USING DATA STORAGE TAGS TO STUDY FISH PHYSIOLOGY AND BEHAVIOUR

By

© Zoe A. Zrini

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Abstract

Data storage tags (DSTs) are implantable devices that record and store physiological, environmental and/or behavioural data from free-living animals. A recently developed DST from Star-Oddi records heart rate (f_H), electrocardiograms (ECGs), tri-axial acceleration and temperature. In my first chapter, I found that parameters of acceleration could predict the swimming speed, tail beat frequency and behaviour of Atlantic salmon (*Salmo salar*), and that the DST could record changes in f_H associated with recovery from surgery, the diurnal (day / night) cycle and temperature. In my second chapter, I used Star-Oddi's micro f_H logger to show that the lumpfish (*Cyclopterus lumpus*) has a low maximum f_H compared to most fishes, experiences tachycardia when acutely exposed to increased hydrostatic pressure and for periods as long as one hour, and that hydrostatic pressure alters their f_H response to decreasing oxygen levels (hypoxia). These data support the use of biologging tags (which have recently been miniaturized and made more affordable) to better understand how aquatic animals respond to changes in their environment, and their ecology.

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hydrostatic pressure in a step-wise protocol (black symbols; n = 23) in Experiment #2. Pressure was initially increased to 20 bar over 2 minutes, then held for 8 minutes. Pressure was then increased in a similar manner to 35, 50, 65 and 80 bar, followed by exposure to 80 bar of pressure for 1 hour. B) The percentage change in f_H (as a % of initial values at 0 bar) was manually calculated from the electrocardiograms. Dissimilar lower-case letters indicate a significant difference ($P < 0.05$) within the pressure-exposed group (no difference existed in the control group), while an asterisk (*) represents a significance difference between the pressure-exposed and control values within a pressure step. Data were recorded every 2 minutes, and the symbols represent means ± S.E.M (n = 5 per fish). 84

Figure 3.9. A) Heart rate (f_H, bpm) in lumpfish exposed to decreasing (at 2ºC h^{-1}; A, C, E) or increasing (at 2ºC h^{-1}; B, D, F) temperature in the IPOCAMP chamber. Prior to the decrease in temperature, lumpfish were held at atmospheric pressure (black symbols; n = 7) or exposed to 80 bar of pressure (grey symbols; n = 7). C, D) The percentage change in f_H (as a % of initial values at 0 or 80 bar) was manually calculated from the electrocardiograms. Relationships between f_H and the percentage change in f_H with temperature were determined by linear regression analysis, and an asterisk (*) indicates a significant difference between the slopes for relationships between control and pressure-exposed groups. E, F) The percentage of 'good' quality ECGs (i.e., with a QI = 0) were provided by the Star-Oddi Mercury software, and significant differences within the control (regular letters) or pressure-exposed groups (bold letters) are represented by dissimilar lower-case letters. No differences existed in the percentage of 'good' quality

List of Abbreviations and Symbols

2P	Second Order Polynomial / Quadratic Equation
3P	Third Order Polynomial / Cubic Equation
AA	Acceleration Activity
ACT	Activity
atm	Atmospheres
BL	Body Lengths
bpm	Beats per Minute
CDRF	Cold-Ocean and Deep-Sea Research Facility
COM-BOX	Star-Oddi Tag-Computer Interface
COT	Cost of Transport
COX	Cytochrome C Oxidase
CT_{MAX}	Critical Thermal Maximum
CT_{MIN}	Critical Thermal Minimum
D	One Phase Decay Equation
DenDF	Degrees of Freedom of the Denominator
DST	Data Storage Tag
EA	External Acceleration
ECG	Electrocardiogram
EG	Exponential Growth Equation
f_H	Heart Rate
HRT	Heart Rate
HRV	Heart Rate Variability

IPOCAMP	Incubatuer Pressurisé pour l'Observation et la Culture d'Animaux Marins Profonds
JBARB	Dr. Joe Brown Aquatic Research Building
LASCCR	Laboratory for Atlantic Salmon and Climate Change Research
LME	Linear Mixed-Effects
LR	Linear Regression
$\dot{M}O_2$	Oxygen Consumption
MS-222	Tricaine Methanesulfonate
NumDF	Degrees of Freedom of the Numerator
ODBA	Overall Dynamic Body Acceleration
OSC	Ocean Sciences Centre
P_{crit}	Critical Oxygen Tension
PQRS	Cardiac electrical activity associated with atrial and ventricular contraction
Q_{10}	Temperature Coefficient
QI	Quality Index
S.E.M.	Standard Error of the Mean
TBF	Tail Beat Frequency
U_{crit}	Critical Swim Speed
VAR	Variation in External Acceleration

Co-authorship Statement

Z. A. Zrini is the principal author, and made the main intellectual and practical contributions to the work reported in this thesis. The development of the research proposal for this work, and experimental design, were completed with assistance from A. K. Gamperl. All practical aspects of the research, data analysis and manuscript preparation were the responsibility of Z. A. Zrini. Assistance with data interpretation and writing of the thesis was primarily provided my supervisor (Dr. A. K. Gamperl), although Drs. W. R. Driedzic and I. J. McGaw (members of my supervisory committee) also made contributions. Z. A. Zrini will be the lead author, and Dr. A. K. Gamperl will be a co-author, of Chapter 2. Ms. R. M. Sandrelli will also be a co-author of the manuscript to come from Chapter 3 given her assistance with conducting the experiments on lumpfish.

