Effects of acoustic telemetry transmitter implantation on survival, growth, resting metabolic rate, and swimming performance in juvenile rainbow trout (Oncorhynchus mykiss) and lake trout (Salvelinus namaycush)

Andrew Paxton Darcy
University of Windsor

Follow this and additional works at: https://scholar.uwindsor.ca/etd

Recommended Citation
Darcy, Andrew Paxton, "Effects of acoustic telemetry transmitter implantation on survival, growth, resting metabolic rate, and swimming performance in juvenile rainbow trout (Oncorhynchus mykiss) and lake trout (Salvelinus namaycush)" (2018). Electronic Theses and Dissertations. 7614.
https://scholar.uwindsor.ca/etd/7614

This online database contains the full-text of PhD dissertations and Masters' theses of University of Windsor students from 1954 forward. These documents are made available for personal study and research purposes only, in accordance with the Canadian Copyright Act and the Creative Commons license—CC BY-NC-ND (Attribution, Non-Commercial, No Derivative Works). Under this license, works must always be attributed to the copyright holder (original author), cannot be used for any commercial purposes, and may not be altered. Any other use would require the permission of the copyright holder. Students may inquire about withdrawing their dissertation and/or thesis from this database. For additional inquiries, please contact the repository administrator via email (scholarship@uwindsor.ca) or by telephone at 519-253-3000ext. 3208.
Effects of acoustic telemetry transmitter implantation on survival, growth, resting metabolic rate, and swimming performance in juvenile rainbow trout (*Oncorhynchus mykiss*) and lake trout (*Salvelinus namaycush*)

by

Andrew Paxton Darcy

A Thesis
Submitted to the Faculty of Graduate Studies
through the Great Lakes Institute for Environmental Research
in Partial Fulfillment of the Requirements for
the Degree of Master of Science at the
University of Windsor

Windsor, Ontario, Canada

© 2018 Andrew Paxton Darcy
Effects of acoustic telemetry transmitter implantation on survival, growth, resting metabolic rate, and swimming performance in juvenile rainbow trout (Oncorhynchus mykiss) and lake trout (Salvelinus namaycush) by Andrew Paxton Darcy

APPROVED BY:

____________________________
N. Hussey
Department of Biological Sciences

____________________________
T. Semeniuk
Great Lakes Institute for Environmental Research

____________________________
T. Johnson, Co-advisor
Great Lakes Institute for Environmental Research

____________________________
A. Fisk, Co-advisor
Great Lakes Institute for Environmental Research

December 17, 2018
DECLARATION OF ORIGINALITY

I hereby certify that I am the sole author of this thesis and that no part of this thesis has been published or submitted for publication.

I certify that, to the best of my knowledge, my thesis does not infringe upon anyone’s copyright nor violate any proprietary rights and that any ideas, techniques, quotations, or any other material from the work of other people included in my thesis, published or otherwise, are fully acknowledged in accordance with the standard referencing practices. Furthermore, to the extent that I have included copyrighted material that surpasses the bounds of fair dealing within the meaning of the Canada Copyright Act, I certify that I have obtained a written permission from the copyright owner(s) to include such material(s) in my thesis and have included copies of such copyright clearances to my appendix.

I declare that this is a true copy of my thesis, including any final revisions, as approved by my thesis committee and the Graduate Studies office, and that this thesis has not been submitted for a higher degree to any other University or Institution.
ABSTRACT

The objectives of this study were to investigate acoustic tag burden in two juvenile salmonid species; rainbow trout \((Oncorhynchus mykiss)\) and lake trout \((Salvelinus namaycush)\), explore the relationship between metabolic rate and swimming performance in tagged and untagged individuals, and investigate effects of surgery and tag implantation on survival and growth. Laboratory experiments measured tag burden effects in fish sizes (e.g., 9-39 g and 105-159 mm (fork length; \(L_f\)) typically stocked by Ontario Ministry of Natural Resources and Forestry (OMNRF) and other natural resource management agencies. The analysis revealed no significant effects of acoustic tagging on survival, growth, oxygen consumption \((\dot{MO}_2)\) (proxy for metabolic rate), and swimming performance \((U_{\text{crit}})\). Rainbow trout \(\dot{MO}_2\) (mass-specific rate of oxygen consumption) increased with time since surgery, and acoustic-tagged rainbow trout had elevated \(\dot{MO}_2\) compared to control fish, but the effect was not significant \((p = 0.024)\). The acoustic-tagged lake trout \(\dot{MO}_2\) were not significantly different from the controls or the other treatments (i.e., PIT, sham, and acoustic-tagged) \((p = 0.011)\). Rainbow trout (i.e., acoustic-tagged and control fish) had a significantly higher \(U_{\text{crit}}\) than lake trout \((p < 0.001)\). Differences in swimming performance between the species was most likely influenced by water temperature and body size. For both species \(U_{\text{crit}}\) was lower in acoustic-tagged fish but the difference was not statistically significant \((p = 0.024)\). Rainbow trout were housed at \(~ 14 \, ^\circ C\) and lake trout at \(~ 11 \, ^\circ C\). This study indicates that specific growth rate, oxygen consumption (via respirometry), and swimming performance \((U_{\text{crit}})\) can be used as novel metrics to assess impacts of acoustic tag burden. The results from this acoustic tagging study suggest tag burden up to 6\% does not have a significant effect on survival, growth, resting \(\dot{MO}_2\), and swimming performance \((U_{\text{crit}})\) in juvenile rainbow trout and lake trout.
ACKNOWLEDGEMENTS

I would like to give thanks to the following people for their contributions to this thesis: my co-advisors Dr. Aaron Fisk, and Dr. Timothy Johnson, committee members Dr. Christina Semeniuk and Dr. Nigel Hussey, Steffi Krause (Chatsworth Fish Culture), the Ontario Ministry of Natural Resources & Forestry (OMNRF) for allowing me access to hatchery-reared lake trout, Dr. Trevor Pitcher for providing resources and access to facilities (Freshwater Restoration Ecology Centre), Graham Raby for project insight and logistical support, numerous colleagues and faculty from GLIER for project assistance, as well as administrative personnel (Mary Lou Scratch & Christine Weisener).

Finally, I would like to acknowledge my loving family and friends for their unconditional support and positive presence in my life throughout this adventure.

Temet nosce.
# TABLE OF CONTENTS

DECLARATION OF ORIGINALITY ................................................................. iii

ABSTRACT ........................................................................................................ iv

ACKNOWLEDGEMENTS .................................................................................. v

LIST OF TABLES .............................................................................................. ix

LIST OF FIGURES ........................................................................................... xi

CHAPTER I: GENERAL INTRODUCTION ......................................................... 1

References ........................................................................................................ 16

CHAPTER II: ASSESSING ACOUSTIC TAGGING EFFECTS ON SURVIVAL, GROWTH,
METABOLIC RATE, AND SWIMMING PERFORMANCE OF JUVENILE RAINBOW
TROUT (ONCORHYNCHUS MYKISS) AND LAKE TROUT (SALVELINUS
NAMAYCUSH) ..................................................................................................... 29

Introduction ....................................................................................................... 29

Methods ............................................................................................................ 32

*Origin and housing of fish* ............................................................................. 32

*Treatment groups and experimental design* .................................................. 33

*Surgical implantation of transmitters* .......................................................... 34

*Experimental timeline* .................................................................................. 35

*Respirometry* ................................................................................................ 36

*Swimming performance* ............................................................................... 38

*Statistical analysis* ......................................................................................... 39
LIST OF TABLES

Table 2.1 Fork length, mass, tag burden, survival, and number of fishes for each treatment group (control, PIT, sham, and acoustic-tagged), of juvenile rainbow trout and lake trout used in experiments. Treatments consisted of control fish to which nothing was done beyond monitoring growth and physiology (“control”, n=30), PIT tagged (“PIT”, n= 24-30), subject to a sham surgery (incision and sutures but nothing inserted into the body cavity) (“sham”, n= 25-30), and acoustic-tagged (“tagged”, n=30). The acoustic tags were either Vemco model V5 (12.7 mm long, 0.67g in air) or V6 (16.5 mm long, and 0.97g in air) (Amirix Corporation, Bedford, Nova Scotia)………………………………………………………………………………………………………………………………………………………………67

Table 2.2 Coefficients of the final model and the significance of each term for lake trout growth model. Utilized the “R” library (MuMln) from Nakagawa and Schielzeth 2013, to obtain F and p-values from the generalized linear mixed model equations. Used linear mixed effects models with treatment, time since surgery, and body mass as fixed effects, with individual fish ID as a random effect to test for treatment effects on specific growth rates (mass and fork length). The “baseline” factor level for treatment = ‘tagged’, and for species = ‘lake trout’, thus the coefficients, p-values, etc. are comparisons against those baseline factor levels………………..69

Table 2.3 Coefficients of the growth model and the significance of each term for rainbow trout and lake trout growth. Utilized the “R” library (MuMln) from Nakagawa and Schielzeth 2013, to obtain F and p-values from the generalized linear mixed model equations. Used linear mixed effects models with treatment and body mass as fixed effects, and individual fish ID as a random effect to test for treatment effects on specific growth rates (mass)…………………………………..70
Table 2.4 Coefficients of the final model and the significance of each term for rainbow trout $\dot{MO}_2$. Utilized the “R” library (MuMIn) from Nakagawa and Schielzeth 2013, to obtain F and p-values from the generalized linear mixed model equations. Used linear mixed effects models with tag burden and time since surgery as fixed effects, and individual fish ID as a random effect to test for treatment effects on oxygen consumption ($\dot{MO}_2$) within the acoustic-tagged group.

Table 2.5 Coefficients of the final model and the significance of each term for lake trout $\dot{MO}_2$. Utilized the “R” library (MuMIn) from Nakagawa and Schielzeth 2013, to obtain F and p-values from the generalized linear mixed model equations. Used linear mixed effects models with treatment and body mass as fixed effects, and individual fish ID as a random effect to test for treatment effects on oxygen consumption ($\dot{MO}_2$).

Table 2.6 Coefficients of the final model and the significance of each term for $U_{crit}$ model. Utilized the “R” library (MuMIn) from Nakagawa and Schielzeth 2013, to obtain F and p-values from the generalized linear mixed model equations. Used linear mixed effects models with treatment and species as fixed effects, and individual fish ID as a random effect to test for treatment effects on swimming performance ($U_{crit}$).
LIST OF FIGURES

Figure 2.1 Boxplot for juvenile rainbow trout \((Onchorhynchus mykiss)\) [left] and lake trout \((Salvelinus namaycush)\) [right] specific growth rates (\% \text{day}^{-1}) (fork length [top] and mass [bottom]) for each treatment group (control [blue], PIT [green], sham [orange], and acoustic-tagged [red]) for each two week growth period (\pm SE). The species x treatment interaction was significant \((p = 0.0005)\), whereby growth was lower in the PIT group compared to the 'baseline' level which was acoustic-tagged fish \((p = 0.0001)\), but only for rainbow trout. Each box has a thick line in the middle which denotes the median (middle 50% value), the lower edge of the box corresponds to the first quartile, while the upper edge of the box corresponds to the third quartile. The middle 50% of the data distribution lies within the box, and the interquartile range \((1.5 \times)\) is represented by the upper and lower whiskers (or the most extreme value, depending on which is closer to the median).

Figure 2.2 Boxplot for juvenile rainbow trout \((Onchorhynchus mykiss)\) oxygen consumption \((\dot{MO}_2 \cdot \text{min}^{-1})\) (mg-O\(_2\)·kg\(^{-1}\)·min\(^{-1}\)) for each treatment group (control [blue], PIT [green], sham [orange], and acoustic-tagged [red]), in relation to days since surgery (\pm SE). There was a significant linear relationship between oxygen consumption and days since surgery in the acoustic-tagged rainbow trout \((p=0.003)\). Each box has a thick line in the middle which denotes the median (middle 50% value), the lower edge of the box corresponds to the first quartile, while the upper edge of the box corresponds to the third quartile. The middle 50% of the data distribution lies within the box, and the interquartile range \((1.5 \times)\) is represented by the upper and lower whiskers (or the most extreme value, depending on which is closer to the median). **R\(^2\) and p-value from model-derived regressions

74

76
**Figure 2.3** Boxplot for juvenile lake trout (*Salvelinus namaycush*) oxygen consumption (\(\dot{M}O_2\cdot \text{min}^{-1}\)) (mg\(O_2\)·kg\(^{-1}\)·min\(^{-1}\)) for each treatment group (control [blue], PIT [green], sham [orange], and acoustic-tagged [red]), in relation to body mass (g) (±SE). There was a significant linear relationship between oxygen consumption and body mass in the control lake trout (\(p = 0.007\)). Each box has a thick line in the middle which denotes the median (middle 50% value), the lower edge of the box corresponds to the first quartile, while the upper edge of the box corresponds to the third quartile. The middle 50% of the data distribution lies within the box, and the interquartile range (1.5 \(x\)) is represented by the upper and lower whiskers (or the most extreme value, depending on which is closer to the median). **R\(^2\) and p-value from model-derived regressions.**

**Figure 2.4** Critical swimming speed (\(U_{crit}\)) (L\(F\)·s\(^{-1}\)) for rainbow trout (*Oncorhynchus mykiss*) and lake trout (*Salvelinus namaycush*) (control [n=11-15] and acoustic-tagged [n=15-16]) (±SE). Every rainbow trout tested had a higher \(U_{crit}\) than fasted lake trout (\(p < 0.001\)). For both species \(U_{crit}\) was lower in acoustic-tagged fish but treatment differences were not significant (\(p = 0.024\)). Each box has a thick line in the middle which denotes the median (middle 50% value), the lower edge of the box corresponds to the first quartile, while the upper edge of the box corresponds to the third quartile. The middle 50% of the data distribution lies within the box, and the interquartile range (1.5 \(x\)) is represented by the upper and lower whiskers (or the most extreme value, depending on which is closer to the median).
CHAPTER 1
GENERAL INTRODUCTION

Studying the movement of fishes has always been a challenge for researchers seeking information about the spatial and temporal preferences of small, cryptic, rare, or hard to capture species (Cooke et al. 2013; Thorstad et al. 2013; Deng et al. 2017). Movement ecology involves the interpretation and evaluation of connections between animal dispersal, immigration, emigration, food availability, and habitat use/preference. The study of animal movement in aquatic environments is logistically and technically difficult due to the nature of the study subject’s biological characteristics (i.e., they survive and exist under water). Fish can travel long distances over a relatively short period of time in an environment that is widely interconnected and constantly changing (Gillanders et al. 2003; Hussey et al. 2015). Understanding how fish move through and around barriers or are blocked and trapped by mad made or natural obstructions in waterways is especially critical for migratory species such as salmonids that must navigate turbulent and fast-flowing tributaries during spawning migrations (Banks 1969; Bjornn & Reiser 1991). River migration and successful reproduction of migratory salmonids could become a potential issue if barriers to movement are not addressed (Farrell 2009).

