba*, and they were closely monitored for several days to ensure that each *B. saida* was injury-free before transport. A minimum of 24 *B. saida*, aged 1–2 years (10–20 cm total length, LT), will ensure sufficient individuals for a brood stock. Males and females cannot be morphologically distinguished and females tend not to survive after egg release (Sakurai et al., 1998; H. Drost, pers. obs.).

Boreogadus saida were transported south by commercial airlines to VAMSC (a distance of c. 2400 km). Prior to air transport, the *B. saida* were starved for 48 h and transferred (a maximum of three *B. saida* per bag) on the day of travel into plastic double-lined polyethylene bags (45·7cm×81·3 cm) that contained 5 l of 0°C seawater to keep them as cool as possible, to increase gas content of water and to reduce toxic waste build up. Transporting *B. saida* in warmer water than 0°C was not tested. The remaining airspace of each bag was filled with oxygen (obtained from the welding shop in town) and sealed using rubber bands (Graham and Hop, 1995; Drost et al., 2014). The bags were placed in coolers packed with ice, which was topped up after ~20 h into transportation from a spare container filled with ice. The water temperature was 0°C upon arrival at VAMSC some 40 h later with no mortality.

At VAMSC, adult *B. saida* were kept in recirculating aquaria with a photoperiod of 10L:14D over a range in holding temperatures. *Boreogadus saida* survived > 6 months prior to experimental work at acclimation temperatures of 3.5 and 6.5°C, as well as at the routine holding temperature of 0.5°C. *Boreogadus saida* are generalist feeders and respond favourably to a varied diet (J. Fisher, pers. obs.). The *B. saida* were fed once daily with a

mixture of appropriately sized pieces of chopped krill species *E. Pacifica* and *E. superba*. Occasionally, Pacific mackerel (*Scomber japonicas* Houttuyn 1782) was added for variety. *Boreogadus saida* are reported to live at 8°C for at least 2 years (J. Marvin, pers. comm.). No *B. saida* held at 6.5 or 8°C, however, have been reported to reproduce. This is the only study that reports successful full life cycle reproduction (when produced larvae grow, mature and reproduce) and was achieved by *B. saida* kept in stenothermal 3.5°C seawater temperature but which had been previously exposed to 6.5°C seawater temperature for 1 month (M. Lo and H. Drost, pers. obs; Drost et al., 2015).

In the Arctic, *B. saida* normally mature sexually during their second or third year and breed under ice during winter months (Bain and Sekerak, unpubl. data). Surprisingly, during the first year of relocation to VAMSC neither mimicking an Arctic photoperiod of the winter season (i.e. no light) nor keeping stenothermal freezing temperatures were required for females to ripen between January and March, i.e. the natural time of reproduction. The longer *B. saida* were kept at VAMSC under these artificial conditions, however, the less pronounced their reproductive season became because, after a few years of captivity, VAMSC staff have found female *B. saida* becoming gravid as late as May. Perhaps, the reproductive season of *B. saida* would remain more predictable if natural seasonal light and water temperature conditions were maintained at the rearing facility (Graham and Hop, 1995).

Adult *B. saida* ripened naturally in recirculating aquaria without the need to resort to hormone stimulation techniques. Ovaplant (Syndel Laboratories Ltd; 2016 www.syndel.com) pellet injections tests were conducted in 2011 to help induce sperm production and synchronicity with gravid females and it was found, over the following 2 years, that successful fertilization was possible with and without artificial hormone stimulation. As long as the males were in the tank with females, particularly during egg development, the natural cues appeared to be sufficient for reproduction synchronicity. Gravid females became visually obvious from their increase in girth. At 0 – 1°C, it took ~20 days for eggs to ripen. Sakurai et al. (1998) reported that mature males change in colour, but this was not seen consistently at VAMSC. Therefore, identifying males for egg fertilization can be difficult and several males may need to be tested by palpitation.

