OXYGEN SUPPLY IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) AND ITS ECOLOGICAL IMPACTS: AN INVESTIGATION OF POOR TRIPLOID PERFORMANCE

by

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Abstract

Acquisition of environmental O_2 and its delivery throughout the body is essential for vertebrates and dictates habitat, life style, variation in anatomical form and function and even survival. Triploid (3N) rainbow trout (*Oncorhynchus mykiss*), which are important to the $0.5 billion British Columbia sport fishing industry, provide an informative model organism to study corporeal O_2 supply limitations and associated effects on survival in the wild. Triploid O_2 supply limitations likely stem from enlarged cells and contribute to poor 3N tolerance of sub-optimal conditions, which, in turn, may lead to high 3N population-level mortality rates in nature.

Therefore, in order to test the hypotheses that corporeal O_2 supply limits aerobic performance of 3N rainbow trout and that aerobic performance, in turn, limits survival in the wild, I compared the cardiorespiratory physiology of diploid (2N) and 3N Blackwater River rainbow trout facing a high temperature challenge in the lab and survival in the wild. I then investigated the potential of a 3N cardiac O_2 supply deficiency, using a modified Krogh diffusion model, and discussed its significance to temperature tolerance, endurance swimming and survival in the wild.

Both of my hypotheses were supported. A slower increase in 3N heart rate with warming suggested reduced O_2 convection through the body of 3N fish at high temperatures. Relating these results and endurance swimming rank with survival and habitat utilization in lakes revealed thermal tolerance and aerobic capacity as important variables influencing lake survival. The Krogh model showed 3N relative to 2N cardiac O_2 supply limitations that were primarily driven by reduced 3N arterial O_2 content, which I showed not to be caused by reduced 3N haemoglobin - O_2 affinity. In supporting the 2 main hypotheses of my thesis, this theoretically predicted 3N cardiac O_2 supply deficiency may explain reduced 3N aerobic swimming capacity and heart rate response to warming.

Thus, my findings are consistent with corporeal O_2 supply limitations to high temperature tolerance and aerobic swimming capacity of 3N rainbow trout, both of which can limit survival in the wild, depending on the biotic and abiotic conditions and physiological state of the organism.

Preface

A version of Chapter 2 has been published as: Verhille C, Anttila K, Farrell AP.A heart to heart on temperature. Impaired temperature tolerance of triploid rainbow trout (*Oncorhynchus mykiss*) due to early cardiac collapse. Comparative Biochemistry and Physiology Part A. Molecular & Integrative Physiology 164(4): 653-657, 2013. I conceived of this experiment in collaboration with Drs. K. Anttila and A.P. Farrell. The experiment was performed by an undergraduate directed studies student (Lubna Lutfa) co-supervised by myself and Dr. Anttila. I performed the majority of the data and statistical analysis and wrote the manuscript with advice and revisions to the manuscript from Drs. K. Anttila and A.P. Farrell. (license number 3106271123947)

A version of Chapter 5 has been published as: Verhille C, Farrell A P. The *in vitro* blood-O_2 affinity of triploid rainbow trout *Oncorhynchus mykiss* at different temperatures and CO_2 tensions. Journal of Fish Biology 81: 1124–32, 2012. I conceived of this experiment in collaboration with Dr. A.P. Farrell and I performed the data collection with the assistance of an undergraduate research assistant (Nancy Gu). I conducted all data and statistical analyses and wrote the manuscript with advice and revisions to the manuscript from Dr. Farrell. This copyrighted material was reproduced with permission from John Wiley & Sons Inc. (license number 3033430514149).

Figure 1-1 was reproduced from a published manuscript, in which it was Figure 2 on page 128: Piferrer F, Beaumont A, Falguière J-C, Flajšhans M, Haffray P, Colombo L. Polyploid fish and shellfish: Production, biology and applications to aquaculture for performance improvement and genetic containment. Aquaculture 293: 125–156, 2009. This copyrighted material was reproduced with permission from Elsevier (license number 3064460169728).

All experiments in this dissertation were approved by the UBC animal care committee (permits: A10-0236, A09-0707).

Table of Contents

List of Tables

List of Figures

List of Symbols and Abbreviations

ABT: Arrhenius breakpoint temperature.
ATU: accumulated thermal units.
BL s^{-1}: body lengths per second.
BPH: Bluey Pothole 2.
British Columbia: BC.
CaO_2: arterial O_2 content.
CT_{max}: critical thermal maximum (temperature at loss of equilibrium).
D: diffusion coefficient.
ECG: electrocardiogram.
fH: maximum heart rate.
fH_{ABT}: heart rate at Arrhenius breakpoint temperature.
fH_{arrhy}: maximum heart rate just before cardiac arrhythmia onset.
fH_{peak}: the highest heart rate, regardless of temperature.
fH_{scope}: scope of heart rate increase.
fH_{10C}: maximum heart rate at 10°C.
fH_{14C}: maximum heart rate at 14°C.
FVTH: Fraser Valley Trout Hatchery.
Hb: haemoglobin.
Hb_{sat}: percent saturation of haemoglobin.
[Hb]: blood haemoglobin content.
IP: intraperitoneal.
Ko_2: Krogh diffusion constant.
ME: mechanical efficiency.
n: Hill's number.
OEC: oxygen equilibrium curve.
P_{50}: partial pressure of O_2 where haemoglobin is 50 % saturated.
PaO_2: partial pressure of oxygen in arterial blood.
pH_e: extracellular pH.
pHi: intracellular red blood cell pH.
Pco_2: partial pressure of CO_2.
Po_2: partial pressure of O_2.
PO: power output.
PO_2mito: cytoplasmic-mitochondrial O_2 pressure gradient.
PO_2req: the O_2 partial pressure (kPa) required to drive O_2 diffusion to the boundary of a tissue cylinder of radius R.
PO_{2cap}: PO_2 of the capillary blood.
PPH: Pete's Pothole
Q: cardiac output.
q_{cor}: coronary flow.
$Q_{10preABT}$: Q_{10} from 10°C to temperature.
R: Krogh tissue cylinder radius.
r_c: capillary radius.
RBC: red blood cell.
T_{crit}: critical temperature (temperature where aerobic scope is zero).
T_{opt}: optimal temperature (temperature where aerobic scope is maximum).

TDS: total dissolved solids.
$Temp_{arrhy}$: temperature of cardiac arrhythmia onset.
VITH: Vancouver Island Trout Hatchery.
VO_2: myocardial O_2 consumption rate.

1N: haploid.
2N: diploid.
3N: triploid.

β: O_2 solubility.
Δabsorbance: difference between absorbance at 435 nm (peak absorbance of deoxygenated Hb) and 390 nm (isobestic point between oxy- and deoxy-Hb).

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My Ph.D. studies would never have been successful without the invaluable advice and constructive criticism of my supervisory committee. Thank you Colin B. for your wisdom and optimism; Jeff R. for always being approachable and easy to discuss science with; Erik P. for sharing your knowledge of fish in the wild; Rick T. for keeping it all in perspective; and especially Tony F. for your patience (I know I have often pushed it!), always being available and guiding me to become a far better scientist than when I began my Ph.D. I would also like to thank my M.Sc. Supervisor Tillmann B. for inspiring me to follow this path.

The UBC zoology department is a unique department of incredible scientists dedicated to supporting each other and their graduate students to produce great science. I feel honored to have been a part of this department over the past 6 years.

The positive influence of this department creates incredible groups of students and postdocs. I would like to thank the graduate students and postdocs of the zoology department, in particular, the Comparative Physiology group and members of the Farrell lab for their support, inspiration and stimulating scientific discussion. Of this group, I would especially like to thank Katja A., an inspirational mentor, incredible scientist and true friend.

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During my Ph.D. I have been lucky to work as a teaching assistant under Agnes L. Thank you Agnes for guiding me as a scientist and a teacher and providing supportive career advice.

Dedication

This thesis is dedicated to my parents.

I am grateful for the opportunities in life that I have received, but many others have no hope of, allowing me to pursue my dreams. This is especially thanks to the unquestioned and never-ending support of my parents.

Thank you Mom and Dad!

Chapter 1 Introduction

Acquisition of O_2 from the environment and delivery of O_2 to the metabolizing tissues is essential for all vertebrates and dictates where (Claireaux and Lefrancois 2007; Domenici et al. 2007; Chabot and Claireaux 2008; Portner and Farrell 2008), how (Jourdan-Pineau et al. 2012) and if they live (Portner 2002; Lennig et al. 2004; Portner and Knust 2007; Chabot and Claireaux 2008; Jacobson et al 2008; Portner and Farrell 2008). Among fishes, anatomical form and function vary enormously in response to the requirement to transport O_2 from the environment to the mitochondria at sufficiently high rates across a wide range of O_2 demand and availability (Johnston and Bernard 1982; Gamperl and Farrell 2004; Solem et al. 2006; Bernier et al. 2007). Triploid (3N) fish represent a special case of altered anatomy and perhaps physiology, which provide an informative model to study O_2 supply to the body and its effects on survival in nature because there are a number of steps along the O_2 cascade where O_2 supply in 3N, relative to diploid (2N), fish is potentially limited. Compared with normal 2N fish, the additional artificially induced set of chromosomes in 3N fish results in enlarged cells throughout the body. In addition, 3N fish are widely regarded as having low tolerance of sub-optimal conditions and thus poor survival in the wild. This poor tolerance is potentially directly related to a limitation in O_2 delivery to the cells of the body, a possibility that was explored in my thesis.

In order to test the hypothesis that corporeal O_2 supply limits the aerobic performance of 3Ns and that aerobic performance, in turn, limits survival in the wild, I compared the cardiorespiratory physiology of 2N and 3N fish using rainbow trout (*Oncorhynchus mykiss*) as a model organism. I also related aerobic swimming capacity of 2N and 3N trout with summer survival. Based on past findings of poor 3N chronic tolerance of high temperature, in Chapter 2 I investigated whether or not 3N impaired thermal tolerance is related to the capacity of the cardiac muscle to respond to increasing O_2 demands with increasing temperature. I then, in Chapter 3, compared endurance swimming capacity, as a proxy for aerobic capacity, of 2N and 3N rainbow trout populations with their survival in the wild and related their lake O_2 and thermal habitat utilization with lab cardiac performance at similar temperatures. Using a modified Krogh diffusion model applied to the ventricle of 2N and 3N rainbow trout, in Chapter

4, I assessed O_2 supply to the myocardium of 3N trout as a mechanism behind reduced 3N maximum cardiac response to warming shown in Chapter 2. Finally, in Chapter 5, I compared the haemoglobin (Hb) O_2 equilibrium curve of 2N and 3N rainbow trout as a prospective mechanism behind reduced arterial Hb-O_2 saturation in salmonids, which was predicted to be the most important contributor to the 3N cardiac O_2 supply deficiency described by the Krogh diffusion model in Chapter 4.

This ecophysiological knowledge obtained in my thesis is important beyond the fundamental examination of animal design. Rainbow trout are the backbone of a $0.5 billion a year sport fishing industry in British Columbia (BC) with 3N rainbow trout being released into over 50 % of stocked lakes throughout the province. Triploid fish are widely used for sport fishing and aquaculture industries because they are sterile and, when used to stock water systems, reduce the genetic impact on wild populations; likewise 3N escapees from aquaculture sites pose reduced genetic threat. Indeed, triploidy is the only practical sterilization technique used at an industrial scale for fish production (Benfey 2001). Despite the benefit of sterility, and at a great cost to sport fishing and aquaculture industries, 3N compared to 2N growth and survival in lakes and hatcheries is often reduced. Therefore, additional knowledge of the mechanisms explaining their limited performance in the wild is useful for industry and fish conservation efforts.

This introduction to my thesis will present the relevant literature justifying my hypothesis that corporeal O_2 supply limits the aerobic performance of 3Ns and that aerobic performance, in turn, limits survival in the wild. I will focus on explaining what a 3N fish is and reviewing the current literature investigating physiological differences between 2N and 3N fish, with a focus on survival, the O_2 transport cascade, aerobic capacity and swimming and high temperature tolerance. I will also describe the current literature on fish locomotion and its relationship with survival in the wild.

Triploid Fish

3N Production

Normal sexual reproduction in fishes involves meiotic division to produce haploid (1N) sperm and ova, which combine to restore diploidy in the progeny. Artificial production of 3N fish most commonly occurs by suppression of the 2nd meiotic division of fertilized eggs by

exposing the egg to a shock (generally through temperature, hydrostatic or chemical treatments) shortly after fertilization (Lou and Purdon 1984). In normal development, eggs are stalled with a 2N chromosome complement at the metaphase stage of meiosis II until contact with a 1N spermatozoon, which initiates the 2nd meiotic division and release of a polar body containing one copy of the maternal chromosome (Figure 1-1). Therefore, joining of the sperm and egg nuclei after the 2nd meiotic division results in an egg with 1N paternal and 1N maternal content. However, a shock to the egg soon after fertilization prevents expulsion of this 2nd polar body, ultimately resulting in an 3N egg with 2N of the maternal and 1N of the paternal chromosome compliment (Figure 1-1). Triploidy is also possible through inter-ploidy crosses between tetraploid and diploid brood, through hybrid crosses between 2 distantly related species (Piferrer et al. 2009) and spontaneously from eggs of poor quality (Thorgaard and Gall 1979). Additionally, reproducing wild populations of 3N fish exist in nature and polyploidization in general has occurred several times during the evolutionary history of fishes (Leggatt and Iwama 2003). My thesis will focus on the physiology of 3N salmonids that were artificially produced by hydrostatic pressure shock soon after fertilization.

There are a number of known effects of artificial triploidization on fish. The hydrostatic pressure shock may have direct, non-genetic effects on the egg. However, as the pressure shock is applied at the one cell stage of development, these effects would be passed to all cells of the adult. Thus, pressure shock effects are most likely fatal during early development and therefore not apparent in post hatch 3N populations. Additionally, the increase in genetic material directly causes a 50 % greater maternal contribution to the genome, sterility, increased cell volume and potentially altered gene expression, all of which likely have whole animal level effects. This introduction will review these known effects with an emphasis on their relationship to 3N corporeal O_2 supply, locomotion, temperature tolerance and general survival.

3N Sterility & Gonad Development

Sterility in 3N fish stems from interference of the 3rd chromosome set in homologous chromosome pairing during early meiosis I (Gui et al. 1992; Carrascoet al. 1998; Krisfalusi and Cloud 1999; Cuñadoet al. 2002), which prevents gonad development and gametogenesis and thus retards ovarian, but not testicular growth (Benfey et al. 1989; Schafhauser-Smith and

Benfey 2003). In female fish, follicle enclosure of the oocyte is dependent on the progression of germ cells through meiosis and necessary for the synthesis and secretion of the sex hormones. As very few 3N oocytes develop to the follicular stage, female 3N fish do not develop endocrine-mediated sexual maturation characteristics and are fully sterile (Lincoln and Scott 1984; Sumpter et al. 1984; Nakamura et al. 1987; Benfey et al. 1989b; Johnstone et al. 1991; Mol et al. 1994; Hussain et al. 1995; Breton and Sambroni 1996). Testes development is not limited by germ cell progress, so, male 3N fish display normal gonad development and sex hormone production. As a result, female 3N fish do not develop secondary sex characteristics, but 3N male fish do. In both sexes only a small proportion of the produced germ cells fully develop (which results in a large absolute number of sperm produced by male 3Ns, but a low probability of any egg production by female 3Ns), but offspring of successful fertilizations involving 3N eggs or sperm are not viable (Penman et al. 1987; Piferrer et al. 1994a; Brämick et al. 1995; Gillet et al. 2001; Perruzi et al. 2009; Feindel et al. 2010).

3N Performance

In addition to the ecological conservation benefits of 3N sterility by limiting genetic influences of stocked non-native fish on native populations, superior performance in terms of growth and survival were expected by removing the tremendous energy investment into gonad production during sexual maturation. However, this benefit has not been realized in 3N salmonids. In fact, the side effects of increased genome size appear to impair 3N performance, with 3N compared to 2N survival in the wild often reduced, especially when conditions are sub-optimal. In lakes, 3N survival is often reported as reduced (Blanc et al. 1992; Simon et al. 1993; Withler et al. 1995; Oppedal et al. 2003), but also sometimes higher (Teuscher et al. 2003; Koenig et al. 2011) or similar (Guo et al. 1990; Withler et al. 1995; Dillon et al. 2000; Oppedal et al. 2003; Teuscher et al. 2003; Wagner et al. 2006) when compared with 2N survival, depending on fish size, age and sex and water conditions. However, when reared at chronically low O_2 and/or high temperature in labs and hatcheries (Ojolick et al. 1995; Myers and Hershberger 1991) and under sub-optimal O_2 and temperature conditions in either lakes (Simon et al. 1993; Koenig and Myer 2011) or marine cage sites (Blanc et al. 1992; Simon et al. 1993; Altimiras et al. 2002), 3N survival, independent of size, age or sex, is low. Observations of

impaired 3N performance may be related to gene dosage-related affects on gene expression or increased cell volume and its downstream affects on anatomy and O_2 supply.

3N Gene Expression

The extra chromosome in 3N cells can directly influence the genotype through increased bulk DNA (2/3 of which is of maternal origin) and alleles at each locus. The resultant increase in allelic diversity is reflected in increased phenotypic variance (Sheerer et al. 1987; Bonnet et al. 1999; Blanc et al. 2001; Friars et al. 2001; Johnson et al. 2004; Johnson et al. 2007). In theory, the 50 % extra DNA in 3N cells increases the gene dose and therefore phenotypic and genotypic variability and heritability. Patterns of heritability and maternal effects provide evidence supporting high gene dosage (Johnson et al. 2007), which is further supported by reports of increased heterozygosity (Allendorf and Leary 1984; Leary et al. 1985), but one study reports no difference in heterozygosity (Garner et al. 2008). On the other hand, similar trends in 2N and 3N gene expression point towards a compensatory decrease in expression (Suresh and Sheehan 1998; Shrimpton et al. 2007; Garner et al. 2008; Pala et al. 2008; Ching et al. 2010) in 3N fish, potentially through incomplete silencing of one of the 3 alleles, and almost certainly not an entire chromosome set (Pala et al. 2008). Considering the high plasticity of gene regulation at the organ level, observed ploidy differences in gene expression are more likely to stem from differences in the physiological state of the organism than from increased quantity of gene loci.

3N Cell Volume

Body and organ sizes have been shown to be similar between 2N and 3N Teleosts of the orders, Cypriniformes, Perciformes, Gadiformes and Salmoniformes (Bonar et al. 1988; Parsons and Meals 1997; Lilyestrom et al. 1999; Felip et al. 2001; Simonot 2005; Feindel et al. 2011; Manor et al. 2012). However, because of maintenance of a constant nucleo-cytoplasmic ratio and a 50 % increase in nuclear material, cells throughout the body of 3N fish are approximately 30 % larger, but fewer in number. Larger but fewer cells throughout the body potentially have multifaceted affects on cellular, organ and whole animal level function. At the cellular level, an increase in surface area to volume ratio of enlarged 3N cells should decrease

metabolic energy investment to maintain trans-membrane electrochemical gradients. Conversely, trans-membrane processes, such as diffusion into cells or signal propagation, should be slower relative to cell volume. In the absence of any compensation, membrane transport and receptor protein densities will be lower in 3N cells, while a compensatory increase in membrane proteins necessarily requires a decrease in other membrane components, with potential positive, negative or neutral effects. Additionally, in anatomical structures where cell shape is not plastic enough to maintain 2N-like intra-cellular distances, transportation and diffusion distances through cells will increase and cellular organization of structures may be altered. All are expected to alter whole organism physiology to some degree; for example, 3N brown trout (*Salmo trutta*) may be more dependent than 2Ns upon intracellular Ca^{2+} stores during cardiomyocyte contraction, which is likely due to reduced surface area to volume ratio in the enlarged 3N cardiomyocytes (Mercier et al. 2002), and may contribute to impaired 3N heart pumping ability.

Effects of enlarged 3N cells are particularly interesting because of the profound effect a small change in diffusion distances can have on O_2 supply to the mitochondria, which I propose as a mechanism behind impaired aerobic performance in 3N, relative to 2N salmonid fish. According to Fick's second law of diffusion, O_2 flux decreases proportionally to the square of diffusion distance and increases linearly with cell surface area. Thus, increased diffusion distances and reduced surface area to volume ratios potentially have profound effects on diffusion through enlarged 3N cells. This could be particularly problematic for O_2 diffusion through: 1) the single layer of gill epithelial cells, impairing O_2 diffusion from the water into the arterial blood; 2) across the surface of red blood cells (RBCs), impeding haemoglobin (Hb)-O_2 loading at the gills; and 3) from the capillaries through the tissue's enlarged cells to the mitochondria, all of which could cause impaired corporeal O_2 supply and thus reduce aerobic capacity in 3N salmonids. A simple example of how this might be problematic is in capillary morphology, which must increase in diameter to accommodate a 30 % larger RBC, potentially resulting in fewer capillaries per unit tissue volume, which would be detrimental to O_2 diffusion. Furthermore, due to enlarged muscle cells, the diffusion distance between the centre of an enlarged 3N capillary and the centre of an enlarged muscle cell is potentially greater. In view of this, it is important to break down the delivery of O_2 to mitochondria from the environment into

its component steps, the O_2 cascade.

Potential Impedance to Corporeal O_2 Supply in 3N Fish (the O_2 Cascade)

Corporeal O_2 supply begins with passive O_2 diffusion across the gills and ends with passive O_2 diffusion through tissue cells to mitochondria. In between, the cardiac muscle propels blood from the gills to the tissue to deliver O_2, taking advantage of Hb contained in RBCs to increase the blood O_2 solubility (Figure 1-2). Corporeal O_2 supply can potentially be impaired at many sites, but the powerhouse of the delivery system, the heart, may be particularly vulnerable because it requires its own O_2 supply, and research on 2N fish has identified the heart as a limiting organ for aerobic performance. For example, during critical swimming velocity tests on chinook salmon (*Oncorhynchus tshawytscha*) (Gallaugher et al. 2001) and rainbow trout (Thorarensen et al. 1996), cardiac output plateaus soon before swimming failure.

The first step of the O_2 cascade in fish is convection of water to and across the surface of gill lamellae, where O_2 diffuses from the water, across the gill epithelium and into the arterial blood. Enlarged 3N epithelial cells potentially increase the boundary layer between the water and blood, decreasing O_2 diffusion rate into the blood. Anatomically, 2N and 3N tench (*Tinca tinca*) gill surface area was similar and 3N cross gill diffusion distance was actually shorter (Flajshans et al. 2006). Measures of gill filament surface area, on the other hand, were smaller for 3N than 2N Atlantic salmon (*Salmo salar*) (Sadler et al. 2001), but did not include lamellae, which is the primary surface of gas exchange and the most plastic gill structure able to respond to the environment. Therefore, gill surface area and diffusion distances suggest 3N fish are equally or better able to transport O_2 across their gills into the blood. This is supported by the observation that arterial PO_2 values did not differ with ploidy in maximally swimming chinook salmon (Bernier et al. 2004).

Though O_2 diffusion across the gills is apparently not limited in 3N salmonids, the blood O_2 content of resting and maximally swimming 3N chinook salmon is 20 to 30 % lower then in 2Ns (Bernier et al. 2004), suggesting a ploidy effect on Hb-O_2 loading. The O_2 carrying capacity of blood is determined by blood Hb content ([Hb]), which is the product of the Hb concentration per RBC and the number of RBCs per volume of blood, which is most often expressed as haematocrit. When compared with 2N conspecifics, [Hb] has been reported to be lower in many

3N species: Atlantic salmon, Coho salmon (*Oncorhynchus kisutch*), Caspian salmon (*Salmo trutta caspius*), rainbow trout, white crappie (*Pomoxis annularis*) and turbot (*Scophthalmus maximus*). However, other studies have reported [Hb] values for 2N and 3N fish to be similar, such as in rainbow trout, brook charr (*Salvelinus fontinalis*), shortnose sturgeon (*Acipenser brevirostrum*), ayu (*Plecoglossus altivelis*), ginbuna (*Carassius auratus langsdorfi*) and shi drum (*Umbrina cirrosa*) (reviewed by Benfey 1999 and Fraser et al. 2012). The only report of elevated 3N blood [Hb] is for transgenic Atlantic salmon (Cogswell et al. 2002). Thus, across a broad range of species, 3N blood O_2 carrying capacity is either reduced or the same compared to their 2N cohorts.

In addition to potentially reduced O_2 carrying capacity, O_2 affinity of Hb in 3N blood may be reduced. In resting and maximally swimming 3N chinook salmon, a 25 % reduction in saturation was seen (Bernier et al. 2004), similar to earlier *in vitro* results for Atlantic salmon blood (Graham et al. 1985). Though previous assessments of 2N and 3N Atlantic salmon Hb found no ploidy differences in Hb-O_2 affinity (Graham et al. 1985; Sadler et al. 2000) or isohaemoglobin components (Sadler et al. 2000), similar blood PO_2 and gill anatomy along with reduced Hb-O_2 saturation provide evidence of reduced Hb-O_2 affinity in 3N blood. These previous studies of blood-O_2 binding characteristics, however, used tonometry techniques that required large volumes of blood (and thus pooled blood due to the small body size of fish used), had small sample sizes and were not always able to test for statistical significance. Therefore, reduced Hb-O_2 affinity has not been conclusively eliminated as the mechanism causing reduced 3N, relative to 2N, arterial O_2 content. Nevertheless, with the potential of reduced [Hb] and Hb-O_2 saturation, the corporeal O_2 supply in 3N salmonids is likely severely impeded.

Any reduction in arterial O_2 content of 3N salmonids could be compensated for by increased cardiac output (arterial O_2 delivery is the product of arterial O_2 content and cardiac output). This would mean that the heart of a 3N fish would routinely work harder and, unless an unlikely compensatory increase in maximum cardiac output occurs, may have a reduce scope for increase to its maximum achievable workload. *In vivo* and *in vitro* measurements of cardiac output and cardiac power output capacities of 3N brown trout were comparable to 2Ns of other salmonid species (Altimiras et al. 2002; Mercier et al. 2002). Unfortunately, the most important comparison to 2N brown trout was lacking in these studies. On the other hand, the less rounded

morphometry of 3N, compared to 2N, aquaculture-reared Atlantic salmon hearts was interpreted to reflect a training effect on 3N hearts resulting from greater work to maintain arterial O_2 convection with a reduced arterial O_2 content (Leclercq et al. 2011). Furthermore, high incidences of cardiac aneurisms and infarcts reported in 3N brown trout that had died during high temperature rearing may have been due to excessive cardiac demands (Mercier et al. 2000). Thus, whether the cardiac muscle compensates for reduced arterial O_2 content to maintain arterial O_2 delivery is unknown. However, given potential reductions in diffusivity of O_2 through the enlarged 3N cardiomyocytes, it seems unlikely that the maximum workload of 3N hearts can exceed that of 2N hearts unless there were major anatomical and physiological cardiac compensations.

The salmonid ventricle makes up a mere 0.1 % of body mass and is necessarily aerobic. Its O_2 supply differs between the two myocardial muscle layers that comprise the ventricle. The cortical compact myocardium is vascularized and derives its highly oxygenated capillary blood supply from the coronary artery, while the inner spongy, trabecular myocardium lacks blood vessels and relies on O_2 -poor venous blood bathing the lumen of the heart after first supplying the rest of the body with O_2. Clearly the spongy myocardium has a more precarious O_2 supply then the compact myocardium because of the lower Po_2 of venous blood compared with arterial blood (Davie and Farrell 1991). Krogh's O_2 supply model (Krogh 1919) can be used to examine the anatomical limits for O_2 supply to the ventricular myocardium.

According to Krogh's O_2 supply model (Figure 1-2), the diffusion rate of O_2 is determined by the O_2 tension (Po_2) gradient, diffusion distance, O_2 consumption rate and Krogh's diffusion constant for O_2 (Ko_2). Regardless of muscle type, O_2 must diffuse from the blood across the endothelium and sarcolemmal membrane and through the sarcoplasm to the mitochondria. Thus, the compact myocardium, which makes up 30 to 40 % of the ventricular mass in rainbow trout (Clark and Rodnick 1998), is supplied with O_2-rich blood via the coronary artery, such that the primary force determining O_2 diffusion to the mitochondria of compact myocardium is capillary Po_2 and capillarity, cell size and mitochondria position within the cardiomyocyte establishes the anatomical distance over which diffusion must occur. Triploidy potentially affects all four of these determinants; however, as mitochondria make up approximately 50 % of the trout cardiomyocyte volume (Vornanen 1998), repositioning is unlikely to affect much change in

diffusion distance. In contrast, in the spongy myocardium, which makes up the majority of the ventricular mass in rainbow trout, the primary force driving O_2 diffusion to the mitochondria of the trabeculae is venous PO_2, and trabecular diameter and mitochondrial position within the cardiomyocytes determine the distance O_2 must diffuse. Triploidy potentially affects trabecular diameter through increased cardiomyocyte volume.

In addition to differences in O_2 supply, the salmonid ventricle shows other types of metabolic zonation between the compact and spongy myocardia. According to LaPlace's Law, wall tension increases with radius and therefore the outer compact myocardium must carry a greater power output burden compared with the inner spongy layer. This would raise O_2 demand of the compact myocardium, which in combination with potential increases in diffusion distance through 3N cardiomyocytes, conceivably may compromise O_2 supply in compact myocardium of 3N salmonids relative to demand. Furthermore, the spongy myocardium may be better-equipped enzymatically to utilize glycolytic pathways to supplement O_2 deficiencies (Ewart and Driedzic 1986; Farrell et al. 1990; Clark and Rodnick 1998). The compact myocardium appears to have a greater capacity for lipid metabolism and mitochondrial O_2 consumption (Poupa et al. 1974; Ewart and Driedzic 1986), suggesting the spongy myocardium may be better able to utilize anaerobic glycolytic metabolic pathways than the compact myocardium, which enzymatically appears to be more committed to aerobic pathways.

Therefore, impaired O_2 supply to the 3N compact myocardium may be predicted to be more detrimental than in the spongy myocardium. This aspect of 3N physiology has never been investigated.

3N Aerobic Capacity

An impediment to O_2 passage through the O_2 cascade should manifest itself in reduced aerobic performance. Few previous studies have compared aerobic performance in 2N and 3N fish, but findings from those that have, are equivocal.

Comparisons of 2N and 3N swimming performance have revealed no differences in aerobic swimming capacity (Small and Randall 1989; Sezaki et al. 1991; Parsons 1993; Stillwell and Benfey 1996; Cotterell and Wardle 2004; Lijalad and Powell 2009; Bernier et al. 2004), but potential differences in anaerobic metabolite production through swimming (Virtanen et al.

1990). For example, clear elevations of anaerobic metabolites occurred after 3-h of sustained swimming (1.5 body lengths (BL) s^{-1}) in 3N, but not 2N rainbow trout (Virtanen et al. 1990), suggesting 3N trout were more dependent on anaerobic metabolism to fuel their swimming muscles. Though Cotterell and Wardle (2004) found the maximum sustained swimming speed did not differ with ploidy in Atlantic salmon, only 28 % of the 3N population, compared to 40 % of the 2N population were able to swim to the level required for the challenge. These findings suggest a smaller proportion of the 3N population could achieve comparable swimming performance to the 2N population. Furthermore, endurance time at swimming speeds above the maximum sustained speed was shorter for the 3N than the 2N population subsamples. Similarly, 53 % of 2N compared to 37 % of 3N ginbuna (*Carassius auratus*) were capable of maintaining a swimming speed of 48 cm s^{-1} for 30 min (Sezaki et al. 1991). Mean critical swimming velocities of 3N rainbow trout, white crappie, brook charr and chinook salmon all tended to be up to 10 % lower compared with 2N populations without reaching statistical significance (Small and Randall 1989; Parsons 1993; Stillwell and Benfey 1996; Bernier et al. 2004), but the same for 2N and 3N Atlantic salmon (Lijalad and Powell 2009).

With few exceptions, resting, routine and maximum metabolic rates of 2N and 3N fish tend to be similar. No difference was found between 2N and 3N resting metabolic rates for three-spined stickleback (*Gasterosteus aculeatus*) (Swarup 1959), Atlantic salmon (Benfey and Sutterlin 1984; Lijalad and Powell 2009), embryonic, 0+ and 1+ rainbow trout (Olivia-Teles and Kaushik 1987 and 1990a,b; Yamamoto and Iida 1994; Scott 2012), brook charr (Hyndman et al. 2003a) or ginbuna (Sezaki et al. 1991), white crappies (Parsons 1993). Metabolic rate of 2N and 3N chinook salmon swimming at 0.40 BL s^{-1} at 15 ^{o}C did not differ, but two strains of 3N brook charr at a similar temperature and swimming speed had lower metabolic rates than their 2N siblings (Stillwell and Benfey 1997). Additionally, 3N brook charr and Atlantic salmon acclimated to temperatures ranging from 12 to 18 ^{o}C had greater metabolic rates then 2Ns when at cool temperatures and lower metabolic rates than their 2N siblings when at higher temperatures (Atkins and Benfey 2008). Maximum metabolic rate of 2N and 3N fish did not differ in Atlantic or chinook salmon (Bernier et al. 2004; Lijalad and Powell 2009), but metabolic rate immediately after being chased to exhaustion was 15 % lower in 3N than 2N brook charr (Hyndman et al. 2003a,).

Aerobic scope, which is the difference between resting and maximum metabolic rate, of 3N chinook salmon was only 40 % of their 2N counterparts (Bernier et al. 2004). In order for 3N aerobic scope to be reduced compared 2N aerobic scope, differences in maximum and/or resting metabolic rate must exist. Thus, the general lack of significant differences in resting and maximum metabolic rate between 2N and 3N fish across several species, including chinook salmon is surprising.

Thus, evidence of reduced aerobic capacity in 3N salmonids is equivocal among studies and species. Yet, evidence exists for a small and sometimes significant reduction of aerobic swimming performance which, combined with reduced aerobic scope, could be taken as circumstantial evidence of an impediment to O_2 passage through the O_2 cascade in 3N salmonids.

3N Hypoxia and Temperature Tolerance

Elevated temperature and environmental hypoxia place burdens on O_2 delivery in fish (Farrell 2009; Portner & Farrell 2008; Farrell and Richards 2009). Research on 2N fish has identified the heart as a limiting organ for high temperature tolerance. For example, while gradually warming swimming sockeye salmon (*Oncorhynchus nerka*), cardiac output begins to plateau soon before swimming failure (Steinhausen et al. 2008). Furthermore, in Coho salmon, aerobic scope closely tracks the heart rate response to warming (Casselman et al. 2012). Given my hypothesis that corporeal O_2 supply limits the aerobic performance of 3N rainbow trout, these burdens are predicted to be exacerbated with triploidy. Indeed, the trends of reduced tolerance of 3N salmonids at the whole fish level for chronic exposure to sub-optimal conditions, especially low O_2 and high temperature, provided further evidence of a corporeal O_2 supply limitation along the O_2 cascade in 3N salmonids. For example, 3N populations experienced higher mortalities than their 2N cohorts when reared under chronically sub-optimal oxy-thermal conditions in either lakes (Blanc et al. 1992; Simon et al. 1993; Koenig and Myer 2011) or marine cage sites (Mercier et al. 2000; Altimiras et al. 2002).

Acute hypoxia tolerance can be reduced in 3N rainbow trout. For example, time to loss of equilibrium in 10 % O_2 saturated water (1.5 mg l^{-1}) is shorter in 3N compared to 2N young of

the year rainbow trout (Scott 2012). However, as water O_2 concentration was gradually lowered from 6 to 1.5 mg l^{-1}, 3N and 2N rainbow trout O_2 consumption rate did not differ until O_2 concentration fell to 1.5 mg l^{-1}, at which 3N O_2 consumption rate became lower than the 2N rate (Yamamoto and Iida 1994). On the other hand, the Po_2 at loss of equilibrium did not differ between 2N and 3N Atlantic salmon (Benfey and Sutterlin 1984).

Direct empirical evidence of reduced 3N tolerance of high temperatures is more tenuous than reduced hypoxia tolerance. Impaired acute thermal tolerance of 3N salmonids has only been demonstrated in conjunction with disease outbreak in rainbow trout (Ojolick et al. 1995). Estimates of the temperature at loss of equilibrium (CT_{max}) during a 2-15 ^{o}C h^{-1} incremental temperature challenge for rainbow trout (Scott 2012) and brook charr (Benfey et al. 1997) and time to loss of equilibrium during a 2 ^{o}C per day incremental temperature challenge (chronic lethal maximum) on rainbow trout and brook charr (Galbreath et al. 2006) reveal no ploidy differences. During high temperature exposures lasting weeks, on the other hand, mortality rates of 3N populations are consistently higher than 2N populations, despite determined or assumed high water O_2 saturation (Myers and Hershberger 1991; Altimiras et al. 2002; Hyndman et al. 2003b). A relationship between impaired 3N high temperature tolerance and cardiorespiratory differences is supported in altered temperature responses of resting metabolic rate between 2N and 3N brook charr and Atlantic salmon. For both species, metabolic rate of 3N compared to 2N fish was significantly higher at cool temperatures (12 and 9 ^{o}C for the salmon and charr, respectively), but lower at warmer temperatures (18 and 15 ^{o}C for the salmon and charr, respectively) (Atkins and Benfey 2008). The same trend was not seen in 2N and 3N embryonic to larval brook charr, which had similar heart rates at 6, 9 and 12 ^{o}C (Benfey and Bennett 2009). Recent theories on high temperature tolerance of ectotherms attribute temperature tolerance in general to be associated with maintenance of O_2 delivery to the body (Farrell 2009; Portner and Farrell 2008). As temperature moves above the temperature at which aerobic scope is greatest (T_{opt}), aerobic scope narrows until becoming zero at the critical temperature (T_{crit}), beyond which survival is temporally limited because it is dependent on either anaerobic metabolism or diversion of aerobic metabolic expenditures from essential processes (Farrell 2009; Portner and Farrell 2008).

In summary, the physiological differences between 3N and 2N cohorts tend to be small or

nonexistent, depending on the variables compared, species and experiment. Moreover, the most convincing differences (impaired 3N aerobic swimming and tolerance of sub-optimal conditions) can be linked to an impediment to O_2 passage through the O_2 cascade. A clear problem exists in arterial O_2 loading for 3N fish, which appears to be due to either reduced Hb-O_2 affinity or reduced blood [Hb] and not impaired cross gill diffusion. Further down the O_2 cascade, reduced arterial O_2 content can combine with anatomical limitations to O_2 diffusion, which might prove particularly problematic in compact myocardium of 3N fish. This would then exacerbate problems associated with any compensatory increase in cardiac output to maintain arterial O_2 transport at 2N-like rates in the face of reduced arterial O_2 saturation. Collectively, these impairments could account for the observed reduced 3N hypoxia and high temperature tolerance and thus survival in the wild and under hatchery conditions, as explained in the next section.