Chapter 1: General Introduction

An urgent, and key, goal for marine scientists is to understand how aquatic animals respond to changes in their environment (Payne et al. 2014), and the ability of scientists to observe and understand these responses in free-swimming animals has been significantly enhanced by technology (Ropert-Coudert and Wilson 2005; Rutz and Hays 2009). Such technologies began as simple capillary tube pressure gauges attached to a harpooned fin whale (Kooyman 2004), but have evolved into sophisticated tools like time-depth recorders, pop-up satellite tags and archival loggers (Ropert-Coudert et al. 2012). The realization that animals can carry foreign objects attached to their bodies, and advancements in technology to transmit information, have led to the development of animal attached electronic devices (Ropert-Coudert and Wilson 2005). Microprocessor development assisted in the miniaturization of electronic recorders and their memory capacity increased throughout the 1900s (Kooyman 2004), allowing smaller sized species to be studied and more variables to be recorded (Ropert-Coudert et al. 2012; Hussey et al. 2015; Wilson et al. 2015). Electronic tagging, or "bio-logging", is now recognized across a wide range of scientific disciplines for its use in providing real-world measurements of free-ranging animals in their natural environment (Thorsteinsson 2002).

The first use of the term "bio-logging" is attributed to Boyd et al. (2004), and many similar definitions have since been used (see Naito 2004; Ropert-Coudert and Wilson 2005; Rutz and Hays, 2009; Ropert-Coudert et al. 2012; Payne et al. 2014; Lowerre-Barbieri et al. 2019). Typically, biologging refers to the use of small animal-borne devices that either log or transmit data (including physiology, behaviour, movement, and / or environmental parameters) collected from the tagged animals. While

biologging includes both logging and transmitting devices, definitions are often further separated into tags which store data in on-board memory (archival loggers or data storage tags) or those that transmit data (biotelemetry) via radio, acoustic or satellite transmission (Ropert-Coudert and Wilson 2005, Cooke 2008; Payne et al. 2014). Each device has pros and cons (reviewed in Cooke et al. 2004a), but ultimately tag choice should be based on the goals of the research being conducted (Arai and Okuyama 2012).

Radio and acoustic transmission tags provide real-time information, but these data cannot be obtained when animals are outside the range of the receivers (Arai and Okuyama 2012). Additionally, radio waves cannot be transmitted through saltwater or at depth in freshwater, and this limits the environments in which these tags can be used (Cooke 2008). While acoustic tags can be used in estuarine or marine environments, their transmission range is < 1 km and acoustic receivers are more challenging and expensive to deploy and maintain (Hussey et al. 2015). Satellite tags eliminate the need for receivers and reduce labor, but satellite communication is expensive and lacks accuracy (Arai and Okuyama 2012). While archival loggers and data storage tags can be expensive, and must be collected to download the data (which is a major disadvantage), they allow for the monitoring of high-resolution data in animals with minimal labor and at sampling frequencies as short as every minute (Cooke et al. 2004a; Payne et al. 2014). All of these technologies provide researchers with the ability to study multiple scales of biology (Cooke et al. 2004a), measure responses of individuals in order to characterize plasticity (Cooke 2008; Chmura et al. 2018), and most importantly, study the response of free-living animals to the abiotic / biotic conditions in their natural environment (Cooke et al. 2004a; Cooke 2008; Ropert-Coudert et al. 2012; Hussey et al. 2015; Wilson et al. 2015).

To date, biologgers have been able to record several different parameters in a diverse range of environments and taxa (Cooke et al. 2004a; Wilson et al. 2015). Ropert-Coudert and Wilson (2005) found papers describing twenty-four different types of biologging sensors, a number which is constantly growing. Among the most popular are those that measure movement (e.g., global location sensors and global positioning systems), activity or energetics (e.g., electromyograms, whole body acceleration, fin and wing beats), physiology (e.g., heart rate and neuro-loggers), environmental parameters (e.g., temperature, pressure, light levels, conductivity, salinity and dissolved oxygen) and on-board imagery (i.e., contain video sensors) (Cooke 2008; Ropert-Coudert et al. 2012). Further, biologging allows for the coupling of multiple sensors or tags to study nearly all aspects of an animal simultaneously (Wilmers et al. 2015), and thus, provides the opportunity to study animals with a more holistic and comprehensive approach (Cooke et al. 2004a; Chmura et al. 2018).

Biologging can further knowledge in a wide range of scientific fields from physiology to ecology and evolution (Payne et al. 2014). A unique extension of biologging is using the animals to monitor the environment in areas that are difficult to survey or have not been surveyed (Block 2005; Ropert-Coudert and Wilson 2005; Rutz and Hays 2009; Wilmers et al. 2015). For example, crabeater (*Loboson carcinophaga*) and leopard seals (*Hydruga leptonyx*) were used to determine temperature profiles in the west Antarctic Peninsula (Costa et al. 2008). Monitoring the environment is clearly a valuable use of biologging tools as 1.4 million temperature and salinity profiles in the World Ocean Database are from animal-borne devices (Wilmers et al. 2015). Further, individual and population level responses to climate change can be monitored, and used

to predict further responses by combining physiological and behavioural data with changing environmental parameters (Ropert-Coudert and Wilson 2005; Chmura et al. 2018). For example, by comparing the depth profiles of Atlantic blue marlin (*Makaira nigricans*) with oxygen concentration data, researchers found that marlin prefer highly oxygenated surface waters, and they used the results to predict the future distribution of marlin in response to expanding oxygen minimum zones (Stramma et al. 2012). As conservation strategies are informed by animal physiology and behaviour (Cooke et al. 2014), biologging studies have the potential to advance our understanding of global climate change and other anthropogenic impacts on animals (Cooke et al. 2004a; Cooke 2008; Wilson et al. 2015). Questions to be addressed in response to human-caused disturbances include changes in the phenology of migration, hibernation and reproduction, the thermal sensitivity and limits of species, mortality rates and causes, and micro / marco-habitat selection (Block 2005; Cooke 2008; Wilson et al. 2015; Chmura et al. 2018).