The field of acoustic telemetry can provide fisheries researchers and biologists with novel methods to evaluate and interpret stocking success, survival rates, fish behaviour, daily/seasonal movements, and foraging patterns (Brown et al. 2006, Klimley et al. 2013; Larsen et al. 2013; Sandstrom et al. 2013; Thorstad et al. 2013; Newton et al. 2016; Ogburn et al. 2017). It is increasingly being used to understand post-stocking behaviour and survival of economically important fishes (e.g., walleye (Sander vitreus), Chinook salmon (Oncorhynchus tshawytscha)) and restoration initiatives (e.g., American eel (Anguilla rostrate), bloater (Coregonus hoyi),
Atlantic salmon (*Salmo salar*) (Gowan et al. 1994; Östergren 2006; Landsman et al. 2011; Béguer-Pon et al. 2015; Krueger et al. 2017; Faust et al. 2018). The breadth of telemetry applications is also growing, and includes several areas of fisheries management, including the assessment of ecological niches (i.e., fine-scale acoustic arrays), species restoration initiatives, and invasive species monitoring (Marsden et al. 1988; Lennox et al. 2016). Due to advancements in acoustic tag size and function, researchers can now track and study smaller fish such as juvenile salmonids (Lucas & Baras 2000; Klimley et al. 2013). There is a need to assess the impact of acoustic telemetry implantation in small fish, where the tag burden (ratio of tag mass to fish mass) is going to be often greater than it is in large fish.

There are several techniques and types of equipment that are utilized for remote location and identification of fish using manual tracking or fixed locations. These include passive integrated transponder (PIT) style tags, acoustic telemetry tags, injectable juvenile salmon acoustic telemetry system tags (JSAT), and radio telemetry tags that can be surgically inserted or externally attached to dorsal tissue (Cooke et al. 2013). Each of these methods has its own advantages and disadvantages. The benefits of acoustic telemetry tags are that they can provide detailed information on animal movement for long periods of time, multiple receiver stations have the capability to generate two or three-dimensional tracks of the study species, and some advanced systems transmit data in real time (Cooke et al. 2013). Benefits of the other methods are that some only require an intramuscular injection with a syringe to implant the tag (i.e., PIT and JSAT), radio telemetry tags can function in shallow water (i.e., < 10 m) and transmit on land via aerial antennae, whereas acoustic tags are typically inserted into the body cavity via surgical procedures, have a finite battery life, and can only be detected using a hydrophone that is submersed under water (Cooke et al. 2013). Intraperitoneal implantation is the preferred method
for long-term acoustic telemetry projects involving fish and involves surgically inserting the acoustic tag into the coelem (i.e., body cavity) (Jepsen et al. 2002; Cooke, Wagner, et al. 2011; Cooke, Woodley, et al. 2011). Radio telemetry tags and acoustic tags can be attached externally but there is potential for decreased swimming performance and tag retention (Wagner et al. 2011; Rub et al. 2014). Although radio telemetry is still the most widely used tracking method, and PIT tagging is used extensively in large scale community and population level research projects, a common approach that is currently being used by many fisheries researchers is acoustic telemetry (Cooke et al. 2013; Smircich & Kelly 2014; Deng et al. 2017).

With the invention of unique coded transmitters in the early 1980’s and miniaturization in the early 1990’s, telemetry studies could begin to investigate large numbers of fish over wide-ranges of area (i.e., returning adult salmonids) (Cooke et al. 2013). The process of collecting acoustic telemetry data typically involves externally or internally attaching an acoustic tag to a specific species or individual of interest. This acoustic tag once activated will emit a series of “pings” on a specific frequency (i.e., 69 or 180 kHz) (Cooke et al. 2013). In the Great Lakes, large-scale acoustic arrays typically operate on the 69 kHz frequency while small-scale arrays in rivers and tributaries generally use the 180 kHz frequency (i.e., higher frequency equates to weaker signal strength) (Pincock & Johnston 2012; Hayden et al. 2016). Individual receivers or receiver arrays are set up in the study area and when a study species that has been tagged passes by the receiver, a time stamp of the animal is recorded. Tags are programmed using a specific ID code, and the signal transmission is set to a desired pulse interval (“ping rate”) and signal repeat rate (Heupel et al. 2006; Cooke et al. 2013). Identifying optimal transmission parameters (i.e., pulse interval and repeat rate) is dependent on study species/size, study design/location, number of receivers in the acoustic array, and environmental conditions such as turbidity, conductivity,
and water flow (Heupel et al. 2006). Even under optimal transmission conditions, there are inadvertently issues with signal collisions (i.e., too many tags in a system) and interference (background noise pollution) that require significant post data-collection processing to detect and eliminate (Cooke et al. 2013).

Acoustic telemetry technology and tag effectiveness have improved significantly over the past decade, and this has led to a subsequent increase in popularity (Cooke et al. 2013; Rub et al. 2014). However, there are also technology-specific challenges associated with acoustic telemetry tags. These include signal range, false detections, fish mortality, and tag loss (Jepsen et al. 2002; Heupel et al. 2006). Many of the issues associated with weak signal range, fish mortality, and tag loss can be mediated by identifying the ideal tag to body weight ratio (Jepsen et al. 2003; Pincock & Johnston 2012). Larger tags are associated with larger batteries, which means longer data collection periods, and increased signal range (Smircich & Kelly 2014). The larger and heavier acoustic telemetry tags can also have environmental and physiological sensors incorporated into the tag (i.e., temperature, depth, and accelerometer features) (Cruz-font et al. 2016). The benefits to identifying maximum tag burden ratios are represented by these two factors (i.e., larger tags are better research tools and provide additional data to a study).

The study of juvenile fish is important because the data acquired from salmon smolts studies for example are used to estimate the amount of returning adult salmon in following years (Chaput et al. 2002; Welch et al 2004; Melnychuk et al. 2007; Drenner et al. 2012; Halfyard et al. 2012; Daniels et al. 2018). Early life history strategies and residency patterns for juveniles of a specific salmonid species can provide insight into future recruitment, migration timing, smolting duration and preferred habitat (Pincock et al. 2009; Melnychuk et al. 2010). This
information is important for management agencies attempting to maximize stocking success and enhance restoration or rehabilitation efforts.

Stocking is used globally to enhance and restore local fish populations. Over 40 million fish stocked in the Great Lakes from 2000-2009, with most of these fish being salmonid species (U.S. Fish and Wildlife Service and Great Lakes Fishery Commission 2010). Little is done to assess the health and survival of juvenile fish after stocking as this is a sensitive time typically associated with high mortality rates (Ersbak & Haase 1983; Berg & Jorgensen 1991; Aarestrup et al. 2005). The fate and influence of stocked juveniles on freshwater ecosystems can be explored with acoustic telemetry techniques (i.e., the observer does not need to interfere or harass the study subject to gather information after initial surgery). Thus, the data attained is relatively untainted from physiological stress caused by handling, air exposure, and displacement. Species such as lake trout and rainbow trout are stocked for either restoration or recreational purposes, respectively (MacCrimmon & Gots 1972; Krueger & Ihssen 1995). These two species are a focus of restoration and enhancement efforts and are of high ecological and recreational importance in the Great Lakes (Gonder 2005; Binder et al. 2016; Wehse et al. 2017).

Rainbow trout are a Pacific salmonid species native to the North American West coast and considered an introduced species in the Great Lakes (Scott & Crossman 1973; Post et al. 2002). The first documented Great Lakes stocking of this species occurred in the AuSable River (1876), which is a tributary of Lake Huron located in Michigan, United States. The remaining Great Lakes (i.e., Ontario, Michigan, Erie, and Superior) were stocked shortly afterwards (1878-1883) (MacCrimmon & Gots 1972), and natural reproduction of rainbow trout was well established within the Great Lakes by the 1920’s. Heavy stocking efforts began in the 1950’s by the Province of Ontario in response to serious stock declines presumably caused by sea lamprey
parasitism and poor environmental conditions (Berst & Wainio 1967; MacCrimmon & Gots 1972). The species made a quick recovery due to successful natural reproduction and effective sea lamprey control, and by the 1960’s rainbow trout were once again well-established throughout the entire Great Lakes basin (MacCrimmon 1971, 1977; Crawford 2001).

Two forms of this species have historically been stocked in the Great Lakes; “rainbow trout” are a smaller and darker coloured stream-dwelling form compared to the larger silver-bodied migratory form referred to as “steelhead” (Crawford 2001). The “steelhead” form is found in large waterbodies and can exist in freshwater and marine environments. Historically, rainbow trout were stocked in smaller creeks/rivers and inland lakes whereas steelhead stocking occurred in the Great Lakes and their tributaries (Scott & Crossman 1973). From 1966 to 1998, there were approximately 174 million rainbow trout introduced into the Great Lakes system (Crawford 2001). One-third of these rainbow trout were stocked into Lake Michigan, and American agencies were responsible for ~ 87% of total stocking efforts (i.e., ~ 151 million fish).

Rainbow trout are mostly potadromous in the Great Lakes, meaning that they require movement through different types of freshwater habitat or environments to complete their lifecycle (i.e., from tributaries and streams/rivers with riffle environments to large freshwater lakes) (Crawford 2001). This species can spend up to three years in a riverine system (i.e., parr stage) before migrating out to the open water (i.e., “smoltification”) (Biette et al. 1981). Growth is rapid once smolts reach the open lake environment and males can mature in as little as a year (MacCrimmon & Gots 1972). The average length for a rainbow trout in Ontario is 53.0 cm with a record of 99.8 cm, and maximum lifespan is estimated at 11 years of age (Holm et al. 2009). Spawning typically occurs in the spring, where females construct “redds” (i.e., nests) in gravel substrate, although some hatchery stock has been documented spawning in the fall.
Females are capable of repeat annual spawning and marked individuals have been reported spawning for 7-9 consecutive years in some undisturbed tributaries of Lake Superior (Gonder 2005).

Lake trout are an inland char species native to North America and the Great Lakes, although they have been widely introduced into inland lakes in the west (Scott & Crossman 1973). Historically, this species has been over-harvested, impacted by competition with non-native species, alewife (*Alosa pseudoharengus*) introduction (i.e., thiamine deficiency), and subject to the devastating effects of sea lamprey (*Petromyzon marinus*) introduction to the Great Lakes via the Welland canal construction during the 1940’s and 50’s (Eschmeyer 1964; Evans & Olver 1995). Due to these issues, naturally occurring populations of lake trout were relatively non-existent in Lake Ontario, Erie, Michigan, and the majority of Huron by the 1960’s (Evans & Olver 1995). Heavy bi-national (i.e., Canadian and American) stocking efforts began in response to this population decline in the Great Lakes, with almost 2 million yearling lake trout stocked into Lake Superior in 1962 (Eschmeyer 1964). The Province of Ontario also developed and started stocking splake (*Salvelinus fontinalis × Salvelinus namaycush*) (i.e., male brook trout x female lake trout cross) as part of their restoration strategy in the 1950’s (Scott & Crossman 1973). Although natural reproduction has been generally weak in the Great Lakes population of lake trout (except in Lake Superior), self-sustaining populations have been documented within many North American inland lakes (Evans & Olver 1995). Natural reproduction has also been reported in Lake Huron and Lake Ontario (Marsden et al. 1988; Krueger & Ihssen 1995).

Lake Superior has the greatest diversification of the lake trout species within the Great Lakes and houses the last remnant populations of naturally occurring lake trout (Eschmeyer 1964; Krueger & Ihssen 1995). Historically up to twelve sub-populations of lake trout in Lake
Superior were documented by commercial and aboriginal fishermen (Eschmeyer 1964). There now remains three general accepted forms of lake trout in the Great Lakes (i.e., “siscowet”, “humper”, and “lean”) (Krueger & Ihssen 1995). The other Great Lakes have all reported the “siscowet” and “lean” forms except for Lake Ontario. The identification of these forms is based on colour and appearance as well as body fat content, time of spawning, and water depth (Eschmeyer 1964; Krueger & Ihssen 1995). The differences in body morphology (i.e., shape) reflects their deep-water or shallow-water habitat preference.

The average age of sexual maturity for lake trout is ~ 5-13 years of age, but this is highly dependant on water temperature, food availability, and environmental conditions (Eschmeyer 1964). Spawning in the Great Lakes generally occurs on shallow-deep rocky reefs and shoals (i.e., 15-90 m deep) in the late summer or fall, although spawning on macrophyte beds (Lake Michigan and Superior), and within rivers (Lake Superior) has been previously documented (Eschmeyer 1964; Evans & Olver 1995). The spawning duration varies but can last up to a month in the Great Lakes, with males arriving on spawning shoals before females to prepare and clean spawning areas (unlike rainbow trout, female lake trout do not construct “redds”, but instead scatter eggs into cracks and crevices within boulders, cobble, rubble or gravel) (Eschmeyer 1964). These eggs can take up to 4 months to fully mature in the cold water, and fish will remain in the refuge of these rocks until about a month until dispersing to deeper open water. The average length for a lake trout in Ontario is 44.5 cm with a record of 130.9 cm, and maximum lifespan is estimated at 50 years of age (Holm et al. 2009). Lake trout prefer cooler water temperatures around 10-12 °C and are typically found in the 30-90 m depth range (Eschmeyer 1964; Scott & Crossman 1973).
The use of acoustic telemetry as a research tool for studying fish behaviour and estimating survival rates of juveniles is a growing trend. Investigating the effects of acoustic tag burden on juvenile fish is a necessary first step before acoustic telemetry studies in the wild (Jepsen et al. 2003). The act of tagging fish introduces a mass-dependent burden that may impair functions (e.g., survival, growth, swimming performance) depending on the size of tags relative to the fish (Cooke et al. 2011). Generally, tag burden less than 2% body mass is the standard in fish telemetry studies, but this has also been questioned, limits applications for smaller fish, and there is the possibility of species-specific variation in burden limits (Winters 1983; Brown et al. 1999; Smircich & Kelly 2014).