Influence of Swimming and Aerobic Capacity on Survival in the Wild

Many researchers have identified swimming performance as an important trait contributing to the survival of fish (Nelson 1989; Plaut et al. 2001; Nelson et al. 2002; Domenici et al. 2010; Oufiero et al. 2011). However, due to difficulties in empirically assessing this relationship, few direct empirical tests of a relationship between fish locomotion capacity and survival in nature have been reported (Reidy et al. 2000). In order for natural selection to be determined to influence a trait, its variance within the population must be consistent through space and time (Jayne and Bennett 1990; Bennett and Huey 1991). Beamish (1978) described three swimming modes: burst (fast speeds sustainable for < 20 s), prolonged (speeds sustainable for more than 20 min and less than 200 min) and sustained (speeds sustainable for more 200 min), with only the later thought to be entirely aerobically powered, though 70 % of prolonged swimming speed may be aerobically powered too. Aerobic and anaerobic swimming performances are heritable (Cano and Nicieza 2006), variable (Kolok 1999; Billerbeck et al. 2001; Langerhans et al. 2004; Walker et al. 2005) and repeatable over time (Reidy et al. 2000; Claireaux et al. 2005; Claireaux et al. 2007; Pedersen et al. 2008; Vandamm et al. 2012) in different groups of fish. Thus both aerobic and anaerobic swimming performances are potentially under natural selection within populations of fish.

Furthermore, according to lab and simulated wild habitat experiments and correlations of population characteristics with and without predation exposure, burst and prolonged swimming performance appear to be important to predation escape and thus survival. Prolonged and burst swimming increase the likelihood of sea bass (*Dicentrarchus labrax*) surviving predation by avian piscivores in simulated estuaries (Handelsman et al. 2010), but acceleration performance of wild and hatchery sea bass stocked into the same mesocosm system for 16 to 24 weeks did not predict survival or growth despite greater than 50 % mortality within the populations (Vandamm et al. 2012). Greater burst swimming speeds have been observed in highly predated upon populations of the Trinidadian killifish (*Rivulus hartii*) (Oufiero et al. 2011), male mosquitofish (*Gambusia affinis*) (Langerhans et al. 2004) and guppies (*Poecilia reticulata*) (Walker et al. 2005) when compared to populations of the same species experiencing low predation. Additionally, high prolonged and burst swimming speeds increase the likelihood of Atlantic silverside (*Menidia menidia*) surviving attacks from piscivorous fish in the lab (Lankford et al. 2001).

Rainbow trout with high prolonged swimming capacity, as assessed through critical swimming velocity, have higher active metabolic rates, aerobic scopes and maximum cardiac outputs than trout with lower critical swimming velocities (Claireaux et al. 2005). The evident importance of aerobic capacity to prolonged swimming capacity and thus predator avoidance suggests sustained swimming (a form of locomotion entirely fueled by aerobic metabolism) may also reflect the ability of a fish to avoid predation.

In addition to improved predator avoidance capabilities through increased prolonged swimming capacity, a high aerobic capacity and scope likely improves fish tolerance of metabolic challenges and recovery time after experiencing metabolic challenges. In fact, a high aerobic scope in European sea bass increased the rate of recovery after a constant acceleration test (Marras et al. 2010). Therefore, a high aerobic capacity can be predicted to support survival in the wild through improved locomotion capabilities and rapid recovery after metabolic challenges such as predator avoidance, competitive interactions or enduring suboptimal conditions.

Because of the above mentioned reductions in 3N aerobic swimming capacity and survival in the wild as well as poor tolerance of sub-optimal O_2 and thermal conditions of 3N compared to

their 2N cohorts, triploidy provides an informative model to investigate the hypothesis of a relationship between endurance swimming (as a proxy for aerobic capacity) and survival in the wild.

As explained earlier, 3N production techniques allow creation of sibling populations of 2N and 3N fish. Sibling populations of different ploidies provides greater variability in swimming performance and survival than within a single ploidy of the same species and or strain, and improves consistency of non-locomotory or metabolic genetic differences compared to what inter-strain or -species comparisons provide (Scott 2012). Furthermore, as the aerobic capacity of 3N salmonids may also be reduced compared to in their 2N cohorts, some inferences of the inter-relationships between aerobic swimming, aerobic capacity and survival in the wild can be drawn.

Experimental Approach

The overarching hypothesis that was tested in my thesis is that O_2 supply to the body limits the aerobic performance of 3N rainbow trout, which in turn limits survival in the wild. To test this hypothesis, I compared thermal tolerance of 3N rainbow trout with respect to cardiac collapse (Chapter 2), and related temperature tolerance and aerobic performance to survival in the wild (Chapter 3). Then, in order to relate impaired thermal tolerance and lake survival to corporeal O_2 supply in 2N and 3N trout, I applied a modified Krogh diffusion model to the compact myocardium of 2N and 3N salmonid fish (Chapter 4) and assessed the potential of reduced 3N Hb-O_2 affinity as a limitation in O_2 loading at the gills (Chapter 5).

More specifically, in light of high mortalities in 3N populations chronically held at high temperatures (Simon et al. 1993; Blanc et al. 1992; Ojolick et al. 1995; Mercier et al. 2000; Altimiras et al. 2002; Koenig and Myer 2011), I investigated thermal tolerance of 3N rainbow trout in Chapter 2 with the hypothesis that impaired 3N high temperature tolerance is due to an early collapse of corporeal O_2 supply. Corporeal O_2 supply limitations could stem from impaired arterial O_2 loading at the gills, O_2 diffusion to the cardiomyocytes' mitochondria and/or cardiac pumping capacity. Despite convincing evidence of impaired 3N tolerance of chronic high temperatures, chronic lethal maximum does not differ and T_{crit} has repeatedly been shown to not differ with ploidy, therefore, considering evidence of reduced 3N cardiorespiratory capacity

discussed above, I performed acute temperature challenges while monitoring heart rate and with cardiac collapse as the end point. This protocol is more informative and sensitive than using loss of equilibrium as an end point.

Chapter 3 then examined the relationships among ploidy, endurance swimming and survival in the wild and tested the primary hypothesis of the thesis while using Chapter 2 data to interpret telemetry-based lake thermal habitat utilization findings. Researchers repeatedly claim the importance of fish locomotion performance to survival in nature (Nelson 1989; Plaut et al. 2001; Nelson et al. 2002; Domenici et al. 2010; Oufiero et al. 2011), but Chapter 3 is the first direct test of this relationship in wild conditions I am aware of. Triploids, with their impaired survival and potentially reduced aerobic swimming capacity compared to 2N conspecifics, provide an informative model to test this relationship. Furthermore, in this Chapter, I compare aerobic swimming performance and, for the first time, habitat utilization of 2N and 3N rainbow trout.

The relationship between cardiac function and impaired 3N trout high temperature tolerance and thus survival shown in Chapters 2 and 3 in conjunction with potential 3N cardiac O_2 supply limitations, lead to the hypothesis of O_2 supply limitations to the 3N compact myocardium. This hypothesis was tested in Chapter 4 with a modified Krogh diffusion model, which probes how O_2 supply to the ventricle might differ as a result of triploidy. The output of this model can provide insights into the role cardiac, and thus corporeal O_2 supply plays in 3N tolerance and survival findings in Chapters 2 and 3.

Finally, and because in Chapter 4 the Krogh model predicted reduced arterial O_2 loading as a more important limiter of O_2 supply to the 3N compact myocardium than enlarged capillaries or cardiomyocytes, in Chapter 5 I eliminated reduced Hb-O_2 affinity as the mechanism behind reduced 3N arterial Hb-O_2 loading. In this chapter I tested the hypothesis that reduced 3N arterial O_2 loading is due to reduced Hb-O_2 affinity. Though, previous investigations found no difference in Hb-O_2 affinity between 2N and 3N blood (Graham et al. 1985; Sadler et al. 2000), technology at the time of these studies was restricted to tonometry techniques, which required large volumes of blood, resulting in small sample sizes and no test of significance. Therefore, the recent availability of the Pwee 50 technology allowing for Hb-O_2 curve measurement on small blood samples, warranted reexamination of 3N Hb-O_2 affinity.

The individual hypotheses of each chapter of my thesis combine into an integrated approach

to test my overall thesis hypothesis that O_2 supply to the body limits the aerobic performance of 3N rainbow trout, which in turn limits survival in the wild. These O_2 supply limitations my occur due to impaired arterial O_2 loading at the gill, diffusional supply to the cardiomyocytes' mitochondria and/or cardiac pumping capacity. My thesis will thus contribute to a better understanding of the mechanisms behind poor performance of 3N salmonid fish and the importance of locomotor performance to the fitness of fish in general.

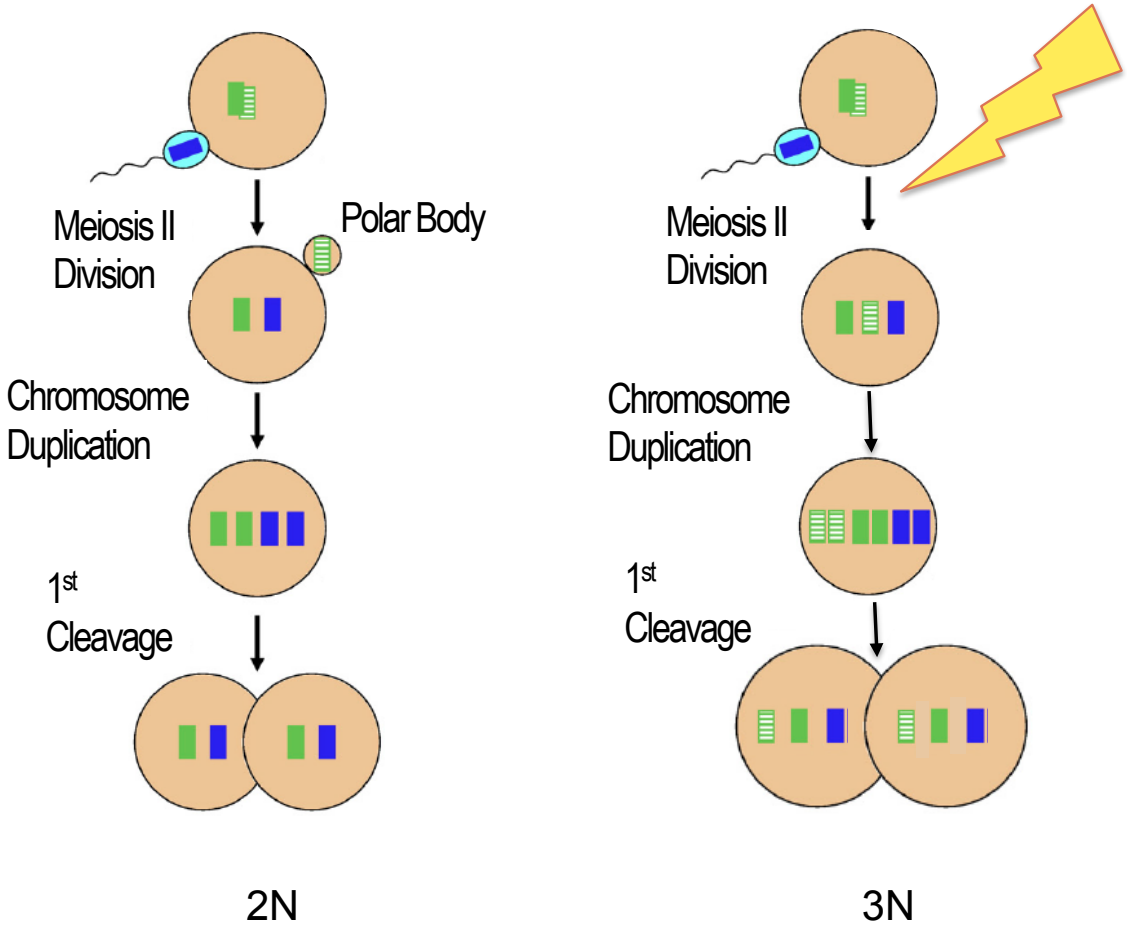

Figure 1-1. Meiotic divisions in fish eggs with fertilization and manipulation to create 3N fish. Adapted, with permissions, from Figure 2 from Piferrer et al. (2009).

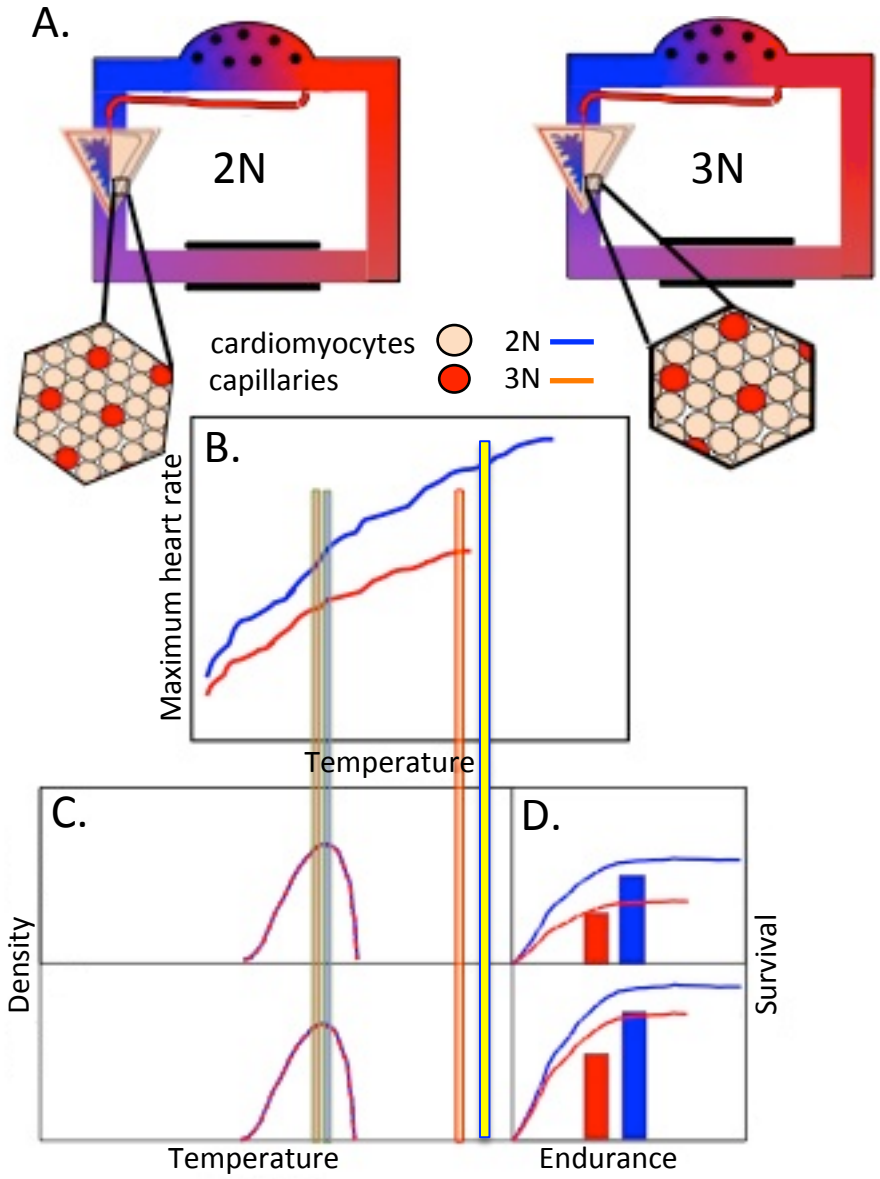

Figure 1-2. O_2 supply to the body limits the aerobic performance of 3N rainbow trout. A. The O_2 cascade of 2N and 3N salmonid fish, illustrating reduced arterial O_2 content and enlarged cardiomyocytes and capillaries in 3N fish. B. The response of 2N and 3N maximum heart rate to warming. (▫) 3N optimal temperature; (▫) 2N optimal temperature; (▫) 3N critical thermal maximum; (▫) 2N critical thermal maximum. C. Kernel density plots of temperature of 2N and 3N rainbow trout equipped with transmitters and stocked into 2 lakes (PPH and BPH). D. Endurance and survival of 2N and 3N rainbow trout in PPH and BPH. Bars represent endurance (x-axis) and survival (y-axis) of the respective ploidy. Lines predict survival based on endurance swimming of the respective ploidy.

Chapter 2 [*]Impaired Temperature Tolerance of Triploid Rainbow Trout (*Oncorhynchus mykiss*) due to Early Cardiac Collapse.

Summary

Triploid (3N) salmonids are of interest to the aquaculture and sport fishing industries, however it has been shown that 3N fish have impaired survival at high temperatures. To test the hypothesis that impaired high temperature tolerance in 3N salmonids is related to impaired maximum performance of the heart, maximum heart rate (fH) was measured in 2N (diploid) and 3N rainbow trout (*Oncorhynchus mykiss*) during an incremental temperature challenge. Maximum heart rate of both ploidies was similar at 10 ^{o}C. However, a significant effect of ploidy on the response of fH to temperature from 10 to 22 ^{o}C was reflected in a significantly lower Q_{10} for 3N individuals. Additionally, all 3N trout developed cardiac arrhythmia by 22 ^{o}C, while 30 % of 2N trout continued to maintain a rhythmic heartbeat. These findings suggest that reduced 3N high temperature tolerance could be due to collapse of the cardiovascular system's ability to deliver O_2 to the body.

Introduction

The thermal tolerance of triploid (3N) salmonids is of interest to aquaculture and sport fishing industries because of a number of fish production and stocking advantages derived from the sterility of 3N fish. Unfortunately, 3N fish populations exhibit high mortality rates when chronically exposed to high temperatures, which is a challenge to industries. Therefore, in this chapter, while characterizing the maximum heart rate response to warming in 2N and 3N rainbow trout, cardiac pumping capacity was investigated as the mechanism behind poor 3N high temperature tolerance.

Several lab- and field-based studies have reported poor 3N performance at high temperature (Myers and Hershberger 1991; Simon et al. 1993; Ojolick et al. 1995; Altimiras et al. 2002; Hyndman et al. 2003; Koenig and Meyer 2011). Chasing to exhaustion at 19 ^{o}C, for example,

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[*] **A modified version of this chapter has been accepted with minor revisions to the Journal of Comparative Biochemistry and Physiology. A. Molecular and Integrative Physiology.**

resulted in nine out of ten 3N brook charr (*Salvelinus fontinalis*) dying, while chased 2N charr and unchased 2N and 3N controls showed no signs of difficulties at this temperature (Hyndman et al. 2003). High mortality of 3N brown trout (*Salmo trutta*) began accumulating after three weeks at 18 ^{o}C and reached 50 % of the population after twelve weeks (Altimiras et al. 2002). Temperature fluctuations from 15.4 to 19.0 ^{o}C triggered 62 % mortality within a 3N rainbow trout (*Oncorhynchus mykiss*) population over six-teen weeks (Altimiras et al. 2002). At 21 ^{o}C, mortality within a 3N rainbow trout population began to accumulate immediately, reaching 68.5 % compared with 38.9 % for the 2N trout population after three weeks (Ojolick et al. 1995). Even so, 3N under yearling and yearling brook charr lost equilibrium at the same temperatures as their 2N conspecifics (from 28.3 to 29.8 ^{o}C for under yearlings and 27.7 to 29.2 ^{o}C for yearlings, depending on warming rate) regardless of age and warming rate (2 ^{o}C hr^{-1} and 15 ^{o}C hr^{-1}; Benfey et al. 1997). Similarly, with brook charr as well as rainbow trout acclimated to 12 to17 ^{o}C, ploidy had no effect on the chronic lethal maximum temperature (from 27.7 to 28.2 ^{o}C for rainbow trout and 27.5 to 28.0 ^{o}C for brook charr) or time to loss of equilibrium when heated at a slower rate of 2 ^{o}C day^{-1} (Galbreath et al. 2006). The basis for this variability among studies remains unclear and may be better informed through study of temperature effects on cardiac pumping, which is essential for maintenance of oxygen supply throughout and thus survival of salmonid fish.

Indeed, recent theories on high temperature tolerance of ectotherms have suggested temperature tolerance in general to be associated with the maintenance of O_2 delivery to the body. This is due to temperature effects on aerobic scope (change in metabolic rate from rest to maximum). As temperature moves away from the optimal temperature (T_{opt} or the temperature range at which aerobic scope is greatest), aerobic scope narrows until becoming zero. This is termed the critical temperature (T_{crit}), beyond which survival becomes temporally limited due to its dependence on anaerobic metabolism and/or diversion of aerobic metabolic expenditures from essential processes (Farrell 2009; Portner and Farrell 2008).

Although few studies have investigated the effects of temperature on the cardiorespiratory system of 3N salmonids, factorial aerobic scope of 3N chinook salmon (*Oncorhynchus tshawytscha*) was smaller than that of 2N salmon (Bernier et al. 2004), providing 3N salmon with less reserves than their 2N counterparts for temperature-mediated reduction in aerobic

scope. Additionally, response curves of resting metabolic rate to acclimation temperature in brook charr and Atlantic salmon (*Salmo salar*) differed between 2N and 3N fish (Atkins and Benfey 2008). On the other hand, heart rate in 2N and 3N brook charr responded similarly to 6, 9 and 12^{o}C temperature exposures at 49-days, 63-days and 99-days post-fertilization (Benfey and Bennett 2009). Cardiorespiratory variables of 3N brown trout have been measured *in vitro* (Mercier et al. 2002) and *in vivo* (Altimiras et al. 2002) at moderate and high temperatures, and while comparable to those of other 2N salmonid fish, a direct comparison with 2N brown trout has not been made. Similarly, the cardiovascular system and aerobic scope of 2N and 3N rainbow trout has never been compared during a temperature challenge. A simplified assessment of the cardiac response to warming has been recently introduced by measuring maximum heart rate in anaesthetized fish (Casselman et al. 2012). Such a comparison may have direct relevance to *in vivo* situations given that the maximum cardiac response to temperature in salmonids appears to be limited by maximum heart rate (Farrell 2008).

Therefore, in order to test the hypothesis that compromised high temperature tolerance in 3N salmonids is related to impaired O_2 delivery throughout the body brought about by an inability to maintain maximum heart rate, maximum heart rate was monitored in 2N and 3N rainbow trout during an incremental temperature challenge, with the transition from rhythmic to arrhythmic heart beats as the measurement endpoint.

Methods

Rearing

The response of maximum heart rate (fH) to temperature was measured in ten 2N and 3N Blackwater rainbow trout (~1 year old) acclimated to 10 ^{o}C and with similar body mass [mean (±SEM) 15.1 ± 0.7 g and 13.5 ± 0.9 g, respectively (Table 2-1)]. This size range was chosen to ensure fish had developmentally switched from cutaneous diffusion to a convective cardiovascular system for O_2 supply and to preclude selection of a more tolerant 3N phenotype through cumulative differential mortality with age. Fish were mixed-sex offspring of wild trout caught from Blackwater River (British Columbia (BC), Canada) by the Freshwater Fisheries Society of British Columbia, who performed hydrostatic pressure shock treatments (Lou and Purdom 1984) to generate 3N eggs and then reared the fish at the Fraser Valley Trout Hatchery (Abbotsford, BC, Canada) until they were transferred to the University of British Columbia

(UBC). At UBC, fish were fed with 2.0 mm pellet BioTrout feed (Bio-Oregon, Vancouver, BC) according to manufacturers feeding guidelines and reared for more than 2 months before challenges began in aerated, flow through tanks supplied with 10 ^{o}C dechlorinated tap water and exposed to Vancouver seasonal daylight hours (October). All protocols were approved by the UBC Animal Care Committee (permit #A10-0236).

Maximum Heart Rate Measurements During Acute Warming

The technique for measuring fH was adopted from that reported in detail by Casselman et al. (2012). Fish were initially anaesthetized in a solution of 75 mg l^{-1} MS222 buffered with 75 mg l^{-1} sodium bicarbonate. Once fish lost equilibrium, they were weighed and placed in a trough with 10 ^{o}C aerated and thermo-regulated water containing a maintenance level of a buffered anaesthetic solution (50 to 60 mg l^{-1} MS222) flowing across the gills and over the body. The trough was a cylindrical PVC pipe with the top removed and a custom-made chromel-A wire electrocardiogram (ECG) electrode and reference probe attached to its base (for more details see Casselman et al. 2012). Electrocardiogram recordings were logged through BIOPAC data acquisition hardware and Acqknowledge software (Biopac. Montreal, PC). Fish were positioned ventral side up in the trough with their heart directly over the ECG electrode in order to maximize ECG signal reception.

Fish were allowed a 1 h equilibration period, after which an IP injection of 1.2 mg kg^{-1} atropine sulphate (Sigma-Aldrich) was administered to the fish to block any vagal inhibition of heart rate. In order to achieve maximum heart rate, after 15 min an IP injection of 4 μg kg^{-1} isoproterenol (Sigma-Aldrich) was administered to maximize β-adrenergic stimulation of heart rate. A preliminary pilot study on two each of 2N and 3N fish was performed to confirm atropine and isoproterenol doses were sufficient to produce maximum heart rate. Once fish had equilibrated to the drug injections (30 min total), the temperature of the water flushing through the trough was increased by increments of 1 ^{o}C at an average rate of 1 ^{o}C per 6 min with continuous ECG monitoring (Casselman et al. 2012 Fig 1a). Temperature was controlled with a recirculating chiller (Fisher isotemp 3016d; precision ± 0.1 ^{o}C) and measured with a thermometer (Fisher Scientific Type K digital thermometer probe; precision ± 0.1 ^{o}C). Water was not monitored for the O_2 level, but continuous aeration ensured saturation as temperature

was increased. The ECG was monitored for signs of a shift from a rhythmic (Casselman et al. 2012 Fig. 1b) to an arrhythmic (Casselman et al. 2012 Fig. 1c) heartbeat, which was the endpoint of the temperature challenge. The temperature was then noted and fish were removed from the trough and euthanized by cervical dislocation. With the breakdown of a rhythmic heartbeat, fH decreased, which was interpreted as the start of cardiac collapse. In addition to the 1 h equilibration period and 0.5 h drug administration period, the duration of the temperature challenge was 1 to 1.5 h, depending on the failure temperature. Blood samples were drawn, smeared on microscope slides and stained with Wright's stain for ploidy confirmation through measurement of the red blood cell (RBC) major nuclear axis (Benfey et al. 1984). The average major axis of RBC nuclei from all presumed 3Ns was larger than from all presumed 2Ns and the standard errors around the averages never overlapped.

Calculations

Using Acknowledge software, fH was calculated from ECG traces at each 1 ^{o}C increment. Typically, the section of trace 30 s before the thermostat began ramping to a new temperature was used to calculate fH (bpm) for each temperature from the time interval between at least ten consecutive R peaks (Casselman et al. 2012 Fig. 1a).

The typical exponential increase in fH with temperature becomes discontinuous prior to arrhythmia (Somero 2011; Casselman et al. 2012). This point is termed the Arrhenius breakpoint temperature (ABT). ABT and fH at ABT (fH_{ABT}) were determined for each individual by plotting the natural log of fH against the inverse of temperature in Kelvin and finding the point of change in slope (Casselman et al. 2012 Fig. 3). Significantly different slopes were determined by comparing the residuals of the regressions of all iterations of high *versus* low temperature groupings using REGRESS (http://www.wfu.edu/~mudayja/prog.html).

The Q_{10} of fH was calculated for each individual across the temperature range 10 to 14 ^{o}C ($Q_{10preABT}$), i.e., below the ABT. The formula used to calculate $Q_{10preABT}$ was ([fH at 14 ^{o}C (fH_{14C})] x [fH at 10 ^{o}C (fH_{10C})]$^{-1}$)^2.5. The scope for fH (fH_{scope}) was calculated as the difference between fH at 10 ^{o}C (fH_{10C}) and the highest recorded fH (fH_{peak}), regardless of temperature. The temperature at which arrhythmia ensued and the associated fH were termed $Temp_{arrhy}$ and fH_{arrhy}, respectively.

Statistical Analyses

Results for individual fish were used to calculate all mean ± SEMs. Ploidy effect on fH response to temperature was tested using a two-way repeated measures ANOVA. Student t-tests were used to compare 2N and 3N values for $Q_{10preABT}$, fH_{peak}, fH_{scope}, fH_{ABT}, ABT, $Temp_{arrhy}$, fH_{arrhy}. All statistical tests were performed using SigmaPlot 12 with an α value of 0.05.

The relationship between temperature and proportion of the arrhythmic cohorts was tested using a generalized linear model with a binomial distribution to model probability of becoming arrhythmic as a function of ploidy and temperature ($p < 0.01$). This was performed using the 'glm' function in R (http://www.r-project.org).

Results

While fH_{10C} was identical for 2N and 3N rainbow trout (67.5 ± 1.3 and 67.1 ± 0.9 bpm, respectively), the $Q_{10preABT}$ was significantly greater for 2N than 3N trout (Table 2-2; Fig. 2-1). This difference resulted in the fH_{peak}, which was reached at 20 ^{o}C for both ploidies, being 13 bpm greater for 2N relative to 3N fish, but this difference did not reach significance (p=0.18). The scope for fH between 10 and 20 ^{o}C was 17 bpm greater for 2N compared with 3N fish, but again this difference did not reach significance (p=0.15) (Table 2-2). Instead, an interaction between ploidy and temperature (two-way repeated measures ANOVA) and Holm-Sidak *post-hoc* testing revealed that 2N fish had a significantly greater fH than 3Ns at 21 ^{o}C (Fig. 2-1).

Despite these subtle differences, the ABT and associated fH_{ABT} were the same at 14.5 ^{o}C (14.3 ± 0.6 and 14.4 ± 0.4 ^{o}C for 2N and 3N, respectively) and 92 bpm (93.9 ± 3.9 and 91.10 ± 3.2 for 2N and 3N, respectively) (Table 2-2). On the other hand, cardiac arrhythmia, which started at the same temperature for 2N and 3N individuals (Fig. 2-1), potentially progressed faster with warming among the 3N individuals. Though no significant difference was detected in this progression, power was low (p = 0.4193). By 22 ^{o}C, 100 % of the 3N population exhibited cardiac arrhythmia, whereas only 70 % of the 2N individuals had become arrhythmic. The most tolerant 2N individual became arrhythmic at 24 ^{o}C, a full 3 ^{o}C higher than the most tolerant 3N fish (Fig. 2-1).

Discussion

Here, I report the first study to elucidate a potential mechanism behind previously reported poor high temperature tolerance of 3N salmonids, in that the effect of temperature on maximum heart rate (fH) significantly differed between ploidies and that cardiac collapse occurred in 100 % of 3N trout at temperatures where hearts of 30 % of 2N trout continued to beat rhythmically. Heart rate is increasingly being used as an index of temperature tolerance in fishes (Farrell 2009) and invertebrates (Somero 2011). Indeed, Casselman et al.'s (2012) findings suggested that the ABT for fH may represent the T_{opt} for aerobic scope for Coho salmon (*Oncorhynchus kistuch*). On the other hand, since fH does continue to increase after the ABT, alternatively the ABT may be a proxy for the lower pejus temperature, above which aerobic scope does not increase appreciably, but below which aerobic scope decreases. While a number of studies have reported the poor tolerance of 3N salmonids to high temperatures (Myers and Hershberger 1991; Simon et al. 1993; Ojolick et al. 1995; Altimiras et al. 2002; Hyndman et al. 2003; Koenig and Meyer 2011) and one that has reported reduced aerobic scope (Bernier et al. 2004), none has unraveled a potential mechanism. The present study breaks this deadlock by demonstrating subtle differences in the response of maximum heart rate to temperature between 2N and 3N rainbow trout.

Reports of heart rate for rainbow trout less than 100 g are lacking. However, the temperature response of routine heart rate was reported for unanaesthetized, 500g rainbow trout acclimated to 15 ^{o}C (Heath and Hughes 1973). At 16 ^{o}C, routine heart rate in conscious rainbow trout was *c.* 82 ± 8 bpm compared to the maximum heart rate of 104 ± 2.6 bpm for anaesthetized trout at 16^{o}C in the present study. However, since routine heart rate approaches a maximum at high temperature, heart rate in conscious trout peaked at 111 ± 5 bpm at 22^{o}C (Heath and Hughes 1973), which compares with 133.2 ± 7.1 bpm at 20 ^{o}C, where heart rate peaked in the present study. Conscious 200 g rainbow trout, acclimated to 20 ^{o}C and treated with atropine and adrenaline, achieved a maximum heart rate of 115 ± 5 bpm (Wood et al. 1979). Size effects, differences in acclimation temperature and potentially aneasthetic effects likely contribute to the differences between the present study and studies with much larger conscious rainbow trout.

MS-222 directly inhibits voltage-gated sodium channels (Frazier and Narahashi 1975, Neumcke et al. 1981) increasing threshold potentials required to achieve action potentials, and therefore, resulting in generalized effects throughout the body including the vasculature and

cardiac muscle. Branchial artery (Hill et al. 2002) and hepatic portal vein (Rothwell and Forster 2006) preparations dilated in response to MS-222 application. Vasodilatory effects were further reported in reduced vascular resistance in perfused chinook salmon tail preparations and *in vivo* reductions in dorsal aortic pressure (Hill and Forster 2004). Reductions in peripheral resistance due to MS-222-induced systemic vasodilation could reduce cardiac afterload and therefore work demands on the heart, but inhibition of voltage-gated sodium channels also affect vagal stimulation of the heart and cardiomyocyte contraction.

In vitro and *in vivo* experiments suggest chronotropic and inotropic effects of MS-222 on the heart that may be overcome with adrenergic stimulation. Perfused heart and cardiac muscle strip preparations show negative inotropic response to MS-222 (Ryan et al. 1993, Wells 1993, Hill et al. 2002). On the other hand, *in vitro* heart preparations with intact vagal stimulation (Hill et al. 2002) and *in vivo* experiments (Randall 1962) show positive chronotropic effects through blockage of vagal transmission (Hill et al. 2002). However, a more recent investigation of the *in vivo* cardiovascular response to MS-222 anaesthetization showed no effect of MS-222 on heart rate, cardiac output or stroke volume (Hill and Forster 2004). The lack of anaesthetic effects *in vivo* is likely due to endogenous regulatory mechanisms. In fact MS-222 did not affect the response of hepatic portal vein preparations to adrenalin application (Rothwell and Forster 2006), suggesting adrenergic stimulation, as was applied in the present study, can override MS-222 inhibition of voltage-gated channels.

In terms of the present experiment, Casselman et al. (2012) and Anttilla et al. (2013) assessed maximum fH response to warming in anaesthetized Coho salmon and rainbow trout, respectively, treated with the same isoproterenol and atropine drug cocktail. Both studies, and compared fH results to the aerobic scope response to warming in conscious fish of the same species and both found the maximum fH response to warming to be similar to the maximum metabolic rate response. Furthermore, Casselman et al.'s (2012) results showed that despite higher fH_{peak} for anaesthetized Coho salmon, maximum fH of conscious and anaesthetized salmon responded similarly to warming. Therefore, in anaesthetized fish with pharmaceutically-induced maximal adrenergic stimulation and vagal blockage, maximum fH may differ from that of conscious fish, but responds similarly to warming.

When compared with the temperature response of similarly sized and temperature-acclimated

Coho salmon tested in the same manner, fH_{10C} for rainbow trout (67.5 ± 1.3 bpm) was 10 bpm lower than in Coho salmon (Casselman et al. 2012). This difference was maintained for the fH_{peak} (133.2 ± 7.1 bpm in the present experiment *versus* 143.2 ± 5.7 bpm in Coho salmon), but at a lower temperature (20.0 ± 1.1 ^{o}C) for rainbow trout in the present study compared with Coho salmon (22.9 ± 0.6 ^{o}C). As a result, the $Q_{10PreABT}$ is similar for rainbow trout and Coho salmon (2.2 ± 0.02 *versus* 2.1 ± 0.02, respectively).

A T_{crit} value of 29 ^{o}C has been reported for rainbow trout acclimated to 10 ^{o}C (Currie et al. 1998) and of 28 to 30 ^{o}C for 2N and 3N brook charr, depending on heating rate and fish age (Benfey et al. 1997). As expected, cardiac arrhythmia (the end point in the present study) occurred at lower temperatures than loss of equilibrium (the traditional endpoint of T_{crit} experiments). Moreover, the cardiac arrhythmia temperature of 20 ^{o}C for 2N rainbow trout was comparable to 22.9 ^{o}C in Coho salmon reported by Casselman et al. (2012).

It was hypothesized that poor 3N tolerance of high temperatures is related to a break down of O_2 supply to the body at high temperatures. The findings of $Q_{10preABT}$ being significantly smaller for 3N rainbow trout and causing maximum fH to become progressively lower than 2N fH at temperatures above 10 ^{o}C until a significant difference emerged at 21 ^{o}C is consistent with this hypothesis. Likewise, the onset of cardiac arrhythmia potentially occurred over a narrower range of temperature for 3N rainbow trout. Although the progression of arrhythmia within the 3N population did not significantly differ from that within the 2N population, the power of the general linear model to detect a significant difference here was low ($p = 0.4193$). Given the importance of fH in setting the response of aerobic scope to temperature, the 10 % lower fH of 3N relative to 2N trout at ABT suggests the aerobic scope of 3N trout is about 10 % lower than 2N trout at T_{opt}. Since maximum oxygen consumption occurs post-exhaustion (Casselman et al. 2012), the post-exhaustion mortality of 3N brook charr acclimated to 19 ^{o}C (Hyndman et al. 2003) could easily be explained by a diminished aerobic scope compared with 2N. Different temperature responses of resting metabolic rate between 2N and 3N brook charr and Atlantic salmon (Atkins and Benfey 2008) also could reflect cardiorespiratory differences, which was, however, not seen in 2N and 3N embryonic and larval brook charr, since they had a similar heart rate as when tested at 6, 9 and 12 ^{o}C (Benfey and Bennett 2009). Consequently, more comparisons of the 2N and 3N cardiorespiratory system are warranted, especially in response to

warming.

Limitations to aerobic scope in 3N fish in relation to limited high temperature tolerance are supported by findings of impaired O_2 supply from the environment to the body of 3N fish. Impaired arterial O_2 loading is apparent in 3N fish with the reduced arterial haemoglobin (Hb) saturation and O_2 content in maximally swimming 3N, compared to 2N chinook salmon (Bernier et al. 2004). Similar arterial O_2 tension in the same individual chinook salmon under the same conditions suggest that reduced O_2 content in 3N arterial blood is not due to impaired diffusion of O_2 across 3N gills (Bernier et al. 2004). Furthermore, Hb-O_2 affinity of 2N and 3N rainbow trout (Verhille and Farrell 2012) and Atlantic salmon (Graham et al. 1985) blood is also similar. Thus the mechanism of reduced arterial O_2 loading does not lie in Hb-O_2 affinity or cross-gill diffusion. Findings for blood Hb concentration are equivocal, with reports of 3N values being lower (Benfey 1999), greater (Cogswell et al. 2002) and equal to (Benfey 1999; Benfey and Biron 2000; Bernier et al. 2004) those in 2N fish. Potentially reduced blood Hb concentration in 3Ns could explain a reduced arterial O_2 content, but not saturation.