Before utilizing the full potential of biologgers to answer such impactful questions, a few limitations must be addressed. For example, the large data sets provided by the tags are both an asset and a hindrance as the data can be difficult to analyze (Cooke et al. 2004a; Rutz and Hays 2009; Payne et al. 2014), and many tags require time-consuming laboratory calibration before field application (Cooke et al. 2004a). Researchers are also concerned about the effects that the tags impose on the animals that transport them (Ropert-Coudert and Wilson 2005; Brown et al. 2013), and the potential abandonment of hypothesis testing caused by an over-abundance of data (Boyd et al. 2004). For these reasons, physiological biologgers are probably underutilized despite their

ability to answer particularly difficult questions (Wilson et al. 2015). Fortunately, advances in the field, such as in data analysis and in laboratory calibrations continue alongside technological development and miniaturization. These advances are being hailed as ushering in the "biologging decade" (2018-2028), in which these tools will: become more popular and multidisciplinary; allow scientists to better understand animals and their environment; and lead to new fundamental theories about the movement, energy use and physiology of animals (Lowerre-Barbieri et al. 2019).

In this thesis, I use biologging to understand two different aspects of fish physiology. Thus, my thesis is organized into two separate data chapters. In Chapter 2, I validated the use of Star-Oddi centi-HRT ACT data storage tags to record heart rate (electrocardiograms) and tri-axial acceleration in Atlantic salmon (*Salmo salar*). This research involved determining the duration of surgical recovery required, the effectiveness (accuracy) of these tags with respect to estimating the swimming speed and behaviour of free-swimming salmon, as well as how the recordings of these parameters change over long periods of time (i.e., up to 6 weeks post-surgery). In Chapter 3, I investigated how pressure (depth) affected the heart rate (f_H) of lumpfish (*Cyclopterus lumpus*), and the f_H responses of the lumpfish to changes in temperature and oxygen levels. In addition, I measured the maximum f_H of lumpfish as induced by exercise and increased temperature, neither of which had previously been determined. Finally, in Chapter 4, I discuss the implications of my results, and highlight the flexibility of data storage tags to study various fields of animal biology, especially in relation to global climate change.

Chapter 2: Validating Heart Rate and Acceleration Data Storage Tags for Use in Atlantic Salmon (*Salmo salar*)

Abstract

Data storage tags (DSTs) record and store information about animals and their environment, and could provide important information relevant to the conservation and welfare of wild and cultured fish. Star-Oddi recently developed a DST that simultaneously records heart rate (f_H), electrocardiograms, tri-axial acceleration and temperature. However, no studies have been performed using these tags in fish, or determined the quality of the data collected. Thus, my research asked: do these DSTs provide reliable and meaningful data? To examine this question, Atlantic salmon (1.4 ± 0.7 kg) were surgically implanted with DSTs, then swam at increasing speeds in a swim tunnel after 1 week of post-surgical recovery. External acceleration (EA, acceleration above normal gravity) and variation in EA (VAR) increased exponentially with both swimming speed (body lengths sec^{-1}) and tail beat frequency (tail beats min^{-1}). The quality index (QI) assigned to ECG recordings (where 0 means great quality, 1 and 2 have decreasing quality and 3 means there is no R-R interval) did not change with increasing swimming speed. However, the accuracy of the Star-Oddi Mercury software in estimating f_H from ECGs was reduced when QI > 0. In a separate experiment, salmon (2.4 ± 0.1 kg) were surgically implanted with DSTs and held in a large tank with conspecifics for 1 week at 11°C or 6 weeks at 8-10°C. Diurnal patterns of f_H and EA were evident from the time the fish were placed in the tank. Heart rate appeared to reach baseline values by 4 days post-surgery in the first experiment, but extended holding showed that f_H declined

for the first 21 days, depending on temperature. During the extended holding period, the tag had difficulty recording low f_H values (i.e., < 30 bpm), and for this reason, in addition to the fact that the tag software can miscalculate f_H when QI > 0, ECGs should be saved when possible for quality control and the manual calculation of f_H. My results indicate that parameters of acceleration can be used to monitor the activity of free-swimming salmon, and that reliable measurements of f_H, including diurnal rhythms and responses to temperature and stressors, can be recorded. However, it is highly recommended that f_H values with QI > 1 be manually calculated.

Introduction

The monitoring of animal physiology and behaviour allows researchers to study how animals cope with changes in environmental conditions, including those associated with global climate change (Block 2005; Wilson et al. 2008; Metcalfe et al. 2016). Historically, the recording of biological data from free-ranging animals has been very difficult. However, the integration of electronic engineering and biology has led to the creation of biologging technologies (Ropert-Coudert and Wilson 2005; Rutz and Hays 2009). Biologging tags allow researchers to study animals that are untethered from stationary equipment and that are free to move in their natural environment (Cooke 2008; Ropert-Coudert et al. 2012). Initially, biologging devices primarily relied on acoustic and radio transmission, however, these technologies do not provide for the recording of high-resolution data for prolonged periods (i.e., up to 15 sec.) due to the bandwidth limitations associated with transmission (Cooke et al. 2004a; Metcalfe et al. 2016). In contrast, archival loggers (also called data storage tags, DSTs) record high resolution data at

sampling frequencies as short as every minute that are stored into the logger, thereby eliminating the need for signal transmission and the manual labor involved in tracking and the installation of hydrophone arrays (Tanaka et al. 2001; Donaldson et al. 2010; Metcalfe et al. 2016; Cooke et al. 2016). However, the fish must be re-captured to obtain the data.