Although acoustic tag burden studies have been carried out for more than 15 years, researchers continue to study and quantify behavioural and physical effects associated with surgically implanted acoustic telemetry tags (Cooke et al. 2011). There is currently an increase in research activities identifying better metrics to quantify and measure tag burden across multiple fish species and sizes (Richard et al. 1999; Bridger & Booth 2003; Jepsen et al. 2003, Brown et al. 2010; Thiem et al. 2011; Carrera-García et al. 2017). Although standard protocols exist for implantation of acoustic tags in fishes, specific surgical techniques and styles vary (e.g., type of needle/sutures, suture pattern, etc.). Standard techniques for acoustic telemetry tag implantation involve sanitization of surgery tools and equipment, some form of anesthetic (i.e., typically MS-222), intraperitoneal implantation, and recovery in fresh water before release. Complete and thorough explanation of surgical procedures and techniques involved with telemetry studies is beneficial for the field and researchers in general (Thiem et al. 2011).

Natural fish behaviour and physiology are ideally kept intact during telemetry studies; however, this may not be the case and further investigation is needed to determine whether the
telemetry data is providing an accurate picture of how the wild fish are behaving (Brown et al. 1999; Jepsen et al. 2003). There is discrepancy when it comes to ideal tag burden ratios and a wide range of tag burden effects and recommended tag burden values are reported in the literature. Brown et al. (1999) reported no significant effects of tag burden (i.e., 6-12 %) in the swimming performance of rainbow trout smolts. Similarly, Anglea et al. (2004), found that the critical swimming speed ($U_{crit}$) of juvenile Chinook salmon was not significantly different among treatment groups (i.e., acoustic tagged, sham-surgery, and control). In a study involving acoustic tag effects on brook trout, heavy tags were associated with slower growth, and the authors advised against anything over 7% body weight due to tag retention issues (Smircich & Kelly 2014). Another study that was investigating the swimming performance of juvenile Chinook salmon found that swimming performance (i.e., $U_{crit}$) decreased as tag burden increased (Perry et al. 2013). Collins et al. 2013, found that higher tag burdens were related to poor swimming performance, survival, and tag retention in sockeye salmon (i.e., $\geq 8\%$ burden had lower $U_{crit}$ values than fish $< 8\%$ burden, only had mortality in fish $> 6\%$ burden, and healing times were longer on fish with larger tags) (Collins et al. 2013). Although past studies have identified a variety of tag burden effects there is discrepancy when it comes to definitive guidelines (Brown et al. 1999). Ultimately, there is likely no ideal value regarding a universal tag burden threshold and individual study objectives, study species/life-stage, and morphology will influence acceptable acoustic tag burden values (Jepsen et al. 2003). It has been suggested that acoustic tags should be neutrally buoyant and that metrics such as tag mass in water and tag volume be incorporated into tag burden investigations to identify impacts on the fish’s ability to maintain buoyancy (Brown et al. 1999; Jepsen et al. 2002, 2003). The addition of multiple performance measures to tag burden studies will help to mitigate the ongoing debate about potential tag
burden effects and suitable tag size/mass for specific sizes and species of fishes. Measuring variables related to swimming performance and metabolic rate have implications for the understanding of vulnerability to predation and resilience/adaptability to environmental conditions or stressors. Swimming performance is an important biological characteristic and can be related to predator avoidance and feeding ability (Anglea et al. 2004; Perry et al. 2013; Walker et al. 2016). Critical swimming speed (i.e., $U_{crit}$) is a standard and commonly used metric to evaluate swimming performance in fishes (Jain & Farrell 1998; Farrell 2008). Swimming performance is important to all fish, specifically for predator avoidance and capturing prey items or food. But this trait is especially relevant for species such as rainbow trout that migrate into river systems during adult spawning events (Biette et al. 1981).

There are two main types of respirometry systems used for measuring anaerobic metabolic rates of aquatic animals (i.e., closed vs. open flow). Closed-flow respirometry systems involve measuring the time course of oxygen in the water of a closed chamber that houses an aquatic animal, whereas open-flow systems measure flow rate and differences in oxygen content (i.e., inside and outside the chamber) (Steffensen 1989). Both techniques have their own unique limitations. Closed-flow systems have issues with build-up of carbon dioxide and excretions from the test subject, as well as concerns with a steady decline in oxygen levels during data collection (i.e., potential compensatory mechanisms in test subject) (Rosewarne et al. 2016). Open-flow systems are less sensitive to changes in $\dot{MO}_2$ and require a state of equilibrium (i.e., well-mixed water) to maintain accurate measurements. Intermittent-flow respirometry is a hybrid system and has been developed to overcome the negatives associated with closed and open-flow respirometry. Intermittent-flow respirometry uses short closed measurement periods and long
flush periods which removes toxic waste by-products and replenishes oxygen concentration in the chamber (Rosewarne et al. 2016; Svendsen et al. 2016).

There are metabolic costs associated with early life history strategy regarding maintaining position in a tributary with high-flow riffle environments (i.e., elevated metabolic rate relative to adults), and any additional stress or burden caused by unnecessarily large tags could be detrimental to the survival and success of an individual or population of fishes (Bjornn & Reiser 1991; Jepsen et al. 2003). Oxygen consumption is correlated with metabolic rate and elevated metabolic rates may reflect increased stress (Chabot et al. 2016). As far as we know, intermittent-flow respirometry has not previously been used to identify physiological consequences of tag burden. This represents a novel approach for evaluating stress physiology following intraperitoneal implantation of acoustic tags in juvenile salmonids.

The connections between aerobic scope and swimming performance regarding individual performance and/or variation in swimming performance can be investigated through the analysis of maximum and standard metabolic rate in relation to critical swimming speed (i.e., $U_{crit}$) (McDonald et al. 1998). Elevated oxygen consumption rates (i.e., increased gill ventilation and cardiac output) caused by high tag burden could be related to increased extraction of oxygen by tissues involved in wound healing (Schreck 1981). Any wound healing will involve a stress which may elevate oxygen consumption, however high tag burden may aggravate that stress (Collins et al. 2013). Wound healing and metabolic rates are interconnected, and the relationship between stressors and changes in metabolic rate have been investigated in previous studies (Farrell 2007; 2008, Metcalfe et al. 2016, Raby et al. 2016). The physiological costs associated with wound healing may require a significant amount of the excess energy that is available to the

I am looking to investigate how juvenile and small-sized fish respond to surgically implanted acoustic telemetry tags. As far as I am aware, such an experiment has not been attempted using this variety of species or evaluation methods, and so would represent a novel and useful test of the following hypotheses.

The first hypothesis I will be testing is whether there is a difference in growth, survival, metabolic rate, and/or swimming performance between four treatment groups (i.e., control, PIT, sham, and acoustic-tagged). The second hypothesis is that these differences in growth, survival, metabolic rate, and swimming performance between the treatment groups will be greater in the short-term (i.e., 2-10 days after surgery) versus long-term (i.e., 20-30 days after surgery).

Hypothesis 1: The act of tagging fish introduces a mass-dependent burden that may impair functions depending on the size of tags relative to the fish. There remain relatively few studies that look at a variety of species, and/or a variety of tagging effects (i.e. survival, growth, metabolic rate, swimming performance), a problem I will contribute to rectifying.

Hypothesis 2: The effects of surgical acoustic tag implantation differ among individuals and salmonid species because of intrinsic biological differences (e.g., physiology, morphology, behaviour). Juvenile rainbow trout are typically found in tributaries while lake trout are found in deep cold lakes due to their habitat preference (i.e. deep, rocky reefs/shoals) (Scott and Crossman 1973). Rainbow trout tend to occupy warmer water (i.e., ~ 15 °C, whereas lake trout are found in colder water temperatures (i.e., ~ 10 °C) (Rao 1968; Beamish et al. 1989; Alsop & Wood 1997). Species such as lake trout have the lowest temperature preference of the two study
species mentioned above (i.e., rainbow trout and lake trout). Because of this temperature
difference, it could be that the low water temperatures the lake trout are housed in may
contribute to slower healing (i.e., temperature will have a measurable effect on $\dot{MO}_2$, etc.). The
effects from tag burden and surgical procedure will presumably be greater than the effects of
individual level variation in performance metrics.

I predict that higher tag burden will result in decreased survival, tag retention, growth,
metabolic rate, and swimming performance. I also predict that there will be greater effects
during the first couple of weeks after surgeries compared to the effects seen after a month (i.e.,
takes approximately four to six weeks for wounds to heal). Wound healing has been investigated
in juvenile salmonids and the literature states that wounds start healing approximately seven days
after surgery and are typically fully-healed in 60 days (Lucas 1989; Ivasauskas et al. 2012; Liss
et al. 2016). However, healing time is highly temperature dependent, as well as influenced by
feeding regime, and other environmental conditions. The metabolic rates and swimming
performance will presumably be negatively impacted by the surgeries and wound healing. If
there is no difference found between the sham surgery group and the control group, I will assume
that the effects of surgery are from the tags themselves, and not from the incision or sutures. The
PIT group is included to investigate the effects of different surgical procedures. Control group
was not exposed to anesthesia, PIT, or acoustic tag surgeries, and will serve as the baseline for
typical metabolic rates and swimming performance. Critical swimming speeds, growth rates, and
metabolic rates that are outside the control range will be compared via statistical analysis and
linear mixed effects models. The final prediction is that there will be differences in sensitivity to
tag burden on an individual level and between species (i.e., rainbow trout and lake trout). Lake
trout for example may experience lower infection rates and rainbow trout faster healing times
due to their individual temperature preferences (i.e., ~ 10-12 °C for lake trout compared to ~ 15 °C for rainbow trout) (Scott & Crossman 1973; Anderson & Roberts. 1975). Although, the physiology of the individual species is optimised at that preferred temperature and therefore healing may be comparable (Schreck 1981).

Rainbow trout have become some of the most commonly used test subjects (i.e., “aquatic guinea pigs”) in fish biology because of their availability from hatchery production facilities, adaptation to life in captivity, and propensity to feed on commercially available fish pellet food (Rao 1968; Rosewarne et al. 2016). Lake trout is an important study species due to its ecological relevance and current restoration efforts in the Great Lakes (Eschmeyer 1964; Krueger & Ihssen 1995; Binder et al. 2016). For the purposes of this experiment, these species represent useful models because they are directly relevant to fisheries, have previously been the subject of tag burden experiments (e.g., rainbow trout), and are closely related to several other economically important species (i.e., most other salmonids). Moreover, these species are part of routine telemetry-tagging programs by academics and fisheries management agencies (new information about tagging effects is directly relevant to these research programs and other research involving juvenile or small-sized fishes).

The project will have linkages to conservation biology and the restoration of native fish species, specifically in the Great Lakes area, though rainbow trout and lake trout are tagged in telemetry projects elsewhere in the world. The knowledge attained from this project will facilitate ongoing restoration and reintroduction efforts that are utilizing acoustic telemetry technology as an assessment tool and enhance the quality of data acquired from future telemetry studies involving small or juvenile fish.
References


Comparing the survival rate of juvenile Chinook salmon migrating through hydropower systems using injectable and surgical acoustic transmitters. Scientific Reports, 7: 42999.


Steffensen, J.F. 1989. Some errors in respirometry of aquatic breathers: how to avoid and correct for them. Fish Physiology and Biochemistry, 6: 49-59.


implantation of transmitters: a call for more complete reporting. Reviews in Fish Biology and Fisheries, 21: 117-126.


CHAPTER 2

ASSESSING ACOUSTIC TAGGING EFFECTS ON SURVIVAL, GROWTH, METABOLIC RATE, AND SWIMMING PERFORMANCE OF JUVENILE RAINBOW TROUT (ONCORHYNCHUS MYKISS) AND LAKE TROUT (SALVELINUS NAMAYCUSH)

Introduction

Acoustic telemetry is a powerful tool that fish biologists now routinely use to study migration, habitat use, behaviours, and assess survival rates in fish at liberty in the wild. (Welch et al. 2004; Brown et al. 2006, Klimley et al. 2013, Larsen et al. 2013, Sandstrom et al. 2013, Thorstad et al. 2013; Newton et al. 2016; Ogburn et al. 2017). The use of telemetry in fish has rapidly grown in recent years and it is now being applied to important problems in fisheries management like the assessment of ecological niches, species restoration, invasive species monitoring, protected area design and management, and the survival of fish after catch-and-release fishing (Cooke et al. 2013; Hussey et al. 2015; Lennox et al. 2016; Crossin et al. 2017). Acoustic telemetry has historically been used to study the movement of large and highly mobile species that typically travel long distances (Cooke et al. 2013). Due to improvements in tag functioning and reductions in size, there have been numerous telemetry studies using small fishes (e.g., < 30 g) in the last ten years, mostly involving juvenile salmonids (e.g., Melnychuk et al. 2007; Drenner et al. 2012; Halfyard et al. 2012).

Telemetry research involves externally or internally attaching a transmitter, here after tags, to an individual from a species or population. Individual receivers or receiver arrays are set up in the study area and when a study species that has been tagged passes by the receiver, the detection is logged with a time stamp. Acoustic tags emit a series of “pings” on a specific
frequency which identify the individual, and with some tags, provide data such as depth, temperature or acceleration at the time of the transmission, when they are within range of a receiver. With recent technological advancements and further miniaturization of acoustic transmitters, there is a trend towards studying smaller species and/or juvenile fish (Pincock et al. 2010; Hussey et al. 2015).