Whether the cardiac muscle can compensate for a reduced arterial O_2 content is unclear. Cardiac output (Q) has not been compared between 2N and 3N fish, although *in vivo* and *in vitro* measurements of Q and cardiac power output capacities of 3N brown trout were comparable to 2Ns of other salmonid species (Altimiras et al. 2002; Mercier et al. 2002). Curiously, the shape of 3N aquaculture-reared Atlantic salmon hearts was more comparable with wild salmon hearts than their 2N cohorts, which was interpreted as a training effect on 3N hearts from which greater work is required to maintain O_2 convection in the face of reduced arterial O_2 content (Leclercq et al. 2011). In fact, the capacity of 3N hearts may be limited due to reduced coronary vascular volume and therefore O_2 supply, as seen in rainbow trout (Simonot and Farrell 2009). This limitation to 3N cardiac O_2 supply would be exacerbated by a potential increase in diffusion distances from capillaries to the mitochondria of the enlarged 3N cardiomyocytes (Mercier et al. 2002 and Johnston et al. 1999 for skeletal muscle cells). Further investigation into the mechanisms behind reduced arterial O_2 content in 3N fish and comparative studies of cardiac output and O_2 diffusion in cardiac and skeletal muscle of 2N and 3N fish will help to elucidate the mechanisms behind impaired aerobic scope and poor tolerance of sub-optimal conditions of 3N salmonid fish.

In summary, the slower rate of increases for maximum fH and a potentially more rapid rate of onset of arrhythmic heartbeats in 3N rainbow trout with warming suggest that limitations to the cardiorespiratory system are important mechanisms behind poor 3N tolerance of high temperatures. Discoveries from this chapter will be applied to thermal habitat utilization observations in Chapter 3 to understand the physiological significance of habitat utilization.

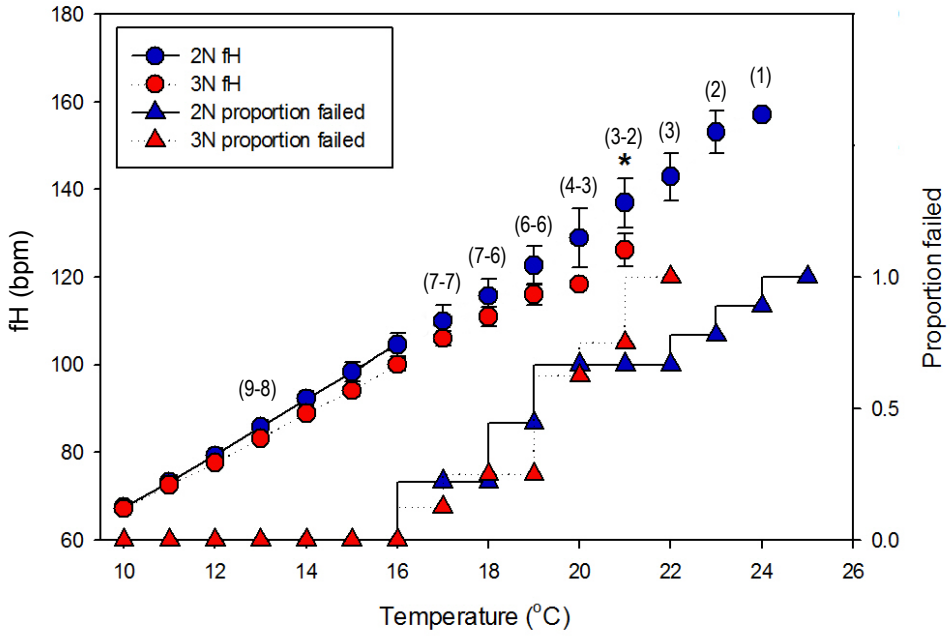


Figure 2-1. Maximum heart rate (fH) and proportion of arrhythmic fish for diploid (2N) and triploid (3N) rainbow trout during an incremental temperature challenge. Numbers above symbols are n values for the corresponding temperature (2N-3N). * demarcates significantly different fH between ploidies.

	2N	3N
Mass (g)	15.1 ± 0.7	14.6 ± 0.9
Length (cm)	11.7 ± 0.2	11.1 ± 0.3
Condition factor	0.95 ± 0.03	0.92 ± 0.02
Ventricle Mass (g)	0.014 ± 0.001	0.013 ± 0.001

Table 2-1. Physical characteristics of experimental fish.

	2N	3N
fH_{10C} (bpm)	67.5 ±1.3	67.1 ±0.9
$Q_{10preABT}$	2.2 ±0.02	2.0 ±0.03*
fH_{peak} (bpm)	133.2 ±7.1	120.6 ±3.5
$TempfH_{peak}$ (^oC)	20.0 ±1.1	19.5 ±0.5
fH_{scope} (bpm)	65.0 ±7.2	53.1 ±3.4
fH_{ABT} (bpm)	93.9 ±3.9	91.10 ±3.2
ABT (^oC)	14.3 ±0.6	14.4 ±0.4

Table 2-2. Indices of temperature response and tolerance in diploid (2N) and triploid (3N) rainbow trout exposed to an incremental temperature challenge. * demarcates significantly different HR between ploidies. $Q_{10preABT}$ is the Q_{10} calculated using change in fH from 10 to 14 ^oC; fH_{10C} is the maximum fH at acclimation temperature, which was 10 ^oC; ABT is the temperature at the Arrhenius break point; fH_{ABT} is the heart rate at the ABT; fH_{peak} is the overall maximum heart rate observed during the challenge; $TempfH_{peak}$ is the temperature at which fH_{peak} occurred. fH_{scope} is the difference between the lowest and highest fH.

Chapter 3 [*]Endurance Swimming is Related to Summer Lake Survival of Rainbow Trout in Warm High Predation Lakes: A Comparison of Diploid and Triploid Swimming Endurance and Survival in the Wild.

Summary

Darwinian fitness of fish in the wild is thought to be influenced by locomotion performance, but empirical evidence supporting its contributions to increased survival in the wild remains sparse. Studies suggest prolonged and sustained swimming capacity of triploid (3N) salmonid fish may be reduced when compared to those of diploid (2N) conspecifics. Poor 3N locomotion performance in conjunction with technology allowing for production of sibling 2N and 3N populations of genetically identical origin resulting in reduced variability among compared populations make 3N trout an informative system to test the hypothesis that fish with high swimming endurance have improved chances of survival. In order to assess the endurance-survival relationship, sibling populations of 2N and 3N rainbow trout were tested and ranked for within population swimming endurance, then assessed for summer survival in two lakes, a deep lake with high predation by birds (Pete's Pothole) and a shallow lake with lower avian predation pressure (Bluey Pothole 2). The ploidy comparison was intended to capitalize on the tendency of 3N salmonids to exhibit poor survival when stocked in lakes, potentially related to reduced aerobic swimming capacity and aerobic scope compared to 2N conspecifics.

The best-fit general linear model predicting survival in Pete's Pothole (PPH) and Bluey Pothole 2 (BPH) across experimental years included the terms temperature, lake and ploidy. Survival in Pete's Pothole (PPH) was nearly 50 % that of the populations in Bluey Pothole 2 (BPH), regardless of ploidy. Differences in survival between lakes are likely explained by different thermal habitats and predation rates experienced by the fish. For example, during peak lake temperatures, fish in PPH spent the majority of their time at 19 to 20 ^{o}C, a temperature at which lab studies suggest little aerobic scope for activity exists for this species. In BPH, fish predominantly selected temperatures 1 to 2 ^{o}C cooler than in PPH. Low 3N survival, ranging from 45 to 71 % of 2N survival depending on year and lake, most likely reflected the high lake

[*] **A modified version of this chapter will be submitted for publication to a yet to be determined journal with Tony Farrell as second author.**

temperature and impaired 3N relative to 2N tolerance of high temperatures shown in Chapter 2. However, there were also ploidy effects on habitat utilization and endurance swimming.

When the endurance-survival relationship was tested, a 3-way interaction among ploidy, endurance and lake made up the generalized linear model best predicting survival in 2008. As predicted, swimming endurance was lower for 3N compared with 2N fish and 3N fish survival in both lakes was consistently lower than 2N survival. In PPH, selective depletion of low endurance fish over time resulted in increased mean swimming endurance for 2N and 3N survivors relative to the initial stock population. However, in BPH, swimming endurance of surviving populations was unchanged when compared with the initial 2N and 3N trout stock populations.

Poor high temperature tolerance and swimming endurance of 3N fish was potentially exacerbated by differences in depth habitat utilization between the ploidies. As assessed by Vemco V9 temperature and depth acoustic biotelemetry, 3N fish in PPH tended to spend more time in warmer surface waters. Thus, though 2N and 3N temperature modes were similar, the increased utilization of warm surface waters in combination with impaired high temperature tolerance could have negatively effected 3N survival.

I concluded that aerobic locomotion can help predict summer survival in a warm lake with piscivorous birds exerting high predation pressure resulting in low fish survival, and especially for 3N rainbow trout. Together, these findings suggest that impaired survival of 3N salmonids may be related to a combination of their reduced aerobic scope and modified behavior compared to 2N conspecifics.

Introduction

Swimming performance is often described as an important trait contributing to the survival of fish (Taylor and McPhail 1985; Nelson 1989; Plaut 2001; Nelson et al. 2002; Domenici et al. 2010; Oufiero et al. 2011), but due to the difficulties in empirically assessing this relationship in the wild, few data exist to support this relationship in natural populations (Reidy et al. 2000). Triploid (3N) salmonid survival is often low in the wild, and aerobic swimming capacity may be inferior when compared to diploid (2N) conspecifics. Thus, 3N salmonids present themselves as an interesting model to investigate the relationships between aerobic swimming performance and survival in lakes.

Three swimming modes of fish were identified by Beamish (1978): burst speeds (sustainable for < 20 s), prolonged speeds (sustainable for more than 20 min, but less than 200 min) and sustained speeds (sustainable for more than 200 min). Only sustainable speeds are thought to be powered entirely by aerobic metabolism, although up to 70 % of maximum prolonged swimming speed (most often assessed using critical swimming velocity challenges) may also be aerobically powered (Burgetz et al. 1998; Lee et al. 2003).

For a trait to be influenced by natural selection, it must vary within a population (Bennett 1991) and the variance must be consistent over space and time (Arnold 1983, Jayne and Bennett 1990, Bennett and Huey 1991). A fish's aerobic and anaerobic swimming performance are variable (Kolok 1999, Billerbeck et al. 2001, Langerhans et al. 2004, Walker et al. 2005) and repeatable over time (Reidy et al. 2000, Claireaux et al. 2005, Claireaux et al. 2007, Pedersen et al. 2008). Thus they are potentially under natural selection within fish populations.

Though I know of only two attempts to correlate swimming performance with fish survival in a simulated natural ecosystem (Handelsman et al. 2010; Vandamm et al. 2012), substantial evidence exists that burst and prolonged swimming performance are important to escape predation and thus survival (Lankford et al. 2001; Langerhans et al. 2004; Walker et al. 2005; Handelsman et al. 2010; Oufiero et al. 2011). For example, prolonged and burst swimming increased the likelihood of sea bass (*Dicentrarchus labrax*) surviving an attack by avian piscivores in simulated estuaries (Handelsman et al. 2010), but another study on the same species in the same simulated estuaries found no significant relationship between acceleration ability and growth or survival (Vandamm et al. 2012). Greater burst swimming speeds were

observed in highly predated upon populations of the Trinidadian killifish (*Rivulus hartii*) (Oufiero et al. 2011), male mosquitofish (*Gambusia affinis*) (Langerhans et al. 2004) and guppies (*Poecilia reticulata*) (Walker et al. 2005) when compared to populations of the same species experiencing low predation. Additionally, high maximum prolonged and burst swimming speeds increased the likelihood of Atlantic silverside (*Menidia menidia*) (Lankford et al. 2001) and Coho salmon (*Oncorhynchus kisutch*) (Taylor and McPhail 1985) surviving attacks from piscivorous fish in the lab. Here, in order to test the hypothesis that aerobic swimming performance influences summer survival, I separately divided 2N and 3N fish populations into quartiles according to their endurance swimming capacity and compared survival in a high and a low predation lake with endurance swimming quartile.

Understanding the mechanisms limiting 2N and 3N rainbow trout survival in natural environments is important because hatchery rainbow trout form the basis of a $0.5 billion a year sport fishing industry in British Columbia (BC) and 3N rainbow trout are stocked in over 50 % of stocked lakes. In lakes, 3N survival is frequently reported as reduced (Blanc et al. 1992; Simon et al. 1993; Withler et al. 1995; Oppedal et al. 2003; Koenig and Meyer 2011), but also sometimes higher (Teuscher et al. 2003; Koenig et al. 2011) or similar (Guo et al. 1990; Withler et al. 1995; Dillon et al. 2000; Oppedal et al. 2003; Teuscher et al. 2003; Wagner et al. 2006) compared with 2N survival, depending on fish size, age and sex and water conditions. However, when reared at chronically low O_2 and/or high temperature in labs/hatcheries (Myers and Hershberger 1991; Ojolick et al.1995) and under sub-optimal oxygen and/or thermal conditions in either a lake (Simon et al. 1993; Koenig and Myer 2011) or a marine cage site (Blanc et al. 1992; Mercier et al. 2000; Altimiras et al. 2002), 3N survival is low regardless of size, age or sex. Elevated temperature and environmental hypoxia place burdens on O_2 delivery in fish (Portner and Farrell 2008; Farrell 2009; Farrell and Richards 2009), thus I hypothesized that reduced 3N survival is related to reduced aerobic capacity and therefore aerobic swimming capacity of 3N fish.

Comparisons of swimming performance for 3N and 2N cohorts for a number of species suggest impaired aerobic swimming capacity in 3N fish. For example, after 3 h of prolonged swimming (1.5 BL s^{-1}), Virtanen et al. (1990) found clear elevations of anaerobic metabolites in 3N, but not 2N rainbow trout. Cotterell and Wardle (2004) identified the top 28 % of 3N and 40

% of 2N Atlantic salmon through prolonged swimming tests and found the maximum sustained swimming speed did not differ with ploidy, but endurance time at speeds above maximum sustained speed was shorter for the 3N than the 2N population subsamples. Though endurance swimming time at 48 cm s^{-1} did not significantly differ between a subsample of 2N and 3N ginbuna (*Carassius auratus*) that were able to maintain this speed for 30 min, only 37 % of 3N, compared to 53 % of 2N individuals met this 30 min sustained swimming criteria (Sezaki et al. 1991). On the other hand, critical swimming velocity of 3N rainbow trout, white crappie (*Pomoxis annularis*), brook charr (*Salvelinus fontinalis*), chinook salmon (*Oncorhynchus tshawytscha*) and Atlantic salmon (*Salmo salar*) did not differ significantly from 2Ns of the same species, despite a tendency of critical swimming velocity of 3N fish to be lower than for 2N fish (Small and Randall 1989; Parsons 1993; Stillwell and Benfey 1996; Bernier et al. 2004; Lijalad and Powell 2009). Whether such small but consistently reduced swimming performance in 3N fish might be amplified into survival consequences in nature is unclear, therefore knowledge of 3N performance in the wild is useful for industry.

Therefore, I examined the relationship between swimming capacity and survival in 2N and 3N rainbow trout in a natural lake setting. The hypothesis that aerobic swimming performance influences survival in the wild was tested in two lakes in which fish were expected to utilize sub-optimal oxygen and thermal habitat and experience different levels of avian predation pressure. I predicted that survival fish with higher swimming endurance fish would be greater than that of fish with lower swimming endurance and 2N and 3N fish of similar endurance would have similar survival in both lakes. In order to account for effects of habitat utilization on the swimming endurance-survival relationship, habitat utilization was also monitored using temperature-depth transmitters surgically implanted into a subsample of each population of fish.

Methods

Hatchery Rearing

In 2007, 2008 and 2009, 2N and 3N Blackwater rainbow trout were screened for swimming endurance to compare 3N and 2N aerobic swimming performance, and stocked into two lakes to assess survival in the wild and its relationship with swimming endurance. Fish were all female offspring of two to three hormonally masculinized genetic female Blackwater rainbow trout

captive broodstock (Bye and Lincoln 1986) and two to three female wild-caught trout from Blackwater River (Cariboo region, BC, Canada) each year. The Blackwater River system has native rainbow trout inhabiting lake and river habitats. All eggs were pooled then divided into two batches before fertilization.

One batch was treated with a hydrostatic pressure shock shortly after fertilization to induce triploidy (Lou and Purdom 1984). Using sibling 2N and 3N populations, allowed for comparisons of populations of identical genetic origin. Though both male and female 3N fish are functionally sterile, only female 3N fish avoid energy investment into gonad production and thus have the potential of superior performance relative to maturing fertile 2N fish. Therefore, using all female 2N and 3N populations, not only minimized variability due to gender differences, but also maximized any potential performance advantages of stocking 3N populations.

Fish were reared at Fraser Valley Trout Hatchery (FVTH, Abbotsford, B.C, Canada) in 10 ^{o}C water and mortalities were low for both ploidies across all years. For example, survival from the green egg stage to stocking was 30 and 36 %, respectively for 3N and 2N trout stocked in 2007 and 43 and 48 %, respectively for 3N and 2N trout stocked in 2009. In 2008, in order to accommodate transmitter implants, fish were transferred from FVTH to Vancouver Island Trout Hatchery (VITH, Duncan, B.C., Canada) after screening trials in November, where winter rearing temperature was warmer (14 ^{o}C compared to 10 ^{o}C at FVTH) and accelerated growth to100 g.

Endurance Swimming Screening

Before lake stocking, fish were screened for endurance swimming in either October (2007) or January (2008) (six or nine months post-fertilization and seven or four months prior to lake stocking). The screening protocol varied slightly each year. In 2007, only 20 g 3N trout destined for Bluey Pothole 2 (BPH) were screened and ranked into the top and bottom halves, which were differentially marked with fin clips. Screening was performed in a 1 m deep circular tank with a plastic tube secured in the middle to delineate the periphery of the tank and a cloth mesh barricade at the downstream end of the 'failure zone' to catch failed fish swept downstream. Water velocity was manipulated using a 24 V electric outboard motor. Although water velocity

was not measured throughout the test, before fish were transferred into the screening tank, the motor setting achieving 15 cm s^{-1} (~1 body length (BL) s^{-1}) was marked and the water velocity at the highest motor setting was predetermined as 45 cm s^{-1} (3.5 BLs^{-1}). Fish were initially acclimated to 15 cm s^{-1} for 15 min before the motor was gradually increased to the maximum setting over approximately 100 min and then held for another 200 minutes, by which time at least 75 % of the fish had failed. Failure was defined as fish drifting into the delineated 'failure zone' and not swimming out when lightly prodded. Failures were netted, measured for weight and length and marked as the top (last to fail) or bottom (first to fail) half of the population.

In 2008 and 2009, rainbow trout destined for Pete's Pothole (PPH) and BPH were separately screened according to ploidy using an improved screening channel, a fiberglass oval channel with a 1 m long straight stretch delineated as the swimming area with plastic mesh at the upstream and downstream ends (Figure 3-1). In this channel, fish were acclimated to speeds of 15 cm s^{-1} for 15 min and speed was gradually increased to the maximum (55 cm s^{-1}) over 125 minutes. During screening of the first group of fish the speed dropped to 40 cm s^{-1} at 250 min, due to loss of battery charge powering the electric outboard motor. Therefore a similar drop was repeated for all subsequent trials. Two groups of 500 fish for each ploidy were screened and ranked by quartile, and endurance was assessed as total time swum for each quartile (Figure 3-2). Very few of the highest endurance quartile fish failed during screening, therefore, only rank without absolute endurance is reported for this quartile. Failed fish were netted, measured for weight and length and marked according to quartile ranking and ploidy status. Fish marking entailed a combination of fin clips (a combination of maxilla, ventral and adipose fins), Visible Implant Elastomere tags (Northwest Marine Technology Inc., Washington) and Pit tags (Biomark, Idaho).

Lake Stocking

Endurance-screened rainbow trout (1+ year old) were stocked into the two lakes in May of each year. Numbers and sizes of fish stocked into each lake in each year and lake characteristics are reported in Table 3-1. The fish biomass stocked into each lake was 20 kg in 2007 and 2009 and 100 kg in 2008. These biomasses are below the calculated sustainable biomass for Pete's Pothole (PPH) (200 kg) and Bleuy Pothole 2 (BPH) (145 kg) based on total dissolved solids and littoral and pelagic areas of the 2 lakes. Both experimental kettle lakes are small mesotrophic

lakes with no natural fish populations and on the Southern interior plateau of BC, Canada. The larger and deeper lake (PPH) was expected to have a much higher fish predation pressure from loons and osprey (Beckman et al. 2006, Biro et al. 2006 and author's observations). In 2007 and 2008, but not 2009, temperature loggers were moored and positioned at 0.5 m depth and in 1 m intervals from 1 m to 6 m depth at the centre of PPH and to 4 m depth at the centre of BPH. Additionally, 1 m interval depth profiles of temperature and O_2 were measured monthly in both lakes using a handheld dissolved O_2 meter (YSI model 550A) with a 20 m probe extension cable.

In 2007 and 2009, fish were transported by road from FVTH to the two lakes (approximately 200 km and 3 h) in 600 L insulated tanks supplied with compressed O_2. Temperature and O_2 were maintained at 10 to 12 ºC and 18 to 12 ppm, respectively. At the lakes, fish were released by hand. During fish release, surface temperature ranged from 15 to 16 ^{o}C for BPH and 15 to 17 ºC for PPH for all stocking years. In 2008, the larger fish used for telemetry were transported from VITH to the lakes (approximately 400 km and 6 h), using a 5 ton truck with an integrated live transport tank. These fish were released into lakes by gravity through hoses from the truck to the lake.

In 2007, while unscreened 2N and 3N trout were stocked into PPH only, endurance-screened 3N rainbow trout were stocked into BPH.

Telemetry

In 2007 and 2008, fish equipped with a VEMCO V9 temperature-depth (TP) transmitter (VEMCO division AMIRIX Systems Inc. Halifax, NS, Canada) were stocked into PPH (in 2007) or both lakes (2008). A pilot study was performed in 2007 using 20 fish (10 each of 2N and 3N) that had surgically implanted transmitters in their abdomens. There were no mortalities during the 2-week recovery period following surgery and fish were released into PPH in September. Transmitters were 39 mm long and 9 mm wide and weighed 2.2 g in water and 4.6 g in air, which averaged 1.5 % of fish body mass in air. Transmitters were programmed to ping temperature and depth measurements every 30 min (estimated battery life 615 days) to a VEMCO VR2W Acoustic Monitoring Receiver moored at the centre of the lake. Previously, radio tags surgically implanted into smaller chinook salmon (16 to 54 g), and adding a larger 2.2

to 5.6 % to body mass compared with 1.5 % in the present telemetry study (1.5 %), caused a 2 % mortality rate (i.e., 1 mortality 36 days after tag insertion) and small reductions in growth rate, which returned to control rates within 58 days post-surgery (Adams et al. 1998a). In this study, fish were stocked into lakes 14 days after surgeries and depletion netting was performed approximately 150 days post surgery. Critical swimming velocity of chinook salmon tagged at similar size with similar radio tags, did not differ from controls by 19 to 23 days post-surgery (Adams et al. 1998b). However, in the same study, though no direct tag-related mortality was observed, tagged fish were more likely than controls to be eaten by smallmouth bass (*Micropterus dolomieu*).

Surgery, which took 7 min on average, involved anaesthetizing fish with an MS222 solution (75 mg l^{-1} MS222 buffered with 75 mg l^{-1} sodium bicarbonate dissolved in distilled water) until they were refractory to a caudal pinch. Then, aerated water at 12 ^{o}C containing a maintenance dosage of buffered MS222 (50 mg l^{-1} MS222) was pumped over the gills while a 1 cm incision through the peritoneum was made approximately 1 cm to the side of and parallel to the linea alba, immediately anterior to the cartilage of the ventral fins for insertion of the transmitter. The incision was then closed with three or four discontinuous #2 silk sutures. Fish were revived in a flow-through, aerated recovery tank before being returned to their stock tank. Transmitters were tested for 24-h in the stock tank and temperature readings were calibrated from these test data. No fish mortality or transmitter loss occurred before lake stocking 2 weeks later.

A similar procedure was used in 2008, using a subsample of four to five fish randomly chosen from each screened quartile destined for each lake for transmitter implants (N=20 fish in total for each ploidy). These fish were stocked into lakes with the rest of the fish in May 2008.

Transmitter Data Analysis

Recordings from transmitters were analyzed for seasonal, diurnal, ploidy and lake differences in temperature and depth habitat utilization of fish. A small campground is located on the edge of PPH, raising concern over human activities on the lake affecting fish behaviour / habitat utilization. As use of this campground tended to be only on weekends and holidays, temperature and depth transmitter recordings from both lakes during these periods were not included in habitat utilization analyses.

Fish were determined to be alive when transmitter depth recordings continued to fluctuate and dead when recordings remained constant across a 24-h period or disappeared, though some error in terms of transmitter loss instead of fish death may have arisen using this criterion. Even so, no transmitter loss occurred in the hatchery during the weeks before lake stocking and only one transmitter was lost during fish transport, so I expect this error to be small.

Temperature and depth habitat utilization of individual fish and ploidies was assessed using kernel density plots of temperature and depth and 24-h plots of temperature and depth *versus* time of day. Weekly kernel density plots for each individual fish in both lakes were created with all half hourly temperature and depth transmitter recordings during that week. The kernel density plot is a non-parametric technique for estimating the probability density function in which each data point is represented by a smoothing kernel of predetermined variance and the area under the probability density function between two temperatures is the probability of the fish being found between those two temperatures. Plots were made using the 'density' function in R (http://cran.r-project.org/) using a bandwidth of 0.5. A weekly density plot was generated for each surviving fish (i.e., functional transmitter) for nearly every week after lake stocking in May until late July (May 27 to June 2; June 5 to 10; June 14 to 20; June 27 to July 2; July 8 to 14 and July 14 to 20) when water temperature had peaked. Although fish were not recaptured until October, low sample sizes (i.e., only one moving transmitter in October) precluded useful analysis post-July.

Plots of 24-h temperature and depth utilization were only analyzed for fish in PPH because by the time inter-individual variability arose in July, only one 3N transmitter fish remained alive in BPH. As all fish spent the entire day at the lake surface at the beginning of the summer and individual differences did not appear until PPH surface temperature exceeded 18 ^{o}C, habitat utilization was compared only for the 6 transmitter fish (three each of 2N and 3N fish) that survived until the end of July.

Seasonal and lake effects on habitat utilization were assessed by creating weekly kernel density plots from the weekly modes of all fish in a lake. Weekly temperature and depth modes for individual fish were determined from the peaks of the weekly temperature and depth density plots, respectively of that fish. Kernel density plots of the modes were then created, again using the 'density' function in R and the same bandwidth of 0.5. Because kernel density plots add a

variance to each data point plotted and surface measurements were obtained from temperature loggers at 0.5 m below the water surface, the fish transmitter temperature recordings and density plot tails sometimes conflicted slightly with reported lake conditions.

Fall Depletion Netting

In the second week of October of each year, summer survival was assessed through depletion gill netting in both lakes. In 2007 and 2009, lethal gill netting was performed over 4 consecutive nights. In 2008, netting was performed over 5 nights, with an initial 3 nights followed by a week of no netting and then 2 additional nights of netting. Gill nets were either 4 m or 8 m deep experimental gill nets with panel sizes ranging from 2.25 to 5.62 cm

In an attempt to explain inter-annual variability in survival, accumulated thermal units (ATU) of air temperature were calculated from the daily mean temperatures reported from the Environment Canada weather station out of Merritt, B.C., which is approximately 40 km from both lakes. Though ATU using air temperature does not allow for determination in inter-lake temperature differences, it was necessary due to failure of lake temperature loggers in 2009 and is expected to be sufficient to predict inter-annual variability in lake temperature.

Statistical Analyses

Effects of lake, ploidy, year and ATU on summer survival were assessed using a generalized linear mixed model with a binomial distribution to predict probability of recapture as a function of ploidy, lake and temperature as fixed factors and year as a random factor. The best-fit model predicting recapture was determined using a top-down approach (Zuur et al. 2009), beginning with the complete model, which included the fixed terms (ploidy, temperature and lake), the single random term (year) and all possible interaction terms. Then, the importance of specific terms was tested by sequentially and individually eliminating terms (beginning with the random term) and testing if the reduced model significantly differed from the complete model using likelihood ratio tests ($p<0.01$). This was performed using the 'glmer' function of the 'lme4' package in R.

The effect of ploidy on fish endurance was tested using a linear model using the 'lm' function

in R ($p < 0.01$).

The relationship between endurance and recapture was tested using a generalized linear model with a binomial distribution to model probability of recapture as a function of ploidy, endurance and lake. This was performed using the 'glm' function in R ($p<0.01$). In order to include endurance as a quantitative variable in the model, the fourth quartile was not included in the analysis because, the endurance of this quartile was not quantified.

RESULTS

Lake Oxygen and Thermal Characteristics

When lake temperatures were highest in July 2008, the volume of favorable oxy-thermal habitat available to fish differed between PPH and BPH, being much greater in PPH (Figure 3-3). Surface temperature in PPH (23 ^{o}C) was warmer than in BPH (20 ^{o}C). In PPH, water temperature remained above 19 ^{o}C to 4 m depth and O_2 concentration remained above 7 mg l^{-1} to 8 m depth where temperature was 10 ^{o}C. Temperature first fell below 14 ^{o}C at 5 m. In BPH, water dropped to 18 ^{o}C by 4 m depth, where O_2 fell below 7 mg l^{-1}. There was no depth in BPH where temperature was below 14 ^{o}C.

In early June surface temperatures of PPH (16 ^{o}C) and BPH (15 ^{o}C) were more similar than they were later in the summer (data not shown). Water temperature dropped to 13 ^{o}C by 2 m in PPH and 1 m in BPH. O_2 concentration first fell below 7 mg l^{-1} at 8 m, where temperature was below 5 ^{o}C, in PPH and 5 m, where temperature was 13 ^{o}C in BPH. Temperature and O_2 depth profiles of early June and late July 2007 were similar to those of 2008, but were not recorded in 2009.

Depth and Temperature Habitat Utilization of 2N and 3N Rainbow Trout

According to kernel density plots of the modes of individual transmitter temperature and depth recordings from 2008 in PPH and BPH, fish tended to use the warmest water in the lake throughout the summer (Figure 3-4). As PPH surface temperature tended to be warmer than that of BPH, transmitter readings from fish in PPH tended to be $\geq$1 ^{o}C warmer than in BPH. For most of the month of June, transmitter temperature readings from fish ranged from 14 to 16 ^{o}C, which is close to the lab-determined optimal temperature of 14 ^{o}C (Table 2-2). However, as

lake temperatures increased in July, transmitter readings from fish increased to 19 to 20 ^{o}C in PPH and 18 to 19 ^{o}C in BPH. At temperatures this high, 25 % or more of 2N and 3N Blackwater rainbow trout had become arrhythmic in the lab (Figure 2-1). As the transition from rhythmic to arrhythmic heart beats signifies a break down in cardiac pumping of O_2 throughout the body, 18 to 20 ^{o}C is expected to be lethal to 25 % or more of the populations of the Blackwater rainbow trout in the PPH and BPH.

The increase in habitat temperature from nearly optimal in June to theoretically lethal in July coincided with transmitter fish mortalities and increased inter-individual and potentially inter-ploidy variability in transmitter depth recordings in BPH (Figure 3-5) and PPH (Figure 3-6). According to weekly kernel density plots of depth recordings of the eighteen (eleven 2N and seven 3N) individual fish with functional transmitters in BPH from May 27 to June 2, depths were unimodal with the modes ranging from 0 to 1 m and an overall range of 0 to 4.5 m (Figure 3-5A). Depth became multimodal with higher temperatures from July 8 to 14 reflected in modes ranging from 1 to 3.5 m and an overall range of 0 to 6 m (Figure 3-5C) for the five (four 2N and one 3N) remaining fish with functional transmitters in BPH. This increase in inter-individual variability from early to mid summer was also reflected in temperature density plots, which, from May 27 to June 2, were unimodal with a mode of 15.5 ^{o}C for all fish and an overall range of 12 to 20 ^{o}C (Figure 3-5B). From July 8 to 14, temperature density plots of most fish became bimodal, while one remained unimodal with modes ranging from 15.5 to 17 ^{o}C and an overall range of 13.5 to 22 ^{o}C (Figure 3-5D).

Inter-individual variability in depth and temperature utilization also increased in PPH as the summer progressed. From May 27 to June 2, the depth density plots of the fourteen (seven each of 2N and 3N) individual fish with functional transmitters in PPH were unimodal with a mode of 1 m for all but two fish, which both had a mode of 0.5 m (Figure 3-6A). From July 8 to 14, six fish (three each of 2N and 3N) with functional transmitters remained in PPH. Depth density plots of some fish remained unimodal and others became bimodal, with modes ranging from 0.5 to 5 m and an overall range of 0 to 7.5 m (Figure 3-6C). Temperature density curves from May 27 to June 2 were also unimodal with a mode of 15.5 ^{o}C for all but one fish, which was a 3N and had a mode of 16.5 ^{o}C (Figure 3-6B). The overall temperature range for the plots at this time of the summer was from 10 to 20 ^{o}C. From July 8 to 14, two fish remained unimodal and all

others became bimodal, with modes ranging from 14 to 19 ^{o}C and an overall temperature range of 10 to 23 ^{o}C (Figure 3-6D).

Depth and temperature recordings from the individual surviving fish with functional transmitters in PPH also showed potential ploidy effects on depth habitat utilization. Plots of temperature and depth versus time of day (Figure 3-7) were created to further investigate subtle ploidy differences in weekly density plots from July, but only for PPH, as only one 3N fish remained in BPH by July 10. Depth preferences of 2N and 3N trout were identical in PPH during late May, but by July, possible ploidy differences in temperature and depth habitat utilization arose (Figures 3-5, 3-6 and 3-7). Twenty-four hour temperature and depth records for individual 2N and 3N fish in May showed no difference between 2N and 3N diurnal movements or temperature or depth habitat utilization in PPH, with trout spending most of their time within the top 2 m of water, where temperatures were between 13.5 and 17 ^{o}C (Figure 3-7). However in July, of the six remaining fish (three 2N and three 3N) with functional transmitters in PPH, two 3N fish consistently spent all of their time within the top 2 m of the lake and their transmitters recorded more time at higher temperatures (14 to 22 ^{o}C) than the three surviving 2N fish (Figure 3-7). This increased time at surface temperatures can be seen in Figure 3-6C as the two 3N depth density plots with higher peaks at lower depth values than the other four plots and in Figure 3-6D as the two 3N temperature plots that are slightly right shifted with slightly higher peaks than all other temperature plots in July. The third 3N fish displayed diurnal behavior, remaining at the surface from dawn (5 am) until noon then spending most of the remaining time until the next dawn at 4.5 to 5 m (Figure 3-7). This individual dove the deepest of all fish in PPH (6 m), and experienced recorded temperatures ranging from 10 to 22 ^{o}C. This behavior can be seen in the density plots (Figure 3-6C and D) as the 3N depth and temperature plots with the lowest peaks and greatest ranges of the all plots for PPH in July. All of the remaining 2N fish in July spent more than 70 % of their time between 3 and 5 m depths, with sporadic trips closer to the surface, and transmitted temperatures ranging from 10 to 22 ^{o}C (Figure 3-7). Thus two of the three surviving 3N trout in July predominantly utilized the surface waters of PPH compared to all three surviving 2N trout, which predominantly utilized deeper waters. However, one surviving 3N fish exhibited more variable behaviour than the other surviving fish of the same ploidy. Though, due to the small numbers and variable habitat utilization of surviving transmitter fish in

PPH in July**,** apparent ploidy differences in depth utilization arising with high lake temperature must be interpreted cautiously, inter-individual variability clearly increased with seasonal increases in lake temperature.

Also, coinciding with increases in lake temperature were sudden transmitter fish losses. When lake surface temperature first reached 18 ^{o}C as the summer progressed, the number of surviving fish with transmitters suddenly decreased in both lakes. In PPH, this occurred between June 14 and 20, when three of the six surviving 2N trout and three of the seven remaining 3N trout were lost (Figure 3-4). BPH surface temperature reached 18 ^{o}C approximately one week later, which was coincident with a loss of five of the eleven surviving 2N and two of the three surviving 3N fish (Figure 3-4). In the lab at 18 ^{o}C, 25 % of 2N and 3N Blackwater rainbow trout acclimated to 10 ^{o}C had become arrhythmic (Figure 2-1), thus this temperature is potentially lethal to 25 % of the fish in PPH and BPH.

Lake Survival of Rainbow Trout: Effects of Ploidy and Endurance

Temperature, lake and ploidy provided the best fit of the general linear model predicting survival in PPH and BPH over the summers of 2007, 2008 and 2009 ($p=0.0325$) (Table 3-2). Recapture of fish from BPH for 2007 was 45 % for 3N (203 fish stocked); for 2008 recapture was 34 % for 3N (406 fish stocked) and 54 % for 2N (384 fish stocked); and for 2009 recapture was 15 % for 3N (397 fish stocked) and 22 % for 2N (400 fish stocked) (Figure 3-8). Recapture of fish from PPH for 2007 was 9 and 20 % for 3Ns and 2Ns, respectively (600 3N and 600 2N fish stocked); for 2008 recapture was 12 and 17 % for 3Ns and 2Ns, respectively (479 3N and 342 2N fish stocked), and for 2009, recapture was 1 and 2 % 3N and 2N, respectively (459 3N and 437 2N fish stocked) (Figure 3-8). Thus, the percentage recapture of 3N trout was always lower, independent of the lake and year they were stocked with 2N trout. As expected for a lake thought to be under higher predation levels, percentage recapture in PPH was lower than in BPH for all three years. Also, the difference between 2N and 3N recapture was greater in PPH, the higher predation lake (with 30 to 60 % recapture), than for BPH (15 to 20 % recapture). Interestingly, in 2009, which was the warmest year (ATU being 10 % higher than the previous two years), the lowest survival (in BPH survival was half of previous years) among years in both lakes and for both ploidies was observed. This small cumulative temperature difference is particularily significant because the biotelemetry recordings from 2008 revealed a preference for

warmer surface temperature, well above lab-measured temperature optimum for Blackwater rainbow trout (Chapter 2).