In fish, DSTs have traditionally been used to study thermal and depth profiles, activity, spatial use and behaviour (e.g., see Tanaka et al. 2000, 2001; Kawabe et al. 2003; Tsuda et al. 2006; Godfrey et al. 2015; Hedger et al. 2017a, b; Algera et al. 2017). However, they have recently been used to measure acceleration and heart rate (f_H) to estimate energy use (Clark et al. 2010; Halsey et al. 2009; Gleiss et al. 2010, 2011; Wright et al. 2014), and to assess when fish are stressed (Laitinen and Valtonen 1994; Cooke et al. 2004b; Prystay et al. 2017, 2019; Brijs et al. 2018, 2019; Wallerius et al. 2019).

Two types of DSTs that measure f_H exist: (1) heart rate recorders which detect and display the number of heart beats in a given sampling period; and (2) electrocardiogram (ECG) readers that record the electrical activity of the heart and store changes in cardiac electrical activity over the complete cardiac cycle (termed the 'complete PQRS profile'; Ropert-Coudert et al. 2012). The visualization of the PQRS complex is more reliable and allows for manual calculation of f_H as well as f_H variability (HRV; Ropert-Coudert et al. 2012; Cooke et al. 2016). Estimating the HRV of fish is important, as f_H can be controlled on a beat-to-beat basis by autonomic innervation, and therefore, provides information on the cardiac output and the control of f_H of free-swimming fish (Priede 1974; Altimiras et al. 1996; Altimiras 1999). Heart rate DSTs have been used to address concerns about the

conservation and welfare of both wild and cultured fish. For example, Donaldson et al. (2010) used ECG loggers to examine the recovery of coho salmon (*Oncorhynchus kisutch*) from predator and fisheries encounters, and Prystay et al. (2017) used f_H DSTs to study the effects of temperature on fisheries interactions in sockeye salmon (*O. nerka*). Most recently, Brijs et al. (2018, 2019) used DSTs to examine the f_H (stress) response of cultured rainbow trout (*O. mykiss*) to common aquaculture practices.

Accelerometers have the potential to inform conservation and management by providing information on the activity, behaviour and energy use of free-swimming fish (Rutz and Hays 2009; Ropert-Coudert et al. 2012; Algera et al. 2017). Accelerometry loggers and transmitters record either partial or whole-animal body acceleration in one, two or three spatial axes with piezoelectric sensors that generate a voltage signal proportional to the acceleration experienced by the sensor (Brown et al. 2013; Payne et al. 2014). This includes both the gravitational and inertial acceleration caused by movement (Brown et al. 2013; Cooke et al. 2016). In several salmonid species, values of acceleration have been related to tail beat frequency and swimming speed (Tanaka et al. 2001; Kawabe et al. 2003; Wilson et al. 2013, 2014). Acceleration can even be used to classify types of behaviours such as routine, resting or burst swimming in male smallmouth bass *(Micropterus dolomieu,* Algera et al. 2017), feeding, escape and spontaneous movements in great sculpin (*Myoxocephalus polyacanthoceaphalus*, Broell et al. 2013), and spawning behaviours in female chum salmon (*O. keta*, Tsuda et al. 2006). However, most commonly, accelerometers are used to predict field metabolism and estimate energy use, due to the relationship between movement and metabolic rate (Clark et al. 2010; Gleiss et al. 2010, 2011). Acceleration DSTs have already been used to inform fisheries

management strategies and to provide information about the welfare of aquaculture species. For example, Brownscombe et al. (2013) assessed the use of recovery bags during bonefish (*Albula vulpes*) angling and Yasuda et al. (2012) monitored field metabolism in net-caged red sea bream (*Pagrus major*).

Despite their potential applications, the use of DSTs is not without its challenges. Some studies have reported problems including technological failure of unknown causes, tag ejection, a chronic inflammatory response and premature mortality (Thorsteinsson 2002; Johansson et al. 2009; Prystay et al. 2017, 2019; Semple et al. 2018). Further, methods for the implantation of these tags, and the tag's settings, should be specific to the fish being studied, due to the anatomical and behavioural differences among species (Thorsteinsson 2002). Therefore, it is highly recommended that effective standardized protocols are developed for each tag type, size, attachment method and species being studied (Thorsteinsson 2002; Campbell et al. 2005; Ropert-Coudert and Wilson 2005; Cooke 2008; Ropert-Coudert et al. 2009; Arai and Okuyama 2012; Chmura et al. 2018). Likewise, feasibility (also called validation / calibration studies) are highly recommended prior to field deployment to assess the usefulness of various tags (Wilmers et al. 2015; Wilson et al. 2015). For example, Thorsteinsson (2002) suggests determining the duration of post-operative recovery because programmable start times can be delayed saving battery and memory in the field. During these validation studies, values of acceleration can be related to swimming speed, tail beat frequency, and swimming behaviours using a swim tunnel (Clark et al. 2010; Gleiss et al. 2011; Wilson et al. 2013). Overall, the goal of validation studies should be to establish effective attachment methods, and to calibrate parameters in order to get the most useful data out of the tags and to prevent false

conclusions caused by 'tag effects' in field situations (Wilmers et al. 2015). However, few studies have assessed the validity of data recorded using DSTs.