Most studies of acoustic telemetry rely on an assumption that the methods used do not systematically affect the behaviour or survival of the study animals such that the conclusions of the research are affected (Brown et al. 1999; Jepsen et al. 2003; Cooke et al. 2011). Therefore, investigating the organism-level effects of acoustic tagging is a necessary first step before large-scale acoustic tagging studies in the wild. The act of tagging fish introduces a mass-dependent burden that may impair functions (e.g., survival, growth, swimming performance) depending on the size of tags relative to the fish (Bridger & Booth 2003; Cooke et al. 2011). In general, larger transmitters have longer battery life and stronger signal transmissions (which increases detection range and efficiency); as such, in most cases it is desirable to use the largest tags possible, taking into consideration the study species morphology and research objectives (Jepsen et al. 2003). A widely-used rule of thumb in fish telemetry studies is that, tag burden (i.e., the mass of the tag relative to the mass of the fish) should be less than 2% of body mass, but this rule of thumb has been questioned for smaller fish, and there are likely to be species-specific variation in burden limits (Winters 1983; Brown et al. 1999; Smircich & Kelly 2014). The “2% rule” was based on several old studies involving buoyancy in fishes (Brown et al. 1999; Jepsen et al. 2003). The swim bladder in freshwater fish can change from ~ 7 to 25% of the total body volume therefore the space taken up in the body cavity (i.e., space for tag) by a tag may restrict the fish’s capacity to regulate its buoyancy (Alexander 1966). Some evidence has emerged in tagging-effects
studies that shows juvenile salmonids may be able to maintain growth, survival, and swimming performance with tags that approach 10% of body mass (Collins et al. 2013). Typical tag burden range in most studies involving juvenile salmonids is 2-10% (Chisholm & Hubert 1985; Brown et al. 2010; Ivasauskas et al. 2012; Newton et al. 2016). There have been several studies that quantified the effects of acoustic telemetry tags and investigated growth, survival, tag retention, wound healing, and swimming performance in single salmonid species (Anglea et al. 2004; Chittenden et al. 2009; Perry et al. 2013; Deng et al. 2017). There are costs and benefits associated with larger tags (i.e., heavier tags could negatively influence fish behaviour and survival/health).

This study will be attempting to evaluate whether the surgical insertion of the acoustic tag, and the recovery post surgery, result in stress (e.g., elevated metabolic rate). The physiological costs associated with wound healing may negatively impact growth and swimming performance (i.e., impairment of locomotion) (Ivasauskas et al. 2012; Liss et al. 2016). I suspect that the tag burden effects will be greater than individual level variation in the parameters used (i.e., growth, metabolic rate, and swimming performance).

The primary objective of this study was to assess the effects of surgical acoustic tag implantation on rainbow trout and lake trout. Transmitters were surgically implanted fish of varying sizes, which meant I could also assess whether effects of the tag varied with tag burden (% of body mass), potentially allowing for the identification of specific-specific tag burden thresholds that cause unwanted effects. The variables I measured included a range of responses relevant to fitness: survival, growth, oxygen consumption rate, and swimming performance. Rainbow trout have become domesticated by the aquaculture industry and, as a result, have become a model species for the study of fish behaviour and physiology, including some use in
previous tag burden experiments (Chisholm and Hubert 1985; Ivasauskas et al. 2012; Sandstrom et al. 2013). In lake trout, on the other hand, for which there have been numerous telemetry studies in adult fish (e.g., Flavelle et al. 2002; Binder et al. 2016; Cruz-Font et al. 2016), there has been no research to date on the effects of acoustic tagging. I predicted surgical implantation of transmitters would impair growth, survival, and swimming performance relative to controls and increase resting metabolic rate because of stress and tag burden. In addition, I predicted that the effects of the tagging would be stronger at higher tag burdens. My study provides novel insights into the interplay between surgically-implanted acoustic tag burden, and fish performance and physiology (i.e., resting metabolic rate and critical swimming speed), which has important implications for future tagging studies and our understanding of how tag burden affects different sizes and species of fish.

Methods

2.1 Origin and housing of fish

Rainbow trout (13-36g and 105-150 mm, in fork length (L_F), n=120) were purchased from a nearby commercial facility (i.e., Rainbow Springs in Thamesville, Ontario) while lake trout (9-39 g and 112-159 mm (L_F), n=120) were donated by Ontario Ministry of Natural Resources and Forestry fish culture facility in Chatsworth, Ontario. Trout were transported by road in 8-12 °C continuously aerated water in an insulated transport tank to the Freshwater Restoration Ecology Center (FREC) in LaSalle, Ontario for housing and experimental trials. While at FREC, fish were held in circular 850 L tanks connected to a recirculation system that continuously filtered, cleaned, and aerated the water (municipal water source) whose temperature was also regulated by a thermostat-controlled chiller. Rainbow trout were housed at 14.0±1.0 °C. Lake trout were housed at 11.0±1.0 °C. Dissolved oxygen levels and pH were monitored daily
with a handheld device and kept at ≥ 85% air saturation, and pH of 7.0 in the holding tank. Food was withheld for 48 h prior to use in respirometry trials or surgery but otherwise fish were fed once daily with EWOS 1.5 mm pellet (Cargill Inc., Minneapolis, MN; https://www.cargill.com/). The lighting in the building was automatically programmed to track the natural photoperiod.

2.2 Treatment groups and experimental design

All fish except controls were individually PIT tagged in the body cavity to track individual performance using a sterilized Biomark MK165 injecting syringe with N165 needle (Biomark mini HPT8 passive-integrated tags (8.4 mm in length; 0.032g in mass), Biomark, Boise, ID; https://www.biomark.com/). Insertion of PIT was on the left side of the fish off the mid-ventral line at the tips of the pleural ribs (between the pyloric caeca and the pelvic girdle). Inter-muscular tagging (dorsal or pelvic) is only recommended for fish > 250 mm, while body cavity tagging is suitable for fish 55-250 mm in size (Biomark, Boise, ID). PIT were inserted into the tissue surrounding the ventral fins on the left side of the fish. Treatments consisted of: 1) control fish to which nothing was done beyond monitoring survival, growth, swimming performance, and oxygen consumption rates (“control”, n=30), 2) PIT tagged (“PIT”, n= 24-30), 3) fish subject to a sham surgery (i.e., anesthetic, incision, and sutures but no acoustic tag inserted into the body cavity) (“sham”, n= 25-30), and 4) acoustic-tagged (“tagged”, n=30). The acoustic tags (Vemco model V5 (12.7 mm long, 0.67g in air) or V6 (16.5 mm long, and 0.97g, Amirix Corporation, Bedford, Nova Scotia; https://vemco.com/) were surgically inserted into the intracoelomic body cavity in the case of the acoustic tag treatment (#4; further details in section 2.3, below). The individual tag burden for both species ranged from ~ 1.0-7.5% of body mass (mass of tag in air/initial mass of fish at surgery) (similar to several previous experiments, e.g., Anglea et al. 2004, Chittenden et al. 2009, Brown et al. 2010, Larsen et al. 2013, Collins et al.)
Tag burden ratios will change as the fish grows (i.e., tag burden will decrease as fish mass increases). It is standard practice in the literature to report tag burdens based on initial mass at the time of surgery. Fish were held in a single tank divided into four sections with mesh screens (one for each treatment) to reduce the likelihood of tank effects. For rainbow trout, each treatment group was housed in the same section throughout the trials. For lake trout, each treatment group was moved to an adjacent section (i.e., clockwise) of the holding tank every two weeks. Fork length and weights of all fish were measured every two weeks without anesthesia (i.e., 2, 4, 6, and 8 weeks), which involved brief handling and air exposure (< 30 s). The rainbow trout experiment occurred between November 23, 2017 and January 18, 2018. For lake trout the experiment was carried out from April 26 to June 7, 2018.

2.3 Surgical implantation of transmitters

The methods used here are standard for insertion of acoustic transmitters into fish (Summerfelt & Smith 1990; Wagner et al. 2011; Liedtke et al. 2012, Rub et al. 2014). Acoustic tags, PIT to be implanted, and surgical equipment were sterilized in betadine solution and rinsed with clean deionized water prior to implantation. All fish were anesthetized using 100 mg·L⁻¹ MS-222 (tricaine methanesulfonate, a.k.a. TMS; buffered with sodium bicarbonate at a ratio of 2:1) and monitored until opercular movements slowed and fish lost response to gentle physical stimuli. Fish were placed on their back in a v-shaped trough for surgery, during which their gills were continuously irrigated with a well-aerated maintenance dose of anaesthetic (buffered MS-222, 30 mg·L⁻¹). A ~ 1.5 cm incision was made at the abdominal midline towards the posterior of the fish, but anterior to the anus using a number 11 scalpel blade. The tag (transmitter) was then removed from a betadine solution and rinsed with deionized water before being inserted into the abdominal cavity. The incision site was then closed using 2 simple interrupted 5–0 Ethicon
Vicryl Plus® absorbable sutures (2-1-1-1 surgeon knot sequence) with RB-1 tapered needle (Ethicon, Cincinnati, OH; https://www.ethicon.com/) at 0.5 cm intervals along the incision line. Fish were monitored in small, well aerated containers of water from the holding tank (same temperature) for post-surgical recovery for up to 1 hour before being returned to their holding tank. The average time individual fish were on the surgery bench was 4.5±0.3 minutes. Survival and tag retention were assessed throughout the entire 8-week trial period. The holding tank was inspected daily for mortalities and/or tag loss. Growth measurements (mass and Lₚ) were taken every 2 weeks.

2.4 Experimental timeline

Fish were organized into two tanks (one for general holding and feeding, and another experimental tank, where no feeding occurred). Fish were transferred to this secondary holding tank 48 hours prior to any respirometry or swim flume trials. A group of fish including individuals from each treatment (i.e., control, PIT, sham, and acoustic-tagged) were transferred to intermittent-flow respirometer chambers for ~ 24 hours of automated measurement of oxygen uptake (~ two measurements per hour) in order to assess impacts of the treatment relative to the control (Loligo Systems, Denmark; http://www.loligosystems.com). All eight respirometry trials ran simultaneously, (7 fish and one empty chamber as a blank, including one control, two PIT, two sham, and two acoustic-tagged fish. The blank chamber was used to correct for background respiration in the system ensuring precise estimates of oxygen consumption (Rodgers et al. 2016). The blank chamber and location of treatment fish amongst the chambers were randomized for each trial. Total sample sizes for respirometry were as follows: 172 rainbow trout; 48 fish per treatment (i.e., PIT, sham, acoustic-tagged), 208 lake trout; 60 fish per treatment, and 27-29 fish for control group. The respirometry trials occurred from ~ 2 hours to 35 days after surgical
procedure. Following each respirometry trial, a series of measurements were taken (L_F and mass), and the fish were returned to the holding tanks.

Swimming performance was assessed in a randomly selected subset (n=15 control and n=15 acoustic-tagged) from the same group of fish as the respirometry trials above, and individually transferred to a 30 L Brett-style swim tunnel to assess swimming performance (Loligo Systems, Denmark; http://www.loligosystems.com). Once respirometry and swim trials were complete (30 and 35 days post-surgery for rainbow trout and lake trout, respectively), all fish were euthanized with an overdose of anesthetic (buffered MS-222).

2.5 Respirometry

Tag burden studies that aim to evaluate fish and stress physiology usually examine the levels of cortisol or some other stress hormone in the plasma of the fish as an indicator of impairment or dysfunction (Smircich & Kelly 2014). As far as we know, intermittent-flow respirometry has not previously been used to identify physiological consequences of acoustic tag burden. This represents a novel approach for evaluating stress physiology following intraperitoneal implantation of acoustic tags in juvenile fishes. Respirometry is a general term used to describe various methods related to estimating metabolic rates in vertebrates and invertebrates (i.e., measurement and analysis of respiration). These techniques can provide valuable information about thermal biology, metabolic trade-offs regarding performance, interactive effects of feeding and exercise on oxygen consumption, and stress physiology. Metabolic rates may be responsive to stress or, in this case, wound healing related to surgery (Alsop & Wood 1997; Clark et al. 2011; Allen et al. 2016).
The respirometry setup consisted of eight individual chambers that were submersed in 200 L tank of aerated water ~ 11-14 °C (dependent on species acclimation temperature). The tank was bleached, rinsed, and drained bi-weekly to minimize bacterial respiration. Respirometry trials involved placing individual fish into sealed, clear, polypropylene chambers (~ 3.1 L in volume) that were submerged in the holding tank. Each chamber had two sets of tubes for (flush and recirculation) of water. The flush tubes allowed well-mixed, aerated water from the ambient tank to be pulled into the chamber at a rate of 12.6 L·min\(^{-1}\), and back out into the tank through an open hose which was elevated above the water surface. Every 28 minutes, the flush line was turned off for 16 minutes, effectively sealing the chamber, to allow a decline in oxygen to occur and be measured. A separate recirculating pump continuously pulled water from the chamber over an optical oxygen sensor (PyroScience, Aachen, Germanyhttp://www.pyro-science.com/), and then back into the chamber, ensuring that the water in the chamber remained well-mixed. Sealed cycles were programmed so that oxygen in the water remained > 80 % air saturation (typically O\(_2\) > 6-9 mg·L\(^{-1}\)). Oxygen sensors were re-calibrated before each new trial (new set of fish). Fish were held in respirometry chambers for 20 to 24 hours, resulting in ~ 40-50 measurements on each fish. One of eight respirometry chambers was kept empty (no fish) at all times so that background (microbial) respiration could be measured. In addition, each chamber was left empty for one measurement (sealed) cycle before and after each fish. Respiration (i.e., a decline in oxygen content during sealed cycles) in the background chamber was used to dynamically adjust oxygen consumption estimate for the fish in the other chambers (by subtracting it; see Rodgers et al. 2016). The mean of the lowest 5 measurements was used to estimate resting \(\dot{MO}_2\) (mass-specific rate of oxygen consumption). \(\dot{MO}_2\) (mg·O\(_2\)·kg body mass\(^{-1}\)·min\(^{-1}\)) was calculated with the following formula (Steffensen 1989):
\[ y = (V_{RE} \cdot W_o^{-1}) \times (dCO_2 \cdot dt^{-1}) \]  

[Equation 1]

where \( V_{RE} \) is the effective respirometer volume (L), \( W_o \) is the mass (kg) of the fish, and \( dCO_2/dt \) is the slope of the linear decrease in oxygen content (measured in mg-O_2·L\(^{-1}\)) during the time (min) the chamber is closed (Svendsen et al. 2016).