Endurance and Survival

In 2008 and 2009, ploidy had a significant effect on endurance swimming time ($p < 0.001$) (data from 2009 not shown). The lowest endurance quartile for 2N rainbow trout swam at 50 cm s^{-1} for 100 ± 50 min compared to 70 ±1 5 for 3N trout (Table 3-3). The highest 2N endurance quartile swam for 272 ± 32 min *versus* 212 ± 7 min for 3N trout. Therefore, quartiles of 2N fish swam at 50 cm s^{-1} 1.3 to 1.7 times longer than the corresponding quartile of 3N fish. Weight, length and condition factor determined after endurance screening tests did not differ among ploidies or endurance quartiles (or halves) in 2007, 2008 or 2009.

The best-fit generalized liner model testing for a significant effect of endurance quartile on fish survival in 2008 included a three-way interaction among ploidy, endurance and lake ($p=0.0475$) (Table 3-2). For BPH, endurance was unrelated to survival within either the 2N or the 3N population (Figure 3-9). Survival was consistently around 50 % for each 2N endurance quartile but around 35 % for all 3N quartiles. However the relationship between endurance and survival was more complex in PPH (Figure 3-9), the higher predation lake, which had half the survival of BPH. In PPH, the lowest endurance quartile for 3N trout had a very low survival of just 7 %. However, survival among the 3N fish in PPH increased with each increasing endurance quartile, resulting with the highest quartile having nearly three-times higher survival (20 %) than the first quartile. A similar survival (20 %) was evident for the lowest endurance quartile of the 2N population in PPH. However, survival of the two highest endurance quartiles of the 2N population was lower, at 13 % in PPH. The apparently paradoxical increase in survival with endurance swimming time for 3Ns and the decrease in survival with endurance for 2N can be resolved if differences in absolute endurance are factored in. Peak endurance time for 3N trout never reached that for 2Ns, and in fact barely exceeded that of the second 2N quartile. Therefore, at a common endurance time of approximately 200 min, 2N and 3N trout had a similar survival of around 20 %. In quartiles of fish unable to swim at 50 cm s^{-1} for 200 min, 2N trout fared no worse than those able to maintain 50 cm s^{-1} swimming speeds, but 3N trout survival was poorer. In terms of survival, 3N performance did not improve beyond this

endurance level, but the 2N fish with greater endurance had a poorer survival in the high predation lake.

When the mean endurance of the 2N and 3N populations stocked into PPH and BPH in 2008 are compared to the mean endurance of the survivors at the end of the summer, selection for high endurance fish in both the 2N and 3N populations in PPH, but not BPH is apparent (Table 3-4). Mean endurance of the stocked 2N population was 1.4 and 1.3 times greater for the stocked 3N population in BPH and PPH, respectively. In PPH, the mean endurance of both the 2N and 3N surviving populations was increased 1.1-fold, relative to the stocked populations. In BPH, the mean endurance of stocked and surviving populations of both ploidies were similar. Thus, increased mean endurance of surviving, relative to stocked 2N and 3N populations in PPH, suggests selection for high endurance fish of both ploidies.

Discussion

As predicted, when assessed for endurance and summer lake survival of 2M and 3N rainbow trout, I showed lower aerobic swimming performance of 3N compared to 2N salmonids and a significant relationship between endurance swimming performance and survival of fish in the wild. An interaction between ploidy, lake and endurance in the endurance-survival relationship reflected differences in survival between the two lakes and survival and endurance between the two ploidies. Ploidy effects on survival and endurance swimming likely reflect limitations to 3N cardiorespiratory capacity, and variability in survival between lakes and years was at least partly explained by lake temperature, but also potentially predation on the fish.

Summer Lake Habitat Utilization

Summer fish survival and habitat utilization was monitored in two dissimilar lakes. PPH is a deep lake with hot surface water temperatures and avian predation on fish. BPH, in the other hand, is shallow with slightly cooler surface temperatures and potentially lower predation levels than PPH. PPH (authors observations, Biro et al. 2006), but not BPH (Beckmann et al. 2006; Biro et al. 2006) is frequented by common loons (*Gavia immer*), which are voracious fish predators. Additionally, O_2 and temperature depth profiles of the two lakes show PPH to be a warmer lake than BPH, but due to its greater depth, it has several meters depth of high O_2, low

temperature habitat, which is not present in BPH.

Reflecting the differences in lake characteristics, rainbow trout habitat utilization differed between BPH and PPH. After mid June, Blackwater rainbow trout in BPH spent no time at the lab-determined optimal temperature of 14 ºC (Table 2-2), and actually spent the majority of their time at temperatures approaching 18 ºC, such that they were spending the entire month of July at supra-optimal temperatures. At 18 ^{o}C, the same lab study showed that 25 % of 2N and 3N fish tested had developed arrhythmic heartbeats when temperature reached 18 ºC (Figure 2-1), which is likely a prelude to cardiac collapse and thus imminent death. With increasing acclimation temperature, high temperature tolerance increases (Brett 1956), so high temperature tolerance of the fish in PPH and BPH in July was most likely greater than for the fish tested in the lab, which were acclimated to 10 ^{o}C. However, salmonid fish are limited in their thermal tolerance plasticity. When acclimation temperature of sockeye salmon was increased from 10 to 20 ^{o}C, the upper lethal limit increased by only 1 ^{o}C from 23 to 24 ^{o}C (Brett 1971). Furthermore, the critical thermal maxima of redband trout (*Oncorhynchus mykiss*) originating from creeks of different temperatures (mean temperatures ranging from 15 to 23 ^{o}C, and maximum temperatures ranging from 18 to 29 ^{o}C) only differed by 0.7 ^{o}C (Rodnick et al. 2004). Thus, high temperature tolerance of the fish in PPH and BPH in July was likely slightly higher than of the fish tested in the lab in Chapter 2. However, this increase in tolerance was unlikely to exceed 1 ^{o}C, suggesting fish were at the limits of the thermal tolerance in the lake in July.

Despite the near lethal temperatures utilized in BPH, fish utilized even hotter temperatures in PPH. After mid-June, fish in PPH similarly spent no time at 14 ºC but spent the majority of their time at 19 to 20 ºC, which is 1 to 2 ºC warmer than in BPH and expected to result in arrhythmia in over 50 % of the stocked populations (Figure 2-1 and Table 2-2). Additionally, maximum heart rate of Blackwater rainbow trout was reached at 20.0 ± 1.1 ºC for 2Ns and 19.5 ± 0.5 ºC for 3Ns, suggesting the resting metabolic requirements at this temperature range require maximum capacity of the cardiovascular system. Thus any metabolic challenges at these temperatures would only be transiently sustainable through anaerobic metabolism. The proximity of these temperatures to the thermal habitat of fish in PPH in July, potentially reflects an inability of fish in PPH to meet the day-to-day metabolic challenges (e.g., foraging, competitive interactions and predator avoidance) required to survive in the wild.

My results suggest potential differences in lake habitat utilization of 3N and 2N Blackwater rainbow trout. Though early in the summer, when lake temperatures were cool, habitat utilization of 2N and 3N trout was similar and surface oriented, but as the lake warmed during summer, 3N trout spent most of their time at the surface while 2N trout preferred slightly deeper and cooler water. Considering the poor tolerance of 3N Blackwater rainbow trout (Chapter 2), it is surprising that they spent more time at the surface in slightly warmer temperatures than their 2N cohorts. This apparently detrimental 3N behavior may be a result of competitive interactions with 2N trout over use the safer deeper and slightly cooler, but still well oxygenated water. Previous behavioral comparisons suggest that 3N fish are less aggressive/dominant than 2N fish (Lincoln and Bye 1987; Stevenson 1991; Sutterlin and Collier 1991; Kavumpurath and Pandian 1992; Galbreath et al. 1994; O'Keefe and Benfey 1997), but 3N behaviour in the wild has never been assessed. Even though all of the 3N fish with transmitters in PPH displayed different behavior compared to their 2N siblings, these trends must be interpreted cautiously because of the small sample sizes of fish with transmitters by the time these differences had appeared. Of course, as most 3N fish with transmitters had died at this point, it is possible that these behavioral differences reflect only those 3Ns able to survive the warm summer water.

Survival in Summer Lakes

Survival of Blackwater rainbow trout was influenced by lake, temperature and swimming endurance. As predicted from the work of others, summer survival in PPH was consistently lower compared to BPH across three years of observations. A study comparing lakes from the same region with and without loon predation showed loons can remove 50 % of all stocked 1-year-old (26 g) rainbow trout from a lake (Beckmann et al. 2006). I used similarly sized fish to stock PPH and BPH. When cannibalism occurred in BPH in 2000, due to stocking large 1+ year old with small 0+ year old rainbow trout, survival of 0+ year olds decreased to 10 to 20 % (Biro et al. 2003). Cannibalism was unlikely to have occurred with the single year class stocking used here, and so generally greater survival was observed here compared with earlier studies in similar lakes.

In addition to predation, high water temperature also correlated with mortality in PPH compared with BPH. Both surface temperature and the temperature fish frequented in PPH were

consistently 1 to 2 ºC higher than in BPH throughout the entire summer. The near maximum heart rates required of rainbow trout to maintain corporeal O_2 supply at these high temperatures reflect a severely reduced aerobic scope, which is the difference between resting and maximum metabolic rates.

Aerobic scope can be thought of as the metabolic reserves available to a fish for responding to the many, usually cumulative, metabolic challenges a fish must successfully navigate in order to survive in the wild. It is the break down in aerobic scope, due to thermodynamic-increases in resting, but decreases to maximum metabolic rate, which dictates fish failure at high temperature (Portner and Farrell 2008; Farrell 2009; Chapter 2). Thus, aerobic scope is especially important in a high temperature lake like PPH.

Evans (2007) found lake trout required 75 % of their aerobic scope for chronic maintenance of life. On the other hand, EMG studies on rainbow trout suggested metabolic costs in the wild rarely exceed 20 % of the aerobic scope for activity (Briggs and Post 1997). However, calibration of EMG readings to metabolic rate was performed on different fish populations than the field EMG recordings were on, this inter-population differences in the EMG-metabolic rate relationship may have introduced error in this estimate. Additionally, field measurements were performed in experimental ponds with no predators. In PPH, predator escape attempts from loons, which can chase their prey for as much as 30 m (Barr 1996), would almost certainly increase metabolic costs in the wild. Thus, in the heat of the summer, the aerobic scope of fish in PPH likely approaches zero but fish are being forced to either expend metabolic energy in escape attempts or become easy prey and die. Thus, the elevated mortality in PPH compared with BPH is likely related to reduced aerobic scope with preference for supra-optimal surface temperatures as a significant contributing factor. Indeed, in both lakes, there was a sudden loss of fish equipped with transmitters coincident with surface temperature first reaching 18 ºC, which occurred a week later in BPH than PPH. Similarly, when reared at 18 ^{o}C, 3N brook charr died after a chase to exhaustion, which 2N charr were able to recover from with no mortalities (Hyndman et al. 2003b). Thus a similar explanation can account for the ploidy differences in survival. In fact 3N survival was consistently nearly 50 % lower than 2N survival in both lakes across all 3 years whenever both ploidies were stocked together.

These observations raise a rather obvious question: Why did Blackwater rainbow trout prefer

near lethal temperatures during the summer? The answer may lie in food availability and field metabolic rates. Food availability is likely tied to photosynthesis and aquatic invertebrates, which are greatest in the surface water of these lakes (Landry 1997). Low aerobic scope requirements would allow fish in BPH to survive well at surface temperatures above optimal. However, in PPH where a greater aerobic scope was required for both predator avoidance and the metabolic consequences of utilizing 1 to 2 ^{o}C higher temperatures, survival was predictably lower than in BPH. Furthermore, the uncharacteristically warm weather in 2009 decreased survival in both PPH and BPH compared with both 2007 and 2008. Thus, trends in summer mortality between lakes, across the summer season and from year to year strongly suggest preference for temperatures above optimal caused significant mortality in both lakes, with predation almost doubling mortality in PPH.

Reduced 3N Swimming Endurance

As predicted, 3N trout had reduced endurance swimming ability compared to their 2N cohorts. I propose this difference reflects a lower aerobic scope in 3Ns because 2N rainbow trout with high prolonged swimming capacity (which, like endurance swimming is primarily aerobically fueled) have greater aerobic scope, maximum O_2 consumption rate and cardiac output (Claireaux et al. 2005). While previous comparisons of fish swimming ability for 2N and 3N cohorts are equivocal, reduced aerobic swimming performance is suggested for 3N Atlantic salmon (Cotterell and Wardle 2004), ginbuna (Sezaki et al. 1991), rainbow trout (Small and Randall 1989), white crappie (Parsons 1993), brook charr (Stillwell and Benfey 1996) and chinook salmon (Bernier et al. 2004). Elevations of anaerobic metabolites in 3N, but not 2N rainbow trout after 3 hours of prolonged swimming (Virtanen et al. 1990) suggests 3N trout lack aerobic capacity and switch sooner to anaerobic energy production to fuel their swimming. Similarly, aerobic scope of 3N chinook salmon was 70 % of 2N siblings' (Bernier et al. 2004). Thus, reduced swimming endurance of 3N Blackwater rainbow trout, was likely due to ploidy effects on aerobic scope and could have contribute to their reduced survival in the wild.

Endurance and Survival

In support of the primary hypothesis, I showed a significant relationship was discovered

between endurance swimming and survival, a relationship that was dependent on lake and ploidy. This significant relationship was further supported with an increase in the average endurance of surviving 2N and 3N populations after a summer in lakes compared to the stocked populations. The interaction between the survival-endurance relationship and lake and ploidy are likely due to biotic and abiotic characteristics of the lakes and behavioral and cardiorespiratory differences between 2N and 3N trout, respectively.

The interaction between lake and the endurance-survival relationship raises the concern of pseudoreplication in this study. This relationship was tested in two dissimilar lakes, in one of which (BPH), recapture rates were high and the endurance-survival relationship was not evident. Though, it appears that the potential predation and high temperatures of PPH explain reduced survival and manifestation of the endurance-survival relationship, replication across many lakes similar to PPH and BPH is necessary in order to confirm this conclusion.

The effect of lake on the endurance-survival relationship is not surprising considering the differences between the two stocked lakes. Reduced survival in PPH is potentially due to predation and high temperature, suggesting strong selection had occurred for high temperature tolerant fish with predator avoidance skills. Previous research has established a link between high temperature tolerance and aerobic scope (Portner and Farrell 2008; Farrell 2009; Cassleman et al. 2012). Rainbow trout with high aerobic swimming capacity have been shown to have greater aerobic scope and maximum O_2 consumption rate and cardiac output than poor swimmers (Claireaux et al. 2005). Therefore, high endurance fish are likely more high temperature tolerant as a consequence of their large aerobic scope.

Though the relationship between sustained swimming capacity and predation escape has never been assessed, fish with high burst (Taylor and McPhail 1985; Lankford et al. 2001; Langerhans et al. 2004; Walker et al. 2005; Handelsman et al. 2010; Oufiero et al. 2011) and prolonged swimming capacity appear to be better at escaping or avoiding predation (Taylor and McPhail 1985; Lankford et al. 2001; Handelsman et al. 2010). Extending these findings to PPH suggests that fish with high swimming endurance were better able to escape predation by loons and therefore had improved chances of survival.

Therefore, due to the importance of aerobic scope to both high temperature tolerance and endurance swimming capacity, selection for high aerobic scope may be even greater than that

for endurance swimming. Furthermore, although endurance swimming was important for survival in a high predation warm lake (PPH), it was not important to survival in a cooler low predation lake (BPH) where aerobic scope requirements are not expected to exceed the available reserves.

The ploidy effect on the survival-endurance relationship is likely due to behavioral and or aerobic metabolic differences between the ploidies. The 30 to 50 % greater survival of 2N relative 3N populations in PPH across 3 years corresponded with a 40 % greater endurance in 2008. Furthermore, a 3-fold increase in endurance doubled survival for 3N fish. Reduced endurance in 3N relative to 2N Blackwater rainbow trout most likely reflects a reduced aerobic scope, which has been shown in 3N chinook salmon (Bernier et al. 2004). Thus, the endurance-survival relationship within the 3N population in PPH further supports the importance of aerobic scope to survival.

Considering 3N salmonids appear to have reduced aerobic scope compared to their 2N cohorts, the relationship between swimming endurance and aerobic scope, and therefore survival may differ between ploidies. Unfortunately, the time requirements to measure aerobic scope make it impossible to test its influence on survival at the population level. Therefore, a lab-based assessment of the relationship between aerobic scope and endurance swimming, which can be more efficiently measured for large populations, would be a more effective first step in confirming the relationship between aerobic scope and survival.

Alternatively, differences between the behavior of 2N and 3N trout, may explain variability in the endurance-survival relationship between ploidies. Altered 3N performance in the presence of 2N fish (Lincoln and Bye 1987; Sutterlin and Collier 1991; Galbreath et al. 1994) may have confounded the endurance ranking of fish in this experiment, because fish were endurance screened with ploidies separate, but stocked into lakes together with 2N cohorts. The potential of inter-ploidy behavioral differences influencing the endurance survival relationship is further supported by evidence of differential habitat utilization by 2N and 3N rainbow trout during the hottest part of the summer. Considering 3N preference of the high temperature surface waters of PPH, it appears that the 3N trout are spending their time in inferior habitat to the 2Ns, which are more frequently in cooler, well oxygenated water. However, the 3N fish may be utilizing the littoral zones where predation risk is low or the surface of the pelagic zone where Daphnoid (an

important food source for rainbow trout in these lakes) density is high (Landry et al. 1999; Post et al. 1999), but so is predation (Biro et al. 2004). For example fish spending more time in the pelagic zone tend to have reduced survival in predator-present, but not predator-free lakes (Biro et al. 2004; Biro et al. 2006). Thus differences in the aerobic scope, endurance swimming capacity and behavior between 2N and 3N Blackwater rainbow trout probably result in different selection pressures which could have profound effects on the endurance-survival relationship.

In summary, endurance swimming was strongly related to summer survival in lakes where sub-optimal conditions existed in the surface water that rainbow trout preferred, likely in search of food. Ploidy effects were discovered for survival and the endurance-survival relationship, which is likely due to variation in selective pressures with ploidy, which in turn, is most likely related to differences in habitat utilization and aerobic scope. Furthermore, differential selection perhaps explains the broader finding that 3N, relative to 2N salmonid survival in sport fishing lakes is often inferior and at a great cost to the industry.

Lake		Bluey Pothole 2 (BPH) 01886NICL			Pete's Pothole (PPH) 01909NICL		
TDS (mg l^{-1})[1]		300 - 500					
[P] (mg l^{-1}) [1]		15 - 30					
Depth (m)		6			20		
Surface Area (ha)		2			4		
Predators[2]		Kingfisher, merganser			Loon, heron, kingfisher, osprey		
Year		**2007**	**2008**	**2009**	**2007**	**2008**	**2009**
3N	Total stocked	203	460	397	600	479	459
	Total stocked per quartile/50 percentile in 2007	88-115	65-115	86-115		57-110	106-119
	Total transmitters stocked		8		10	10	
	Weight (g)	19±7	111±35	27±1	19±3	104±34	30±1
	Length (mm)	119±1	215±12	137±1	125±2	208±23	138±1
	cf	1.1±0.1	1.1±0.1	1.0±0.1	1.1±0.1	1.1±0.1	0.9±0.1
2N	Total stocked		384	400	600	342	437
	Total stocked per quartile		81-130	98-104		96-130	100-115
	Total transmitters stocked		11		10	10	
	Weight (g)		102±28	29±1	20.1±8	99±30	31±2
	Length (mm)		211±19	140±1	133±20	208±21	140±1
	cf		1.0±0.1	1.0±0.1	1.1±0.2	1.1±0.1	0.9±0.1
Date stocked		May 29	May 26	May 25	May 29	May 26	May 25
Temperature (°C)		15	16	16	15	17	17
Date recapture		Oct 15-18	Oct 5-8	Oct 5-8	Oct 15-18	Oct 5-8	Oct 5-8
July average surface temperature (°C)		19.1±2	19.3±2		20.8±2	20.5±2	
Peak summer surface temperature (°C)		22	20.5		22	22	

Table 3-1. Lake characteristics and fish stocking details for Pete's Pothole (PPH) and Bluey Pothole 2 (BPH) for 2007, 2008 and 2009. [1] Post et al. 1999; [2] Bechman et al. 2006; Biro et al. 2006; personal observation.

Response Variable	Fixed Effects Terms	Random Effects Terms	Significance
Inter-annual Recapture	Ploidy + Temp.	Year	<0.001
Recapture 2008	Ploidy * Endur. * Lake		<0.001

Table 3-2. Significant terms in statistical models predicting survival in PPH and BPH. The inter-annual recapture model was based on the terms year, ploidy and temperature (generalized linear mixed effects model). The 2008 recapture model, aimed at testing for endurance swimming effects on survival, was based on ploidy, endurance and lake (general linear model). * signifies an interaction.

	1st Quartile (min)	2nd Quartile (min)	3rd Quartile (min)
2N	100±50	197±17	272±32
3N	70±15	118±13	212±7

Table 3-3. Endurance (mean ± SEM) of the 1st, 2nd and 3rd 2N and 3N quartiles to fail during endurance screening tests in 2008 (n=1000).

Population	BPH2 – low predation		PPH – high predation	
	2N	3N	2N	3N
Pre-stock	204	142	178	139
Survivors	203	139	193	158

Table 3-4. Mean endurance swimming of stocked and recaptured populations of 2N and 3N Blackwater rainbow trout in BPH and PPH during the summer of 2008. Units are in cm.

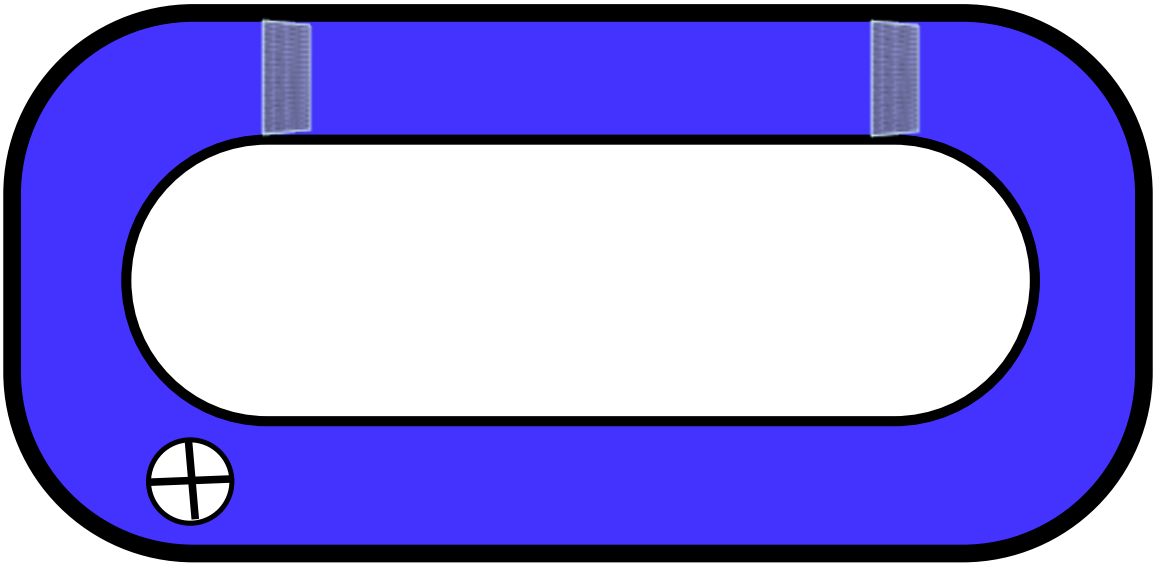


Figure 3-1. Endurance screening raceway used in 2008 and 2009 with electric outboard motor controlling water velocity and a swimming channel delineated with mesh screens at either end of the top 2 m long straight stretch

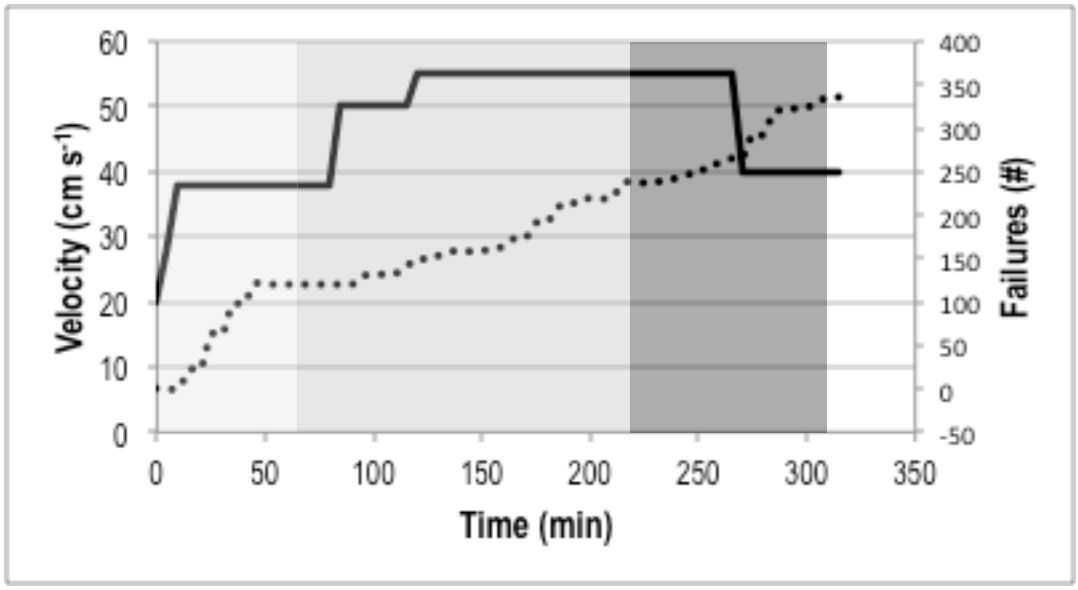


Figure 3-2. Endurance screening protocol for rainbow trout. (—) water velocity, (· ·) cumulative failures. The increasingly shaded regions delineate each failure quartile.

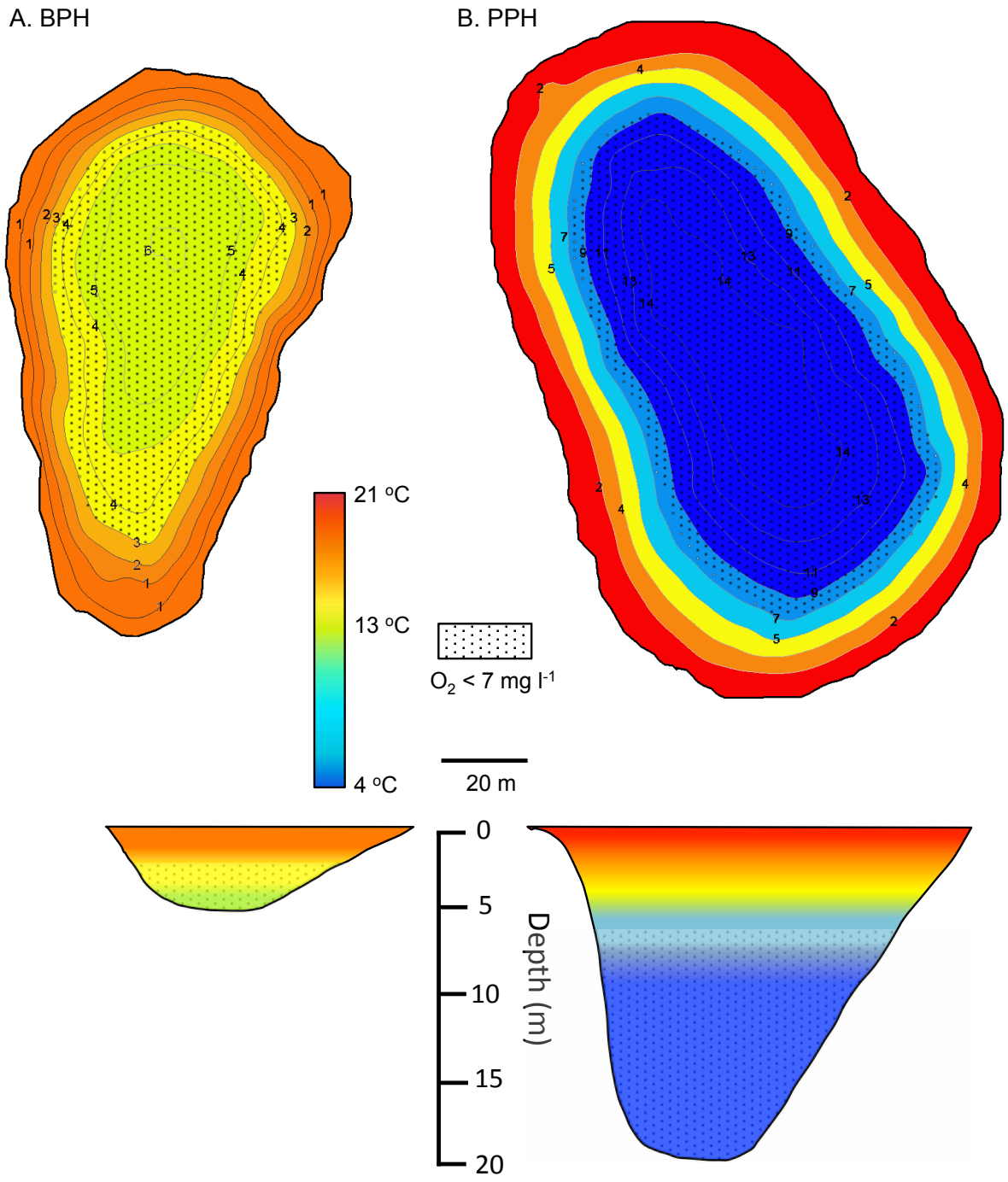


Figure 3-3. Temperature depth profiles of Bluey Pothole 2 (BPH) (A) and Pete's Pothole (PPH) (B) on July 10 2008. In top profiles, depth is illustrated with contour lines and numbers delineating depth (m). Bottom profiles are cross sections of lake depth. ( ) O_2 below 7 mg l^{-1}.

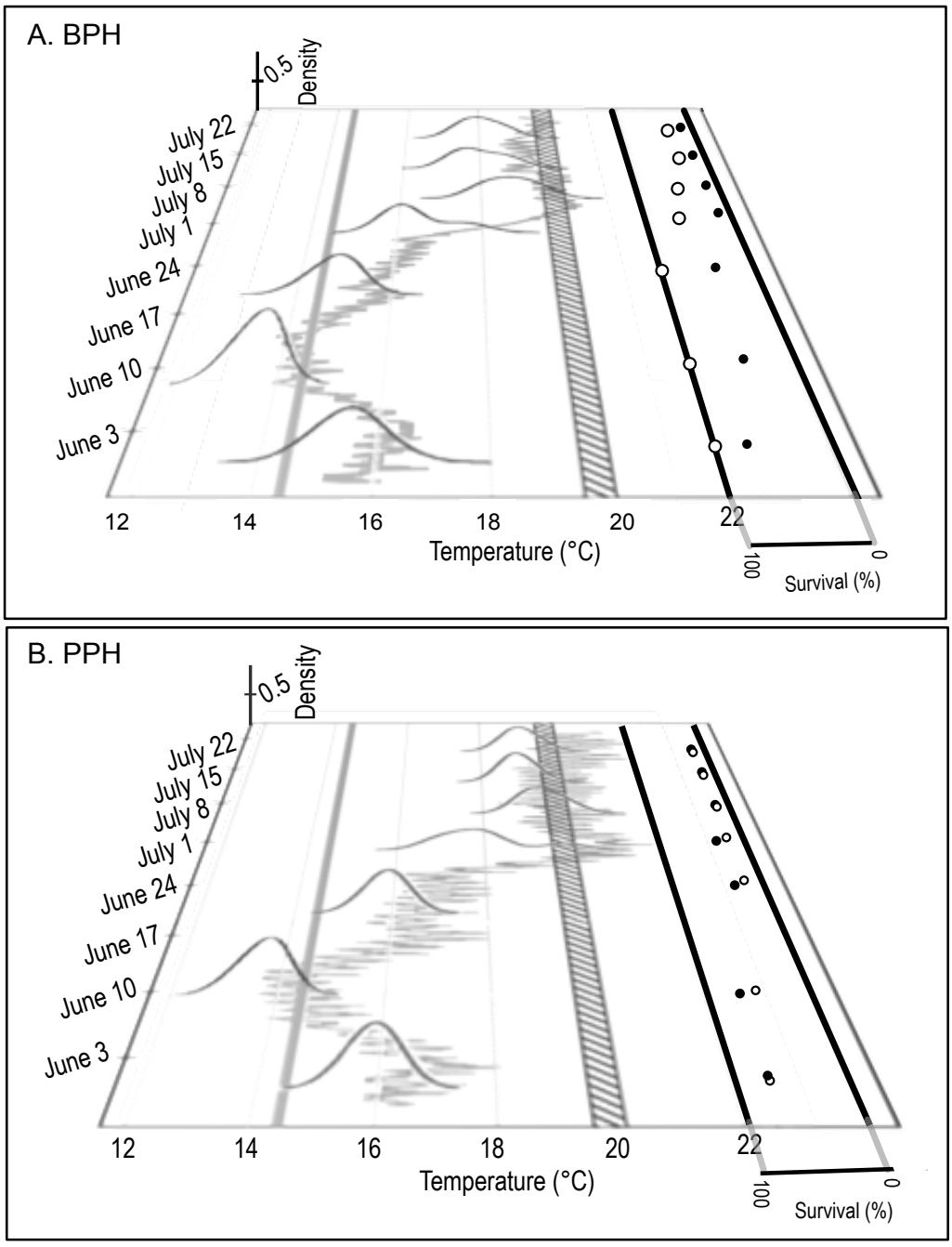


Figure 3-4. Kernel density plots of temperature modes from individual fish equipped with temperature transmitters and % transmitter fish survival in BPH (A) and PPH (B) from May to August 2008. In chronological sequence, the n of each plot for BPH is 18 (11 2N and 7 3N), 17 (11 2N and 6 3N), 15(11 2N and 4 3N, 7 (6 2N and 1 3N), 6 (5 2N and 1 3N) and 5 (4 2N and 1 3N) for the last 2 date ranges and for PPH is 14 (7 2N and 7 3N), 13 (6 2N and 7 3N), 7 (3 2N and 4 3N), 7 (3 2N and 4 3N) and 6 (3 2N and 3 3N) for the remaining 3 time periods. ( ) temperature at 0.5 m below the lake surface. ( ) temperature at which maximum heart rate plateaus and optimum temperature ( ) for 2N and 3N Blackwater rainbow trout determined in the lab (Chapter 2). Survival of 2N (○) and 3N (●) transmitter fish is illustrated with survival (%) on the x-axis beyond 22 °C.

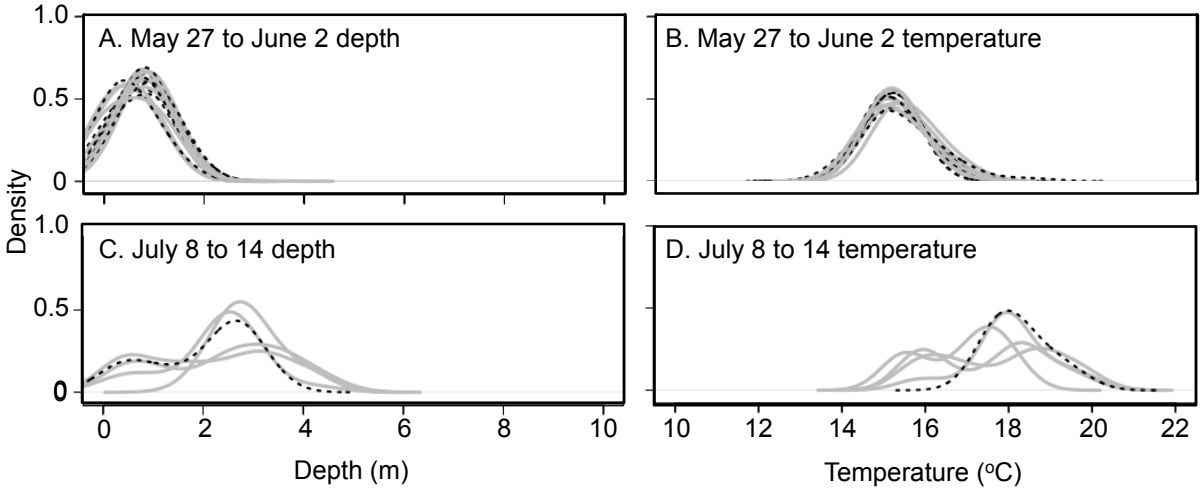


Figure 3-5. Kernel density plots of water temperatures depths for fish in BPH in 2008 determined through telemetry (Vemco VP TP transmitters) of 2N (▬) and 3N (▪▪▪) Blackwater rainbow trout from May 27 to June 2 (A and B) and July 8 to 14 (C and D). From May 27 to June 2, n was 11 for 2N and 7 for 3N. From July 8 to 14, n was 4 for 2N and 1 for 3N.

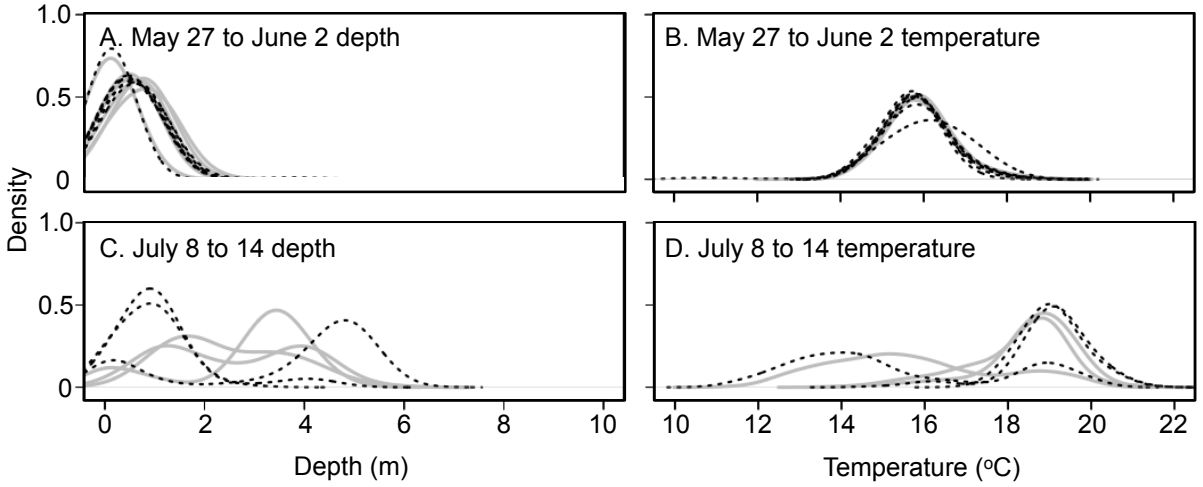


Figure 3-6.. Kernel density plots of water temperatures depths for fish in PPH in 2008 determined through telemetry (Vemco VP TP transmitters) of 2N (▬) and 3N (▪▪▪) Blackwater rainbow trout from May 27 to June 2 (A and B) and July 8 to 14 (C and D). From May 27 to June 2, n was 7 for 2N and 3N. From July 8 to 14, n was 3 for 2N and 3N.