Many companies are producing biologging equipment / sensors that require validation (Ropert-Coudert et al. 2012). A new DST developed by Star-Oddi (Iceland, https://www.star-oddi.com) can simultaneously measure f_H, ECGs, tri-axial acceleration and temperature. There are few tags available with simultaneous measurement of both physiological and behavioural parameters, and only one study exists in which a tag recorded both parameters in fish. Clark et al. (2010) established the relationships between acceleration, f_H, tail beat frequency, energy expenditure and swimming speed in free-swimming sockeye salmon. However, the data logger used in their study (iLogR, B.D. Taylor, La Trobe University, Melbourne, Australia) is not commercially available. While a few studies have used Star-Oddi milli HRT tags to measure f_H in fishes (Prystay et al. 2017, 2019; Ekström et al. 2018; Brijs et al. 2018, 2019; Wallerius et al. 2019), it is not currently known how accurate or effective Star-Oddi centi-HRT ACT tags are in monitoring the f_H and activity of free-swimming fish.

This chapter evaluated the validity and reliability of Star-Oddi HRT-ACT tags for recording the physiology and behaviour of free-swimming Atlantic salmon (*Salmo salar*) by addressing four questions. First, do the acceleration parameters calculated with Star-Oddi software produce meaningful estimates of swimming speed, tail beat frequency and swimming behaviour? Second, how long does it take fish to recover from the effects of surgery? Third, can the tags record subtle changes in fish physiology and behaviour (e.g., diel patterns)? And finally, over long periods of time (i.e., weeks to months), does the

initial tag placement / orientation change and do tags continue to accurately record f_H and acceleration?

Methods

Animal Husbandry

The Atlantic salmon used in the below studies were supplied by the Dr. Joe Brown Aquatic Research Building (JBARB) at the Ocean Science Centre (OSC), Memorial University. All experimental work described was approved by the Institutional Animal Care Committee of Memorial University of Newfoundland (Protocol #17-95-KG), and followed the standards and guidelines outlined by the Canadian Council on Animal Care.

Data Storage Tag Implantation / Attachment

Several Star-Oddi DSTs were used in these experiments (see Table 2.1.). The milli-F (depth and temperature) tag was used simultaneously in some experiments with the centi-HRT ACT tag (combined mass ≤ 31 g in air), whereas in other experiments only the centi-HRT ACT tag or milli-HRT tags were used (see below). In all experiments, the tags did not exceed 2% of the fish's body mass, and therefore, the weight of the tags was not expected to disrupt fish behaviour, growth or activity (Lacroix et al. 2004; Snobl et al. 2015). Prior to implantation, DSTs were inserted into the tag-computer interface (COM-BOX) provided by Star-Oddi (this unit connected to a laptop computer), and the start time, start date, and sampling intervals were set using the tag's corresponding computer software (Mercury for HRT and HRT ACT tags, and SeaStar for the milli-F tags).

Table 2.1. Specifications of the Star-Oddi data storage tags used in Chapter 2 experiments.

Tag Type	Parameters Recorded	Length (mm)	Diameter (mm)	Mass in Air (g)
Centi-HRT ACT	Heart Rate, ECGs, acceleration and temperature.	50	15	19
Milli-HRT	Heart Rate, ECGs, and temperature.	42	13	12
Milli-F	Pressure / depth and temperature.	39.4	13	12

Atlantic salmon were implanted with centi-HRT ACT tags in one of two orientations: 1) with the positive and negative sensors facing the body wall ("sensors up"); or 2) with the label facing the body wall ("label up"). Preliminary trials determined that while the "label up" method was best for fish ranging in size > ~ 2 kg, implanting fish less than 1.7 kg with this method resulted in artefacts in the ECG recordings. Thus, fish less than 1.7 kg were implanted with tags in the "sensors up" orientation. Milli-HRT tags were always implanted in the "sensors up" orientation. To prepare the tags for implantation, two pieces of black, braided, non-absorbable and non-sterile, silk suture (2-0) were tied around the body of the tag in the "sensors up" or "label up" orientation (Figure 2.1.). All tags and surgical equipment were cleaned thoroughly and sterilized in 70% ethanol between uses.

Previous studies have described methods for implanting tags into fish, and provided the basis for the methods detailed below (e.g., see Rikardsen and Thorstad 2006; Clark et al. 2010; Korsøen et al. 2012; Prystay et al. 2017). Fish were anaesthetized in seawater containing 0.2 g L^{-1} tricaine methanesulfonate (MS-222). After losing equilibrium, the salmon were moved to a wetted surgical sponge where their gills were irrigated with aerated, 6 °C, seawater containing 0.1 g L^{-1} MS-222. Beginning at the posterior margin of the pectoral fins, a small (~ 3 cm) mid-ventral incision was made through the skin and muscle using a scalpel. Blood from the muscle was cleaned and clotted with light pressure from Q-tips. Centi-HRT ACT or milli-HRT tags were then inserted blunt end towards the posterior of the fish, then pulled forward to within 0.5 cm of the pericardium. The sutures attached to the tag were then passed through the body wall at the anterior and posterior margins of the incision using a curved surgical needle,