Egg fertilization employed clean and dry containers for the fertilization procedure and three buckets of seawater at ≤ 3°C, one of which contained anaesthetic (50–100 mg)$^{-1}$ Tricaine

methanesulphonate (MS-222), Sigma-Aldrich Products;www.sigmaaldrich.com). The other buckets of seawater were used to revive sedated *B. saida* after stripping and to transport stripped *B. saida* back to holding aquaria. *Boreogadus saida* were only lightly sedated for a maximum of 5 min and were handled only when insensitive to a tail pinch. The underside of each *B. saida* was dried to prevent any seawater contamination of the eggs and sperm during hand stripping them into separate clean, dry receptacles (for details on various egg stripping techniques, see Rottmann et al., 1991). Egg abundance was similar to the values (~25 000 per *B. saida*) reported by Graham and Hop (1995). The eggs and sperm, from one female and one male, were maintained separately at ≤ 3°C until they were combined using 500 ml of seawater to help mix and activate the sperm. Fertilization occurred while the eggs sperm and seawater were kept in cold storage for at least 45 min before the eggs were released into a rearing aquarium. The eggs and larvae were reared at 3·5°C because two earlier studies (Sakurai et al., 1998; H. Drost, pers. obs.) indicated a tolerance to higher temperatures and faster growth rate. Moreover, the higher rearing temperature increased the survival and mobility of the live prey items used for food.

The rearing facility was part of the larger VAMSC Arctic holding system, which is made up of local seawater that is initially filtered (25μm) by gravity fed sand filters. The rearing tank for developing eggs was 300 l (80 cm diameter×60 cm deep), circular with re-circulating water and aeration. The rearing tank had a central internal cylindrical fine meshed screen that was plumbed through the bottom to an external standpipe, which set the height of the water and fed effluent water back to the sump. A daily minimum of 10% water change and daily water quality testing ensured that nitrogen species (NH_3, NO_2 and NO_3) and orthophosphate were kept at or below detection levels, while salinity (28·3) and pH (7·8) remained constant. The water was chilled using a 1 hp air-cooled chiller Delta Star DS-9 (Aqualogic; www.aqualogicinc.com). A small submersible pump, located in the sump for the main aquarium, provided a constant $1 min^{-1}$ flow with a turnover rate of 5 h. A side stream trickle filter with bio-balls was used for biological filtration and organic wastes were removed by a foam fractionation protein skimmer (AquaC EV-240; www.marinedepot.com) and 200–300 μm filter socks were placed over the intake pipes. The inflow of water was provided at the subsurface level during egg development so as not to disturb the mono layer of viable eggs on the surface. Most eggs were neutrally buoyant below the surface layer but a few viable

ones sank to the bottom. Tank cleaning was kept to a bare minimum during egg development without compromising water quality. Only obviously non-viable eggs (pure white) were removed daily. After the eggs hatched, a small micro-sprinkler was added to the surface of rearing tank to prevent the formation of a surface tension film (which larvae can get stuck in). This surface film can also harbour bacteria, which can be deleterious to growing larvae (Igarashi et al., 1991). Further investigation into the use of probiotics and antimicrobial rearing techniques is required and would perhaps lead to reduced egg and larval mortality (Skjermo and Vadstein, 1999).

At 3.5°C, hatching started 29 days after fertilization (i.e. ~100 accumulated thermal units; see Fig. B.2). The eggs hatched with the yolk sac still attached, and the yolk sac was depleted inc.7–13 days at 3·5°C. Even so, *B. saida* have been observed trying to feed within a day of hatching (Graham and Hop, 1995). Wild *B. saida* larvae are directly influenced by the variability in the stages of zooplankton development and abundance (Bradstreet et al., 1986; Lønne and Gulliksen, 1989; Matley et al., 2013). Therefore, feeding the right size, type and amount of food at the right time to developing larvae is the most critical part of successful rearing of fish species from eggs. The first mark of rearing success occurs when fish have graduated from live feed cultures to final frozen food items, which in this case were mainly frozen bio-engineered copepods (Cyclopeeze brand, Argent; www.argent-labs.com) and *E. pacifica*. The second marker is when fin ray formation is complete and juvenile pigment and other morphological characteristics are expressed. Full life cycle success is achieved when the reared first-generation fish become reproductive.