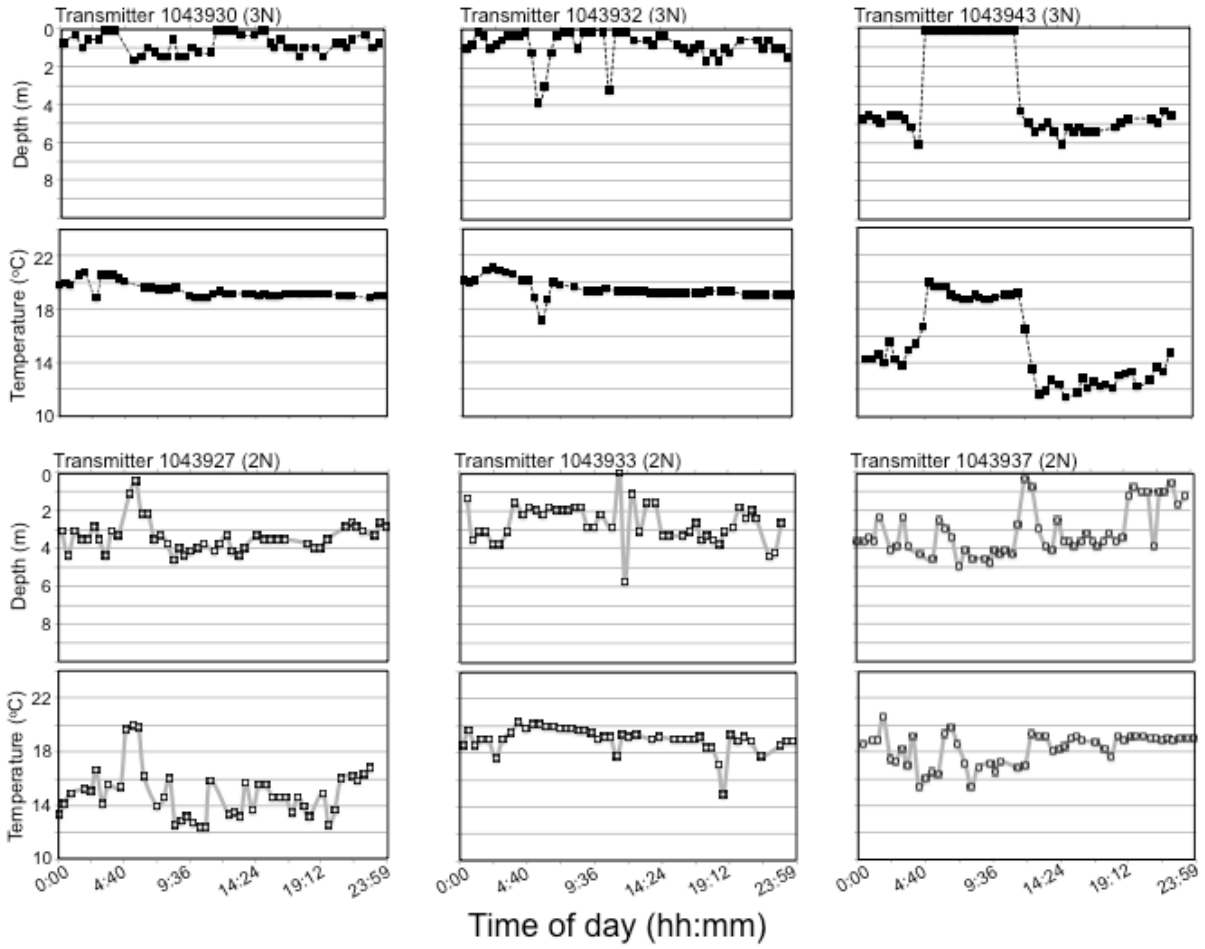


Figure 3-7. 24-h temperature and depth habitat utilization in PPH on July 10, 2008 as determined through telemetry (Vemco VP TP transmitters) of three 2N (○) and three 3N (■) Blackwater rainbow trout.

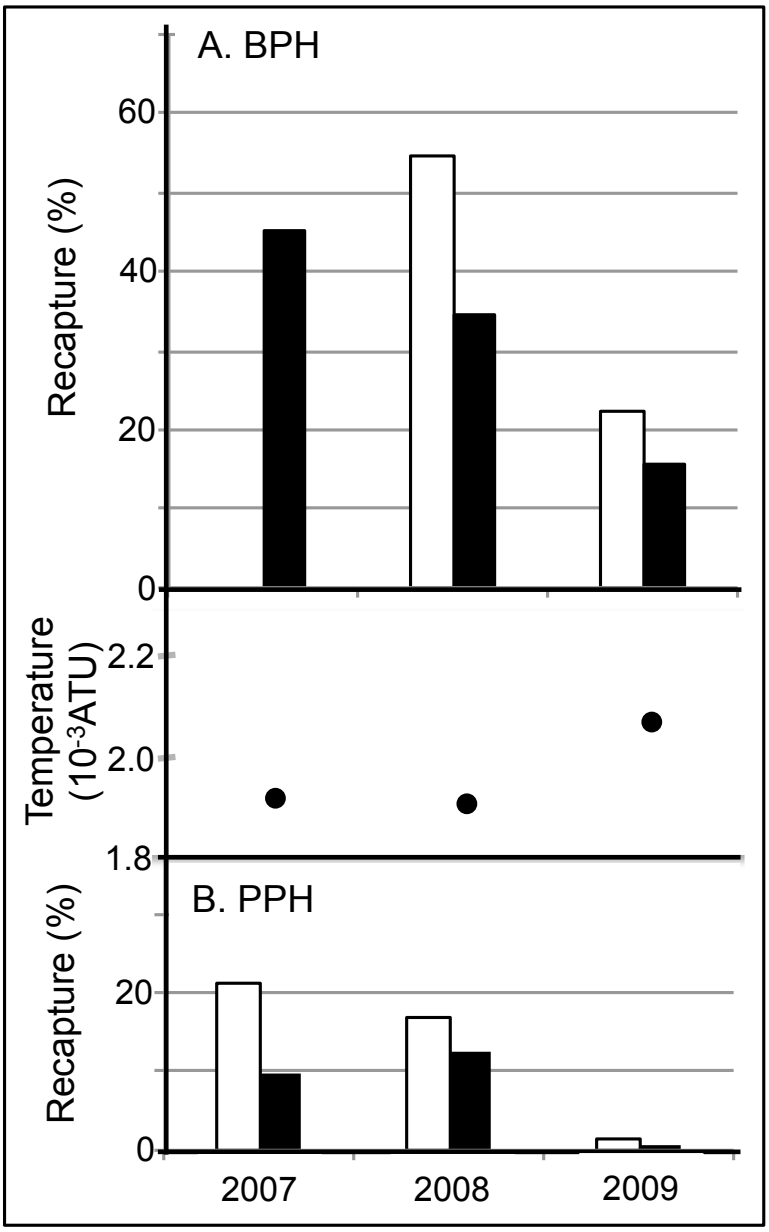


Figure 3-8. 2N (□) and 3N (■) survival in PPH (a warm, high predation lake) (A) and BPH (a cool, low predation lake) (B) in the summers of 2007, 2008 and 2009. (●) accumulated thermal units (ATU) calculated through June, July and August of each year based on Merritt, British Columbia air temperatures obtained from the Environment Canada database.

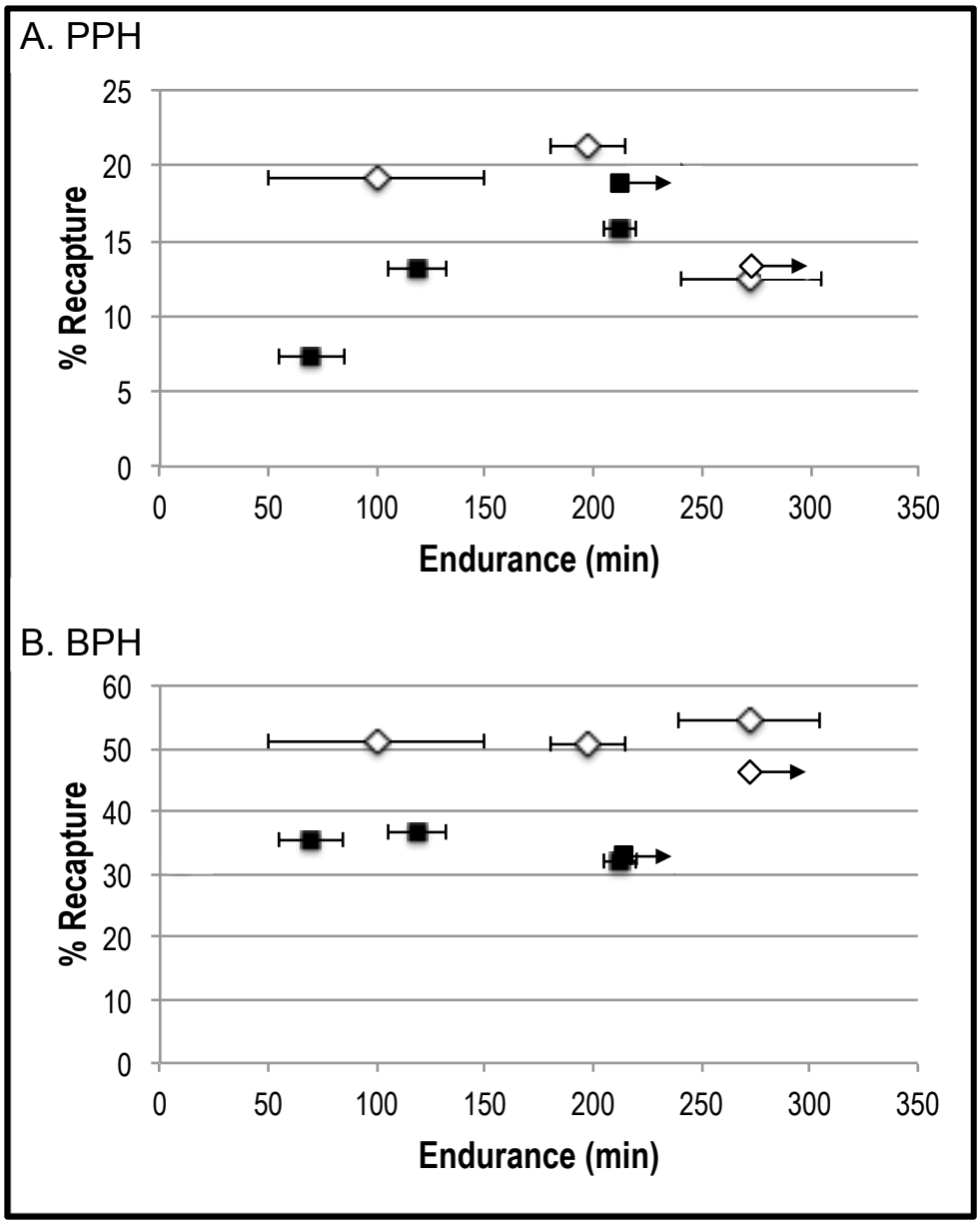


Figure 3-9. Summer 2008 lake recapture and average endurance swimming of endurance-screened 2N (◇) and 3N (■) quartiles of Blackwater rainbow trout (*Oncorhynchus mykiss*) in PPH (a warm, high predation lake) (A) and BPH (a cool low predation lake) (B). Arrows represent the 4th endurance quartile, mean endurance swimming of which is unknown, but greater than that of the 3rd quartile of the same ploidy.

Chapter 4 *Predicted Capillary Oxygen Supply Limitations to the Triploid Salmonid Ventricle Using a Modified Krogh Diffusion Model

Summary

Triploid (3N) salmonids have enlarged cardiomyocytes and a potentially larger diffusion distance between coronary capillaries and the mitochondria of the heart muscle cells. This larger diffusion distance could then impede cardiac O_2 supply, which would be magnified by the known reduced arterial O_2 content (CaO_2) in 3N salmonids. In order to test the hypothesis that O_2 supply to the 3N heart is impaired, a modified Krogh model was applied for the first time to the capillary O_2 supply of the rainbow trout ventricle while varying the Krogh diffusion constant (Ko_2), CaO_2, coronary flow (q_{cor}), myocardial O_2 consumption (VO_2), capillary radius (r_c), haemoglobin (Hb)-O_2 affinity, and the cytoplasmic-mitochondrial O_2 pressure (PO_2mito) gradient across physiological ranges relevant to both 2N and 3N rainbow trout. The assessment was based on calculating the Krogh tissue cylinder (R), which is the radius of tissue sustainably supplied with O_2 from its centrally located capillary, and assessing whether an anatomically realistic R of 10 μm for 2N and 11 μm for 3N (based on a 1.1 fold increase in R over that of 2Ns) hearts is supported by blood O_2 to the end of an anatomically realistic 60 μm long capillary.

The application of physiological values for the model input variables predicted sufficient myocardial O_2 supply of a minimal 10 μm radius Krogh cylinder (R) of tissue along a 60 μm long capillary for resting 2N hearts, but a modest O_2 supply deficiency in hearts of maximally swimming 2N fish. The output of the resting model was only sensitive to VO_2, compared to the exercising model, which was highly sensitive to VO_2 and Ko_2, and moderately sensitive to CaO_2, q_{cor}, P_{50} and r_c.

When the modified Krogh model was applied to hearts of resting and maximally swimming 3N fish by reducing CaO_2 and increasing r_c and comparing the output R to the required diffusion distance (R) of 11 μm, a severe O_2 supply shortfall was revealed in hearts of maximally swimming, but not resting 3N salmonids. This O_2 supply deficiency was rectified by modeling a

* **A modified version of this chapter will be submitted for publication to a yet to be determined journal with Tony Farrell as second author.**

decrease in myocardial workload, but this would likely require a reduction in cardiac output and/or aortic output pressure. In fact, this model output supports previously reported evidence of reduced pressure generation capabilities of rainbow trout hearts experiencing myocardial O_2 supply limitations due to experimentally ligated myocardial coronary blood supply. Alternatively, 3N salmonids may potentially compensate with more dense vasculature in their myocardia to match O_2 demand or achieve lower maximum swim intensity, as was reported for 2N rainbow trout with experimentally ligated coronaries, to minimize the required increase to myocardial work. Literature reports of reduced 3N vascularity suggest vascular compensation does not occur. Thus, contractility of 3N salmonid hearts is almost certainly impaired due to the conundrum faced by 3N angiogenic regulatory mechanisms in that increasing capillarity to prevent loss of contractility during periods of high cardiac demand would be at the expense of myofiber cross sectional area and therefore contractility.

Introduction

As pointed out in the main introduction, the characteristically enlarged cardiomyocytes of triploid (3N) fish have the potential to create a larger diffusion distance between coronary capillaries and the mitochondria of the ventricular muscle cells. If increased diffusion distance limits O_2 supply to the heart, which is obligatorily aerobic (Davie and Farrell 1991), convective O_2 supply to the body would become compromised. Similarly, the reduced arterial O_2 content (CaO_2) observed in 3N fish would reduce convective O_2 supply to the body without a compensatory increase in routine cardiac output (Q), which would add a load to the heart or reduce the heart's scope for work.

Such limitations are unlikely to be manifest under routine conditions, but rather when fish either are exercising and cardiac O_2 demand is increased 2 to 4-fold (Graham and Farrell 1990), or faced with environmental hypoxia and arterial Po_2 is reduced. In Chapters 2 and 3, I showed impaired high temperature tolerance of 3N rainbow trout, which at least partly explains poor survival in nature (Chapter 3), is related to a more rapid onset of cardiac collapse within 3N populations experiencing incrementally increasing temperatures. In turn, an early cardiac collapse may reflect O_2 supply limitations to 3N hearts experiencing high O_2 demands due to thermodynamic increases in metabolism. However, empirical measurements of such effects are

near impossible. Instead, the Krogh O_2 diffusion model for capillaries (Krogh 1919) is an effective modeling tool to elucidate the roles myocardial and vascular anatomy and circulatory convection of O_2 play in meeting myocardial O_2 demands. Here, this model is modified and applied to the ventricular myocardium of salmonids. By applying the presumed consequences of enlarged cells to capillaries and the reduced CaO_2 of 3N salmonid fish to this model, I can provide novel information on the needs and limits for myocardial capillarity. Thus, 2N and 3N physiological input variables were used in a version of the Krogh diffusion model to test the hypothesis that triploidy leads to impaired O_2 supply to the salmonid ventricle.

Larger cells in all 3N tissues examined to date have been reported across a large number of fish species (Benfey 1999; Johnstone et al. 1999; Mercier et al. 2002; Bjornevik et al. 2004). This comes about because 3N fish are produced by manipulating fish eggs shortly after fertilization, causing them to retain the 2nd meiotic polar body and have 3 sets of chromosomes in all mature nucleated somatic cells, compared to two sets in 2N conspecific cells. The result is 50 % more DNA in nucleated cells and thus 30 % larger cells. All the same, larger cell size tends to be compensated for by a reduction in cell number, resulting in an animal of similar size with similar sized organs to their 2N cohorts. As a result, relative ventricular mass is similar for 3N and 2N salmonids (personal observations; Simonot and Farrell 2009).

Cardiac muscle, which comprises a mere 0.1 % body mass, requires an essentially uninterrupted O_2 supply. In salmonids, O_2 is supplied to the heart in two ways, via the coronary artery and via the venous blood returning to the heart. The coronary artery supplies the vascularised, cortical, compact layer of the salmonid ventricle while the avascular, inner spongy layer is supplied with the venous blood bathing its trabeculae (Davie and Farrell 1991). Ploidy is not known to affect the proportion of compact myocardium (personal observation; Simonot 2005). But as shown below, unless there is a compensation in terms of capillary density, enlarged cardiomyocytes could effect a small change in diffusion distances between capillaries in the compact myocardium and mitochondria, which might then impair O_2 supply under conditions of high O_2 demand or reduced environmental O_2 availability. Evidence that cardiac compensations for enlarged 3N cardiomyocytes are likely necessary comes from the observation that 3N brown trout are more dependent on intracellular Ca^{2+} stores during cardiomyocyte contraction compared with 2Ns, which is likely related to a reduced surface area to volume ratio

of enlarged 3N cardiomyocytes (Mercier et al. 2002). A larger capillary diameter to accommodate a larger 3N RBC may compound this problem. While cardiac capillarity has not been measured in 3N salmonids, there is sufficient information to create a Krogh diffusion model.

Diffusion is particularly problematic for trout cardiomyocytes because the mitochondria are centrally located (Vornanen 1998) and, according to Fick's second law of diffusion, O_2 flux decreases proportionally to the square of diffusion distance. The centrally located mitochondria in 2N rainbow trout compact myocardium have been measured at 13 μm from the capillaries on average (Clark et al. 2004), suggesting closest neighbor capillaries would be maximally separated by 3 cardiomyocytes, if cardiomyocytes are 8.5 μm wide (Vornanen 1998; Clark et al. 2004). If changes to cardiomyocyte shape are similar to changes reported in 3N Atlantic salmon RBCs (i.e., 1.2-fold increase in length and 1.1-fold increase in width, Benfey et al. 1984), then 3N cardiomyocytes can be predicted to increase in width from 8.5 to 9.4 μm (Vornanen 1998, Clark et al. 2004) and length from 30 to 37 μm (Figure 1 from Vornanen 1998), resulting in a cardiomyocytes volume increase from 6800 to 10300 μm^3. These estimates are based on the main body of the ellipsoidal cardiomyocyte before the ends begin to taper. If capillarity is maintained, the inter-capillary distance associated with the 10 % increase in diameter of the same 3 cardiomyocytes would increase from 26 to 28 μm, which corresponds to a 1.1-fold increase in diffusion distance from 13 to 14 μm and thus, according to Fick's second law of diffusion, a 1.2-fold increase in diffusion time between the capillary and centre of the cardiomyocyte. *In vivo* however, 3-D radial O_2 diffusion and metabolic O_2 removal as it diffuses through the tissue preclude such a simplistic approach, which lead to the advent of the O_2 diffusion model proposed by August Krogh and Agner Erlach (Krogh 1919), which provides a simple theoretical framework under which O_2 supply to metabolizing tissue may be examined.

The Krogh diffusion model derives the PO_2 tension gradient required to adequately drive radial diffusion of O_2 over a radius of R from the centre of the capillary of radius r_c, through tissue with an O_2 solubility of β and diffusion coefficient of D while it is metabolizing at a rate of VO_2 (Figure 4-1). Thus, compact myocardial O_2 supply could become limited by either the primary force for O_2 diffusion, the O_2 pressure gradient between blood and mitochondria, which is approximated by arterial PO_2, or the anatomical distance over which O_2 must diffuse, which is

set by capillarity and cell size. In contrast, the spongy myocardium, which makes up the majority of the ventricular mass in rainbow trout and lacks capillaries, has venous Po_2 as the primary force driving O_2 diffusion to the trabecular mitochondria, and trabecular radius setting the anatomical distance O_2 must diffuse. A low venous Po_2 gradient could create O_2 supply problems in the spongy myocardium. Salmonids may, however, circumvent this problem by arranging the trabecular myocytes in sheets rather than tubes, thus maintaining small O_2 diffusion distances (Pieperhoff et al. 2009). The spongy myocardium might also benefit from its inner location in the ventricular wall because Laplace's Law states that wall tension increases with radius. Therefore, in generating a lower wall tension compared with the outer compact myocardial layer, the inner wall of the ventricular chamber likely carries a lower power output burden and hence O_2 demand than the outer compact layer. Observed differences in metabolic enzyme activities between compact and spongy myocardia of fishes (Poupa et al. 1974; Ewart and Driedzic 1986; Farrell et al. 1990; Clark and Rodnick 1998) suggest that an important metabolic zonation may exist in the salmonid heart, but how this relates to O_2 supply and demand is unclear at this time. What is clear, however, is that the higher O_2 demand of the compact myocardium in combination with potential increases in diffusion distance from coronary capillaries through 3N cardiomyocytes of the compact myocardium create a potentially fatal short falling in O_2 supply under some circumstances. Therefore, the focus of the present model was on the compact myocardium.

To test the hypothesis that enlarged 3N cardiomyocytes impair the O_2 supply to 3N cardiomyocyte mitochondria, which contributes to reduced cardiac pumping ability and thus aerobic capacity of 3N rainbow trout, I applied a modified Krogh diffusion model to 2N and 3N compact myocardia. The model was applied while varying the model input parameters according to literature values for myocardial O_2 consumption rate, coronary flow rate, capillary size, arterial O_2 content, haemoglobin-O_2 affinity and required mitochondrial-cytoplasmic PO_2 gradient.

Methods

A modified Krogh model was used to generate predictions of the radius of the Krogh cylinder of tissue supplied by O_2 along the capillaries of the compact myocardium. The model

was applied with physiologically relevant input parameters obtained from the literature for resting and maximally swimming 2N and 3N salmonids (Tables 4-1 to 4-3).

Iterative Determination of the Krogh Cylinder

In the Krogh-Erlach equation, Po_2 is the dependent variable with R as an independent variable and rearrangement of the model to solve for R is not simple. However, the present experimental question requires solving for R as the dependent variable with a known Po_2 as one of the independent variables. Therefore, in order to solve for R, an iterative input of an incrementally increasing R was applied to the Krogh model until the Po_2 required was equal to the Po_2 of the capillary blood supplying the Krogh tissue cylinder. Downstream blood Po_2 was then adjusted with respect to the metabolic O_2 removal that had occurred upstream and the iterative determination of R was repeated for subsequent 1 μm increments of capillary length.

Iterations of the Krogh-Erlach equation (Equation 4-1), which solves for the capillary Po_2 required to drive O_2 diffusion through an infinitely short tissue cylinder of radius R, were performed with R increasing by 0.001 μm increments for each iteration until either the required Po_2 equaled the predetermined capillary Po_2 or R exceeded 15 μm. An important assumption of the Krogh model is that O_2 supply to the Krogh cylinder is solely derived from the central capillary. Personal observations of inter-capillary distances of 30 μm in rainbow trout compact myocardium, suggest that diffusive O_2 from adjacent capillaries will overlap by 15 μm away from a capillary, thus a maximum limit of 15 μm for R, was introduced to the model in order to account for O_2 supply from adjacent capillaries. In Equation 4-1, Po_2req is the O_2 partial pressure (kPa) required to drive O_2 diffusion out of a capillary of radius r_c (μm) through a cylinder of radius R (μm) if the Krogh diffusion constant and O_2 consumption rate of the tissue making up the cylinder are Ko_2 (μl O_2 s^{-1} μm^{-1} kPa^{-1}) and VO_2 (μl s^{-1} μm^{-3}), i.e., the Po_2 required to provide just enough O_2 to meet the O_2 demand for a given radius from a point source.

Equation 4-1

$$Po_{2req} = \frac{Vo_2}{2K}\left\{R^2 \times \ln\left[\frac{R}{r_c}\right] - \frac{R^2 - r_c^2}{2}\right\}$$

Axial Changes to Capillary Blood O_2

The capillary Po_2 driving O_2 diffusion into the Krogh cylinder of tissue was determined using Equation 4-2, which converted the haemoglobin (Hb)-O_2 saturation of the blood into Po_2 based on the Hb-O_2 curve using physiological values for P_{50} and the Hill coefficient, as determined in Chapter 5 and from literature values.

Equation 4-2

$$Po_{2cap} = \frac{Hb_{sat} \times P_{50}^{Hill}}{100 - Hb_{sat}^{1/Hill}}$$

The metabolic removal of O_2 from the capillary was determined at 1 μm increments along an anatomically realistic 60 μm capillary (Clark et al 2004). For each 1 μm increment, the volume of O_2 consumed by metabolism per second was determined, based on the Krogh cylinder volume for that increment and the O_2 consumption rate specific to the tissue cylinder volume. This calculated volume of O_2 was removed from the amount of capillary blood O_2 reaching the next 1 μm capillary increment per second, as an updated capillary O_2 flow (Equation 4-3). The updated capillary O_2 flow rate calculated from Equation 4-3 was then used in Equation 4-4 to update blood O_2 content, and generate a new percent saturation using an O_2 carrying capacity of 118 μl O_2 ml^{-1}. Percent saturation was then used in Equation 4-2 to determine the PO_2 for the next 1 μm capillary increment.

Equation 4-3

$$O_2 \text{ Flow} = \pi \times Co_2 \times q_{cor} \times (10200\text{-}60r_c^2) - \pi \times Vo^2 \times (R^2\text{-}r_c^2)$$

Equation 4-4

$$Co_2 = \frac{O_2 \text{ Flow}}{\pi \times q_{cor} \times (10200\text{-}60r_c^2)}$$

Sensitivity Analysis

The Krogh diffusion model assessed ventricular O_2 supply in the resting and maximally exercising state (Table 4-3). A sensitivity analysis for both states was performed for each of the Krogh model input variables across its entire physiological range to determine the most influential variables in coronary O_2 supply. Sensitivity analyses involved varying one input variable across its physiological range while using a mid-physiological range base value for all other input variables (Tables 4-1 to 4-4). The results of this analysis are displayed in Figures 4-2 to 4-6 and described below.

Rest and Exercise Conditions for the Model

Exercise and resting scenarios of 2N and 3N fish were compared using the base input values for the model, as described below.

Myocardial VO_2

A variety of studies have measured myocardial VO_2 in salmonid hearts using a variety of tissue and organ preparations. These measurements are summarized in Table 4-1. Myocardial VO_2 is dependent foremost on workload and secondarily on mechanical efficiency, which changes with workload and temperature, but tends to be 15 % at rest and 25 % at maximum workloads (Graham and Farrell 1990; Farrell and Jones 1992). This range of values is encompassed in the sensitivity analysis for VO_2. Maximum cardiac workload is also dependent on temperature (Farrell and Jones 1992). For my model, I was particularly interested in using a resting and maximal cardiac power output for a salmonid heart at 15 ^{o}C, which are 1.5 and 5 to 7 mW g ventricular mass $(VM)^{-1}$, respectively (Kiceniuk and Jones 1977; Claireaux et al. 2005).

Two data sets are available for rainbow trout myocardial VO_2 using working, perfused hearts, one with the coronary artery perfused and the other without, but relying on a supra-physiological perfusate Po_2 to drive O_2 from the lumen of the heart to the outer compact myocardium.

Though perfused mammalian hearts tend not to achieve *in vivo* maximum power outputs, this is not necessarily the case with salmonid hearts. Maximally working *in vitro* salmonid hearts, even without coronary perfusion (Graham and Farrell 1990, Claireaux et al. 2005), can achieve

in vivo maximum power outputs of 5 to 7 mW gVM^{-1} (Kiceniuk and Jones 1977) provided the Po_2 of the perfusate is supraphysiological. A heart working at a power output of 7 mW gVM^{-1} with a mechanical efficiency of 20 % was predicted to require a VO_2 rate of 17.4 $x10^{-13}$ µl O_2 μm^{-3} s^{-1} (Graham and Farrell 1990). For a heart working at the same mechanical efficiency of 20 %, but with a resting power output of 1.5 mW gVM^{-1}, a VO_2 rate of 3.7 $x10^{-13}$ µl O_2 μm^{-3} s^{-1} was predicted (Graham and Farrell 1990). Rainbow trout hearts working with and without the coronary artery perfused (Agnisola et al. 2003), but at a slightly lower routine power output (1.2 and 1.4 mW gVM^{-1} for hearts with and without coronary perfusion, respectively) required similar VO_2 rates (3.5 to 5.0 $x10^{-13}$ µl O_2 μm^{-3} s^{-1} with and without coronary perfusion, respectively), and therefore operated at a lower mechanical efficiency (14 and 12 % with and without coronary perfusion, respectively) compared to in Graham's work. However, maximum cardiac performance was not considered in Agnisola's study. Therefore, base VO_2 input values for the Krogh model scenarios for hearts of resting and maximally swimming fish were 3 $x10^{-13}$ and 20 $x10^{-13}$ µl O_2 μm^{-3} s^{-1}, respectively, to reflect hearts working at 1.5 mW gVM^{-1} with 20 % efficiency and 7 to 9 mW gVM^{-1} with 20 % efficiency, respectively.

Based on the above literature values (Table 4-1), the sensitivity analysis for the Krogh diffusion model varied resting myocardial VO_2 between 1.9 $x10^{-13}$ and 7.5 $x10^{-13}$ µl O_2 μm^{-3} s^{-1}, which reflect hearts working at 1.5 mW gVM^{-1} with 40 and 10 % efficiency, respectively. For maximally swimming fish, myocardial VO_2 was varied from 10 $x10^{-13}$ to 35 $x10^{-13}$ µl O_2 μm^{-3} s^{-1}, which reflected hearts working at 5 mW gVM^{-1} with 20 % efficiency and 7 mW gVM^{-1} with 10 % efficiency, respectively (Table 4-3).

Arterial O_2 Delivery Rate

The two components of arterial O_2 delivery to the compact myocardium are coronary flow rate (q_{cor}) and arterial O_2 content (CaO_2).

q_{cor}

Coronary flow to the salmonid ventricle has been measured *in vivo* with flow probe placement and microsphere injection, and has been estimated through *in vitro* perfused coronary preparations. These measurements are summarized in Table 4-2. Coronary blood flow tends to

divert 1 % of cardiac output to the compact myocardium in normoxic resting salmonids (Axelsson and Farrell 1993; Gamperl et al. 1994; Gamperl et al. 2003) and can more than double in response to increased dorsal aortic blood pressure (Farrell 1987; Axelsson and Farrell 1993; Gamperl et al. 1994; Gamperl et al. 2003). Resting q_{cor} *in vivo* ranges from 7.1 to 10.0 $x10^{-15}$ ml s^{-1} μm^{-3} in rainbow trout (Gamperl et al. 1994) and Coho salmon (Axelsson and Farrell 1993), respectively. Microsphere injection similarly estimated resting q_{cor} as 12.5 $x10^{-15}$ ml s^{-1} μm^{-3}. *In vitro* q_{cor} to perfused resting rainbow trout ventricles is several-fold higher than *in vivo* values, ranging from 50 $x10^{-15}$ ml s^{-1} μm^{-3} for saline perfused (Farrell 1987; Agnisola et al. 2003; Jensen and Agnisola 2004) to 370 $x10^{-15}$ ml s^{-1} μm^{-3} for RBC (Jensen and Agnisola 2004) perfused hearts. The difference between q_{cor} *in vivo* and *in vitro* is in part related to the lower viscosity of perfusate compared with blood. In addition, there may be tonic control of the coronary vascular resistance *in vivo*. Indeed, *in vivo* coronary vasoconstriction, induced with adrenaline perfusion, resulted in rainbow trout q_{cor} (11 $x10^{-15}$ ml s^{-1} μm^{-3}) similar to resting values (Gamperl et al. 1994).

Coronary flows have not been measured in maximally swimming fish, but, in rainbow trout, flow doubled to 14.3 $x10^{-15}$ ml s^{-1} μm^{-3} with swimming at 1 BL s^{-1} (Gamperl et al. 1994). Farrell (1987), based on manipulation of coronary perfused working *in vitro* rainbow trout heart preparations, predicted maximally dilated flows to be 50 $x10^{-15}$ ml s^{-1} μm^{-3} which is over double the *in vivo* measurement of fully dilated q_{cor} (25 $x10^{-15}$ ml s^{-1} μm^{-3}) in Coho salmon (Axelsson and Farrell 1993), which, in turn, is nearly double the 1 BLs^{-1} swimming rainbow trout q_{cor} described above. During bouts of spontaneous activity, Coho salmon q_{cor} increased only 1.5-fold to 15.3 $x10^{-15}$ ml s^{-1} μm^{-3} (Axelsson and Farrell 1993). Based on these literature values, for the sensitivity analysis on q_{cor}, values ranging from 5 to 10 $x10^{-15}$ ml s^{-1} μm^{-3} for resting fish and 15 to 25 $x10^{-15}$ ml s^{-1} μm^{-3} for maximally swimming fish were applied to the Krogh diffusion model with base resting and maximum values of 7.5 $x10^{-15}$ and 20 $x10^{-15}$ ml s^{-1} μm^{-3}, respectively (Table 4-3).

CaO_2

The range of CaO_2 applied to the Krogh diffusion model is reported in Table 4-3. For resting rainbow trout and chinook salmon (*Oncorhynchus tshawytscha*), CaO_2 ranges from 76 to 116 µl

ml^{-1} (Kiceniuk and Jones 1977; Perry and Reid 1994; Thorarensen et al. 1996; Holk and Lykkeboe 1998; Bernier et al. 2004; McKenzie et al. 2004). At critical swimming velocity, CaO_2 is either maintained or falls with reported values ranging from 82 to 101 μl ml^{-1} (Kiceniuk and Jones 1977; Thorarensen et al. 1996; Bernier et al. 2004; McKenzie et al. 2004).

Therefore, for the sensitivity analysis on CaO_2, resting and maximally swimming fish CaO_2 was varied across the same literature value-based range of 82 to 117 μl ml^{-1}. However, a resting base value of 117 μl ml^{-1} was used and 96.9 μl ml^{-1} was used for the maximally swimming base value (Table 4-3).

Krogh's Diffusion Constant (Ko_2)

Reported literature values for Ko_2 varied 3 fold from 9 $x10^{-11}$ to 26 $x10^{-11}$ μl s^{-1} μm^{-1} s^{-1} for skeletal and cardiac muscle of frog (Grote and Thews 1962) and skeletal muscle of fish (Desaulniers et al. 1996). Thus, Ko_2 values ranging from 5 $x10^{-11}$ to 26 $x10^{-11}$ μl s^{-1} μm^{-1} s^{-1} were applied for the sensitivity analysis of resting and maximally swimming fish with a base value of 15 μl s^{-1} $\mu m^{-1} s^{-1}$ (Table 4-3).

Mitochondrial PO_2

The PO_2 at the mitochondrial membrane in isolated cardiomyocytes has been reported as 0.27 kPa (Takahashi and Asano 2002), but there is still considerable debate as to its exact value *in vivo* (Brown 1992, Wagner 2012). For the sensitivity analysis of the effect of the PO_2 gradient across the mitochondrial membrane on R, an extra term was added to the model. This term accounts for a PO_2 greater than zero at the distal border of the Krogh cylinder. Mitochondrial PO_2 values ranging from 0 to 0.27 kPa were used in the sensitivity analysis and a base value of 0 was used (Table 4-3).

Hb-O_2 Dynamics

The affinity of blood Hb for O_2, quantified as P_{50}, can influence the Po_2 of blood, and therefore the tension gradient driving O_2 diffusion out of the capillary, as blood O_2 drops along the capillary due to metabolic O_2 removal. P_{50} of rainbow trout blood at 12 to 15 ºC exposed to

0.25 to 0.30 kPa CO_2 ranged from 2.50 to 3.29 kPa (Eddy 1971; Milligan & Wood 1987; Rummer 2010) and ranged from 2.76 to 4.61 kPa at a Pco_2 of 0.50 kPa (Holk & Lykkeboe 1998; Rummer 2010). In Chapter 5, I found P_{50} of rainbow trout blood at 11 ºC was 2.63 kPa at a Pco_2 of 0.25 and 3.82 kPa at a Pco_2 of 0.50 kPa. The sensitivity analysis of Hb-O_2 affinity effects on O_2 supply to the compact myocardium used P_{50} values ranging from 2 to 3 kPa with a base value of 2.3 kPa for the resting model scenario and values ranging from 2.5 to 5.0 kPa with a base value of 4.0 kPa for the swimming scenario (Table 4-3).