2.6 Swimming performance

Swimming performance is an important biological characteristic and can be related to predator avoidance and feeding ability (Anglea et al. 2004; Perry et al. 2013; Walker et al. 2016). \( U_{crit} \) is a standard and commonly used performance metric to evaluate swimming performance in fishes (Jain & Farrell 1998; Farrell 2008). For swimming performance trials in this study, the swim tunnel was continuously flushed with fresh water at the fish’s acclimation temperature. Fish were placed in the working section of the swim tunnel (rapid transfer using a dipnet, < 10 s air exposure) and allowed to recover for 45 minutes at a water speed at ~ 0.5 body lengths per second (BL·s\(^{-1}\)) (minimal effort required to hold station in swim tunnel). Each fish then completed a practice swim during which the speed was slowly increased up to 40 cm/s (~ 3-4 BL·s\(^{-1}\)) over 3 minutes, and the fish was then encouraged to continue swimming at that speed for an additional 15 minutes (Lee et al. 2003). After another 45-minute rest period (Jain et al. 1998), the \( U_{crit} \) swim was started. It involved gradually ramping the speed up to ~ 60% of expected \( U_{crit} \) (based on pilot test trials with earlier fish) over the course of 10 minutes and using that speed (46 cm/s) as the initial 20-minute conditioning interval. Speed was then ramped up in a sequential fashion in steps of 6 cm/s (~ 0.5 BL·s\(^{-1}\)) every 20 minutes. The front of the working section of the swim tunnel was darkened with black plastic, and a light shone on the downstream end to encourage the fish to remain at the front. A metal grid at the downstream end of the working section was occasionally electrified (8 V) for 1-2 seconds to motivate the fish to swim and
prevent it from resting against it. If the fish remained on the back grid for more than 10 seconds (despite attempts at motivation using shocks) or was only able to resume swimming for ≥ 20 second periods before resting on the downstream grid, the swimming trial was ended, and the time noted.

2.7 Statistical analysis

2.7.1 Data processing/analyses

2.7.2 Growth

PIT tagging enabled estimation of individual fish growth rates for fish in the PIT, sham, and acoustic-tagged treatment groups. Specific growth rate (SGR, %·d⁻¹) was calculated as:

\[
\text{(SGR), } \% \text{ day}^{-1} = \left[ \frac{\ln W_2 - \ln W_1 \times 100}{t_2 - t_1} \right]
\]

where \( W_2 \) and \( W_1 \) were the weights (g) of the fish at time \( t_2 \) and \( t_1 \) (d). Growth measurements were taken every two weeks for each species, equating to three observations for rainbow trout and four for lake trout. PIT ID enabled tracking individual fish growth in all but the control treatment (no PIT). A random subset of control fish (n=15) was measured at each time point; these data were used to provide an estimate of the mean growth rate for control fish for each time interval and are presented alongside the growth data for the other groups. The growth rates for mass and \( L_F \) were transformed into % per day, as a function of the initial mass and \( L_F \).

Exploratory analyses revealed notable differences in growth rates between species and among time intervals, so to standardize growth rates and ensure variance was heterogeneous across species/times, growth rates were converted to Z scores for statistical analyses, based on the mean and standard deviation for growth for that species and time interval (across the three tagging treatments – acoustic-tagged, sham, PIT). To test for treatment effects of specific growth rate
(mass and fork length assessed in separate tests), I used linear mixed effects (LME) models with treatment, time since surgery, and body mass as fixed effects, with individual fish ID as a random effect. Likelihood ratio tests were used to assess the overall significance of each fixed effect to model fit by comparing AIC (Akaike Information Criterion) scores among nested models (i.e., with and without each predictor variable).

2.7.3 Oxygen consumption ($\dot{MO}_2$)

Resting metabolic rate was estimated from the mean of the lowest five $\dot{MO}_2$ measurements per individual per trial, which represented, on average, ~ 10-12% of the total $\dot{MO}_2$ measurements. Using the minimum estimates would more closely represent resting metabolic rate where differences among individuals and treatments would reflect differences in stress or healing rather than spontaneous activity that may occur within the respirometer. Each species was analysed separately using LME models with treatment, time since surgery, and body mass as fixed effects and individual fish ID as a random effect to test for treatment effects on resting metabolic rate. A separate analysis included tag burden for the acoustic-tagged treatment group only.

2.7.4 Swimming performance

$U_{crit}$ (L·s$^{-1}$) was estimated from:

$$U_{crit} = U_f + [(T_f/t) \times U_v]$$  \hspace{1cm} [Equation 3]

where $U_f$ is the speed (cm/s) of the last interval swam before fatigue, $T_f$ is the duration (s) the fish swam at the final velocity before fatigue, $t$ is the length of time (1200s) at each speed increment at that velocity and $U_v$ is the velocity increment (5cm·s$^{-1}$) used throughout the test. (Brett 1965; Tierney 2011). Results were analysed using LME models with species, treatment,
time since surgery, and condition as fixed effects to test for treatment effects on swimming performance \((U_{crit})\). Species was used as an effect in this model because it was clear from visualizing the data that the rainbow trout achieved higher swimming speeds than lake trout. A species-specific condition index was used in place of body mass because the latter was strongly correlated with species (the rainbow trout had higher mass-at-length). To ensure standardization across species, condition was calculated as the regression residual for each fish relative to the line of best fit for the relationship between fork length (mm) and body mass (g) for each species. Likelihood ratio tests were used to assess the overall significance of each fixed effect to model fit by comparing nested models (i.e., with and without each predictor variable).

2.7.5 Overall modelling details

All statistical analysis and modelling were conducted using R version 3.0.1 (R Development Core Team (2012). Labchart reader 8.1.9 software was utilized to analyze and isolate slopes of oxygen decline (Adinstruments, Colorado Springs, CO; https://www.adinstruments.com/). To minimize the likelihood of type I error, \(\alpha\) was set to 0.008 (0.05 / 2 species = 0.025; 0.025 / 3 fixed effects – treatment, time since surgery, body size).

Results

3.1 Survival and growth

Survival for both species was 100% in control fish, 97-100% for PIT and sham treatments (100% in rainbow trout and 97% in lake trout), and 88-97% for acoustic-tagged fish (88% in rainbow trout and 97% in lake Trout) (See Table 2.1 for details). Each of the lake trout mortalities was related to a non-treatment event: two fish got caught in the respirometer outflow and one got stuck under the tank divider. The sole rainbow trout mortality is unexplained. The
PIT group (p = 0.31) and sham surgery groups (p = 0.87) did not differ in Lₕ growth (across species) from the acoustically tagged fish (Table 2.2). However, there was a main effect of species (higher growth in rainbow trout, p = 0.006), and an interaction with the PIT treatment whereby the PIT-tagged rainbow trout grew slower than did the other two treatments (p < 0.001; Fig. 2.1). Body mass of rainbow trout was lower in days 13-27 (for clarification, this is compared to control fish). For body mass, there was a weak (p = 0.044) overall effect of treatment on model fit, Lₕ with the sham group (across species) tending to exhibit lower growth rates compared to acoustically tagged fish (p = 0.02; Fig. 2.1) (Table 2.3).

3.2 Metabolic rate and swimming performance

For rainbow trout the best model (lowest AIC) for resting $\dot{M}O_{2}$ only included an overall effect of treatment (See Table 2.4), but none of the individual treatment-to-treatment differences were significant within the model. There was a tendency for higher $\dot{M}O_{2}$ in the acoustic-tagged rainbow trout group compared to control fish, but the effect of tagging treatment was not significant (p = 0.024). Likewise, $\dot{M}O_{2}$ tended to increase with time since surgery in the acoustic-tagged group, but the effect was not significant (p = 0.011) (Figure 2.2).

In lake trout, the best model included body mass, treatment, and their interaction (Table 2.5) with $\dot{M}O_{2}$ decreasing with body mass in all treatments, but the effect was not significant (p = 0.011), except in the PIT group where it increased with body mass (Figure 2.3). However, separately examining the relationship between body mass and $\dot{M}O_{2}$ for each group revealed the only significant relationship occurred in the control fish (linear regression; Figure 2.3). The acoustic-tagged lake trout $\dot{M}O_{2}$ was not significantly different from the controls or the other treatments (p > 0.05). Within the acoustic-tagged group (n = 28 unique individuals, n = 53 resp
trials), tag burden (p = 0.48), time since surgery (p = 0.28), and their interaction (p = 0.72) had no effects on resting metabolic rate.

Every rainbow trout I tested had a higher $U_{crit}$ than fasted lake trout (p < 0.001) (Figure 2.4). For both species $U_{crit}$ was lower in acoustic-tagged fish but the treatment differences were not significant. (p = 0.024) (Table 2.6).

Discussion

This study found negligible effects of acoustic tagging on survival, growth, resting $\dot{M}O_2$, and $U_{crit}$ for juvenile rainbow trout and lake trout. Growth and survival were not statistically different between controls and acoustically-tagged fish. For swimming performance ($U_{crit}$), there was a numerical decline of 3-10% in performance in the tagged fish (vs. controls) in both species, but overall the effect of tagging was only marginally significant. In resting metabolic rate ($\dot{M}O_2$) there were no main effect differences, and one interaction whereby in rainbow trout there was a tendency for higher oxygen consumption with increasing time since surgery but only in the acoustically-tagged fish. Within the acoustically-tagged group, tag burden (as a continuous variable, ranging from 1-7.5%) did not have significant effects on any of the responses we measured. Overall, my results suggest that acoustic tagging with a tag burden in the range of 1-7.5% may not have substantial fitness impacts for juveniles of either species; further replication would be needed to confirm the subtle, context-specific effects we did find, and to clarify their ecological relevance.

There were small variations in specific growth rates, swimming performance, and resting metabolic rate. These differences are most likely related to individual variation in size, metabolic
rate, and/or species biology and physiology. Body size and water temperature have a major impact on metabolic rate in fishes (Brett 1965; Tang & Boisclair 1995; Gillooly et al. 2011).

Tag burden, growth, and survival

Tag burden greater than 12% in juvenile rainbow trout has been shown to have a negative effect on growth and survival (Welch et al. 2011). Dummy acoustic tags (8 mm diameter x 24 mm long; 1.4 g) with a PIT (12 mm) embedded inside were implanted in the body cavity of rainbow trout pre-smolts (L_F 110-170 mm). The authors suggested that current acoustic tag sizes can be implanted in juvenile salmonids greater than or equal to 120 mm L_F, however cautioned that fish in the 120-130 mm size range experienced combined tag loss and mortality rates of 33-40% over a 7-month period. Fish in the 140-150 mm size range experienced tag loss and mortality rates of less than 15% and growth rates after surgery were close to control fish indicating that an acoustic tag burden of ~ 3-4% has a negligible effect on tag loss and survival. The rainbow trout and lake trout in my study experienced no tag loss during the 8-week experimental trial period. My acoustic tags were smaller (~ 1.0 g) and generated a smaller tag burden than those used by Welch et al. (2011) (~ 1.4 g), and thus the tag burden (%) was lower. The rainbow trout in my study experienced an acoustic tag burden of 1.0-5.3% and the lake trout experienced a tag burden of 2.4-7.5%. My results indicate that these tag burdens are suitable for tagging juvenile salmonids.

The growth rates for both species in my study were comparable to those reported in the literature (i.e., similar fish sizes, water temperatures, and food availability) (Eschmeyer 1964; Stewart et al. 1983; Gregory & Wood 1999). The rainbow trout in my study experienced a specific growth rate (SGR) of ~ 0.4-2.0 (% BM·day\(^{-1}\)), and the lake trout experienced a SGR of ~ 0.1-0.4 (% BM·day\(^{-1}\)). The rainbow trout may have experienced somewhat higher SGR because
they were held in warmer temperatures (14 vs 11 °C). These temperatures are near the reported preferred temperatures for each species (McCauley & Tait 1970; Hokanson et al. 1977). The rainbow trout acoustic-tagged, sham, and PIT treatment groups exhibited similar trends which suggests that surgical procedures and/or PIT injection may cause short-term effects (i.e., 8-week study period) on growth rates (i.e., less than control fish) in juvenile salmonids. There were no significant growth effects within the lake trout treatment groups.

I saw similar growth trends in my acoustic-tagged fish compared to control fish (i.e., differences in growth rates) which is consistent with other studies. A previous study by Gregory & Wood (1999), illustrated differences in growth rates of juvenile rainbow trout (e.g., 5.23-5.73 grams in size) based on feeding rations (i.e., 0.5-2.0%·day⁻¹). The overall specific growth rates for the four treatment groups were 0.11-1.79 (% body mass BM·day⁻¹) (Gregory & Wood 1999). My fish were fed ad libitum (~ 1.0 BM·day⁻¹) and experienced similar growth rates even though my rainbow trout were approximately twice the size. A study by Martinelli et al. (1998) reported that relative growth rates (% BM·day⁻¹) for juvenile Chinook salmon (i.e. L₉ 100 mm–154 mm) were ~ 1.3-1.9%, and that growth rates for treatment groups that underwent surgery had similar values to control fish initially, but after three weeks, the surgery group had significantly lower growth. The lower growth rates are presumably linked to wound healing and potentially inflammation associated with surgery and/or sutures.

The average annual growth rate for wild juvenile lake trout at preferred water temperature (i.e. 10 °C) is approximately 7.62 cm per year (i.e. (SGR), length % day⁻¹ = ~ 0.56), and 200 grams per year (i.e. (SGR), mass % day⁻¹ = ~ 1.45 for juvenile fish 1 year after stocking) (Eschmeyer 1964; Stewart et al. 1983). The lake trout used in my study were of similar age but experienced slightly poorer growth. Differences from other studies could be explained by genetic
differences, differences in the food, differences in the temperature, or something else about the environment that affected the propensity of the fish to feed (e.g., noise, light, non-lethal pathogens). In the natural environment different species are known to exhibit variability in growth rates. This variability is usually the result of environmental constraints such as resource availability (i.e., mainly food and temperature) (Filbert & Hawkins 1995). It is also possible that certain individuals are naturally more aggressive and active, and thus able to obtain more food. Studies have shown that individuals of the same species that are cared for in a lab environment and are supplied with equal food allocation can also display differences in growth rates most likely due to genetic differences or individual fitness (Sumpter 1992; Mangel & Stamps 2001; Johnston & Bennett 2008). The difference in growth rates between the lake trout and rainbow trout can most likely be attributed to higher water temperatures with the rainbow trout, plus they have a different life history with associated differences in natural growth rates, feeding rates, digestive physiology, bioenergetics, etc. – lake trout are slower growing and mature later (Scott & Crossman 1973).