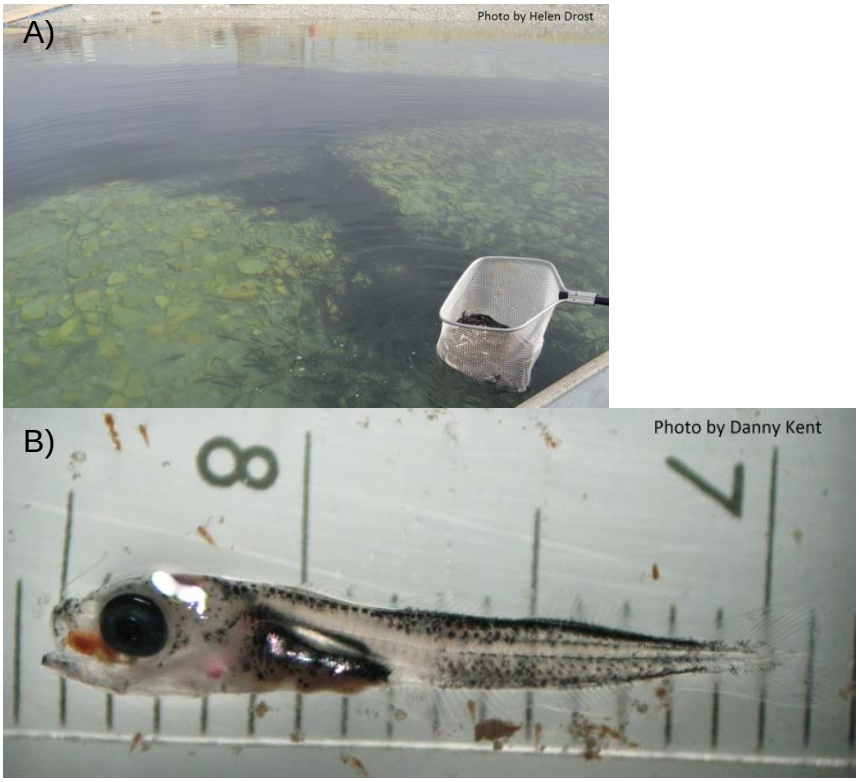

Figure. B.1 Pictures of *B. saida* adults in the wild and larvae in the laboratory
A) Usage of a dip-net to collect swarming *Boreogadus saida* during ice break-up near Cambridge Bay, Victoria Island, Nunavut, July 2012. **B)** Larvae are 16·5 mm in fork length (LF) on day 110 post-fertilization. This image shows that their guts are full of frozen copepods (Cyclopeeze brand, Argent) and the first dorsal-fin rays are developing.

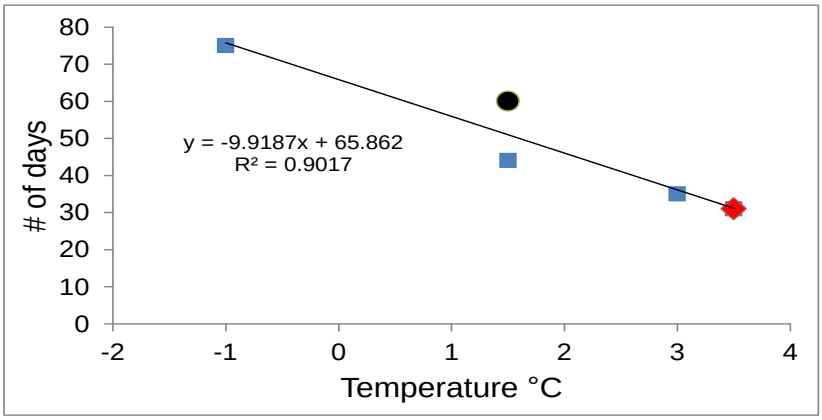

Figure B.2 Number of days taken to reach ~ 50% hatching
of *Boreogadus saida* as a function of water temperature. The curve was fitted by: $Y = -9.9187x+65.862$ ($R^2 = 0.90$). Data from Sakurai et al. (1998); Graham and Hop (1995); this study.