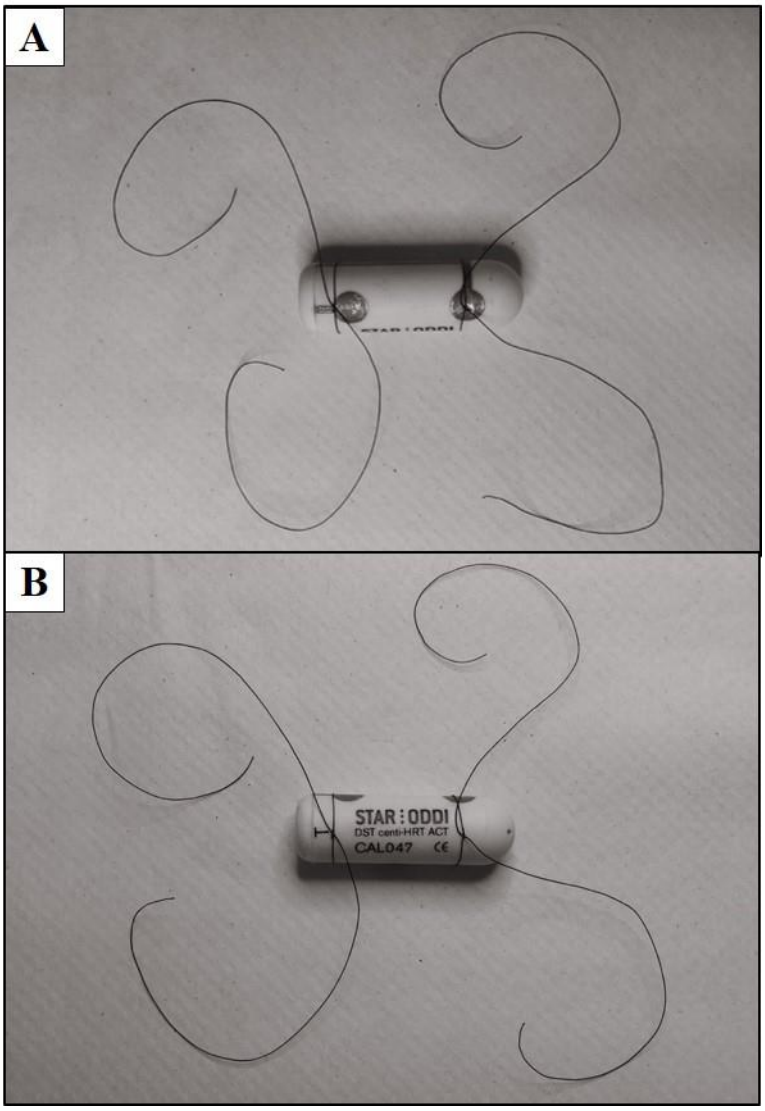

Figure 2.1. The Star-Oddi centi-HRT ACT tag, which records heart rate, electrocardiograms, tri-axial acceleration and temperature, was prepared for implantation by tying two pieces of braided, non-absorbable and non-sterile, 2-0 silk suture around the tag (one near the front and one near the back). This was done with the tag either in the "sensors up" (A) or "label up" (B) orientation.

and tied to hold the DST in place and to start closure of the incision. Finally, the remaining incision was closed using continuous stitches (2-0 silk) and super glue was applied to the incision and allowed to dry.

In some fish (see Experiments #2 and #3), milli-F tags were then attached to the fish externally using a "tag holder kit" provided by Star-Oddi and stainless steel wire (0.02" diameter); the kit consisting of two silicone pads and two plastic molds (Figure 2.2). Tags were prepared for attachment by looping pre-sterilized stainless steel wire over the tag and passing the ends of the wire through a silicone pad and the pre-drilled holes in the plastic mold. Four pre-sterilized stainless steel hypodermic needles (15 gauge, 3.5" long) were then passed through the skin and muscle below the dorsal fin to allow the stainless-steel wire to be guided through. Then, the hypodermic needles were removed and the 4 wires exiting the muscle were passed through the other silicone pad and plastic mold, and the wires were twisted together (Figure 2.2). When fish were not equipped with the DST milli-F tag, two coloured beads or disc tags were attached near the dorsal fin for individual identification.

After surgery, the salmon were recovered in anaesthetic-free water and returned to their holding tank (see below). Following all experiments, the fish were euthanized in seawater containing 0.3 g L^{-1} MS-222 in order to perform post-mortem dissections and to recover the tags / data. Post-mortem dissections were conducted to record incision length, the distance from the front of the tag to the pericardium, to note any signs of infection or inflammation, and to determine sex when possible. Data was retrieved using the COM-BOX and Mercury or SeaStar software.