The rainbow trout in my study experienced an acoustic tag burden of 1.0-5.3% and the lake trout experienced a tag burden of 2.4-7.5%, which fall into the typical range used previous studies of tagging effects, most of which aim to challenge the “2 % rule” (Ivasauskas et al. 2012; Sandstrom et al. 2013; Makiguchi & Kojima 2017). Juvenile rainbow trout housed at 10-15 °C and with a tag burden of 1.1-3.4% experienced no mortalities or significant effects on growth, although dummy-tagged fish did exhibit slower growth than control fish in one experiment (Ivasauskas et al. 2012). Sandstrom et al. (2013) reported no significant differences in growth (mass or L_F) among the control or dummy-tagged treatment groups) (i.e., Vemco V7 (1.3-2.3% tag burden) or V9 (3.4-6.6% tag burden) in juvenile steelhead trout (L_F 180-225 mm and 71.0-
141.0 grams in size). Additionally, there were no significant differences found in the tag retention rate in relation to tag burden over the duration of the study. Finally, a study by Makiguchi & Kojima (2017) suggested that tag burden ratios > 3% in juvenile and adult rainbow trout had short term negative effects on feeding behaviour, and that fish with a tag burden of ~ 6.0% were expected to have a 10% poorer survival rate than in controls (survival rate was negatively correlated with tag burden, fish mass however was not). The authors also reported no effects on physiological indicators of stress (i.e., plasma lactate levels) and concluded that a tag burden of 2% is likely conservative and suitable for adult and juvenile rainbow trout.

Domesticated fish are typically artificially selected for rapid growth and aggressive feeding, whereas the lake trout possess more “wild” traits, and so should be more cautious when it comes to foraging. The rainbow trout in my study were obtained from a commercial fish hatchery, and the lake trout were sourced from a Provincial fish hatchery, which means there could be species differences related to genetics, exposure/experience and life history traits. The rainbow trout were larger (i.e., mass and fork length) in general and had a higher condition factor than the lake trout. Condition factor (i.e., the relative health/robustness of fish) is closely correlated with growth rate (e.g., Martinelli et al. 1998). It is possible that these intrinsic morphological differences between the two species could have affected results. The lower growth rates in the rainbow trout PIT treatment group relative to the control group could potentially be due to some type of quadrant-based tank effect or experimental artefact. The rainbow trout PIT group always occupied the tank quadrant where the aeration and water inflow were situated. Because of this, the PIT treatment group possibly altered environmental conditions and potentially food availability. Waterflow and aeration were turned off during feeding, however, there was still some movement of food pellet out of that quadrant. The lake trout were
moved from quadrant to quadrant in a clockwise fashion every two weeks when growth measurements were taken, whereas the rainbow trout were not.

*Resting metabolic rate*

I saw differences between species, but within a species my data are comparable to other previously published results. Water temperature and body size are highly correlated with oxygen consumption rates (Gibson & Fry 1954; Fry 1971; Cossins & Bowler 1987; Norin & Malte 2011). Thus, you would expect the $\dot{M}O_2$ of either species to increase with elevated temperatures. The rainbow trout in my study were housed at ~ 14 °C and the lake trout at ~ 11 °C. There is a strong allometric (body size) effect on metabolic rate so as fish get larger the specific (i.e., weight adjusted mg-O$_2$·kg$^{-1}$·min$^{-1}$) rates should decrease. There was an overall tendency for an increase in $\dot{M}O_2$ over time in the acoustic -tagged group for rainbow trout but not for lake trout. This could be an indicator that healing is occurring in the acoustic-tagged group, or that the acoustic tag was increasingly causing stress (e.g., via infection) in the acoustic-tagged group over time. The lake trout model suggested that body mass and treatment had the most significant interaction, indicating decreasing $\dot{M}O_2$ rates associated with body mass in the control group, and that this negative body-mass relationship was significant in the PIT group. It is unclear what caused these disparate effects of body mass, which were generally weak (low $R^2$), and these group-specific relationships were not replicated in the rainbow trout experiment.

The absolute rates of oxygen consumption in my study were consistent with values from the literature for both species. The $\dot{M}O_2$ for the juvenile rainbow trout (1.0-3.8 mg-O$_2$·min$^{-1}$·kg$^{-1}$) in my study were comparable to oxygen consumption rates found in the literature (Rao 1968; Alsop & Wood 1997). A study by Alsop & Wood (1997), found that juvenile rainbow trout (i.e., 6-12 grams in size) fed to satiation (which would be expected to elevate $\dot{M}O_2$ significantly
because of specific dynamic action) had an oxygen consumption rate of 2.1-3.7 mg-O\textsubscript{2}·min\textsuperscript{-1}·kg\textsuperscript{-1}. While another similar study reported that juvenile rainbow trout (i.e., ~ 23-196 grams) housed at 5-15 °C, and fasted for < 30 hours prior to measurements, had resting oxygen consumption rates of 0.95 mg-O\textsubscript{2}·kg\textsuperscript{-1}·min\textsuperscript{-1} at 5 °C, and 1.9 mg-O\textsubscript{2}·kg\textsuperscript{-1}·min\textsuperscript{-1} at 15 °C (Rao 1968). My study was designed to evaluate acoustic tag burden effects on $\dot{M}O_2$. I found no significant effect of treatment on $\dot{M}O_2$, and therefore my results should be comparable to other studies where no tagging or surgery occurred.

The $\dot{M}O_2$ for the lake trout (1.2-2.8 mg-O\textsubscript{2}·kg\textsuperscript{-1}·min\textsuperscript{-1}) in my study were consistent with oxygen consumption rates found in the literature (Gibson & Fry 1954; Stewart et al. 1983; Beamish et al. 1989). A study by Beamish et al. (1989), determined average resting oxygen consumption or (= metabolic rate) (RMR) for juvenile lake trout (10-20 grams) housed at 10 ±1 °C to be ~ 1.8 mg-O\textsubscript{2}·kg\textsuperscript{-1}·min\textsuperscript{-1}. Gibson and Fry (1954) reported a lower RMR of 0.78 mg-O\textsubscript{2}·kg\textsuperscript{-1}·min\textsuperscript{-1} for lake trout housed at 10 °C. A higher value for RMR of lake trout (i.e., 2.3 mg-O\textsubscript{2}·kg\textsuperscript{-1}·min\textsuperscript{-1}) was predicted from a regression relating metabolism, body weight, temperature and swimming speed (Stewart et al. 1983). The correlation between mass and oxygen consumption was evident in juvenile lake trout from my study, and that relationship was significant in control fish. There are no previous studies evaluating tag burden effects on juvenile lake trout oxygen consumption.

There are natural tendencies for larger fish to consume more oxygen (mg-O\textsubscript{2}·min\textsuperscript{-1}) (i.e., in absolute terms, larger fish consume more oxygen than small fish), but on a weight-specific basis (mg-O\textsubscript{2}·kg\textsuperscript{-1}·min\textsuperscript{-1}) small fish consume more oxygen than large fish, and therefore have higher $\dot{M}O_2$ rates (Rao 1968; Brett 1972). Previous studies have indicated a strong correlation between swimming performance and oxygen consumption, such that increased swim
performance is highly correlated with higher oxygen consumption rates (Brett 1965; Rao 1968). Thus, the higher $U_{\text{crit}}$ and resting oxygen consumption rates for rainbow trout could be related to warmer acclimation temperatures and the slightly larger size of the rainbow trout relative to the lake trout. However, individuals with a naturally high resting metabolic rate may also be more efficient and robust swimmers (Rosenfeld et al. 2015; Allen et al. 2016).

**Swimming performance**

My study found that an acoustic tag burden of ~ 2-6% had no significant effect on the swimming performance of juvenile rainbow trout (13-36 g and 105-150 mm, in fork length). Brown et al. (1999) reported that a tag burden of 6-12% in juvenile rainbow trout (5-10 g), did not alter swimming performance in sham or dummy-tagged treatment groups. There were no significant relationships between mass of the fish and $U_{\text{crit}}$ among treatment groups and the authors suggested that a different metric be used for future tag burden studies (i.e., volume of tag and/or weight of tag in water). Chinook salmon (*Oncorhynchus tshawytscha*) (6.7-23.1 g) with a tag burden of 3.1-10.7% were found to have a $U_{\text{crit}}$ of 4.3-4.7 L·F·s⁻¹ (47.5-51.2 cm·s⁻¹), and no difference was found in swimming performance or growth rates between control, sham, and dummy-tagged fish (Brown et al. 2006).

The $U_{\text{crit}}$ values for both species in my study were comparable to the $U_{\text{crit}}$ range found in the literature for juvenile rainbow trout and lake trout (Rao 1968; Alsop & Wood 1997; Burden et al. 1998; Gregory & Wood 1999; Katopodis & Gervais 2016). Rainbow trout in my study were able to maintain swimming speeds of 6.0-7.8 L·F·s⁻¹ or 74.5-92.0 cm·s⁻¹ and lake trout in my study were able to maintain swimming speeds of 3.1-5.1 L·F·s⁻¹ or 43.0-66.7 cm·s⁻¹. Based on a review of fish swimming performance by Katopodis & Gervais (2016), the average $U_{\text{crit}}$ for rainbow trout (average total length of 116 mm, range: 22-420 mm) at ~ 11.8 °C is 43.6 cm·s⁻¹ or
3.8 L_F·s⁻¹. Rainbow trout with a mean body mass of 2.59 g and total length of 59.7 mm, were
defined to have a \( U_{crit} \) value of \( \sim 71.1 \text{ cm·sec}^{-1} \) or 11.9 BL·s⁻¹ (Burden et al. 1998). A study by
Gregory and Wood (1999), identified rainbow trout (e.g., 5.23-5.73 grams in size) absolute \( U_{crit} \)
(cm·s⁻¹) to be 28.37-44.21, and relative \( U_{crit} \) (BL·s⁻¹) to be 4.23-3.42 based on four different
feeding regimes (e.g., 0.5- 2.0 % BM·day⁻¹). My fish were fed ad libitum (~ 1.0 BM·day⁻¹) but
fasted for 24-48 hours prior to any swim trial. Alsop & Wood (1997) found that juvenile rainbow
tROUT (i.e., 6-12 grams) had a \( U_{crit} \) of 3.0-10.0 L_F·s⁻¹, and an earlier study conducted by Rao
(1968) reported that juvenile rainbow trout (i.e., ~ 30-150 grams; no L_F were listed in the paper)
can maintain swimming speeds of 57.5-72.7 cm·s⁻¹. Finally, Farrell (2008), suggested that
juvenile rainbow trout housed at water temperatures of 9.0-11.0 °C can maintain critical
swimming speeds of ~ 1.0-1.5 L_F·s⁻¹ or 40-70 cm·s⁻¹. The average swimming speed of adult lake
trout tracked in Opeongo Lake, Ontario was 13.0-19.1 L_F·min⁻¹ or 0.22-0.32 L_F·s⁻¹ in the spring
and summer of 2001 (Janoscik 2001). This \( U_{crit} \) value is much lower because these values are
from adult fish (i.e., < 250mm) (larger fish have lower \( U_{crit} \) relative to size). Beamish et al.
(1989), reported \( U_{crit} \) values of ~ 76.5-95.4 cm·s⁻¹ for juvenile lake trout (122-129 mm total
length). Based on a review of fish swimming performance by Katopodis & Gervais (2016), the
average \( U_{crit} \) for lake trout (average total length of 181 mm, range: 115-225 mm) at ~ 12.1 °C is
85.6 cm·s⁻¹ or 4.7 L_F·s⁻¹. These critical swimming speeds are slightly higher than the \( U_{crit} \) values
for my lake trout, however the fish used in my swimming trials were larger in size. Regarding
swimming performance, there is a large degree of natural variation within species and
individuals (Tierney 2011). The feeding regimes, water temperatures, and fish sizes in my study
were comparable to the above-mentioned studies.
Although there was a tendency towards decreased swimming performance in acoustic-tagged individuals in comparison to controls for both species, the final model did not include the treatment effect, and instead utilized a species effect, as the differences in $U_{crit}$ between rainbow trout and lake trout are most likely species-related. Lower $U_{crit}$ performance in lake trout is most likely related to life history traits and physiology (i.e., lake trout do not undergo tributary spawning migration like rainbow trout do, and lake trout tend to occupy colder water). The results from my tagging study suggest you can acoustically tag small fish across a range (~ 2-6% tag burden) with no significant effect on $U_{crit}$.

Conclusion and implications

There are many reasons to continue to pursue and advance acoustic telemetry techniques as a tool for species restoration and monitoring. Identifying ideal stocking rates and targeting specific release locations for native species restoration purposes provides a challenge for stocking agencies and captive husbandry facilities such as fish hatcheries (Seddon et al. 2007; Ogburn et al. 2017). Juvenile mortality and lack of natural reproduction are considered major threats to restoration or stocking programs (Ersbak & Haase 1983). Measuring the success or survival of the juveniles involved in these stocking programs could be facilitated by acoustic telemetry (Pincock et al. 2010). Stocking programs continue to be the most common strategy for restoring and rehabilitating native fish populations (U.S. Fish and Wildlife Service and Great Lakes Fishery Commission 2010; Wehse et al. 2017). The results from this acoustic tagging study provide evidence juvenile salmonids can be implanted with acoustic transmitters (~ 2-6% tag burden by mass) with negligible effects on survival, growth, resting metabolic rate (RMR), and swimming performance ($U_{crit}$).
References


Makiguchi, Y., & T. Kojima. 2017. Short term effects of relative tag size and surgical implantation on feeding behaviour, survival rate, plasma lactate and growth rate in juvenile to adult rainbow trout (Oncorhynchus mykiss). Fisheries Research, 185: 54-61.


Steffensen, J.F. 1989. Some errors in respirometry of aquatic breathers: how to avoid and correct for them. Fish Physiology and Biochemistry, 6: 49-59.