Table B.I Key development stages for *Boreogadus saida* larvae reared at 3.5° C.

Key stages	**Details**	**Date**	**# of days post fertilization**
Egg take		26 Sept. 2011	0
Blastula stage		03 Oct. 2011	7
Hatching	Choroid pigmented eyes only. No visible mouth or swim bladder.	25 Oct. 2011	29
Yolk sac depleted	Pre-feeding, guanine pigmented eyes, mouth and small swim bladder visible	01 Nov. 2011	36
Eating rotifers	Some yolk still present (opaque material ventrally below full gut). Swim Bladder visible.	07 Nov. 2011	42
Eating *Artemia* nauplii	No visible fin ray formation yet. 8mm L_F	21 Nov. 2011	56
High mortality		02 Dec. 2011	67-72
Caudal fin ray visible		12 Dec. 2011	77
Pelvic fins buds visible		20 Dec. 2011	85
Dorsal (2^{nd} & 3^{rd}) and anal fin forming	Swim bladder looks more elongated	26 Dec. 2011	91
High mortality		07 Jan. 2012	103-105
Eating frozen copepods and krill	Start of 1^{st} dorsal fin ray. 16.5 mm L_F	14 Jan. 2012	110
Eating pellets	Pigmentation increasing. 19 mm L_F	19 Jan. 2012	115
All fins formed		19 Feb. 2012	146
Juvenile	34 mm L_F	05 Mar. 2012	161
Adult fully pigmented		03 Nov. 2012	404
Gravid females	Full life cycle achieved	01 Mar. 2014	887

Lf. fork length

The feeding regime of developing *B. saida* larvae was labour-intensive. Live rotifers *Brachionus* spp. And *Artemia* sp., which are the main food items for newly hatched larvae, were cultured for food during egg development. Feeding started on a small scale, as soon as

they hatched and reached an average of three feeds per day. An auto-feeder is recommended for at least one late night feed. Aronovich et al. (1975) found that although the newly hatched larvae do not successfully feed until the yolk is mostly reabsorbed and their mouth is sufficiently developed, there is increased mortality due to starvation if food is added only when the yolk is almost gone. Once the larvae started feeding, 24 h of light was supplied using a LED clamp light. A reduced tank hygiene regime before hatching also allowed harpacticoid copepods to opportunistically colonize the walls and bottom of the rearing tank. The young copepods unintentionally acted as an additional food source for the early larval stages. The introduction of small amounts of the various sized live feedstock, before the larvae could consume it, appeared to be an important step in developing a feeding behaviour. An early introduction of larger feed potentially acts as a visual cue for larvae (Conceicao et al., 2010). VAMSC provided newly hatched *B. saida* larvae initially with rotifers that had guts full of a solution of frozen copepods (Cyclopeeze brand, Argent) emulsified with fish flakes and concentrated microalgae paste (Innovative Aquaculture, Starter Formula; www.innovativeaqua.com). Once the larvae were eating rotifers, small amounts of *Artemia* sp. were introduced. Overlapping the types of feed rather than an abrupt transition is recommended. The biggest challenge to rearing *B. saida* was the transition from *Artemia* sp. to frozen food and Otohime pellets (Marubeni Nisshin Feed; www.mn-feed.com). The protracted transition stage encompassed the greatest number of larval mortality events (see Table B.I). They finally graduated to frozen finely shaved *E. pacifica*, frozen copepods and pellets between 110–115 post-fertilization days [Fig. B.1(b)]. The population stabilized thereafter and after 1–2 years of low mortality, they became reproductively active (Table B.I). In summary, successful full life cycle rearing of *B. saida* required a dedicated, labour intensive feeding regime, continuous precise measures of water quality which allowed for flexibility in the application of techniques based on the unique requirements of *B. saida* and limited resources.