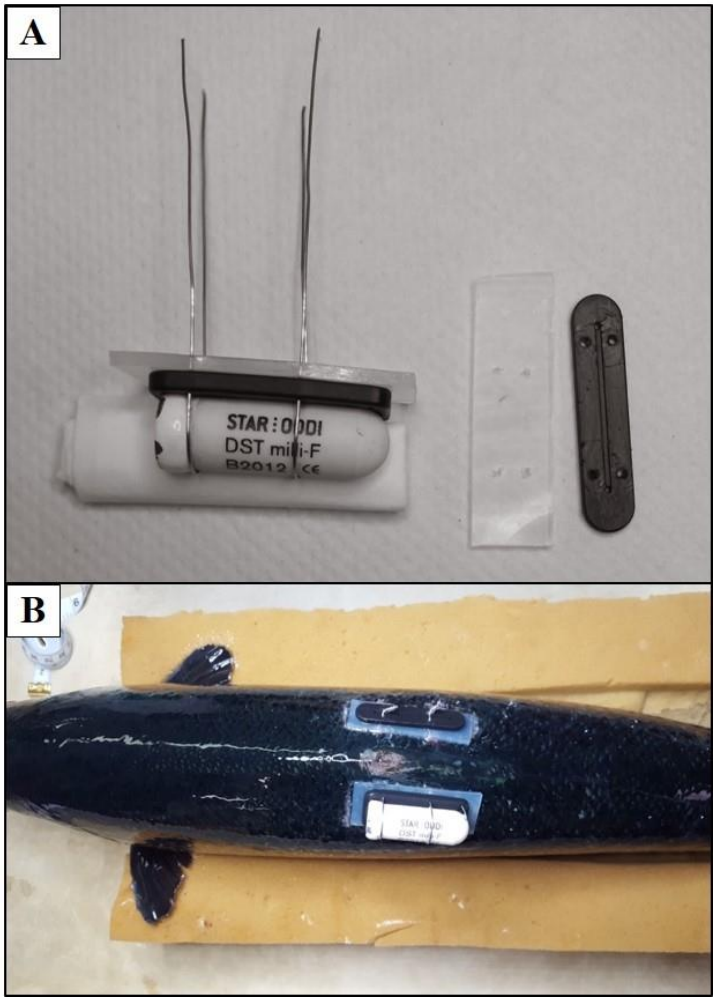

Figure 2.2. A) A Star-Oddi milli-F tag, which measures depth and temperature, shown with the attachment kit (i.e., plastic mold and silicone pad) and the stainless steel wires that were used to secure the attachment kit to the tag. B) The tag was attached to the Atlantic salmon by inserting 15 gauge stainless steel needles through the fish's dorsal musculature just below the dorsal fin, passing the stainless steel wire through the needles to the other side of the fish and then through another silicone pad and plastic mold, and then twisting the pairs of wires together so that the tag was firmly attached to the fish.

Experiment #1: Relationship Between Accelerometry Parameters and Swimming Speed

The Atlantic salmon used in this experiment (range ~ 0.94 to 1.52 kg) were held in the Laboratory for Atlantic Salmon and Climate Change Research (LASCCR) at the OSC. The fish were maintained in 2.2 m^3 tanks supplied with seawater at 11-13°C and 100-120% air saturation, and a 14h light:10h dark photoperiod. The fish were fed daily, the amount of feed 1.5 x what they would eat in a single meal. This feed was provided by automatic feeders that distributed pellets every 30 minutes from 9:00 AM to 5:00 PM.

These fish (n = 8; means ± S.E.M.; 1.35 ± 0.74 kg, 46.70 ± 0.79 cm in total length) were implanted with a centi-HRT ACT tag and returned to their tank in the LASCCR to recover for 6 days. The pre-programmed centi-HRT ACT tags were off for the first 5 days and began saving ECGs and recording f_H (at 100 Hz for 6 seconds), tri-axial acceleration (at 1 Hz for 60 seconds) and temperature at a sampling frequency of 2 minutes on the sixth day at 12:00 PM. At 1:00 PM, the fish were netted, lightly anaesthetized in seawater containing 0.1 g L^{-1} MS-222 and transferred to an 81 L Blazka-type swim tunnel. The swim tunnel was maintained at ~ 11°C by a temperature controlled, and aerated, external reservoir and set to a low current velocity (~ 0.2 body lengths sec^{-1}, BL sec^{-1}; ~ 10 cm sec^{-1}). This allowed the fish to rest on the bottom of the swim tunnel and maintain an upright position. The front of the swim tunnel was covered with black plastic to encourage the fish to seek refuge near the front of the tunnel.

At 11:00 AM the following day (i.e., after 21-22 hours of acclimation), a critical swim speed (U_{crit}) test was performed on the fish. Specifically, the water velocity was increased to 0.6 BL sec^{-1} (a velocity at which the fish would start swimming), and then

increased by 0.2 BL sec^{-1} every 10 minutes until the fish fatigued and could no longer swim. When the fish's tail entered the back $1/6^{th}$ of the tunnel, the back of the swim tunnel was tapped to encourage the fish to swim forward. In some cases (n = 5), after fish had reached their U_{crit}, they were given a short (~ 5 minute) rest period at low current velocity, and then the water velocity was rapidly increased back up and fish were briefly able to swim at speeds above their U_{crit}. Only accelerometry data was used from these latter trials. The fish were continuously monitored during these swim trials, and only periods when fish were actively swimming were used in data analysis. Fish were given 1 hour of recovery after the swim trial, then they were removed from the swim tunnel and euthanized. At each swimming speed, video was recorded for 30 seconds from the side of the swim tunnel using a GoPro (Model HERO5; San Mateo, CA) mounted on a tri-pod. From these videos, the number of full tail oscillations in 10 seconds was recorded, which were multiplied by 6 to get the fish's tail beat frequency (TBF) in beats min^{-1}. Three triplicate values were averaged to calculate the mean TBF of fish at each swimming speed.

Experiment #2: Heart Rate and 'Activity' of Free-Swimming Fish for Seven Days Post-Surgery

Atlantic salmon (range ~ 2.10 to 2.95 kg) were held in the JBARB of the OSC in a 2.64 m diameter x 2.50 m deep tank (Figure 2.3 A). These tanks were supplied with seawater at 10-11°C and 100-120% of air saturation, and a 12h light: 12h dark photoperiod. The fish were fed a maintenance ration (1.0% body mass) of commercial salmon diet every other day.