Variables Reflecting Triploid Coronary O_2 Supply

Triploid fish CaO_2 is reduced and RBCs are enlarged. These differences were inputted into the model to reflect 3N O_2 supply. Both resting and maximally swimming CaO_2 was lower in 3N chinook salmon compared to 2N, being 79 and 67 μl ml^{-1}, respectively (Bernier et al. 2004). Additionally, enlarged 3N RBCs are expected to require wider capillaries, but capillary radius has never been assessed in 3N fish. Based on 3N Atlantic salmon RBC dimensions, which are 1.1-fold wider than for 2Ns (Benfey et al. 1984), the effect of increasing r_c by 1.1-fold from 2.6 to 2.8 μm on the Krogh model was assessed. Furthermore, enlarged 3N cardiomyocytes are expected to increase the required O_2 diffusion distance through myocardial tissue. As detailed in the introduction of this chapter, diffusion distances in 3N compact myocardium were predicted to increase 1.1-fold, resulting in an increase in minimum distance from 10 to 11 μm. These ploidy effects were assessed in both the resting and maximally swimming scenarios of the Krogh model using the above described base values for Ko_2, VO_2, q_{cor}, P_{50} and mitochondrial Po_2 and 3N values of 84.3 and 67 μl ml^{-1} for resting and swimming CaO_2 and 2.8 um for capillary radius (Table 4-3). Additionally, due to the large influence the sensitivity analysis showed VO_2 to have on R, resting and swimming models were run varying VO_2 3-fold around the base VO_2 of 20 $x10^{-13}$ μl O_2 μm^{-3} s^{-1} with a low value of 10 $x10^{-13}$ μl O_2 μm^{-3} s^{-1} and a high value of 35 $x10^{-13}$ μl O_2 μm^{-3} s^{-1}.

Results

Diploid

Sensitivity Analysis for the Resting Scenario

For the resting scenario, the Krogh model output was insensitive to all input variables across the physiological range (Figure 4-2), except myocardial VO_2. Varying Ko_2, P_{50}, PO_2mito, r_c, q_{cor} and CaO_2 by 5-, 1.5-, 2-, 2-, 2- and 1.4-fold, respectively had no effect on the 15 μm R along the entire capillary length seen with the base condition (Figure 4-2B, only output for Ko_2 shown).

Resting Cardiac Metabolic O_2 removal (VO_2)

In the resting scenario, the only variable the Krogh model was sensitive to was myocardial tissue VO_2 (Figure 4-2A). Applying the physiological range of resting myocardial VO_2, which differed nearly 5-fold from low (1.9×10^{-13} μlO_2 μm^{-3} s^{-1}) to high (10×10^{-13} μlO_2 μm^{-3} s^{-1}), resulted in an R of 15 μm at the capillary origin across the entire physiological range. For the mid range to low extreme VO_2 values, which corresponded to a cardiac workload of 1.5 mW gVM^{-1} with mechanical efficiency ranging from 20 to 40 %, an R of 15 μm was maintained along 60 μm capillary length. However, the high physiological extreme VO_2 value, corresponding to 10 % efficiency, resulted in a drop in R below 10 μm at 51 μm downstream of the capillary origin.

Sensitivity Analysis for the Exercising Scenario

The swimming model output was highly sensitive to Ko_2, VO_2, q_{cor} and CaO_2, moderately sensitive to P_{50} and r_c and negligibly sensitive to PO_2mito. Varying VO_2, Ko_2, CaO_2, q_{cor}, P_{50}, r_c and Po_2mito from high to low extremes resulted in 53 %, 23 %, 47 %, 39 %, 10 %, 6 % and no change, respectively in the length of capillary over which an R greater than 10 μm was supported. Thus, the swimming Krogh model scenario was most sensitive to myocardial tissue VO_2 (Figures 4-3 and 4-4).

Exercising Metabolic O_2 removal by Myocardia (VO_2)

Applying the physiological range of myocardial VO_2 during exercise, which varied nearly

3.5-fold from low (10 x10^{-13} µlO_2 µm^{-3} s^{-1}) to high (35 x10^{-13} µlO_2 µm^{-3} s^{-1}), to the exercising model scenario resulted in an R of 15 µm at the capillary origin for the entire physiological range, but variability in the downstream decline in R (Figure 4-3A). For the highest VO_2, which corresponded to hearts working at a power output of 7 mW gVM^{-1} with a mechanical efficiency of 10 %, R began to fall by 15 µm downstream, and fell below 10 µm at 28 µm downstream. The low extreme of the physiological range of VO_2, which corresponded to a mechanical efficiency of 20 % with a workload of 5 mW gVM^{-1}, resulted in an R of 15 µm along a capillary length of 60 µm. Thus, only for the combination of the high mechanical efficiency and lowest cardiac workload were the O_2 demands associated with the cardiac power outputs of maximally swimming fish hearts met.

Exercising Krogh's Diffusion Constant (Ko_2)

Unlike for the resting model scenario, the Krogh cylinder for the maximum swimming model scenario was extremely sensitive to varying Ko_2 across the physiological range, which differed 5 fold from the low (5 x10^{-11} µl s^{-1} µm^{-1} kPa^{-1}) to high (26 x10^{-11} µl s^{-1} µm^{-1} kPa^{-1}) extremes (Figure 4-3B). Applying the physiological range of Ko_2 resulted in an R of 15 µm at the capillary origin across the entire range, but variability in the downstream fall in R. The low extreme of the physiological range resulted in an R of nearly 10 µm along 60 µm of capillary length. The midrange value and high extreme resulted in a 15 µm R to 37 and 42 µm downstream, respectively, but R of both rapidly fell to 10 µm by 48 and 45 µm, respectively.

Exercising Arterial O_2 Delivery Rate

R in exercising fish was highly sensitive to changes within the physiological range of both q_{cor} and CaO_2.

Exercising qcor

Coronary flow had a strong impact on the swimming scenario Krogh model output (Figure 4-4A). Increasing q_{cor} 2-fold from the low (15 x10^{-15} ml s^{-1} µm^{-3}) to high (25 x10^{-15} µlO_2 µm^{-3} s^{-1}) physiological extremes resulted in no change in R at the capillary origin, but greatly affected the downstream decline in R. For the low extreme of the physiological range of exercising q_{cor}, R

first fell below 15 μm at 27 μm downstream of the capillary origin and fell below 10 μm at 35 μm downstream. The initial decline from an R of 15 μm was delayed to 49 μm for the upper exercising q_{cor} extreme, for which R first fell below 10 μm at 57 μm downstream.

Exercising CaO_2

The exercising scenario of the Krogh model was also highly sensitive to varying CaO_2 1.4-fold from the low (82 μl ml^{-1}) to high (117 μl ml^{-1}) physiological extremes (Figure 4-4B). Similar to q_{cor}, an output R of 15 μm was achieved at the capillary origin for the entire physiological range of CaO_2, and decreasing CaO_2 resulted in a downstream decrease in R. Applying the low physiological CaO_2 extreme resulted in R dropping below the physiologically realistic 10 μm range at 39 μm downstream of the capillary origin, compared to at 55 μm for the upper physiological extreme value of CaO_2.

Exercising P_{50}

P_{50} had a moderate impact on the Krogh cylinder (Figure 4-3C). As with all variables applied to the exercise Krogh model scenario, applying the physiological range of arterial P_{50}, which differed 2-fold from the low (2.5 kPa) to high (5 kPa) extreme, had no effect on R at the capillary origin, which was 15 μm. However, the low extreme P_{50} maintained an anatomically realistic R of 10 μm to 50 μm downstream of the origin, compared to 45 μm for the high extreme of P_{50}.

Exercising r_c

Capillary radius had a moderate impact on the Krogh cylinder of swimming fish hearts (Figure 4-3E). Applying the physiological range of r_c, which differed 2-fold from the low (1.6 μm) to high (3.1 μm) extreme, supplied an R of 15 μm at the capillary origin, regardless of r_c. Increasing r_c reduced the downstream distance over which an anatomically realistic R of 10 μm was maintained from 50 μm for the low extreme of r_c to 47 μm for the high r_c extreme.

Exercising Mitochondrial PO_2 Gradient

Introduction of a required PO_2mito across the mitochondrial membrane had a negligible effect on R for the exercise model (Figure4-3D). Applying the physiological range of PO_2mito, which differed from the low (0 kPa) to mid value (0.13 kPa) to high (0.27 kPa), supplied an R of 15 μm at the capillary origin, regardless of PO_2mito. Furthermore, R dropped below the anatomically realistic value of 10 μm at 46 μm downstream of the capillary origin regardless of the physiological PO_2mito value applied. As the Krogh model was relatively insensitive to PO_2mito, the extra term was not applied to further modeling.

Diploid Rest versus Exercise Scenarios

When the rest and exercise model scenarios using base input values were compared, O_2 supply was sustainable for the resting, but not the swimming model (Figure 4-5). For the resting scenario, the base VO_2 value corresponded to a power output of 1.5 mW gVM^{-1} at a mechanical efficiency of 20 %, and supplied an R of 15 μm along the anatomically realistic capillary length of 60 μm. Only when a resting VO_2 corresponding to a low mechanical efficiency of 10 % was applied to the model, did supply become insufficient to meet demands, with R falling below the anatomically realistic value of 10 μm at 51 μm downstream of the capillary origin.

For the exercising scenario, the base VO_2 value, which corresponded to a power output of 7 mW gVM^{-1} with a mechanical efficiency of 20 %, supplied a 10 μm R only to 46 μm downstream of the capillary origin, and thus O_2 supply was insufficient, but only slightly. By reducing cardiac workload 30 % from 7 to 5 mW gVM^{-1} and maintaining an efficiency of 20 %, an R of 15 μm was sustained along the entire 60 μm long capillary.

Triploid Rest and Exercise Scenarios

When physiological values for 3N r_c and CaO_2 were applied to the resting and swimming scenarios of the Krogh model, O_2 supply was sufficient to the 3N compact myocardium of resting, but not swimming fish (Figure 4-6). The 30 % reduction in CaO_2 of resting 3N arterial blood combined with a 1.1-fold increase in 3N r_c (Table 4-3) had no effect on R which was maintained as 15 μm along the entire capillary length, as in the 2N resting scenario.

For the 3N maximum swimming model scenario, the 30 % reduction in CaO_2 along with a 1.1-fold increase in 3N r_c resulted in more severe O_2 supply limitations than for the 2N scenario. For the 3N scenario, like in the 2N scenario, capillary origin R was 15 μm, but by 31 μm downstream, R fell below the 3N anatomically realistic value of 11 μm. For the 2N exercise scenario, on the other hand, R first fell below the 2N anatomically realistic R of 10 μm at 46 μm downstream of the capillary origin. By decreasing cardiac workload to 5 mW gVM^{-1} and maintaining an efficiency of 20 %, VO_2 was just sustainable in 3N fish, with R falling to 13 μm at the capillary terminus. There was no fall in R below the capillary origin value of 15 μm for the 2N scenario with the same low power output and high efficiency.

Therefore, though maximally exercising 2N hearts were slightly O_2 deficient, the model output suggested O_2 supply to the heart of maximally swimming 3N fish was insufficient to support 2N-like cardiac O_2 demands unless maximum cardiac power output was 30 % lower in 3N than in 2N salmonid fish.

Discussion

A modified Krogh diffusion model was applied to the compact myocardium of salmonid hearts in order to predict the radius of tissue (R) supplied with O_2 from its central capillary and along the entire capillary length. The resting scenario model shows 3N ventricular vascularity and O_2 supply was able to support 2N-like resting myocardial performance. The swimming fish model output, on the other hand, suggests vascularization of and O_2 supply to the heart of maximally swimming 3N fish was insufficient to support 2N-like cardiac O_2 demands. Sensitivity analyses showed the resting 2N model to be insensitive to all variables but VO_2, while the output of the swimming 2N model was highly sensitive to VO_2, Ko_2, q_{cor} and CaO_2, moderately sensitive to P_{50} and r_c and relatively insensitive to Po_2mito.

2N Resting and Swimming Scenarios

As expected, the base 2N resting model scenario output suggested sustainable O_2 supply to the compact myocardium, but the maximum swimming scenario was unsustainable. When the entire physiological range of VO_2 reflecting a power output of 1.5 mW gVM^{-1} and mechanical

efficiency ranging from 10 to 40 % was applied to the resting scenario model, even the low efficiency of 10 % was nearly sustainable. Thus, as expected, O_2 supply to the resting heart model just exceeds demand.

For the exercise scenario model, when myocardial O_2 demand was increased nearly 3-fold by increasing power output 3.3- to 4.7-fold from 1.5 to 5 mW gVM^{-1}, O_2 supply fell just barely short of demand. O_2 supply was only sustainable with the highest physiologically realistic efficiency at power outputs of 5 mW gVM^{-1}. The more precarious match of O_2 demand with supply in the heart of maximally exercising fish was not entirely surprising, as maximum swimming tends to be a temporally limited state and is considered to be limited by cardiovascular capacity. This is supported by the plateau in cardiac output, with increasing swimming speed at 70 % of critical swimming velocity. Therefore a slight short-falling of O_2 supply in the base 2N maximum swimming model supports previous suggestions that cardiac capacity limits maximum swimming performance and together with resting O_2 supply just exceeding demand suggests the model and input parameters are realistic.

Sensitivity of R to Physiological Variables

Metabolic O_2 Removal by Myocardia (VO_2)

The resting model was modestly sensitive to myocardial VO_2 and the swimming model scenario was highly sensitive to VO_2. For the resting model, decreasing efficiency 4-fold, increased VO_2 5-fold and reduced the sustainable capillary length by 15 %, with O_2 demand of only the lowest efficiency exceeding supply. Varying all other input variables across their physiological range had no effect on sustainable capillary length, therefore the resting scenario was only sensitive to VO_2 and, as expected, O_2 supply to the resting heart model barely exceeded demand.

The exercising scenario model, on the other hand, was highly sensitive to VO_2. Myocardial VO_2 rates for hearts working at 5 to 7 mW gVM^{-1} and with efficiency ranging from 10 to 25 % were applied, with only the lower workload of 5 mW gVM^{-1} combined with a moderate efficiency of 20 % requiring sufficiently low amounts of O_2 to be met by physiological O_2 supply. Varying VO_2 3.5-fold across the physiological range for maximally working fish hearts caused a 53 % change in sustainable capillary length, which is greater than all other variables

which ranged from 47 % change to no effect. Thus the exercise scenario model was most sensitive to myocardial VO_2 and, as expected, was barely sustainable for even the lowest physiological demand.

Ko_2

The swimming, but not resting, model scenario was highly sensitive to Ko_2, which enhanced O_2 supply when the low physiological extreme was applied to the model. Low Ko_2, which reflects a low O_2 diffusion rate through the tissue, resulted in a smaller R along the majority of the capillary length. Though a small R may seem detrimental to O_2 supply, when R was close to the anatomically realistic R of 10 μm, compared to the model limit of 15 μm, the reduced metabolic O_2 removal from the capillary blood conserved O_2 for delivery to downstream tissue. Therefore, the maximally swimming scenario model output here suggests that low diffusivity of muscle tissue in fish can serve to regulate capillary O_2 levels along the entire capillary length, avoiding rapid O_2 loss at the capillary origin.

Arterial O_2 Delivery

The exercise, but not resting, Krogh model scenario was highly sensitive to arterial O_2 delivery, the product of q_{cor} and CaO_2, and similarly sensitive to q_{cor} and CaO_2. Increasing q_{cor} 1.7-fold or CaO_2 1.4-fold across the physiological ranges resulted in 1.6- and 1.4-fold, respectively, increases in the distance downstream from the capillary origin where R was greater than 10 μm. Thus, as expected, q_{cor} and CaO_2 were equally effective in increasing myocardial O_2 supply to match demand.

P_{50} and r_c

Though the 2N resting Krogh model scenario was insensitive to P_{50} and r_c, the exercise scenario was moderately sensitive to both variables. Increasing Hb-O_2 affinity by reducing P_{50} 2-fold across the physiological range for arterial blood of exercising fish, moderately increased the distance downstream of the capillary origin over which an R of 10 μm was sustainable. This finding is contrary to traditional interpretations of P_{50}, which suggest a high P_{50}, or low Hb-O_2 affinity, conserves a high blood PO_2 despite a drop in blood O_2 content due to metabolic

removal of O_2. Maintenance of a high PO_2 in turn maintains a high gradient for O_2 diffusion out of the blood and into the tissue, which would maintain a large R. However, here, the Krogh model output suggests that, by supporting a large R at the capillary origin, which results in metabolic depletion of capillary O_2 close to the capillary origin, a high PO_2 gradient between the blood and tissue results in ineffectively rapid removal of O_2 from the blood at the capillary origin leaving insufficient O_2 to supply downstream tissue. This finding correlates with findings here that a low Ko_2, and thus tissue O_2 diffusivity, is more effective than high Ko_2 for sustainable myocardial O_2 supply along the entire length of the capillary.

Capillary radius also had moderate effects on the 2N exercise scenario, but no effects on the resting scenario of the Krogh model. Smaller r_c size increased R downstream of the capillary origin, thus a large capillary radius was detrimental to myocardial O_2 supply.

Assumptions of the Modified Krogh Diffusion Model

As with any theoretical model, the applied modified Krogh model is dependent upon a number of important assumptions, most of which have been identified by Hoofd (1992). These assumptions are discussed below.

O_2 consumption rate is homogeneous throughout the heart. While this may be a problem in skeletal muscle where mixed fiber types exist (Johnston et al. 1975, Higgins 1990) and motor units can be recruited, this issue does not apply to the heart, which contracts as a syncytium with each heartbeat. However, the law of Laplace dictates that the inner myocardium performs less work than the outer myocardium. Because myocardial VO_2 was derived for the whole heart, VO_2 of the outer compact myocardium may have been underestimated, which would push the resting scenario model output closer to an O_2 supply limitation and increase the O_2 supply deficiencies of both 2N and 3N maximally working hearts.

At the subcellular scale, heterogeneous tissue VO_2 may occur due to heterogeneous distribution of mitochondria, such as the centrally located mitochondria within salmonid cardiomyocytes. Because overall O_2 consumption per volume of tissue (or one cardiomyocyte) is not affected by this assumption, the maximum error in predictions of the Krogh cylinder radius introduced by this assumption would be less than half the diameter of a cardiomyocyte, or 4 to 5 μm. Over the initial and final 2 to 4 μm of cardiomyocyte sarcoplasm, which likely

remove very little O_2 due to low densities of mitochondria, the Krogh model applies artificially high O_2 removal rates. However, this error is fully corrected by an underestimation of O_2 removal by the mitochondrial-dense centre of the cardiomyocytes. Therefore, Krogh model-based estimates of R may be slightly underestimated if the predicted R is positioned between the myocyte membrane proximal to the capillary and the mitochondrial dense center of the cardiomyocyte, or slightly overestimated if R lies on the distal side of the cardiomyocyte centre.

Capillaries are anatomically straight and parallel to each other with constant dimensions and homogeneous distribution throughout the myocardium. There is no reason to think that this is not the case during diastole, when q_{cor} is greatest. However, because fish increase SV during exercise, capillaries are likely to be lengthened under exercise conditions. Thus, the resting condition may overestimate the role of capillary length.

Additionally, it is impossible to fill a tissue with Krogh cylinders without some overlap, so the assumption of radial symmetry of the Krogh cylinder is a valid criticism and has been shown to potentially introduce significant error to predictions of O_2 supply (Hoofd 1992).

The time for O_2 to diffuse through the capillary wall is negligible. The addition of O_2 diffusion resistance through the capillary wall would decrease R in the model output, therefore increasing severity of supply deficiencies where they were already predicted and possibly introducing more deficiencies where sufficient supply was predicted. However, the capillary wall is much smaller than R and given the negligible effect of Ko_2 observed on O_2 supply predicted by the model, this is a reasonable assumption. Furthermore, as the primary function of capillaries is gas exchange at the tissue, it would be surprising to see a large diffusion limitation across the capillary wall. Therefore, this is most likely a reasonable assumption.

Perfusion is continuous and unidirectional. Coronary blood flow is unidirectional, but not continuous. Indeed, fluctuations in transmural pressure within cardiac muscle during the contraction/relaxation cycle result in most of q_{cor} occurring in diastole (Axelsson and Farrell 1993). While overall coronary flow rate is not affected, the residence time of blood in capillaries may be, which would then result in an underestimate of the importance of q_{cor} in the model, particularly under the exercise condition. Maximum literature values, which likely reflect diastolic high coronary flow rates were applied to the model, therefore O_2 flow through the

capillaries may be over estimated.

PO_2 is equal throughout the cross sectional area of the capillary. With the tight squeeze of 7 to 9 μm wide RBCs (Benfey et al. 1984) through 5 to 8 μm wide capillaries (Clark et al. 2004), sufficient mixing of the blood is expected to occur ensuring an equal Po_2 through the cross sectional area of the capillary. Even with a heterogeneous Po_2 within the capillary, R model output is unlikely to be affected as rapid diffusion facilitated through Hb-O_2 interactions as well as convective mixing within the capillary would likely result in an effectively homogenous capillary PO_2.

Krogh's diffusion constant is not significantly altered through changes in cardiomyocyte composition (e.g., myoglobin concentration, lipid density, mitochondrial density) and facilitated diffusion does not occur. Ko_2 does change with tissue composition. Desaulniers et al. (1996) reported a doubling of Ko_2 in striped bass with temperature acclimation (5 *versus* 25 ^{o}C). They attributed these changes to altered cellular lipid content. The model sensitivity analysis for Ko_2 showed these changes to have negligible influence on the resting model output, but to strongly influence the exercising model output.

Regardless of these assumptions, some of which potentially add significant error to predictions of cardiac O_2 supply, all should apply equally to the 2N and 3N conditions, and thus conclusions regarding 3N relative to 2N myocardial O_2 supply are unaffected.

O_2 Supply during Rest and Swimming in 3N Compact Myocardium

Anatomical diffusion distance in compact myocardium of the rainbow trout ventricle is reported as 10 to 15 μm, depending on acclimation conditions and life stage (personal observation, Clark et al. 2004). In order to allow passage of 1.1-fold wider 3N RBCs (Benfey et al. 1984), 3N capillary diameters are also likely enlarged 1.1-fold from 10 to 11 μm. Additionally, capillary length is reported as 60 μm for rainbow trout myocardium (Clark et al. 2004). Therefore, the Krogh model output of a ventricle with sufficient O_2 supply should predict a minimal R of 10 or 11 μm for 2N and 3N hearts, respectively, for the entire length of a 60 μm long capillary.

O_2 supply to the resting, but not maximally swimming 3N fish heart was sustainable

according to the Krogh diffusion model of O_2 supply to compact myocardium. Though, O_2 supply to the heart of maximally swimming 2N fish was slightly deficient, O_2 supply to hearts of maximally swimming 3N fish fell far short of 2N-like demand. In the 2N base swimming scenario, the radius of sufficiently oxygenated tissue fell below the anatomically realistic radius of 10 μm at 50 μm downstream of the capillary origin, which is just 10 μm short of the anatomically realistic 60 μm capillary length. For 3N hearts, on the other hand, the anatomically realistic Krogh cylinder radius was predicted to be 11 μm, and for the exercising scenario, the radius fell below 11 μm at just 30 μm downstream of the capillary origin. With an anatomically realistic capillary length of 60 μm, half of the 3N cardiac tissue was predicted to suffer from O_2 shortage if the 3N hearts performed at the same workloads as 2N hearts.

The 3N myocardial O_2 supply deficiency was rectified when modeling a decrease in myocardial workload, but this would require a reduction in cardiac output and/or aortic pressure. Reduced blood pressure in coronary ligated rainbow trout swimming at sub-maximum velocities suggests a reduction in at least the latter (Steffensen and Farrell 1998). In 2N chinook salmon, coronary ligation reduced maximum swimming velocity (Farrell and Steffensen 1987), presumably to minimize the required increase in myocardial work in the face of deficient O_2 supply to the compact myocardium. A similar reduction in maximum swimming velocity would be expected with reduced maximum cardiac workload of 3N fish. In fact, though previous studies have never shown 3N critical swimming velocity to be significantly slower than that of 2Ns, the magnitude of mean 3N critical swimming velocity is consistently lower than for 2Ns (Small and Randall 1989; Parsons 1993; Stillwell and Benfey 1996; Bernier et al. 2004).

Alternatively, 3N salmonids potentially compensate by increasing vasculature density in their myocardia to match O_2 demand. The O_2 supply deficiency under the exercising scenario, was primarily a manifestation of reduced 3N arterial O_2 loading with only moderate influences of the predicted increased capillary radius and negligible effect of the predicted increase in anatomical diffusion distance.

An apparently simple solution for maintaining O_2 supply to mitochondria in 3N hearts is to increase the capillary to fiber ratio. With proposed 3N cardiomyocyte diameters of 10 μm compared to a Krogh radius of 11 μm and centrally located cardiomyocyte mitochondria, it is certainly possible for 3N hearts to reduce the required diffusion distances to these levels by

limiting capillary separation by 2 or fewer cardiomyocytes. However, the resultant increase in vascular volume required to support an increased 3N capillary to fiber ratio necessarily results in either an enlarged heart or reduction in other components of the compact myocardium. The most troublesome, but also most likely target for reduction would be that of the contractile myofibers, the cross sectional surface area of which determines tension generation capacity and thus contractility of the heart. Therefore 3N hearts face a trade off between losing cardiac contractility due to reduced myofiber area or losing contractility due to O_2 supply insufficiencies. 3N hearts appear to choose an O_2 supply insufficiency over reduced contractility, as they may be less vascularized than hearts of their 2N cohorts (Simonot and Farrell 2009). Though reduced 3N vascular volume must be concluded with caution, as 3N hearts were smaller than the 2N hearts in this experiment, and the response of vascularization to myocardial growth is unknown. Nevertheless, due to an O_2 transport limitation in 3N salmonid fish, my modified Krogh model predicts reduced contractility of 3N hearts.

This short falling in myocardial O_2 supply in maximally swimming 3N fish, corresponds with reports of reduced aerobic swimming capacity (Virtanen et al. 1990; Sezaki et al. 1991; Cotterell and Wardle 2004; Chapter 3), tolerance of sub-optimal conditions (Myers and Hershberger 1991; Ojolick et al. 1995; Mercier et al. 2000; Altimiras et al. 2002; Hyndman et al. 2003; Chapters 2 and 3) and survival in the wild (Blanc et al. 1992; Simon et al. 1993; Withler et al. 1995; Oppedal et al. 2003; Teuscher et al. 2003; Koenig et al. 2011; Chapter 3). It also points to cardiac O_2 supply limitations as the mechanism behind Chapter 2 findings of slower increases in 3N, relative to 2N rainbow trout heart rate with warming and potentially more rapid onset of cardiac within 3N compared 2N trout populations as water approached lethally high temperatures (Figure 2-1). Thus this predicted mismatch in 3N myocardial O_2 supply and demand under maximally swimming conditions supports my hypothesis that O_2 supply to the 3N heart is impaired.

In summary, anatomically realistic Krogh radii were predicted for 2N, but not 3N hearts using a resting and exercising scenario of a modified Krogh model with physiologically realistic input values. According to the model, myocardial O_2 supply of resting fish was insensitive to all input variables but VO_2. However, the exercising model scenario was highly sensitive to VO_2,

Ko_2, q_{cor} and CaO_2, moderately sensitive to r_c and P_{50} and insensitive to PO_2mito. A small O_2 supply demand mismatch was predicted for hearts of maximally swimming 2N trout, suggesting that workloads performed by hearts of maximally swimming fish are limited by myocardial O_2 supply. This mismatch in O_2 supply to demand was magnified in hearts of swimming 3N fish, supporting the hypothesis that O_2 supply to the 3N heart is impaired. These limitations were primarily due to reduced 3N arterial O_2 content, but also moderately influenced by enlarged capillaries accommodating enlarged 3N RBCs. Though 3N O_2 supply limitations may be compensated for by angiogenic decreases in diffusion distance, due to resultant decreases in myofiber cross sectional area, this is not expected to circumvent impaired cardiac contractility.

	$x10^{-13}$ µl $s^{-1}µm^{-3}$	Original value	Original units	PO (mW gVM^{-1})	%ME	Coronary	Temp (°C)	Species
Rest Rainbow Trout Perfused	3.7	0.37[1]	µl s^{-1} g^{-1}	1.5	20	N	15	Rainbow trout
	7.5	0.75[1]	µl s^{-1} g^{-1}	1.5	10	N	15	Rainbow trout
	3.0	0.30[1]	µl s^{-1} g^{-1}	1.5	25	N	15	Rainbow trout
	1.9	0.19[1]	µl s^{-1} g^{-1}	1.5	40	N	15	Rainbow trout
	3.45	21[2]	µl min^{-1} g^{-1}	1.7	24	Saline	10	Rainbow trout
	5	30[3]	µl min^{-1} g^{-1}	1.2	12	N	10	Rainbow trout
	5	30[3]	µl min^{-1} g^{-1}	1.4	14	Saline	10	Rainbow trout
	0.28	1.7[4]	µl min^{-1} g^{-1}	0	NA	Saline	15	Rainbow trout
	1.23	7.5[4]	µl min^{-1} g^{-1}	0	NA	RBC	15	Rainbow trout
Max Rainbow Trout Perfused	12.5	1.25[1]	µl s^{-1} g^{-1}	5	20	N	15	Rainbow trout
	24.9	2.49[1]	µl s^{-1} g^{-1}	5	10	N	15	Rainbow trout
	10.0	1.00[1]	µl s^{-1} g^{-1}	5	25	N	15	Rainbow trout
	17.4	1.74[1]	µl s^{-1} g^{-1}	7	20	N	15	Rainbow trout
	34.9	3.49[1]	µl s^{-1} g^{-1}	7	10	N	15	Rainbow trout
	13.9	1.39[1]	µl s^{-1} g^{-1}	7	25	N	15	Rainbow trout
	22.4	2.24[1]	µl s^{-1} g^{-1}	9	20	N	15	Rainbow trout
Non Salmonid Fish	3.2	0.316[5]	µl s^{-1} g^{-1}	1	16.2	N	10	Sea raven
	5.0	0.5[6]	µl s^{-1} g^{-1}	1.5		N	15	Tilapia
	12.0	1.2[6]	µl s^{-1} g^{-1}	5		N	15	Tilapia
Whole Fish	$x10^{-13}$ µl s^{-1} $µm^{-3}$	Original value	Original units	State			Temp (°C)	Species
	0.10	25[7]	µmol kg^{-1} min^{-1}	Rest				Rainbow trout
	0.32	5[8]	µmol g^{-1} hr^{-1}	Rest				Rainbow trout
	0.38	6[9]	µmol g^{-1} hr^{-1}	Rest				Rainbow trout
	0.14	72[10]	mg kg^{-1} hr^{-1}	Routine			14	chinook salmon
	0.74	193[7]	µmol kg^{-1} min^{-1}	Maximum				Rainbow trout
	1.33	21[9]	µmol g^{-1} hr^{-1}	Post chase to exhaustion				Rainbow trout
	0.71	360[10]	mg kg^{-1} hr^{-1}	Maximum			14	chinook salmon
Mammal	$x10^{-13}$ µl s^{-1} $µm^{-3}$	Original value	Original units	Scale				Species
	2.33	1100[11]	µmol $180g^{-1}$ min^{-1}	*In vivo* heart				human
	0.76	2[12]	µmol g^{-1} min^{-1}	Perfused heart				sheep

Table 4-1. Literature values for myocardial and whole body O_2 consumption rate of fish and mammals. Units used for the Krogh model were $x10^{-13}$ µl $s^{-1}um^{-3}$. PO is myocardial power output. %ME is the mechanical efficiency of the heart calculated as 100 x 0.0498 PO x VO_2^{-1}, with PO units of mWg^{-1} and VO_2 units of ml $g^{-1}s^{-1}$. RBC is red blood cell. Literature sources, indicated with superscript number adjacent to original value are 1. Graham and Farrell 1990; 2. Agnisola et al. 1998; 3. Agnisola et al. 2003; 4. Jensen and Agnisola 2003; 5. Farrell et al. 1985; 6. Lague et al. 2012; 7. Kiceniuk and Jones 1977; 8. Alsop and Wood 1997; 9. Scarabello et al. 1992; 10. Bernier et al. 2004; 11. Lin et al. 2011; 12. Portman et al. 1995.

	$x10^{-14}$ ml s^{-1} μm^{-3}	Original value	Original units	State	Species
In vivo	0.71	0.14[1]	ml min^{-1} gVM^{-1}	Rest	Rainbow trout
	1.00	0.20[2]	ml min^{-1} gVM^{-1}	Rest	Coho salmon
	1.43	Rest+200 %[3]		1 BLs^{-1}	Rainbow trout
	1.53	Rest+153 %[2]		Spontaneous activity	Coho salmon
	2.5	0.1[2]	ml min^{-1} $kgBM^{-1}$	Peak of biphasic flow	Coho salmon
	2.00	Rest+200 %[2]		Max vasodilation	Coho salmon
	1.07	0.24[1]	ml min^{-1} gVM^{-1}	Adrenalin	Rainbow trout
	1.10	0.22[1]	ml min^{-1} gVM^{-1}	Hypoxia	Rainbow trout
	2.20	Rest+220 %[2]		Hypoxia	Coho salmon
Perfused Heart	7.00	1.4[4]	ml min^{-1} $kgBM^{-1}$	Rest (saline perfused)	Rainbow trout
	37.00	7.4[4]	ml min^{-1} $kgBM^{-1}$	Rest (RBC perfused)	Rainbow trout
	4.00	0.8[5]	ml min^{-1} $kgBM^{-1}$	Rest (saline perfused)	Rainbow trout
	5.00	1[6]	ml min^{-1} gVM^{-1}	Rest (saline perfused)	Rainbow trout
Micro-spheres	0.12	2.5[7]	ml min^{-1} $100gBM^{-1}$	Rest	Rainbow trout

Table 4-2. Literature values for coronary flow rate of salmonid fish. Units used for the Krogh model were $x10^{-13}$ ml $s^{-1}\mu m^{-3}$. RBC is red blood cell. Literature sources, indicated with superscript number adjacent to original value are 1. Gamperl et al. 1994; 2. Axelsson and Farrell 1993; 3. Gamperl et al. 2003; 4. Jensen and Agnisola 2004; 5. Agnisola et al. 2003; 6. Farrell 1987; 7. Taylor et al. 1992. BLs^{-1} is swimming speed in body lengths per second.

	Rest					Max Swimming				
	High	Med/ Opt	Low	2N	3N	High	Med	Low	2N	3N
VO_2 x 10^{-13} ($\mu l\ s^{-1}\ \mu m^{-3}$)	7.5	**3.0**	1.9	3.0	3.0	35	**20**	10	20	20
Ko_2 x10^{-11} ($\mu l\ s^{-1} \mu m^{-1}\ kPa^{-1}$)	26.0	**15**	5	15	15	25	**15**	5	15	15
q_{cor} x 10^{-15} ($ml\ s^{-1}\ \mu m^{-3}$)	10	**7.5**	5	7.5	7.5	25	**20**	15	20	20
CaO_2 ($\mu l\ ml^{-1}$)	**117**	90	82	117	84.3	117	**96.9**	82	96.9	67
r_c (µm)	3.1	**2.6**	1.6	2.6	2.8	3.1	**2.6**	1.6	2.6	2.8
P_{50}	3.0	**2.3**	2.0	2.6	2.6	5.0	**4.0**	2.5	2.6	2.6
mito PO_2	0.27	0.13	**0**	0	0	0.27	0.13	**0**	0	0
Hill	**2.5**					**2.5**				

Table 4-3. Input values for the variables of the modified Krogh diffusion model. Ko_2 is the Krogh diffusion coefficient; q_{cor} is the coronary flow rate, VO_2 is myocardial O_2 consumption rate; r_c is the capillary radius; CaO_2 is arterial O_2 concentration; Hill is Hill's Hb coefficient of cooperativity; P_{50} is the blood O_2 partial pressure at which Hb is 50% saturated. See methods and Tables 1 and 2 for literature sources. Values highlighted and underlined combined to form the minimally sustainable state.

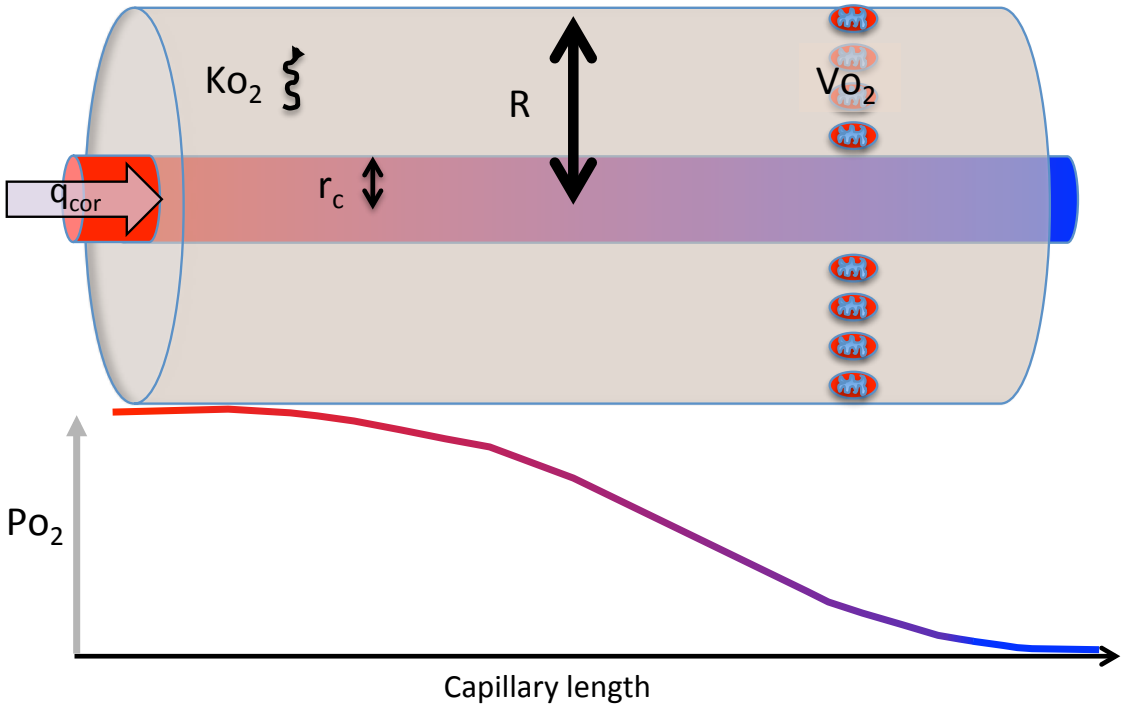


Figure 4-1. The modified Krogh model of O_2 supply to a cylinder of tissue with radius R. KO_2 is Krogh's diffusion constant, r_c is capillary radius, q_{cor} is coronary flow and VO_2 is myocardial O_2 consumption rate.