A rapidly changing Arctic food web could create a mismatch between developing fish larvae and their critical food sources (Fortier et al., 1995; Edwards and Richardson, 2004). However, the increased rate of larval development observed when reared in seawater temperature of 3.5°C could help *B. saida* adapt, over generations, to the rapidly changing

food webs associated with ice melting in the Arctic Ocean (Cushing and Horwood, 1994; Wassmann, 2011; Hop and Gjøsæter, 2013).

A.P.F. was funded by the Natural Sciences and Engineering Research Council of Canada. A.P.F. holds a Canada Research Chair. H.E.D. was funded by the Northern Students Training Programme (2011 and 2012). The authors would like to thank those from the remote community of Cambridge Bay for their considerable support (F. Buchan-Corey, B. Sitatak, J. Pzanioyak, M. Townsend, J. Puglik, M. Havioyak, A. Townsend and T. Rutherford) and in 2011, the captain and crew of the Canadian Coast Guard ice-breaker Sir Wilfrid Laurier. I thank E. Carmack and S. Dockerill for their help and inspiration, the ongoing support provided by the Nunavut Arctic College (Kitikmeot campus). At the Vancouver Aquarium we thank the BC waters staff, J. Nightingale, D, Thoney, E. Solomon, S. Young, J. Wong and S. Balfry. We also thank M. Lo and J. Marvin, Directrice de la collection animale/ General Curator, Aquarium du Québec, for sharing their observations.

References:

Aronovich, T. M., Doroshev, S. I., Spectorova, L. V. and Makhotin, V. M. (1975). Egg incubation and larval rearing of navaga (*Eleginus navaga* Pall.), polar cod (*Boreogadus saida* lepechin) and Arctic flounder (*Liopsetta glacialis* Pall.) in the laboratory. *Aquaculture* **6**, 233–242.

Bradstreet, M. S., Finley, K. J., Sekerak, A. D., Griffiths, W. B., Evans, C. R., Fabijan, M. F. and Stallard, H. E. (1986). Aspects of the biology of Arctic cod (*Boreogadus saida*) and its importance in Arctic marine food chains. *Canadian Technical Report of Fisheries and Aquatic Sciences* No. **1491**, viii–193

Christiansen, J. and George, S. (1995). Contamination of food by crude oil affects food selection and growth performance, but not appetite, in an Arctic fish, the polar cod (*Boreogadus saida*) *Polar Biology* **15**, 277–281.

Christiansen, J. S., Hop, H., Nilssen, E. M. and Joensen, J. (2012). Trophic ecology of sympatric Arctic gadoids, *Arctogadus glacialis* (Peters, 1872) and *Boreogadus saida* (Lepechin, 1774), in NE Greenland *Polar Biology* **35**, 1247–1257.

Conceicao, L. E. C., Yufera, M., Makridis, P., Morais, S. and Dinis, M. T. (2010). Live feeds for early stages of fish rearing *Aquaculture Research* **41**, 613–640.

Cushing, D. H. and Horwood, J. W. (1994). The growth and death of fish larvae. *Journal of Plankton Research* **16**, 291–300.

Davison, W., Franklin, C. E. and McKenzie, J. C. (1994). Hematological changes in an Antarctic teleost, *Trematomus bernacchii*, following stress. *Polar Biology* **14**, 463–466.

Davison, W., Axelsson, M., Forster, M. and Nilsson, S. (1995). Cardiovascular responses to acute handling stress in the Antarctic fish *Trematomus bernacchiiare* not mediated by circulatory catecholamines. *Fish Physiology and Biochemistry* **14**, 253–257.

Drost, H. E., Carmack, E. C. and Farrell, A. P. (2014). Upper thermal limits of cardiac function for Arctic cod *Boreogadus saida*, a key food web fish species in the Arctic Ocean. *Journal of Fish Biology* **84**, 1781–1792.

Dunn, J. R. and Vinter, B. M. (1984). Development of larvae of the saffron cod, *Eleginus gracilis*, with comments on the identification of gadid larvae in Pacific and Arctic waters contiguous to Canada and Alaska. *Canadian Journal of Fisheries and Aquatic Sciences* **41**, 304–318.