Table 2.1 Fork length, mass, tag burden, survival, and number of fishes for each treatment group (control, PIT, sham, and dummy-tagged), of juvenile rainbow trout and lake trout used in experiments. Treatments consisted of control fish to which nothing was done beyond monitoring growth and physiology (“control”, n=15), PIT tagged (“PIT”, n= 24-30), subject to a sham surgery (incision and sutures but nothing inserted into the body cavity) (“sham”, n= 25-30), and acoustic-tagged (“tagged”, n=30). The acoustic tags were either Vemco model V5 (12.7 mm long, 0.67g in air) or V6 (16.5 mm long, and 0.97g in air) (Amirix Corporation, Bedford, Nova Scotia).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rainbow trout</th>
<th>Lake trout</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control Fish (n=)</strong></td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>(L_F) (mm)</td>
<td>113-172</td>
<td>122-171</td>
</tr>
<tr>
<td>(Mean\ L_F) (mm) ((\pm) SD)</td>
<td>136.91 +/- 13.58</td>
<td>140.36 +/- 12.92</td>
</tr>
<tr>
<td>Mass (g)</td>
<td>15.91-66.01</td>
<td>17.01-48.73</td>
</tr>
<tr>
<td>(Mean\ Mass) (g) ((\pm) SD)</td>
<td>30.79 +/- 10.55</td>
<td>27.26 +/- 6.78</td>
</tr>
<tr>
<td>Tag Burden (%)</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>(Mean\ Burden) (%) ((\pm) SD)</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Survival (%)</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td><strong>PIT Fish (n=)</strong></td>
<td>24</td>
<td>30</td>
</tr>
<tr>
<td>(L_F) (mm)</td>
<td>114-150</td>
<td>108-166</td>
</tr>
<tr>
<td>(Mean\ L_F) (mm) ((\pm) SD)</td>
<td>137.08 +/- 12.17</td>
<td>136.53 +/- 11.97</td>
</tr>
<tr>
<td>Mass (g)</td>
<td>16.05-36.30</td>
<td>8.85-44.48</td>
</tr>
<tr>
<td>(Mean\ Mass) (g) ((\pm) SD)</td>
<td>30.71 +/- 9.15</td>
<td>22.72 +/- 6.80</td>
</tr>
<tr>
<td>Tag Burden (%)</td>
<td>0.09-0.20</td>
<td>0.07-0.36</td>
</tr>
<tr>
<td>(Mean\ Burden) (%) ((\pm) SD)</td>
<td>0.11 +/- 0.03</td>
<td>0.15 +/- 0.05</td>
</tr>
<tr>
<td>Survival (%)</td>
<td>100.0</td>
<td>96.7</td>
</tr>
<tr>
<td><strong>Sham Fish (n=)</strong></td>
<td>25</td>
<td>30</td>
</tr>
<tr>
<td>(L_F) (mm)</td>
<td>105-176</td>
<td>112-155</td>
</tr>
<tr>
<td>(Mean\ L_F) (mm) ((\pm) SD)</td>
<td>136.77 +/- 15.28</td>
<td>140.34 +/- 9.17</td>
</tr>
<tr>
<td>Mass (g)</td>
<td>13.00-66.62</td>
<td>12.28-35.55</td>
</tr>
<tr>
<td>(Mean\ Mass) (g) ((\pm) SD)</td>
<td>31.40 +/- 10.60</td>
<td>24.79 +/- 5.11</td>
</tr>
<tr>
<td>Tag Burden (%)</td>
<td>0.05-0.24</td>
<td>0.09-0.26</td>
</tr>
<tr>
<td>(Mean\ Burden) (%) ((\pm) SD)</td>
<td>0.11 +/- 0.04</td>
<td>0.13 +/- 0.03</td>
</tr>
<tr>
<td>Survival (%)</td>
<td>100.0</td>
<td>96.7</td>
</tr>
<tr>
<td><strong>Acoustic-tagged Fish (n=)</strong></td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>(L_F) (mm)</td>
<td>115-178</td>
<td>120-163</td>
</tr>
<tr>
<td>(Mean\ L_F) (mm) ((\pm) SD)</td>
<td>139.91 +/- 15.03</td>
<td>139.52 +/- 9.42</td>
</tr>
<tr>
<td>Description</td>
<td>Mass (g)</td>
<td>Mean Mass (g) (±) SD</td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>----------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>Mass (g)</td>
<td>16.70-70.21</td>
<td>34.25 +/- 12.51</td>
</tr>
<tr>
<td>Mean Mass (g) (±) SD</td>
<td>13.00-39.97</td>
<td>24.05 +/- 5.50</td>
</tr>
<tr>
<td>Tag Burden (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Burden (%) (±) SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Survival (%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2.2 Coefficients of the final model and the significance of each term for rainbow trout and lake trout growth model. Utilized the “R” library (MuMIn) from Nakagawa and Schielzeth 2013, to obtain F and p-values from the generalized linear mixed model equations. Used linear mixed effects models with treatment, time since surgery, and body mass as fixed effects, with individual fish ID as a random effect to test for treatment effects on specific growth rates (mass and fork length). The “baseline” factor level for treatment = ‘tagged’, and for species = ‘lake trout’, thus the coefficients, p-values, etc. are comparisons against those baseline factor levels.

<table>
<thead>
<tr>
<th></th>
<th>Coefficient +/- SE</th>
<th>Degrees of freedom</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>(Intercept)</strong></td>
<td>0.1168 +/- 0.0906</td>
<td>396</td>
<td>1.2897</td>
<td>0.1979</td>
</tr>
<tr>
<td><strong>Treatment (PIT)</strong></td>
<td>-0.2157 +/- 0.1159</td>
<td>154</td>
<td>-1.8618</td>
<td>0.0645</td>
</tr>
<tr>
<td><strong>Treatment (Sham)</strong></td>
<td>-0.1336 +/- 0.1154</td>
<td>154</td>
<td>-1.1578</td>
<td>0.2487</td>
</tr>
<tr>
<td><strong>Species (RBT)</strong></td>
<td>0.0031 +/- 0.0960</td>
<td>154</td>
<td>0.0318</td>
<td>0.9747</td>
</tr>
</tbody>
</table>
Table 2.3 Coefficients of the growth model and the significance of each term for rainbow trout and lake trout growth. Utilized the “R” library (MuMIn) from Nakagawa and Schielzeth 2013, to obtain F and p-values from the generalized linear mixed model equations. Used linear mixed effects models with treatment and body mass as fixed effects, and individual fish ID as a random effect to test for treatment effects on specific growth rates (mass).

<table>
<thead>
<tr>
<th></th>
<th>Coefficient ± SE</th>
<th>Degrees of freedom</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>0.1491 ± 0.0741</td>
<td>396</td>
<td>2.0133</td>
<td>0.0448</td>
</tr>
<tr>
<td>Treatment (PIT)</td>
<td>-0.2023 ± 0.1037</td>
<td>155</td>
<td>-1.9500</td>
<td>0.0530</td>
</tr>
<tr>
<td>Treatment (Sham)</td>
<td>-0.2380 ± 0.1033</td>
<td>155</td>
<td>-2.3027</td>
<td>0.0226</td>
</tr>
</tbody>
</table>
Table 2.4 Coefficients of the final model and the significance of each term for rainbow trout $\dot{MO}_2$. Utilized the “R” library (MuMIn) from Nakagawa and Schielzeth 2013, to obtain F and p-values from the generalized linear mixed model equations. Used linear mixed effects models with tag burden and time since surgery as fixed effects, and individual fish ID as a random effect to test for treatment effects on oxygen consumption ($\dot{MO}_2$) within the acoustic-tagged group.

<table>
<thead>
<tr>
<th></th>
<th>Coefficient +/- SE</th>
<th>Degrees of freedom</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.9045 +/- 0.2390</td>
<td>23</td>
<td>3.7840</td>
<td>0.0010</td>
</tr>
<tr>
<td>Tag Burden</td>
<td>-0.0939 +/- 0.0567</td>
<td>21</td>
<td>-1.6552</td>
<td>0.1128</td>
</tr>
<tr>
<td>Surgery Time</td>
<td>0.0117 +/- 0.0050</td>
<td>21</td>
<td>2.3421</td>
<td>0.0291</td>
</tr>
</tbody>
</table>
Table 2.5 Coefficients of the final model and the significance of each term for lake trout $\dot{M}O_2$.

Utilized the “R” library (MuMIn) from Nakagawa and Schielzeth 2013, to obtain F and p-values from the generalized linear mixed model equations. Used linear mixed effects models with treatment and body mass as fixed effects, and individual fish ID as a random effect to test for treatment effects on resting oxygen consumption ($\dot{M}O_2$).

<table>
<thead>
<tr>
<th></th>
<th>Coefficient +/- SE</th>
<th>Degrees of freedom</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>2.6768 +/- 0.2239</td>
<td>105</td>
<td>11.9543</td>
<td>0.0000</td>
</tr>
<tr>
<td>Treatment (Acoustic-tagged)</td>
<td>-0.8451 +/- 0.2984</td>
<td>105</td>
<td>-2.8318</td>
<td>0.0055</td>
</tr>
<tr>
<td>Treatment (PIT)</td>
<td>-1.4228 +/- 0.2785</td>
<td>105</td>
<td>-5.1086</td>
<td>0.0000</td>
</tr>
<tr>
<td>Treatment (Sham)</td>
<td>-0.6856 +/- 0.3109</td>
<td>105</td>
<td>-2.2055</td>
<td>0.0296</td>
</tr>
<tr>
<td>Mass</td>
<td>-0.0376 +/- 0.0091</td>
<td>70</td>
<td>-4.1492</td>
<td>0.0001</td>
</tr>
<tr>
<td>Mass (Acoustic-tagged)</td>
<td>0.0298 +/- 0.0121</td>
<td>70</td>
<td>2.4558</td>
<td>0.0165</td>
</tr>
<tr>
<td>Mass (PIT)</td>
<td>0.0478 +/- 0.0115</td>
<td>70</td>
<td>4.1491</td>
<td>0.0001</td>
</tr>
<tr>
<td>Mass (Sham)</td>
<td>0.0214 +/- 0.0128</td>
<td>70</td>
<td>1.6763</td>
<td>0.0981</td>
</tr>
</tbody>
</table>
Table 2.6 Coefficients of the final model and the significance of each term for $U_{crit}$ model used for rainbow trout and lake trout. Utilized the “R” library (MuMln) from Nakagawa and Schielzeth 2013, to obtain F and p-values from the generalized linear mixed model equations. Used linear mixed effects models with treatment and species as fixed effects, and individual fish ID as a random effect to test for treatment effects on swimming performance ($U_{crit}$).

<table>
<thead>
<tr>
<th></th>
<th>Coefficient +/- SE</th>
<th>Degrees of freedom</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>4.2565 +/- 0.1317</td>
<td>54</td>
<td>32.312</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Species (RBT)</td>
<td>2.6028 +/- 0.1552</td>
<td>54</td>
<td>16.775</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Treatment (Acoustic-tagged)</td>
<td>-0.3495 +/- 0.1552</td>
<td>54</td>
<td>-2.252</td>
<td>0.0284</td>
</tr>
</tbody>
</table>
Figure 2.1 Boxplot for juvenile rainbow trout (Oncorhynchus mykiss) [left] and lake trout (Salvelinus namaycush) [right] specific growth rates (%day⁻¹) (fork length [top] and mass [bottom]) for each treatment group (control [blue], acoustic-tagged [red], PIT [green], and sham [orange]) for each two week growth period (±SE). The species x treatment interaction was significant (p = 0.0005), whereby growth was lower in the PIT group compared to the 'baseline' level which was acoustic-tagged fish (p = 0.0001), but only for rainbow trout. Each box has a thick line in the middle which denotes the median (middle 50% value), the lower edge of the box corresponds to the first quartile, while the upper edge of the box corresponds to the third quartile. The middle 50% of the data distribution lies within the box, and the interquartile range (1.5 x) is
represented by the upper and lower whiskers (or the most extreme value, depending on which is closer to the median).
Figure 2.2 Boxplot for juvenile rainbow trout (*Oncorhyncus mykiss*) oxygen consumption ($\dot{M}O_2$) (mg-O$_2$·kg$^{-1}$·min$^{-1}$) for each treatment group (control [blue], PIT [green], sham [orange], and acoustic-tagged [red]), in relation to days since surgery (±SE). There was a significant linear relationship between oxygen consumption and days since surgery in the acoustic-tagged rainbow trout ($p=0.003$). Each box has a thick line in the middle which denotes the median (middle 50% value), the lower edge of the box corresponds to the first quartile, while the upper edge of the box corresponds to the third quartile. The middle 50% of the data distribution lies within the box, and the interquartile range (1.5 x) is represented by the upper and lower whiskers (or the most extreme value, depending on which is closer to the median). **R$^2$ and p-value from model-derived regressions.
Figure 2.3 Boxplot for juvenile lake trout (Salvelinus namaycush) oxygen consumption ($\dot{M}O_2$) (mg-$O_2$·kg$^{-1}$·min$^{-1}$) for each treatment group (control [blue], PIT [green], sham [orange], and acoustic-tagged [red]), in relation to body mass (g) (±SE). There was a significant linear relationship between oxygen consumption and body mass in the control lake trout ($p = 0.007$). Each box has a thick line in the middle which denotes the median (middle 50% value), the lower edge of the box corresponds to the first quartile, while the upper edge of the box corresponds to the third quartile. The middle 50% of the data distribution lies within the box, and the interquartile range (1.5 x) is represented by the upper and lower whiskers (or the most extreme value, depending on which is closer to the median). $**R^2$ and p-value from model-derived regressions.
Figure 2.4 Critical swimming speed ($U_{crit}$) ($L_F \cdot s^{-1}$) for rainbow trout ($Oncorhynchus mykiss$) and lake trout ($Salvelinus namaycush$) (control [n=11-15] and acoustic-tagged [n=15-16]) ($\pm$SE).