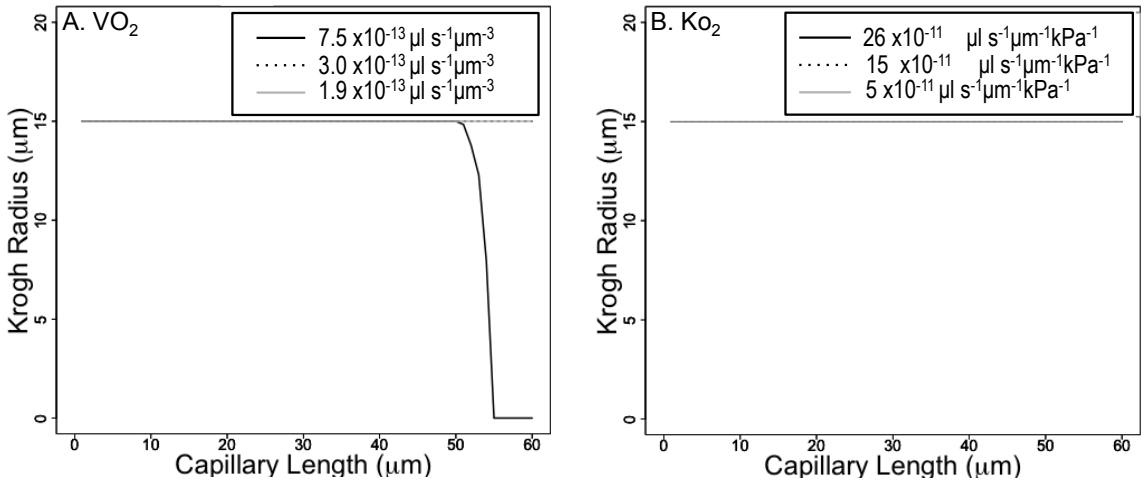


Figure 4-2. Sensitivity of the resting scenario ventricular Krogh diffusion model to physiological ranges of A. VO_2 and B. Ko_2, as seen in the predicted radius of tissue supplied with sufficient O_2 to meet metabolic demands along the anatomically realistic 60 µm of capillary length. The physiological range of VO_2 was based on a resting fish myocardial power output of 1.5 mW gVM^{-1} with mechanical efficiency ranging from 10 to 40 % (Table 4-1,-3). The physiological range of Ko_2 was based on values reported for heart and skeletal muscle of fish acclimated to temperature ranging form 5 to 25 ^{o}C.

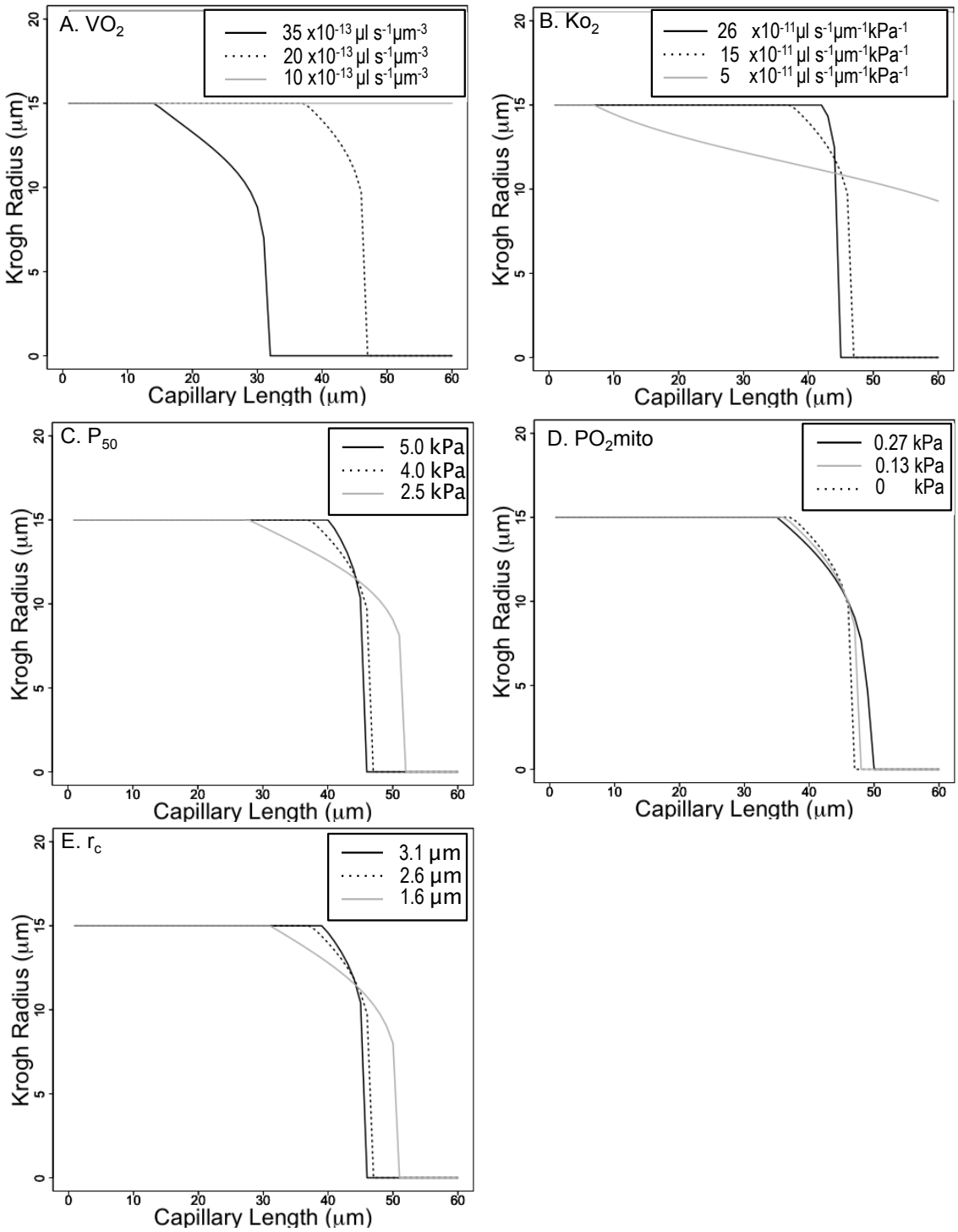


Figure 4-3. Sensitivity of the maximally swimming scenario ventricular Krogh diffusion model to physiological ranges of A. VO_2, B. Ko_2, C. P_{50}, D. PO_2mito and E. r_c as seen in the predicted radius of tissue supplied with sufficient O_2 to meet metabolic demands along the anatomically realistic 60 µm of capillary length. The physiological range of VO_2 was based on a reported maximum *in vivo* and *in vitro* myocardial power output of 5 to 9 mW gVM^{-1} with mechanical efficiency ranging from 10 to 25 % (Table 4-1,-3). The physiological range of Ko_2 was based on values reported for heart and skeletal muscle of fish acclimated to temperature ranging from 5 to 25 ^{o}C. P_{50} values were based on reported values for arterial blood with exercising PCO_2 levels and r_c and PO_2mito values were based on literature values.

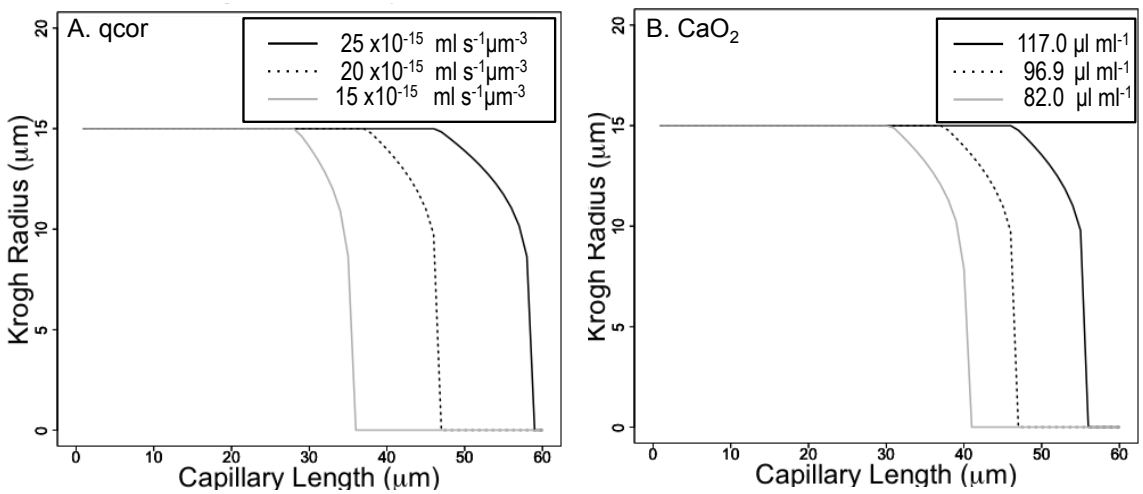


Figure 4-4. Sensitivity of the maximally swimming scenario ventricular Krogh diffusion model to physiological ranges of A. q_{cor} and B. CaO_2 as seen in the predicted radius of tissue supplied with sufficient O_2 to meet metabolic demands along the anatomically realistic 60 µm of capillary length.

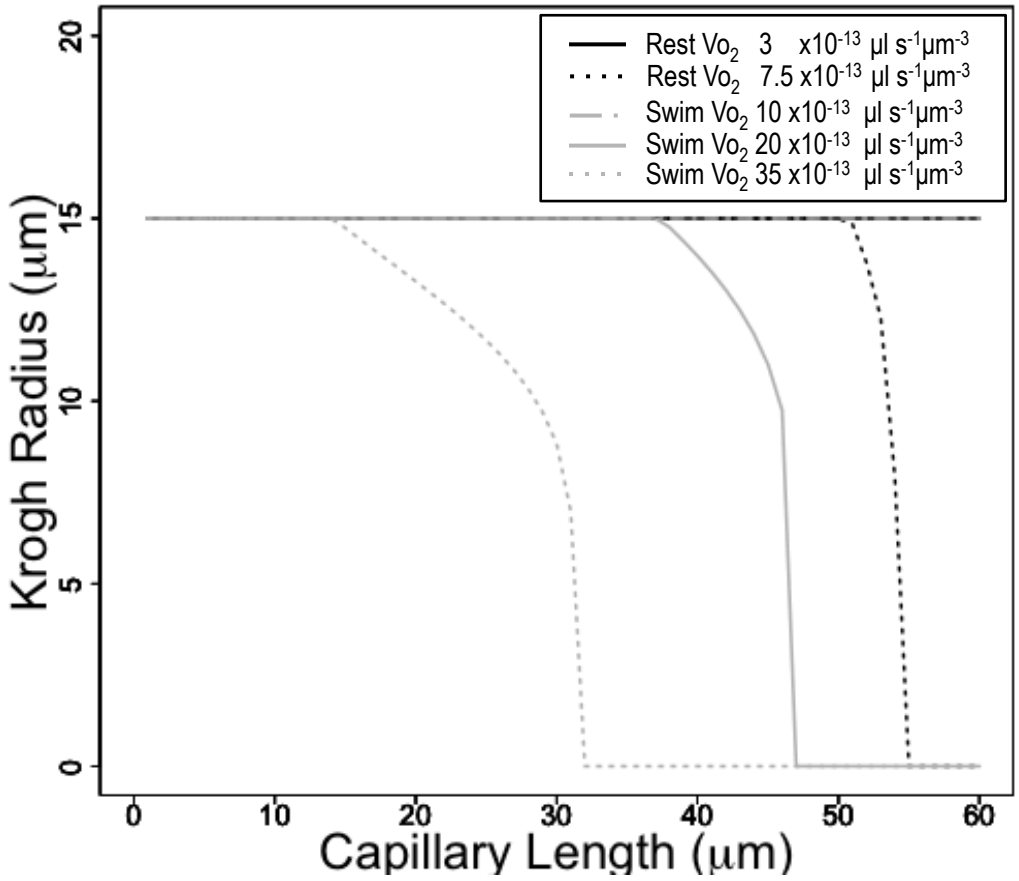
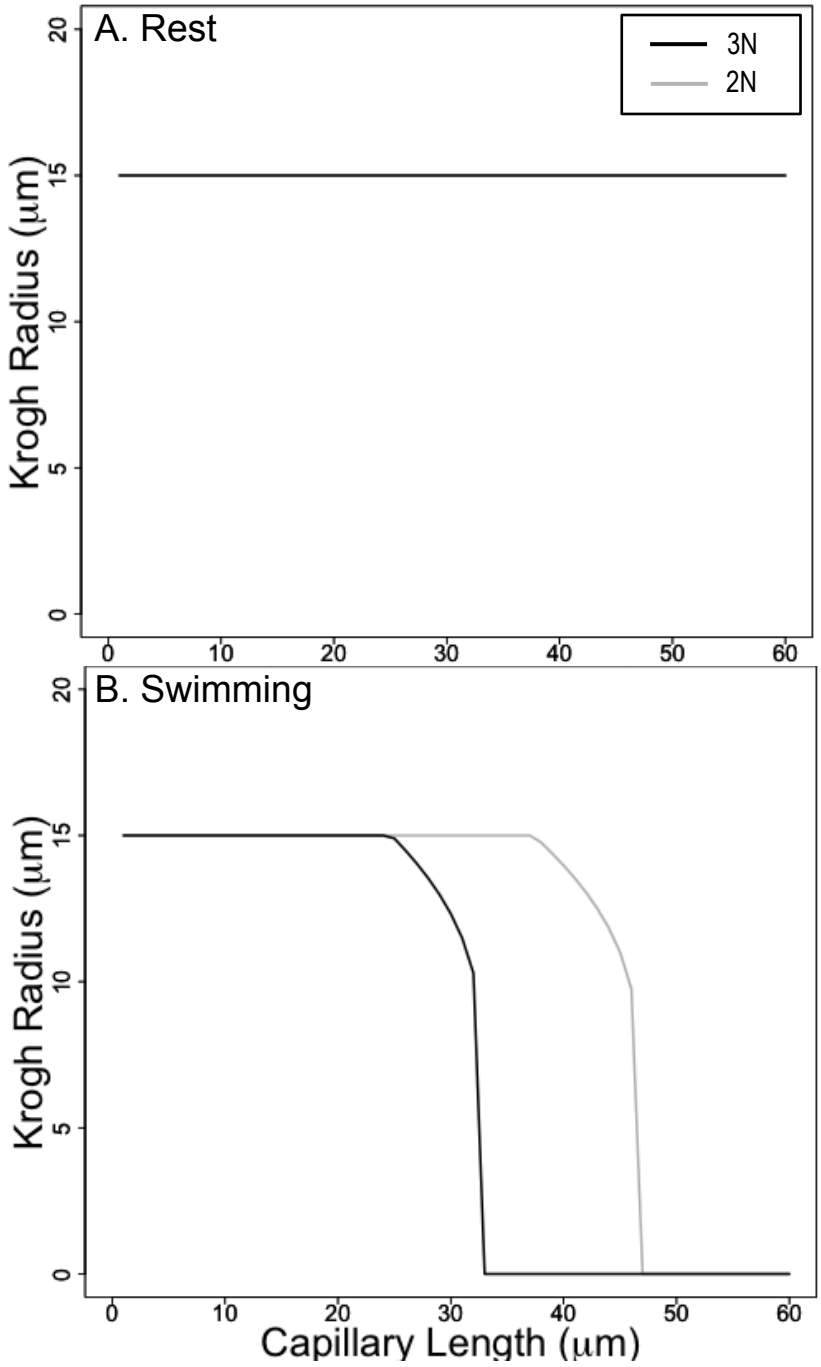


Figure 4-5. Predicted Krogh tissue cylinder radius for resting and maximally swimming scenarios of the Krogh model using base input variables (Table 4-3) As both the resting and maximally swimming model scenarios were most sensitive to myocardial VO_2 inputs, resultant output from applying the physiological high and low extremes of VO_2 are plotted here in addition to the base value. Resting VO_2s were based on a myocardial power output of 1.5 mW gVM^{-1} with efficiencies from 10 to 40 % as expected in a resting

fish. Swimming VO_2s were based on *in vivo* and VO_2s reports of maximum myocardial power outputs ranging from 5 to 7 mW gVM^{-1} with efficiencies from 10 to 25 %.



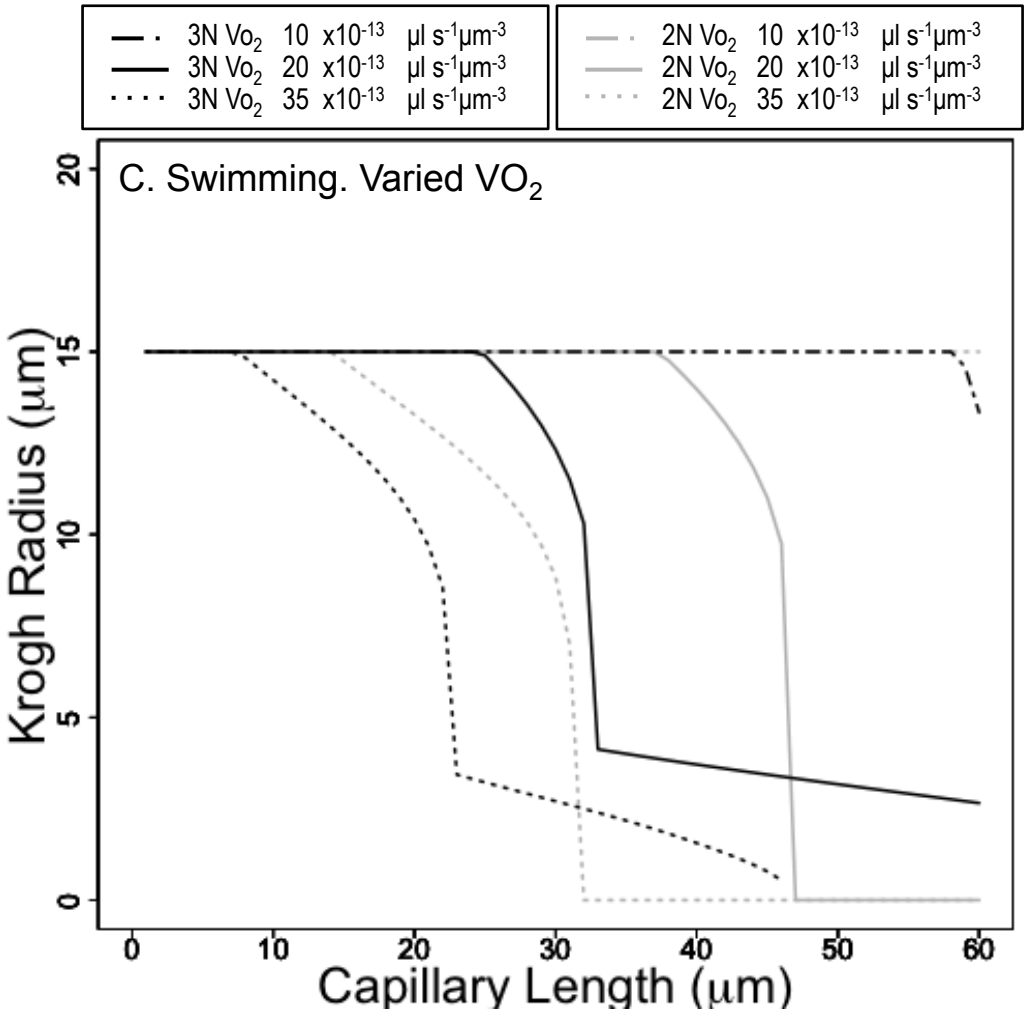


Figure 4-6. Effect of triploidy on predicted Krogh tissue cylinder radius of the ventricular compact myocardial A. resting and B & C. maximally swimming fish. As both the resting and maximally swimming model scenarios were most sensitive to myocardium VO_2 inputs, in C, in addition to the base VO_2 value output, the outputs from the physiological high and low extremes of VO_2 were plotted. Resting VO_2s were based on a myocardial power output of 1.5 mW gVM^{-1} with efficiencies from 10 to 40 % as expected in a resting fish. Swimming VO_2s were based on *in vivo* and VO_2s reports of maximum myocardial power outputs ranging from 5 to 7 mW gVM^{-1} with efficiencies from 10 to 25 %.

Chapter 5 [*]The *In Vitro* Blood-Oxygen Affinity of Triploid Rainbow Trout (*Oncorhynchus mykiss*) at different Temperatures and Carbon Dioxide Tensions

Summary

When O_2 affinity of diploid (2N) and triploid (3N) rainbow trout, *Oncorhynchus mykiss,* blood was compared under varying temperature and CO_2 partial pressure combinations, ploidy did not affect either blood-O_2 affinity, Hill number or predicted arterial O_2 saturation. These results suggest that previous observations of reduced aerobic capacity, hypoxia tolerance and warm water tolerance for 3N salmonids compared with 2N conspecifics is not due to altered blood-O_2 affinity. Further investigation into intracellular pH, O_2 transport capacity and O_2 diffusion from capillary blood into tissues of 3N fishes as well as the effects of enlarged 3N red blood cells (RBCs) on gas diffusion into and out of RBCs, may prove more fruitful in unraveling the mechanisms behind reduced tolerance of 3N salmonids to sub-optimal environments.

Introduction

As detailed in the introduction (Chapter 1) to my thesis, one unanswered question regarding the mechanism behind poor tolerance of 3N fish to sub-optimal conditions is whether or not the affinity of haemoglobin (Hb) for O_2 is altered by triploidy. If Hb-O_2 affinity is reduced, it could explain lower 3N arterial O_2 content (CaO_2) (Bernier et al. 2004), which would impede O_2 supply to 3N ventricular myocardium. Chapter 4 of my thesis shows that an O_2 supply demand mismatch in the resting and maximally swimming 3N salmonid heart is primarily due to reduced 3N arterial O_2 content, which may be due to reduced Hb-O_2 affinity in 3N fish. Thus reduced Hb-O_2 affinity has the potential to explain poor 3N performance in general.

Survival of triploid (3N) fishes in the wild (Simon et al. 1993; Cotter et al. 2000; Koenig and Meyer 2011; Koenig et al. 2011) and under sub-optimal laboratory or aquaculture environments (Yamamoto and Iida 1994; Ojolick et al. 1995; Withler et al. 1995; Altimiras et al. 2002;

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Hyndman et al. 2003) tends to be inferior when compared to diploid (2N) conspecifics under identical conditions (but Dillon et al. 2000; Teuscher et al. 2003). The physiological mechanisms behind poor 3N performance are of great importance to aquaculture and sport fishing industries, which struggle to increase profits through the benefits of 3N sterility. In fact, approximately 50 % of British Columbia (BC) small lakes are stocked with 3N salmonids for sport fishing, making high 3N mortality rates very costly for this industry. Poor hypoxia and warm temperature tolerance of 3N fishes (Lilyestrom et al. 1999; Yamamoto and Iida 1994) suggests elevated mortality rates may be related to deficiencies in O_2 loading at the gills. This possibility is further supported by findings that arterial O_2 saturation of haemoglobin in 3N fishes is only 75 % of that in 2N fishes (Bernier et al. 2004; Graham et al. 1985). While low saturation may be due to an O_2 diffusion limitation across the gill epithelium, arterial partial pressure (Po_2) was unchanged in maximally swimming 2N and 3N chinook salmon (*Oncorhynchus tshawytscha*) (Bernier et al. 2004). Consequently, Hb-O_2 reactions could differ between 2N and 3N fishes and contribute to poor O_2 saturation of the blood in 3N fishes and poor survival in sub-optimal environments.

Blood-O_2 binding dynamics are critical for effective O_2 loading in the blood at the gills. This process is hampered at high water temperature because of a shift of the equilibrium of the exothermic Hb-O_2 binding reaction, which reduces blood-O_2 affinity. Reduced Hb-O_2 affinity can decrease the amount of O_2 loaded into arterial blood at the gills. Triploid blood-O_2 affinity has never been assessed at high temperatures even though triploid brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) appear to be sensitive to temperatures above 18 ºC (Altimiras et al. 2002, Hyndman et al. 2003).

Previous comparisons between 2N and 3N Atlantic salmon (*Salmo salar*) blood-O_2 binding characteristics revealed no ploidy effect (Graham et al. 1985, Sadler et al. 2000), a result that is difficult to reconcile with findings of reduced blood-O_2 saturation in 3N chinook and Atlantic salmon. However, these previous studies of blood-O_2 binding characteristics used tonometry techniques that required large volumes of pooled blood, had small sample sizes and did not always test for statistical significance. Given that a better technological approach for measuring blood-O_2 binding characteristics is now available, a more thorough evaluation of the intrinsic blood-O_2 affinity of 3N salmonid fish is warranted.

Methods

Sibling 2N and 3N, 4-year old, female Black Water rainbow trout (*Oncorhynchus mykiss*), from Fraser Valley Trout Hatchery (Abbotsford, BC, Canada) were reared in flow through tanks supplied with 10-14 °C dechlorinated city water. Diploid and triploid fish weighed (mean±S.E.) 97±5 and 101±6 g and were (mean±S.E.) 206±4 and 204±5 mm long, respectively. Eggs designated to become triploid fish were treated with a pressure shock soon after fertilization and their ploidy was confirmed by nuclear size in blood smears (Benfey et al. 1984). Blood samples were drawn from anaesthetized fish (0.1 g l^{-1} MS222 buffered with 0.01 g l^{-1} $NaHCO_3$) using caudal puncture into heparinized syringes within 2 minutes of netting fish. The blood sample was immediately placed on ice where it was stored until measurements were completed.

Each oxygen equilibrium curve (OEC) was determined in a modified version of the Pwee50 (http://www.latrobe.edu.au/) typically within 24 h (between 2 and 32 h) after blood collection, based on the methods of Clark et al. (2008). Measurements of skipjack tuna (*Katsuwonus pelamis*) blood P_{50} do not differ after 24 h storage, but 48 h storage did alter the P_{50} of yellow fin tuna (*Thunnus albacares*) blood (Brill and Bushnell 1991). Additionally, it was confirmed that there was no storage effect on P_{50} by comparing measurements on blood from one 2N and one 3N rainbow trout at 1 and 32 h post-collection (data not shown). Briefly, the Pwee50 is a microdiffusion chamber equipped with a spectrophotometer that measured relative blood-O_2 saturation at constant temperature (11 or 20 °C) and Pco_2 (0.25 or 0.5 kPa) as Po_2 was increased incrementally (0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 10, and 21 % O_2 replaced the N_2 to create N_2 : O_2 : CO_2 gas mixtures) (Figure 5-1). The Pco_2 conditions were chosen to reflect a resting rainbow trout arterial Pco_2 of 0.22 kPa (Thomas et al. 1994) and swimming arterial Pco_2 of up to 0.51 kPa in rainbow trout (Brauner et al. 2000) and 0.53 and 0.46 kPa for 2N and 3N chinook salmon (Bernier et al. 2004), respectively. The temperatures tested were near optimal (11 °C) and supra-optima (20 °C), at which 3N brook charr and brown trout exhibit high mortality rates (Altimiras et al. 2002; Hyndman et al. 2003). Blood-O_2 saturation was determined by measuring the difference between absorbance (Δabsorbance) at 435 nm (peak absorbance of deoxygenated Hb) and 390 nm (isobestic point between oxy- and deoxy-Hb where absorption is independent of Hb oxygenation). These Δabsorbances were then compared to the Δabsorbance for 100 % blood-O_2 saturation to calculate % saturation of blood. Due to the Root effect in the presence of CO_2, it is possible that Hb will never completely saturate with O_2, even when exposed to very high Po_2

levels. In order to ensure Δabsorbance for 100 % saturation was precisely recorded, Δabsorbance was measured with 40 % O_2 in the absence of CO_2. Additionally, any machine drift during measurements was compensated for by assessing 100 % saturation at the beginning and end of each OEC, and regressing the Δabsorbance with time. The regression slope was then applied to all % saturation points. One OEC or 2 to 3 replicate OEC were generated for individual blood samples from four to nine fish per temperature-Pco_2 combination. A fresh blood sample was used for each replicate OEC and these replicates were averaged to represent the OEC for an individual fish.

Individual OECs were interpolated to determine the oxygen partial pressure for 50 % haemoglobin O_2 saturation (P_{50}) and estimate arterial blood saturation (i.e., using a Po_2 of 11.18 kPa) for each fish sample. The P_{50} and Hill's number (n) were determined from Hill Plot equations created in Excel using log-transformed values for Po_2 and blood saturation, but only values from the linear portion of the OEC (between 20 and 80 % saturation). Blood saturation at a Po_2 of 11.18 kPa was interpolated after the OEC was fitted to a 4-parameter sigmoidal curve ($Y=Y_o+(a/(1+e^{((X-X_o)/b)})$) using the Dynamic Fit Wizard function in SigmaPlot 11 (http://www.systat.com):

Where Y is saturation (%) and X is Po_2 (kPa). Y_o and X_o are the saturation when Po_2 is zero and the Po_2 where saturation becomes zero, respectively. a and b are constants. The equation was then solved for Y when X is 11.18.

Results

Effects of ploidy on P_{50}, arterial saturation and n were determined using a 2-way ANOVA with 41 residual d.f. and 48 total d.f.. The Holm-Sidak pairwise multiple comparison procedure ($\alpha = 0.05$) was then used to determine effects of temperature and Pco_2 independent of ploidy. An $\alpha < 0.05$ was used to determine statistical significance.

Ploidy had no significant effect ($P>0.05$; 2-way ANOVA) on the P_{50} of rainbow trout blood equilibrated at either 11 or 20 °C and with a Pco_2 of either 0.25 or 0.5 kPa (Figure 5-2). There was no interaction between ploidy and these conditions ($P>0.05$). As expected, temperature and Pco_2 ($P<0.001$) significantly increased P_{50}, independent of ploidy. Increasing temperature from 11 to 20 °C at a constant Pco_2 significantly increased P_{50} by 0.95 ($P<0.001$) and 1.36 kPa

(P<0.001) at a Pco_2 of 0.25 and 0.50 kPa, respectively. Likewise, increasing Pco_2 from 0.25 to 0.5 kPa at constant temperature significantly increased P_{50} by 1.11 (P<0.001) and 1.51 kPa (P<0.001) at 11 and 20 °C, respectively.

There was also no ploidy effect (P>0.05; 2-way ANOVA) or interaction between ploidy and test conditions (P>0.05) for n at any of the tested combinations of temperature and Pco_2 (Figure 5-3). For blood at 11 ^{o}C and a Pco_2 of 0.25 kPa, n was 2.4-2.5. With an increase in either Pco_2 to 0.50 kPa or temperature to 20 ^{o}C, n significantly decreased to 1.6-2.1. When Pco_2 and temperature were increased together to 0.50 kPa and 20 ^{o}C, respectively, n was significantly reduced to 1.4 to 1.5, which suggested additive effects.

Similar to P_{50} and n, ploidy had no significant effect (P>0.05) on the predicted arterial saturation at a Po_2 of 11.18 kPa at either 11 or 20 °C with Pco_2 of either 0.25 or 0.50 (Figure 5-4). There was no interaction between ploidy and these conditions (P>0.05). The predicted arterial saturation remained around 92 to 98 % independent of Pco_2 at 10 °C and with a Pco_2 of 0.25 kPa at 20 °C. In contrast, a Pco_2 of 0.50 kPa significantly reduced the predicted arterial saturation to approximately 85 % at 20 °C (P<0.001).

Discussion

This study is the first comprehensive comparison of the blood-O_2 affinity of 2N and 3N salmonid fish under tightly regulated conditions that represent optimal and supra-optimal temperatures and at representative values for arterial Pco_2. Although the increases in P_{50} with temperature and Pco_2 were as expected, P_{50} was unaffected by ploidy under any of the conditions tested. Similarly, no ploidy effect was seen for O_2 saturation at a predicted arterial Po_2. Therefore, despite potential methodological and statistical shortcomings of previous studies, this study confirms that a difference in blood-O_2 affinity of rainbow trout at optimum temperatures cannot account for the reduced tolerance of 3N salmonids to sub-optimal environments. The same is true for the blood-O_2 affinity at the supra-optimal temperature of 20 °C, which is a novel result.

Our measured values for P_{50} correspond well with literature values. P_{50} of rainbow trout blood at 12 to 15 ºC exposed to 0.25 - 0.30 kPa CO_2 ranged from 2.50 to 3.29 kPa (Eddy 1971; Milligan and Wood 1987; Rummer 2010) and ranged from 2.76 to 4.61 kPa at a Pco_2 of 0.50

kPa (Holk and Lykkeboe 1998; Rummer 2010). Here P_{50} at 11 ºC was 2.63 kPa at a Pco_2 of 0.25 and 3.82 kPa at a Pco_2 of 0.50 kPa. P50 ranged from 2.63 to 3.55 kPa when measured at 20 ºC with a Pco_2 of 0.25 kPa (Eddy 1971; Tetens et al. 1981) and was 3.55 kPa with the same Pco_2 of 0.25 kPa here. Published P_{50} values for Rainbow trout blood at 20 ºC with a Pco_2 of 0.50 kPa were not found.

The affinity of Hb for O_2 is reduced in the presence of H^+ and CO_2 through the Bohr and/or Root effects, which cause an increase in P_{50} and a decrease in saturation, even at high O_2 tensions in the case of the Root effect. The increase in P_{50} through the Bohr and Root effects observed here is in good agreement with the literature. Rummer (2010) found a 45% elevation in P_{50} of rainbow trout blood at 12 ºC when Pco_2 was raised from 0.25 to 0.50 kPa, which corresponded to a decrease in extracellular pH (pH_e) from 8 to 7.81. Here the same change in Pco_2 at a similar temperature increased P_{50} by 1.11 kPa, which is a 55 % increase. For the same pH_e change used by Rummer (2010), Vorger (1985) found the P_{50} of rainbow trout blood at 10 ºC to increase from 2.24 to 2.89 kPa, a 30 % increase.

The expected reduction in blood-O_2 affinity with increasing temperature was also in good agreement with the literature (Irving et al. 1941; Weber et al. 1976; Weber & Fago 2004). Irving et al. (1941) reported a 1.45 kPa increase in the P_{50} of rainbow trout blood when temperature was increased from 10 to 17 ºC at a constant Pco_2 of 0.13 kPa. Here, when temperature was increased from 11 to 20 ºC at a Pco_2 of 0.25 kPa, P_{50} increased by 0.92 kPa.

Although temperature tolerance and preference varies with life history and among species, the preferred temperature of 1 year old, hatchery-reared 2N rainbow trout has been reported as 13 ºC, regardless of acclimation temperature (Garside and Tait 1958). For the same species, the heart achieves its maximum power output between 15 and 18 ºC (Farrell et al. 1996) and the upper lethal temperature is approximately 24 ºC (Black 1953). Triploid trout show elevated mortality rates at 18 ºC across a number of different life histories and species, including brown trout, brook charr and rainbow trout (Altimiras et al. 2002; Hyndman et al. 2003; Ojolick et al. 1995). Clearly, blood-O_2 affinity did not differ between ploidies at temperatures ranging from optimal to upper tolerable limits and with Pco_2 tensions that bracketed the resting and maximum swimming arterial levels for rainbow trout. This suggests that reduced tolerance of high temperature by 3N trout is not due to ploidy-related thermodynamic effects on blood-O_2 affinity.

It is difficult to reconcile a lack of ploidy effect on blood-O_2 affinity with 3N fishes being less tolerant of hypoxic water (Lilyestrom et al. 1999; Yamamoto & Iida 1994) and having a 31 % lower factorial metabolic scope and 23 % lower arterial-O_2 saturation (Bernier et al. 2004). Interestingly, the results for saturation did not reveal the ploidy effect of reduced arterial blood O_2 saturation observed *in vivo* by Bernier et al. (2004). It could be that the allosteric effects of CO_2 and H+ on Hb are more subtle than could be examined here, or more likely that allosteric factors that can alter *in vivo* blood-O_2 affinity in 3N fishes are not present under the controlled *in vitro* conditions tested here. Bernier et al. (2004) found the intracellular red blood cell (RBC) pH (pHi) of swimming 3N chinook salmon (7.42) was slightly, but significantly, lower than that of maximally swimming 2N salmon (7.47), which would favour reduced saturation in 3N fish. The similar P50 and n of 2N and 3N blood here suggests that either this pHi difference was not present or, if it was, either another factor was countering it (e.g., increased cooperativity due to tighter packing of Hb within the 3N RBC) or the reduction was insufficient to change P_{50}.

Regardless, these results suggest that altered blood-O_2 affinity alone cannot explain previous observations of reduced aerobic capacity, hypoxia tolerance and warm water tolerance for 3N salmonids compared with 2N conspecifics. But to further determine if there is any effect of triploidy on blood-O_2 affinity, future studies should investigate the effects of small changes in pHi on 2N and 3N OECs and arterial O_2 loading as well as pHi regulation via catecholamines (Nikinmaa 1983; Nikinmaa et al. 1990) and the effects of enlarged 3N RBCs on the kinetics of gas diffusion into and out of RBCs to explain previous reports of reduced *in vivo* blood-O_2 saturation. Additionally, further investigation into O_2 transport capacity and O_2 diffusion from capillary blood into tissues of 3N fishes, may prove more fruitful in unraveling the mechanisms behind reduced tolerance of 3N salmonids to sub-optimal environments.

In view of this negative result, it is clear that reduced 3N Hb-O_2 affinity is not the mechanism behind a predicted O_2 supply demand mismatch in 3N ventricles stemming from reduced arterial O_2 content.