Edwards, M. and Richardson, A. J. (2004). Impact of climate change on marine pelagic phenology and trophic mismatch. *Nature* **430**, 881–884.

Fortier, L., Ponton, D. and Gilbert, M. (1995). The match mismatch hypothesis and the feeding success of fish larvae in ice-covered southeastern Hudson Bay. *Marine Ecology Progress Series* **120**, 11–27.

Gade, H. G., Lake, R. A., Lewis, E. L. and Walker, E. R. (1974). Oceanography of an Arctic bay*. Deep Sea Research and Oceanographic Abstracts* **21**, 547–571.

Graham, M. and Hop, H. (1995). Aspects of reproduction and larval biology of Arctic cod (*Boreogadus saida*). *Arctic* **48**, 130–135.

Hop, H. and Gjøsæter, H. (2013). Polar cod (*Boreogadus saida*) and capelin (*Mallotus villosus*) as key species in marine food webs of the Arctic and the Barents Sea. *Marine Biology Research* **9**, 878–894.

Hop, H., Tonn, W. M. and Welch, H. E. (1997). Bioenergetics of Arctic cod (*Boreogadus saida*) at low temperatures. *Canadian Journal of Fisheries and Aquatic Sciences* **54**, 1772–1784.

Igarashi, M. A., Romero, S. F. and Kittaka, J. (1991). Bacteriological character in the culture water of penaeid, homarid and palinurid larvae. *Nippon Suisan Gakkaishi* **57**, 2255–2260.

Lannig, G., Storch, D. and Pörtner, H. O. (2005). Aerobic mitochondrial capacities in Antarctic and temperate eelpout (*Zoarcidae*) subjected to warm versus cold acclimation. *Polar Biology* **28**, 575–584.

Lønne, O. J. and Gulliksen, B. (1989). Size, age and diet of polar cod, *Boreogadus saida* (Lepechin 1773), in ice covered waters. *Polar Biology* **9**, 187–191.

Matley, J. K., Fisk, A. T. and Dick, T. A. (2013). The foraging ecology of Arctic cod (*Boreogadus saida*) during open water (July-August) in Allen Bay, Arctic Canada. *Marine Biology* **160**, 2993–3004.

Pörtner, H. O., Van Dijk, P. L. M., Hardeqig, I. and Sommer, A. (2000). Levels of metabolic cold adaptation: tradeoffs in eurythermal and stenothermal ectotherms. In *Antarctic Ecosystems: Models for Wider Ecological Understanding*, Eds. Davison, W., Howard-Williams, C.& Broady, 109–122. Christchurch: Caxton Press.

Rottmann, R. W., Shireman, J. V. and Chapman, F. A. (1991). Technique for taking and fertilizing the spawn of fish. *SRAC Publication* **426**, 1–6.

Sakurai, Y., Ishii, K., Nakatani, R., Yamaguchi, H., Anma, G. and Jin, M. (1998). Reproductive characteristics and effects of temperature and salinity on the development and survival of eggs and larvae of Arctic cod (*Boreogadus saida*). *Memoirs of the Faculty of Fisheries*, Hokkaido University **44**, 77–89.

Seebacher, F., Davison, W., Lowe, C. J. and Franklin, C. E. (2005). A falsification of the thermal specialization paradigm: compensation for elevated temperatures in Antarctic fishes. *Biology Letters* **1**, 151–154.

Skjermo, J. and Vadstein, O. (1999).Techniques for microbial control in the intensive rearing of marine larvae. *Aquaculture* **177**, 333–343.

Stroeve, J., Holland, M. M., Meier, W., Scambos, T. and Serreze, M. (2007). Arctic sea ice decline: faster than forecast. *Geophysical Research Letters* **34**, L09501. doi:10.1029/2007GL029703.

Wassmann, P. (2011). Arctic marine ecosystems in an era of rapid climate change. *Progress in Oceanography* **90**, 1–17.