Every rainbow trout tested had a higher $U_{crit}$ than fasted lake trout ($p < 0.001$). For both species $U_{crit}$ was lower in acoustic-tagged fish but treatment differences were not significant ($p = 0.024$). Each box has a thick line in the middle which denotes the median (middle 50% value), the lower edge of the box corresponds to the first quartile, while the upper edge of the box corresponds to the third quartile. The middle 50% of the data distribution lies within the box, and the interquartile range (1.5 x) is represented by the upper and lower whiskers (or the most extreme value, depending on which is closer to the median).
CHAPTER 3
RESEARCH IMPLICATIONS AND FUTURE DIRECTION

Overview

The goal of this research was to investigate acoustic tag burden effects in small-sized or juvenile fish by using rainbow trout and lake trout as proxy species. Acoustic telemetry tags were surgically inserted into the intracoelomic body cavity of juvenile trout following standard implantation techniques and procedures (Summerfelt & Smith 1990; Wagner et al. 2011; Liedtke et al. 2012, Rub et al. 2014). Survival, growth, tag retention, resting metabolic rate (via intermittent-flow respirometry), and swimming performance ($U_{crit}$) were measured as response variables. Metabolic rate and swimming performance are frequently used to assess the effects of environmental variables or stressors on fishes (Brett 1965; Tierney 2011; Chabot et al. 2016). There have been several studies that quantified the effects of acoustic telemetry tags on single salmonid species in one genus (i.e., Oncorhynchus), such as juvenile Chinook, coho, or sockeye salmon. This research, however, evaluated the effect of different tag burdens (mass of the tag as a proportion of the body mass of the fish) in additional species within two genera (i.e., Oncorhynchus and Salvelinus) and used different techniques (i.e. respirometry and resting metabolic rate) to assess possible effects of tag burden. This work will lead to an improved best practice for field-based telemetry studies with respect to tag burden values for small fish. As suggested by Cooke et al. (2011), there is a need to study multiple aspects of acoustic tagging and surgical procedures before an accurate estimate of tag burden effects can be identified. Respirometry and evaluation of metabolic rate in small-sized or juvenile fishes could provide additional insight into acoustic tag burden dynamics.
The problem that was addressed with my work is whether certain sizes (i.e., juveniles or small-sized fish) have a different sensitivity to acoustic tag burden than larger fish which have been more extensively studied with respect to tag burden. My research objectives were to determine how body size and tag burden influences the growth, survival, metabolic rate, and swimming performance of juvenile rainbow trout and lake trout and evaluate what the short-term vs. long-term effects are on each study species. Early life history strategies and residency patterns for juveniles can provide insight into future recruitment, seasonal movement, and preferred habitat for different life stages. Acoustic telemetry can provide answers to many of these questions, and several studies have used this technology to estimate migration and survival rates of several fish species (Chittenden & McKinley 2009; Klimley et al. 2013; Ogburn et al. 2017). Similarly, the energetic cost of activity and movement in fishes can be explored with acoustic telemetry (Cruz-Font et al. 2016). There is potential for using acoustic tagging as a conservation tool, however sensitivity of study species must first be identified and addressed before accurate estimates of survival or restoration success can be measured (Sandstrom et al. 2013). Acoustic telemetry can be utilized to help managers and biologists identify optimal habitat and management strategies for species of concern (Crossin et al. 2017; Ogburn et al. 2017).

Summary of Results

The rainbow trout in my study experienced a SGR of ~ 0.4-2.0 (% BM·day\(^{-1}\)), and the lake trout experienced a SGR of ~ 0.1-0.4 (% BM·day\(^{-1}\)). There was no effect of treatment on SGR and the observed growth rates for both species were within the range reported in the literature (i.e., similar water temperatures and food availability) (Eschmeyer 1964; Stewart et al. 1983; Gregory & Wood 1999). Rainbow trout were able to maintain critical swimming speeds of
6.0-7.8 L\(_F\)·s\(^{-1}\) or 74.5-92.0 cm·s\(^{-1}\)) while lake trout achieved swimming speeds of 3.1-5.1 L\(_F\)·s\(^{-1}\) or 43.0-66.7 cm·s\(^{-1}\). Again, there was no effect of treatment on \(U_{crit}\) and the observed values for both species were comparable to the \(U_{crit}\) values found in the literature for juvenile salmonids under similar environmental conditions (Rao 1968; Alsop & Wood 1997; Burden et al. 1998; Gregory & Wood 1999; Katopodis & Gervais 2016). Finally, oxygen consumption (my proxy for metabolic rate) did not differ with treatment, and the observed values (1.0-3.8 mg-O\(_2\)·min\(^{-1}\)·kg\(^{-1}\) for rainbow trout and 1.2-2.8 mg-O\(_2\)·min\(^{-1}\)·kg\(^{-1}\) for lake trout) were once again comparable to values found in the literature (Gibson & Fry 1954; Rao 1968; Stewart et al. 1983; Beamish et al. 1989; Alsop & Wood 1997).

There were no occurrences of tag loss for either species during the entire 8-week experiment. Tag expulsion has been previously reported for juvenile rainbow trout and is related to tag burden (Chisholm & Hubert 1985; Welch et al. 2007; Ivasauskas et al. 2012; Sandstrom et al. 2013). My study indicates that tag retention for juvenile rainbow trout and lake trout is very high (i.e., 100 %) when tag burden values are kept under 6%. In summary, the results of my study indicate that an acoustic tag burden of 2-6% by mass has no significant effect on survival, growth, resting metabolic rate, or swimming performance of juvenile rainbow trout or lake trout (9-39 g and 105-159 mm (L\(_F\))).

**Impact on the Field**

The results of this study contribute valuable information to future acoustic telemetry studies involving small or juvenile fish and provide insight into the physiology and performance of acoustically tagged fish. Although this research focused on two juvenile salmonid species, the results provide initial guidance for other fish species and life stages. As previous studies have indicated, the “2% rule” is a very conservative measure for estimating suitable tag burden even
in small and juvenile fish (Brown et al. 1999; Richard et al. 1999; Smircich & Kelly 2014). There is discrepancy when it comes to ideal tag ratios and a wide range of results are reported in the literature. High tag burden (> 6%) has been associated with reduced growth (Smircich and Kelly 2014), swimming performance (Perry et al. 2013, Collins et al. 2013), higher mortality (Collins et al. 2013), and longer healing times and tag loss (Collins et al. 2013). My results provide further evidence to support the idea that 2% tag burden is a very conservative threshold even for small fishes (< 100mm (Lf)), and that a tag burden range of 5-6% is more acceptable and reasonable for acoustic-tagged juvenile or small-sized fishes.

In general, most studies recommend keeping tag burden values below 10% to ensure minimal negative effects on survival, tag retention, growth, and swimming performance of acoustic-tagged fishes (Collins et al. 2013; Perry et al. 2013; Smirchich & Kelly 2014). This research also provides reference information (e.g., specific growth rates, resting metabolic rate (RMR), and $U_{crit}$) for future studies involving rainbow trout and/or lake trout and tagging studies regarding comparisons with similar treatment groups (e.g., acoustic-tagged, sham, PIT, and control fish). It has been suggested that multiple performance measures should be taken into consideration when attempting to address the physical and physiological effects of tag burden (Brown et al. 1999; Cooke et al. 2011). The addition of metabolic rate (via respirometry) as a tool to measure stress responses to acoustic tag implantation and wound healing. These performance metrics address physiological aspects of tag burden effects and will be useful performance measures for future studies involving acoustic tag burden in fishes. The relevance of swimming performance to navigation and predator avoidance has been well established in previous literature (Adams et al. 1998; Wolter & Arlinghaus 2003; Anglea et al. 2004; Janak et al. 2012). Thus, adding to the knowledge base regarding $U_{crit}$ values for juveniles of a specific
species, is very useful for researchers evaluating impacts of acoustic tag burden on navigation and movement rates.

Future Research Direction

Recent literature recommends more thorough reporting of surgical procedures and tagging techniques in future telemetry studies (Thiem et al. 2011). Future work involving surgically implanted acoustic tag burden should include all details of surgical procedures and methods in a thorough and complete manner as to better facilitate an understanding of different surgery techniques. This advancement of knowledge will also help lead the field to a more standardized universal procedure for implantation of acoustic telemetry tags. This study focused on tag mass (in air) as a metric which is the standard used in most tagging studies. It would be beneficial for future research involving acoustic tag burden in small or juvenile fish to consider the effects of other tag specific metrics or measurements such as mass in water, and tag volume or length instead of the standard mass calculation (Brown et al. 1999; Cooke et al. 2011). This is especially true in small fish where the available body cavity volume is relatively much smaller than it is in larger fish. My study looked at an acoustic tag burden range of ~ 2-6%. Longer term studies will require larger and heavier tags, thus extending tag burden for small-sized fish will be valuable for future telemetry studies involving long-term tracking projects.

Aerobic scope and MMR values could have provided additional insight and precision into the oxygen consumption rates associated with tag implantation and wound healing processes (Killen et al. 2017). The swim flume used in experimental trials was not appropriately scaled to accurately estimate maximum metabolic rate values (MMR). Future research involving respirometry as a tool to assess acoustic tag burden should also consider including multiple variations of metabolic rate (i.e., MMR, SMR, RMR) in their experimental design. This will
allow for researchers to better isolate the fine-scale physiological effects of tag burden and help distinguish between individual variability in oxygen consumption and effect of tag burden on oxygen consumption/metabolic rate. My recommended priority would be to explore surgical methods (i.e., that may affect healing times via respirometry (i.e., investigate elevated oxygen consumption and resting metabolic rate). Additionally, the effects of acoustic tagging on reproductive success, feeding ability and other ecologically relevant behaviours such as navigation ability would be helpful for the acoustic telemetry field in general (Jepsen et al. 2003; Cooke et al. 2011). These behaviour-based metrics could provide researchers with additional insight and information about potential effects of surgical procedures and tag implantation. In-situ experiments are often overlooked and dismissed due to the logistics and feasibility of some projects. It has been suggested that lab-based tag burden studies may underestimate effects on survival, growth, and swimming performance (Rub et al. 2014). However, researchers should also consider these types of experiments when developing their experimental design and protocol to try and better understand wound healing rates and the physiology associated with acoustic tag burden in fishes within natural environments.

The effects of environmental variation and water temperature increase are becoming significant in the Great Lakes and other aquatic systems worldwide (Farrell 2009). Increasing water temperatures could result in higher rates of infection and increased inflammation in acoustic-tagged fish, resulting in behavioural or navigational impacts (Anderson & Roberts 1975; Farrell 2009). This is another reason for future research to consider ecologically relevant behaviours and environmental factors including the effects of temperature physiology in tag burden studies.
The results of this study are relevant to other sizes and species of fish, however, additional research involving diverse families of fish such as cyprinids (i.e., minnows) could be beneficial. Identifying individual species physiological tolerances for tag burden is important (Brown et al. 1999; Cooke et al. 2011). The cyprinids represent a large group of fish with a lot of diversity. Several invasive species are included in this family (e.g., rudd (Scardinius erthrophthalmus) and Asian carp spp.). The potential to use acoustic telemetry for studying the behaviour and movement of invasive species has recently been identified (Lennox et al. 2016). Using acoustic telemetry to study the movement and distribution of juvenile invasive species and non-minnow cyprinids such as Asian carp (i.e., bighead carp (Hypophthalmichthys nobilis) and grass carp (Ctenopharyngodon idella) would be a very valuable tool for fisheries managers and agencies in the Great Lakes.

The next logical step in tag miniaturisation and small fish tagging studies would be to start acoustic telemetry tagging projects with small forage fish such as the cyprinid family. A study by Jones (1982) suggested that anaerobic relationships associated with exercise in teleost fish, is much different for salmonids than it is for cyprinids (i.e., the metabolic scope for salmonids is larger than it is for cyprinids). The dynamics between oxygen consumption and swimming performance are therefore different for each group of species. This is something to consider when attempting to compare tag burden in different species of fish or applying similar techniques to an acoustic tag burden evaluation. Further work to examine the effects of larger tags on the internal anatomy of fishes and specifically the swim bladder should also be undertaken to validate whether the original “2% rule” (Winter 1983) has any merit regarding available body cavity space in different species and sizes of fish. Body size and shape (i.e., morphology) is highly variable depending on species and life stage of the fish (Jepsen et al.
Fish with gas-filled swim bladders can regulate buoyancy and maintain neutral position via the release or absorption of gases in the swim bladder (McNeill 1993). In primitive fish such as salmonids (i.e., physostomatous fish), buoyancy is maintained by taking air at the surface and releasing as needed through an open connection in the gut (i.e., connective tube between swim bladder and stomach) (Bone & Marshall 1985). Most teleost fish tend to lose this ability as they mature (i.e., physoclistous fish), and their swim bladder is typically filled with gas before the complete closure of the open connection occurs (Bone & Marshall 1985; Jepsen et al. 2003). It has been suggested that physostomatous fish remain neutrally buoyant, while physoclistous fish are negatively buoyant for the most part, which helps to facilitate vertical migrations in the water column (Arnold & Walker 1992). Future research should attempt to fill in the current knowledge gaps related to buoyancy regulation, and the pathological and physiological effects associated with long-term (1-3 years) intracoelomic acoustic tag implantation (i.e., long-term wound healing rates and impact of heavy tags on internal anatomy).

In conclusion, there are a variety of methods and techniques available for researchers to examine and investigate the effects of surgical procedures and tag burden on fishes in a lab-based setting. This study indicates that specific growth rate, resting metabolic rate, and swimming performance ($U_{crit}$) can be used to assess impacts of acoustic tag burden in small or juvenile fishes and that a tag burden of 2-6% is suitable and acceptable for small-sized or juvenile fishes.
References


insertion of transmitters and telemetry methods in fisheries research. American Journal
of Veterinary Research, 75(4): 402-416.

Sandstrom, P.T., A.J. Ammann, C. Michel, G. Singer, E.D. Chapman, S. Lindley, R.B.
MacFarlane, & A.P. Klimley. 2013. Growth, survival, and tag retention of steelhead
tROUT (Oncorhynchus mykiss) and its application to survival estimates. Environmental
Biology of Fishes, 96: 145-164.

Smircich, M.G., & J.T. Kelly. 2014. Extending the 2% rule: the effects of heavy internal tags
on stress physiology, swimming performance, and growth in brook trout. Animal
Biotelemetry, 2: 16.

tROUT, Salvelinus namaycush: application to the Lake Michigan population. Canadian
Journal of Fisheries and Aquatic Sciences, 40(6): 681-698.


reporting of tagging procedures for fish telemetry studies that have used surgical
implantation of transmitters: a call for more complete reporting. Reviews in Fish
Biology and Fisheries, 21: 117-126.

experiments, (51).


Andrew Paxton Darcy was born in 1982 in Burlington, Ontario. He graduated from Waterdown District High School in 2001. From there he went on to Fleming College to obtain an advanced diploma in Ecosystem Management Technology, and then Trent University where he obtained a B.Sc. in Biology and Natural & Resource Science in 2016. He is currently a candidate for the Master's degree in Environmental Science at the University of Windsor. Andrew was also awarded the Alex S. Davidson Great Lakes Stewardship award in 2017.