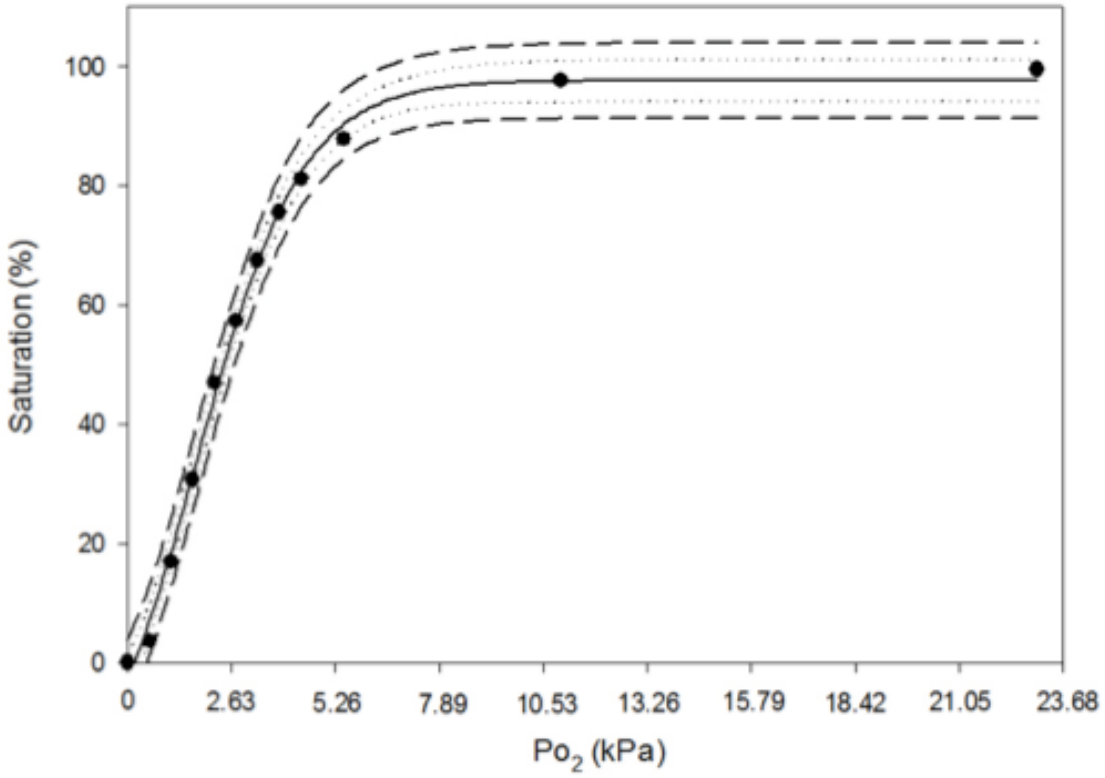

Figure 5-1. An example of an OEC resulting from a 4-parameter sigmoidal curve fitted to the blood O_2 saturations determined with the Pwee50 system. Solid dots represent data points. The solid line is the line of best fit, dotted line is the 95 % confidence band and the dashed line is the 95 % prediction band.

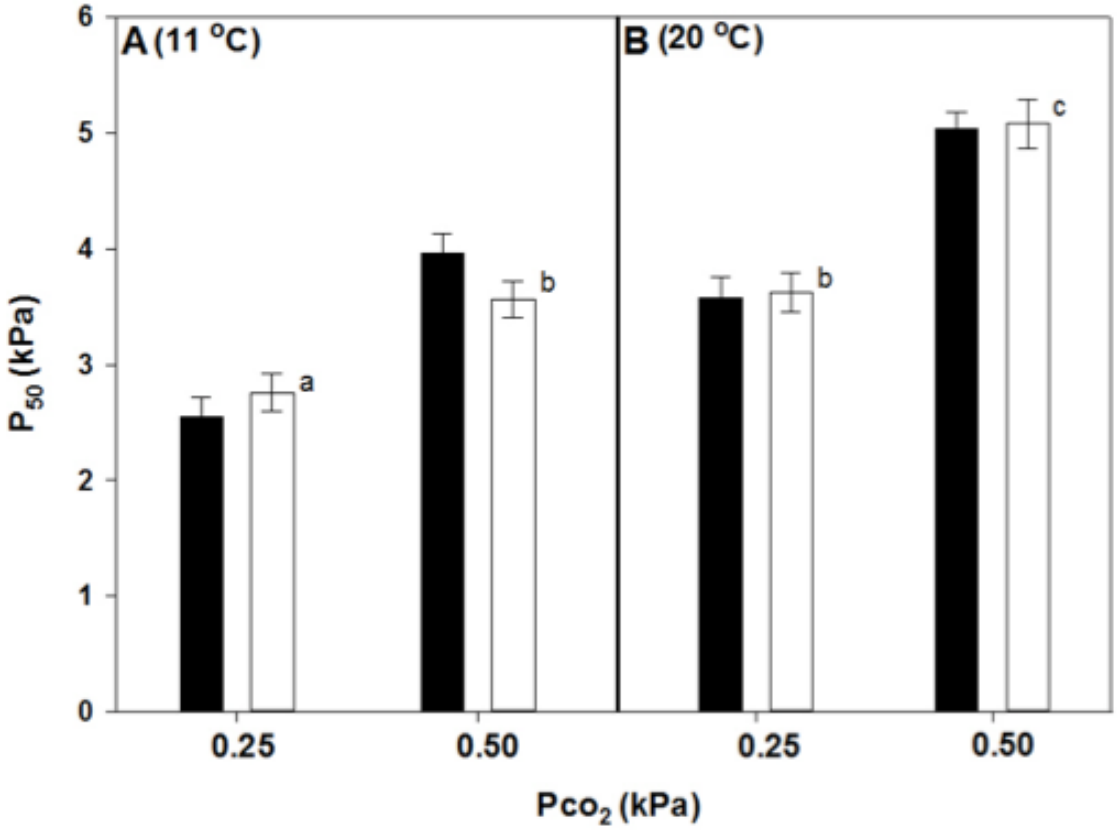

Figure 5-2. P_{50} values for 2N and 3N rainbow trout blood incubated at varying temperature (11 °C (A) and 20 °C (B)) and Pco_2 combinations. Black bars represent 2N and open bars represent 3N means. Error bars are standard errors around means. Differing letters demarcate significant differences in P_{50} values between conditions, with ploidies pooled. Sample sizes were 6 (2N 0.25 kPa 11 °C), 7 (3N 0.25 kPa 11 °C), 6 (2N 0.50 kPa 11 °C), 7 (3N 0.50 kPa 11 °C), 5 (2N 0.25 kPa 20 °C), 6 (3N 0.25 kPa 20 °C), 8 (2N 0.50 kPa 20 °C), and 4 (3N 0.50 kPa 20 °C).

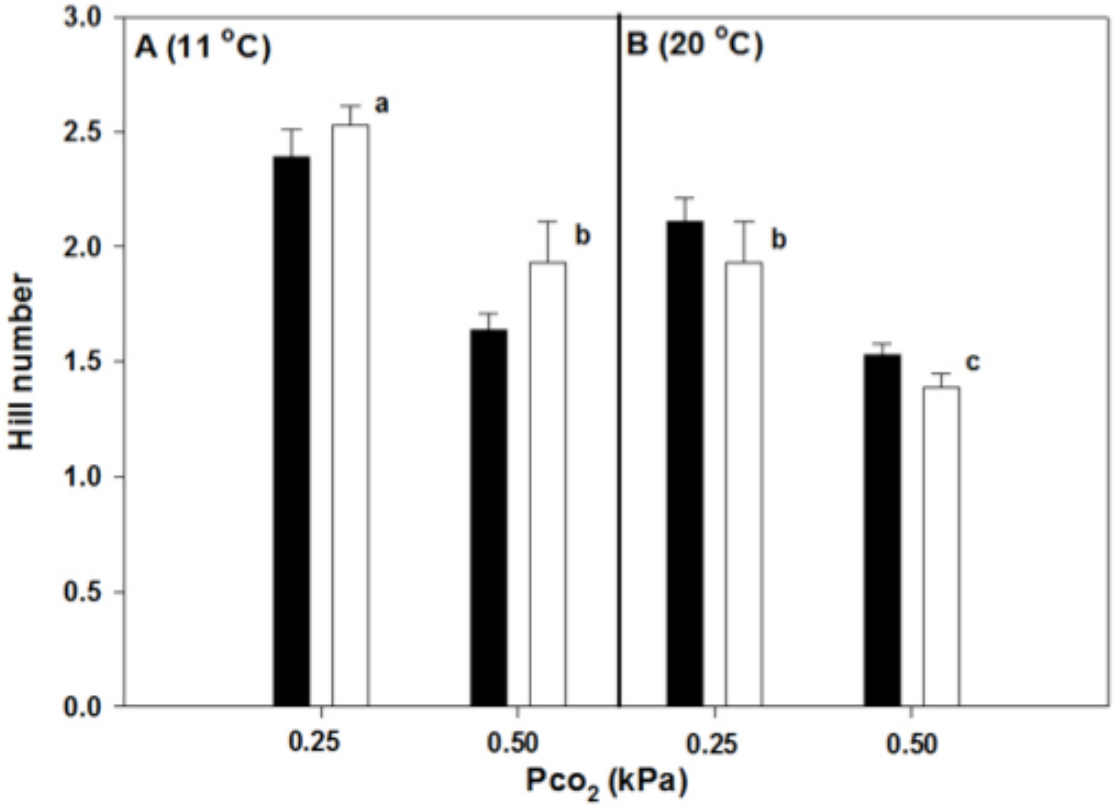

Figure 5-3. Hill values for 2N and 3N rainbow trout blood incubated at 11 °C (A) and 20 °C (B) with a Pco_2 of either 0.25 or 0.50 kPa. Black bars represent 2N and open bars represent 3N means. Errors bars are standard errors around the mean. Differing letters demarcate significant differences in Hill values between conditions, with ploidies pooled. The sample sizes are as in Figure 5-2.

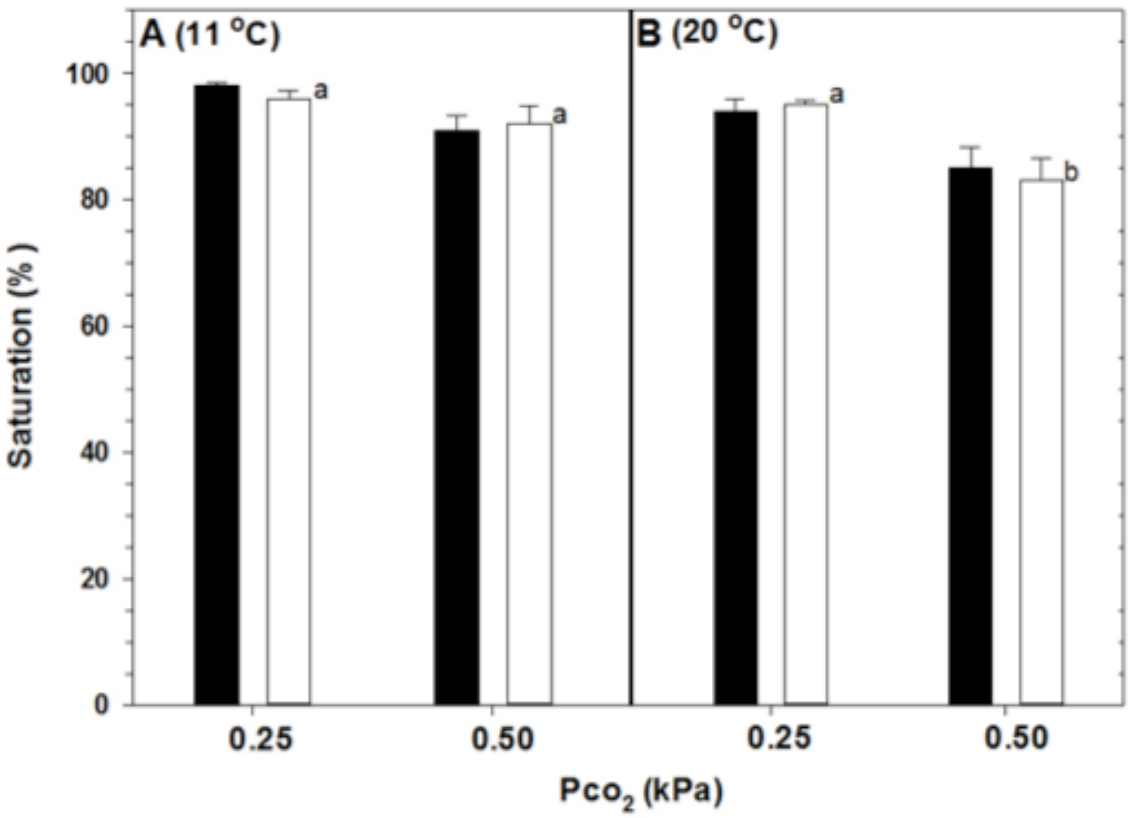

Figure 5-4. Fig. 4. Effect of Pco_2 on predicted O_2 saturation of arterial blood (Po_2 = 11.18 kPa) of 2N and 3N rainbow trout blood incubated at 11 °C (A) and 20 °C (B). Black bars represent 2N and open bars represent 3N means. Errors bars are standard errors around the mean. Differing letters demarcate significant differences in arterial saturation between conditions, with ploidies pooled. The sample sizes are as in Figure 5-2.

Chapter 6 Conclusions

Rainbow trout are the backbone of a $0.5 billion a year sport fishing industry in British Columbia (BC) that relies heavily upon triploid (3N) rainbow trout, which are released into over 50 % of stocked lakes across the province. Triploid rainbow trout are particularly valued because their secondary sexual characteristics, which are deemed undesirable by anglers, remain under-developed throughout their lives, and their sterility limits the extent of genetic interactions between stocked and native rainbow trout. Unfortunately, 3N survival in sport fishing lakes is often inferior relative to that of diploid (2N) fish, which represents a great cost to the industry. Consequently, knowledge of 3N performance in the wild is useful for industry.

The objective of my thesis was to investigate the role of O_2 supply limitations throughout the body in salmonid fish tolerance of high temperature conditions and survival in nature by focusing on comparisons between 2N and 3N rainbow trout. I hypothesized that corporeal O_2 supply limits the aerobic performance of 3N rainbow trout and that aerobic performance, in turn, limits tolerance of sub-optimal conditions and survival in nature. In order to test these hypotheses, in Chapter 2 I looked for ploidy differences in maximum heart rate response to warming, optimal temperature (T_{opt}) and upper critical thermal maximum (CT_{max}). These data were then used to interpret the findings in Chapter 3, which examined survival in the wild and tested the primary hypothesis of the thesis. Then in Chapter 4, due to the clear relationships between cardiac function and impaired 3N trout high temperature tolerance and survival seen in Chapters 2 and 3, I modified the Krogh diffusion model to investigate how O_2 supply to the ventricle might be limited as a result of triploidy. Finally, because Chapter 4 model output predicted reduced arterial O_2 loading as a more important limiter of O_2 supply to the 3N compact myocardium than enlarged capillaries or cardiomyocytes, in Chapter 5, I investigated a mechanism behind reduced 3N arterial Hb-O_2 loading. Based on previous research, which described reduced arterial O_2 content, but similar arterial O_2 partial pressure in 3N relative to 2N salmon (Bernier et al 2004), reduced Hb-O_2 affinity in 3N arterial blood was a likely mechanism behind reduced 3N arterial Hb-O_2 loading. Surprisingly, in Chapter 5, I was able to eliminate this previously proposed reduction in Hb-O_2 affinity as one potential mechanism behind reduced 3N arterial Hb-O_2 loading.

Analysis and Integration of My Thesis Conclusions with Current Literature

Does Maximum Heart Rate of 2N and 3N Trout Respond to Temperature Increase Similarly?

Meeting the high metabolic demands of living at elevated temperatures can require maximum O_2 delivery capacities in fish (Portner and Farrell 2008; Farrell 2009; Farrell and Richards 2009). Given my thesis hypothesis that corporeal O_2 supply limits the aerobic performance of 3N rainbow trout, high O_2 delivery rates required of fish during high temperature exposure can be predicted to maximize or even exceed the cardiorespiratory O_2 supply capacity of 3N fish. Indeed, previous reports of reduced 3N salmonid tolerance of chronic, but not necessarily acute, exposure to sub-optimal conditions (Myers and Hershberger 1991; Blanc et al. 1992; Simon et al. 1993; Yamamoto and Iida 1994; Ojolick et al. 1995; Altimiras et al. 2002; Mercier et al. 2000; Koenig and Myer 2011; Koenig et al. 2011), especially low O_2 and high temperature, support this prediction. Based on past findings of poor 3N tolerance of chronic high temperature exposure, I investigated whether 3N impaired thermal tolerance is related to the capacity of the 3N cardiac muscle to respond to increasing O_2 demands with increasing temperature. Atkins and Benfey (2008) showed evidence of ploidy effects on the metabolic response to temperatures ranging from 9 to 18 ^{o}C in adult salmonid fish, which were not seen in the embryonic salmonid heart rate response to acclimation temperatures ranging from 6 to 12 ^{o}C (Benfey and Bennett 2009). This suggests that impaired thermal tolerance of adult 3N salmonids is due to altered cardiorespiratory status. Lab-based comparisons of 2N and 3N thermal tolerance have revealed no differences in chronic lethal maximum (Galbreath et al. 2006) or the upper critical maximum temperature (CT_{max}) (Benfey et al. 1997; Scott 2012), all of which used loss of equilibrium as end points.

In Chapter 2, based on the evidence that impaired 3N thermal tolerance stems from altered cardiorespiratory physiology, I tested the hypothesis that impaired high temperature tolerance in 3N salmonids is related to impaired maximum performance of the heart. By monitoring maximum heart rate of 2N and 3N rainbow trout during incremental warming with arrhythmia onset as an end point, I used a more informative and sensitive test of acute thermal tolerance. I showed an inability of 3N maximum heart rate to increase with warming at a comparable rate with 2N heart rate, potentially resulting in impaired O_2 circulation within the 3N, relative to the 2N population at high temperatures. Thus, my thesis provides the first empirical evidence of a

mechanism behind previous reports of impaired 3N high temperature tolerance in reduced ability of 3N rainbow trout to increase heart rate with warming. These findings raise questions concerning the impact of reduced thermal tolerance and cardiac pumping capacity on survival of 3N fish in nature.

Is There a Relationship between Swimming Endurance and Survival in Lakes? Does this Relationship Explain Reduced 3N Survival in the Wild?

Researchers have repeatedly identified fish locomotion performance as an important trait determining survival (Nelson 1989; Plaut et al. 2001; Nelson et al. 2002; Domineci et al. 2010; Oufiero et al. 2011), but here is one of the first direct tests of this relationship in nature. According to lab and mesocosm experiments and correlations of population characteristics with and without predation exposure, burst and prolonged swimming performance appear to be important to predation escape and thus survival of a large range of species including Coho salmon (Taylor and McPhail 1985), sea bass (*Dicentrarchus labrax*) (Handelsman et al. 2010), Trinidadian killifish (*Rivulus hartii*) (Oufiero et al. 2011), male mosquitofish (*Gambusia affinis*) (Langerhans et al. 2004), guppies (*Poecilia reticulata*) (Walker et al. 2005) and Atlantic silverside (*Menidia menidia*) (Lankford et al. 2001). Therefore, despite the paucity of direct evidence through assessment of survival in nature, there is convincing evidence that anaerobic and aerobic swimming performance is important to survival in the presence of predators. I tested the hypotheses that aerobic swimming performance influences survival in the wild by assessing endurance swimming and then summer lake survival of 2N and 3N rainbow trout populations.

In testing the endurance-survival hypothesis, endurance swimming of 2N and 3N populations was assessed and compared. Previous sustained and prolonged swimming challenges revealed no differences between 2N and 3N aerobic swimming capacity (Small and Randall 1989; Sezaki et al. 1991; Parsons 1993; Stillwell and Benfey 1996; Cotterell and Wardle 2004; Lijalad and Powell 2009; Bernier et al. 2004), despite greater accumulation of anaerobic metabolites after sustained swimming (Virtanen et al. 1990) and shorter endurance time at speeds above maximum sustained swimming speed (Cotterell and Wardle 2004) for 3N fish. However, the only two studies to compare sustained swimming performance of 2N and 3N fish were designed in such a way to prevent conclusive determination of reduced 3N aerobic swimming performance (Virtanen et al. 1990; Sezaki et al. 1991; Cotterell and Wardle 2004).

Despite previously equivocal findings for ploidy effects on aerobic swimming performance, my endurance swimming experiments showed endurance swimming time of 3N Blackwater rainbow trout to be shorter than of 2N trout. Thus, I concluded that 3N aerobic swimming performance was reduced compared to 2N cohorts. This result is also consistent with evidence of reduced 3N aerobic capacity seen in the reduced maximum heart rate response to warming in 3N compared to 2N fish reported in Chapter 2.

Furthermore, Chapter 3 of my thesis reports the first direct evidence of a relationship between survival and endurance swimming. This relationship was straightforward for the 3N population, for which survival in a high temperature lake with potentially high predation increased with previously measured swimming endurance. For 2N populations, the relationship was more complicated, in that survival of the poorest swimming half of the 2N population was double that of the better endurance swimmers. This shape of the 2N endurance-survival relationship was perplexing, but as the highest 50 % of the 2N population had greater endurance than the highest endurance of the 3N population, it appears that survival increased with endurance up to a point; however, further increases in endurance were disadvantageous to fish survival. It is impossible to explain the reduced survival of the highest endurance fish with the data available, but two potential mechanisms could be high endurance fish having more risky behaviour (e.g., under food limiting conditions sea bass with a high resting metabolic rate are more likely to partake in risky behaviour than those with a low resting metabolic rate (Killen et al. 2011)) or fitness tradeoffs between high endurance and other important traits to survival in nature (e.g., some studies show tradeoffs between aerobic and burst swimming performance; reviewed by Langerhans and Reznick (2009)) resulting in proportionally more predation on the highest endurance fish. This relationship also appeared to depend on the lake habitat sufficiently stressing the fish to bring about high mortalities and therefore selection. In the lake where the endurance-survival relationship was demonstrated, fish were threatened with high predation from loons and telemetry of O_2 and thermal habitat showed fish spending the majority of time at temperatures that, in the laboratory, caused cardiac collapse in over 50 % of both populations as reported in Chapter 2. Therefore, as predicted, I concluded that aerobic performance can limit survival in high temperature lakes, which are frequently visited by fish predators, which supported my hypothesis of an endurance-survival relationship in nature. Furthermore, and also

as I had predicted, based on my Chapter 2 findings of an impaired 3N maximum heart rate response to warming and reduced 3N aerobic swimming performance in Chapter 3, I concluded that 3N poor survival is directly related to reduced aerobic capacity, thus supporting the main hypothesis of this thesis.

Is O_2 Supply to the 3N Salmonid Heart Sufficient During Maximum Cardiac Work?

In order to investigate a connection between impedance of 3N corporeal O_2 supply and reduced cardiac pumping capacity observed in Chapter 2 and poor survival observed in Chapter 3, I applied a modified Krogh diffusion model to the compact myocardium of 2N and 3N salmonids in Chapter 4. Once O_2 is conveyed to the tissue capillaries, it must diffuse through the tissue cells to their mitochondria. Though, diffusion distances may be greater in 3N fish due to enlarged 3N cell volumes, to the best of my knowledge, no empirical or theoretical assessment of diffusive O_2 supply to 3N tissues has been published. Krogh and Erlach developed a theoretical model to assess the volume of tissue surrounding and dependent upon a centrally located capillary, as a cylinder with the radius of that cylinder being the maximum distance from the capillary O_2 can sustainably be supplied (Krogh 1919). This distance is determined by the Po_2 of the capillary blood (i.e., the gradient between the capillary and the wall of the Krogh tissue cylinder), O_2 flow rate through the capillaries, tissue O_2 consumption rate and the tissue properties influencing O_2 diffusivity from the capillary to the mitochondria. Though this model could be applied to any tissue under any conditions, I chose to apply it to the compact myocardium of the 3N ventricle and assess whether an O_2 supply demand mismatch exists in the maximally working 3N heart for 2 reasons: 1. Due to the single coronary artery feeding the compact myocardium with blood and available literature values for coronary blood flow, O_2 transport rate to the heart is easily estimated and 2. Chapter 2 findings of impaired 3N cardiac pumping under conditions of extremely high O_2 demand (i.e., high temperature) may be explained by an O_2 supply limitation to the 3N heart (Figure 2-1 and Table 2-2). A Krogh model modified to include the effects of metabolic O_2 removal along the length of the capillary on the downstream PO_2, revealed an O_2 supply limitation in maximally working 3N relative to 2N hearts when 2N and 3N physiological values were applied as input variables (Figure 4-6). This limitation was primarily due to reduced arterial O_2 content and thus arterial O_2 delivery rate and

likely results in reduced maximum cardiac workload, and thus aerobic swimming capacity. Though 3N hearts have the potential to anatomically compensate by increasing capillary density, whether this plasticity exists is questionable, as increasing vascularity will almost certainly reduce muscle fiber area and thus heart contractility. Regardless, the modified Krogh diffusion model I developed and applied to the compact myocardium of 2N and 3N salmonid hearts suggests a mismatch between O_2 supply and demand due to reduced 3N arterial O_2 content, which likely reduces the 3N cardiac power output capacity.

Is O_2 Loading at the Gills Impaired in 3N Fish?

In Chapter 5 of my thesis, based on my Chapter 4 finding that reduced 3N arterial O_2 content was more influential in limiting O_2 supply to the mitochondria of the compact myocardial than enlarged capillaries or increased diffusion distance, I investigated the mechanism behind reduced 3N arterial O_2 content.

When assessing the O_2 transport cascade of 3N fish, the first inquiry is whether O_2 loading at the gills is limited in 3N fish. In fact, before this thesis, strong evidence of impaired 3N blood O_2 loading existed. Similar chinook salmon arterial Po_2 (Bernier et al. 2004), but reduced *in vivo* chinook salmon and *in vitro* Atlantic salmon blood and Hb-O_2 saturation (Graham et al. 1985, Bernier et al. 2004) suggest O_2 loading is limited by Hb-O_2 affinity and not cross gill diffusion. In addition to similar arterial Po_2, gill anatomy suggests the absence of gill diffusion limitations for 3N fish (Flajshans et al. 2006). Therefore, despite previous assessments of 2N and 3N Atlantic salmon Hb showing no ploidy differences in Hb-O_2 affinity or isohaemoglobin components (Graham et al. 1985; Sadler et al. 2000), the logical explanation of reduced arterial Hb-O_2 saturation remains a reduction in Hb-O_2 affinity. The above equivocal evidence becomes important to clarify considering my Chapter 4 conclusion that reduced 3N compared to 2N arterial O_2 content creates an O_2 supply limitation to the hearts of 3N salmonid fish

Therefore, using improved technology for Hb-O_2 curve determination, I confirmed similar Hb-O_2 affinity of 2N and 3N rainbow trout under temperature and Pco_2 conditions reflective of optimal and supra-optimal temperatures and resting arterial and venous (or maximally exercising arterial) blood never tested before (Figures 6-2 and 6-3). Therefore, I have shown that 3N limitations at the blood O_2 loading step of the O_2 cascade are not due to ploidy effects on inherent Hb-O_2 affinity and others have shown cross gill diffusion is not impaired in 3N fish.

In summary, I concluded that corporeal O_2 supply does limit high temperature tolerance of 3N rainbow trout, which along with other performance limitations can limit survival in the wild, depending on the biotic and abiotic conditions and physiological state of the organism.

Strengths and Limitations of My Ph.D. Research

I feel the strongest and most novel aspects of my Ph.D. thesis are the use if 3N fish as a model organism, hypothesis testing across multiple scales of morphological and functional organization and laboratory-based experiments designed to augment field-based experimental observations.

Diploid and 3N cohorts provide a useful organismal model to investigate the mechanisms behind differential performance. Comparative physiology experiments most often use paired species or strains to investigate the physiological mechanisms anatomical structures causing differential performance, but production of 2N and 3N sibling cohort groups of identical genetic origin allows comparisons between less genetically dissimilar populations. Although this is not done in all studies in my thesis, 2N and 3N sibling populations of genetically identical origin can by produced by pressure shocking only a portion of an egg batch resulting in reduced variability among compared populations. Unfortunately, the high level of genetic similarity between 2N and 3N cohorts of the same species contributes to low statistical power to detect significant differences between ploidies, a limitation of my thesis.

The hypothesis that O_2 supply limitations in 3N fish explain poor tolerance of sub-optimal conditions and reduced survival in the wild of 3N compared to 2N fish was tested across several physiological scales providing more thorough support of my hypotheses. For example, fish performance was assessed at the level of the heart (Chapter 2 characterized maximum heart rate response to warming), whole animal (Chapter 3 assessed endurance swimming time of fish) and population (Chapter 3 assessed survival according to the variability in swimming performance within lake-stocked cohort groups). Additionally, limitations to O_2 supply through the body were investigated empirically in Chapter 2, by assessing the break down of rhythmic heart beats, and thus convective O_2 transport and in Chapter 5, through comparison of Hb-O_2 affinity of 2N

and 3N blood and theoretically in Chapter 4, at all levels of the O_2 transport cascade using the Krogh diffusion model. Testing of multiple hypotheses related to 3N O_2 supply at various physiological scales allowed for comparisons of findings and assessment of consistency in conclusions.

An additional strength of my thesis is the design of experiments to allow direct links of results from laboratory-based and field-based experiments. Characterization of the cardiovascular response to warming in Chapter 2 allowed for determination of optimal and upper lethal temperatures as well as predictions of the metabolic scope available to fish for activity at lake temperatures. These data, combined with telemetry data from fish equipped with surgically-implanted temperature loggers provided the means to form inferences about the physiological state of fish in lakes. Furthermore, linking endurance swimming tests in the hatchery to survival of the same fish in lakes allowed for novel empirical testing of an endurance-survival relationship in nature.

Limitations to my thesis include pseudoreplication, especially for the fieldwork (i.e., low replication across lakes and years), low power (in most results of no significant ploidy effect) and the absence of a direct comparison of 2N and 3N aerobic capacity (i.e., maximum O_2 consumption and or arterial O_2 delivery).

The problem of pseudoreplication was especially problematic for my conclusion that a relationship exists between endurance swimming performance and survival in nature, which was observed for 3N trout in one lake for one year. It is difficult to imagine the strong relationship seen among all 4 3N endurance quartiles and survival being due to chance, but for irrefutable confidence in this relationship, it must be demonstrated to be repeatable across years and lakes. An attempt to replicate this finding was made in 2009, but due to above average temperatures, high mortalities occurred and insufficient numbers of fish from each endurance quartile were recaptured to asses the survival-endurance relationship. This conclusion would have far greater impact if the relationship had been demonstrated across multiple species in multiple lakes.

Low power for statistical tests was an additional limitation in my thesis. Low power was especially problematic in Chapter 2 of my thesis. Despite a significantly slower rate of fH change in 3N relative to 2N fish and identical fHs at acclimation temperature, no significant

differences in scope for change in fH ($p = 0.15$), peak fH ($p = 0.18$) or temperature of arrhythmia ($p = 0.4193$) onset were detected. However the power for the ploidy comparisons of all of these variables was low. This suggests small samples sizes or effect sizes or large variation could have prevented detection of significant differences.

Finally, despite finding convincing evidence supporting my thesis hypotheses that corporeal O_2 supply limits the aerobic performance of 3Ns and that aerobic performance, in turn, limits survival in the wild, aerobic capacity of 3N and 2N fish was not directly compared in my thesis. I relied on literature and inductive reasoning to connect tested hypotheses and assumptions to deduce reduced 3N aerobic capacity.

Applications of This Research

The novel knowledge arising from my thesis has applications to the sport fishing and aquaculture industries and general understanding of the cardiorespiratory system of fish and its limitations.

Triploid rainbow trout are important to sport fishing industries across Canada and triploid salmon are important to the aquaculture industry in Europe. The increased cost of 3N production in conjunction with high mortality losses among 3N fish populations underscores the importance of elucidating the mechanisms behind poor 3N performance for these industries. My findings suggest hatcheries could perform mass early development endurance swim screenings on 3N fish populations in order to identify 3N fish with high tolerance to sub-optimal conditions and thus reduce mortality rates or stock fish into more appropriate lakes matched to their habitat tolerance.

Future Directions

As with any good scientific research, I feel that my thesis has introduced as many questions as answers.

In terms of 3N salmonids, though I believe I have provided the most convincing evidence yet of impedance of the O_2 cascade as a mechanism behind 3N poor performance, a great deal more research is required to thoroughly understand the mechanisms behind their poor performance. As reduced arterial O_2 content is predicted to be the most influential impedance to 3N corporeal

O_2 supply, it is important to understand the mechanism behind reduced 3N arterial Hb-O_2 saturation. I have conclusively eliminated the inherent characteristics of 3N Hb as this mechanism. Therefore, future directions investigating *in vivo* effects on Hb-O_2 affinity are recommended. Considering findings of slight, but significantly, reduced red blood cell intracellular pH (Bernier et al. 2004), 3N acid base regulation and catecholamine response and their effects on Hb-O_2 affinity are promising next steps to understanding this particular ploidy difference. Furthermore, replicate experiments measuring 3N arterial blood O_2 and resting and maximal O_2 consumption rates are necessary to confirm findings of similar arterial Po_2, but reduced Hb saturation and reduced aerobic scope for 3N fish (Bernier et al. 2004).

Alternative hypotheses to the O_2 supply limitation hypothesis, such as increased metabolic investment into ion regulation or diffusion limitations of metabolic substrates, explaining poor 3N performance should also be considered. In an attempt to maintain corporeal O_2 supply in the face of reduced arterial O_2 content, 3N salmonids may increase gill perfusion. Increased gill perfusion has the potential to increase energy investment into ion regulation due to cross-gill ion (in fresh water) or water (in salt water) loss. Fish chronically exposed to ion-poor water develop high densities of lamellar and gill filament ionocytes, and in turn increased gill water-blood diffusion distance (Perry et al. 1996). Although 3N chinook salmon did not have reduced arterial Po_2, thickening of the blood water barrier would inhibit the ability to uptake O_2 into the blood. Similar anatomical adjustments may be seen in 3N fish losing ions due to chronic high gill perfusion rates; even if O_2 diffusion limitations do not arise, energy expenditure through recovering ions lost across the gills may affect tolerance of sub-optimal conditions and survival of 3N fish.

Additionally, enlarged 3N cell volumes may impair diffusion of important molecules. Although this thesis focused on O_2 diffusion limitations with enlarged 3N cells, evidence exists that diffusion of other important molecules may be impaired. Triploid brown trout cardiomyocytes may be more dependent on intracellular Ca^{2+} stores during cardiomyocyte contraction compared with 2N. This enhanced dependence on intra-cellular stores is likely related to a reduced surface area to volume ratio of enlarged 3N cardiomyocytes (Mercier et al. 2002). Little attention has been dedicated to trans-membrane ion movement or intracellular metabolic substrate movement (e.g., ATP) in enlarged 3N cells.

My thesis work, investigating the relationship between endurance swimming and survival in the wild is the only work I know of to directly assess the influence of swimming performance of fish on survival in the wild. Unfortunately, the lack of spatial and temporal replication in this experiment limits the general application of this knowledge. Though high aerobic capacity and performance is often suggested to improve Darwinian fitness (e.g., Plaut 2001; Claireaux and Lefrancois 2007), there is a large gap in the fish literature supporting this claim. Follow up research should attempt to establish a conclusive link between swimming endurance, aerobic capacity and survival in more lakes of variable characteristics and across years in the same lakes.

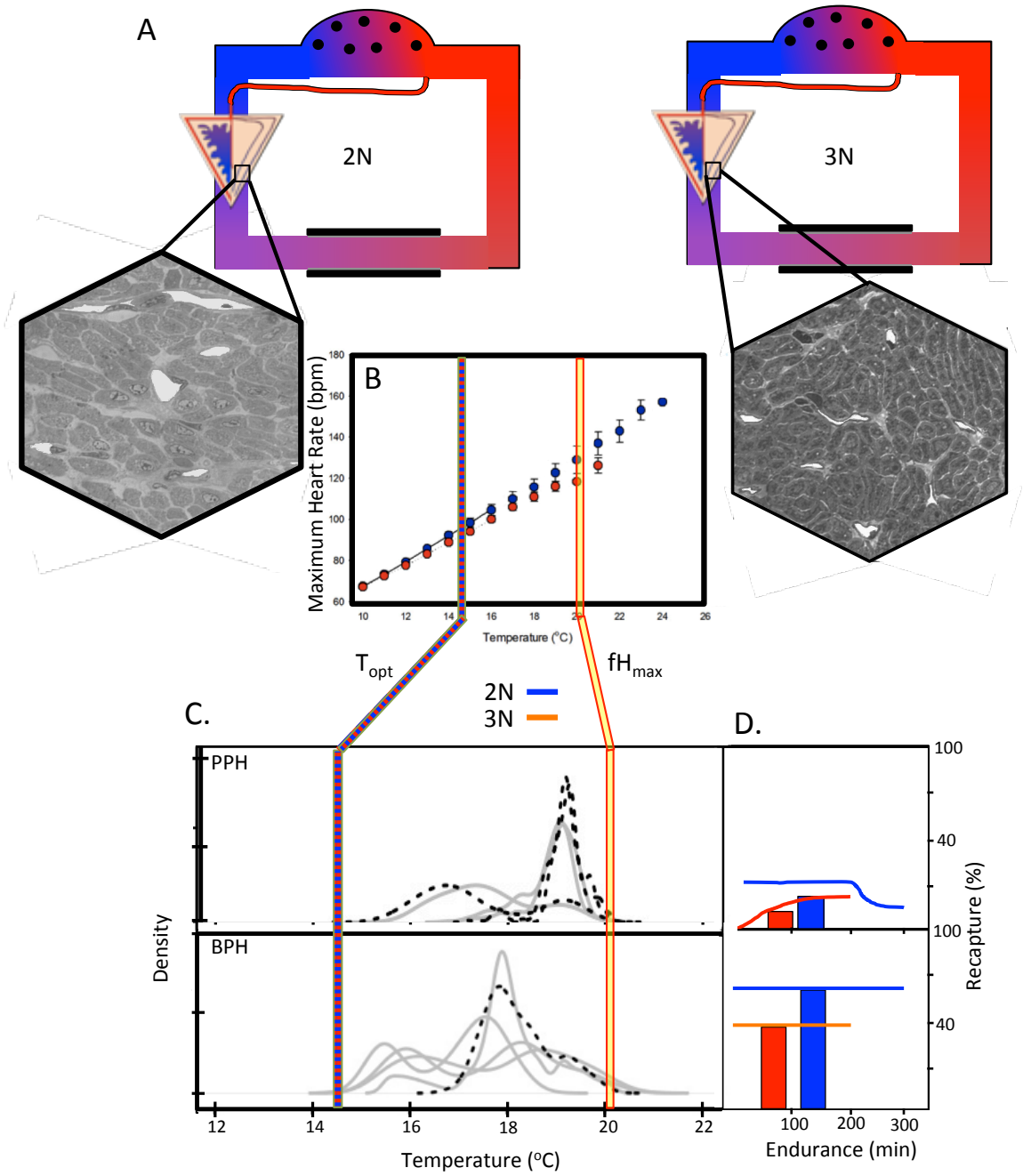

Figure 6-1. O_2 supply to the body limits the high temperature tolerance and survival of 3N rainbow trout. A. The O_2 cascade of 2N and 3N salmonid fish, illustrating reduced arterial O_2 content and enlarged cardiomyocytes and capillaries in 3N fish. B. The response of 2N and 3N maximum heart rate to warming. ( ) 2N and 3N optimal temperature (T_{opt}); ( ) 2N and 3N temperature at maximum heart rate (fH_{max}). C. Kernel density plots of temperature of 2N and 3N rainbow trout equipped with transmitters and stocked into 2 lakes (PPH and BPH). D. Endurance and survival of 2N and 3N rainbow trout in PPH and BPH. Bars represent endurance (x-axis) and survival (y-axis) of the respective ploidy. Lines predict survival based on endurance swimming of the respective ploidy.

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