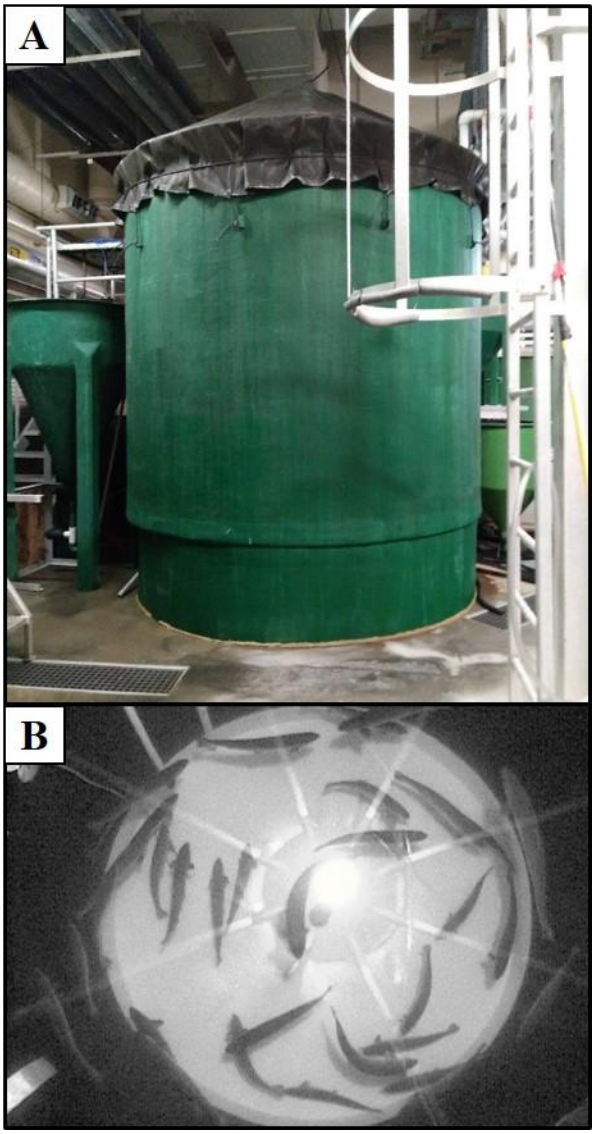

Figure 2.3. A) A 2.64 m diameter x 2.50 m deep tank in the Dr. Joe Brown Aquatic Research Building at the Ocean Science Centre, Logy Bay, Newfoundland. B) Top view of the inside of the tank showing Atlantic salmon recovering from implantation / attachment of Star-Oddi centi-HRT ACT and milli-F tags. Fish were held in this tank for 7 days with 30-35 conspecifics.

These fish (n = 10; 2.54 ± 0.92 kg; 62.23 ± 1.00 cm in total length) were implanted with centi-HRT ACT and milli-F tags and returned to their tank to recover for 7 days with 30-35 conspecifics (Figure 2.3 B). Two fish were tagged weekly between January 18th and March 7th, 2018. The tags were pre-programmed to save ECGs and record f_H (at 100 Hz for 6 seconds), tri-axial acceleration (at 1 Hz for 60 seconds) and temperature at a sampling frequency of 10 minutes, while the milli-F tags were set to record depth and temperature at a sampling frequency of 1 minute. On the 7th day, the fish were netted from their tank and euthanized.

Experiment #3: Heart Rate, 'Activity' and Tag Retention in Free-Swimming Fish for Six Weeks Post-Surgery

Atlantic salmon (range ~ 1.09 to 3.25 kg) were held in the JBARB of the OSC in a 2.64 m diameter by 3.78 m deep tank with a volume of 30 m^3. These tanks were supplied with seawater at 8-12°C and 100-120% of air saturation, and a 12h light: 12h dark photoperiod, and the fish were fed a maintenance ration (1.0% body mass) of commercial salmon diet on Mondays, Wednesdays and Fridays.

These fish (2.27 ± 0.25 kg; 59.29 ± 1.38 cm in total length) were implanted with centi-HRT ACT and milli-F tags (n = 4), centi-HRT ACT tags alone (n = 4), or milli-HRT tags (n = 2). Following implantation, fish were held for 6 weeks with 20-25 conspecifics. Five fish were tagged on September 17th, 2019 and another 5 on December 12th, 2019. The centi-HRT ACT tags were pre-programmed to store ECGs and record f_H (at 100 Hz for 6 seconds), tri-axial acceleration (at 1 Hz for 60 seconds) and temperature at a sampling frequency of 2 hours, the milli-HRT tags were set to store ECGs and record

f_H (at 100 Hz for 6 seconds) and temperature at a frequency of 2 hours, and the milli-F tags were set to record depth and temperature at a frequency 1 hour. At the end of 6 weeks, the tagged fish were netted from their tank and euthanized. In these experiments, both centi-HRT ACT and milli-HRT tags were used as the latter tag was kindly provided by Star-Oddi for preliminary testing.

Calculation of Heart Rate Parameters

All measurements of f_H were provided with a unitless measurement known as the quality index (QI) determined by the Mercury software; this measurement representing the quality of the ECG signal where 0 means great quality, 1 and 2 have decreasing quality and 3 means there is no R-R interval. In Experiment $^{\#}$1, manual calculations from the stored ECGs were performed on all reported f_H data. However, based on the results of Experiment $^{\#}$1, manual calculations in Experiments $^{\#}$2 and $^{\#}$3 were only performed on f_H measurements less than 15 beats per minute (bpm) or greater than 85 bpm and when the associated QI values were greater than 0. All f_H measurements between 15 and 85 bpm and with a QI value of 0 were accepted. To manually calculate f_H from the stored ECGs, the time between R wave peaks was measured (in seconds) and averaged, and then 60 was divided by the average to obtain the fish's f_H in bpm (Figure 2.4). Manual calculations of f_H were not possible when there was only one QRS complex or when ECG artefacts made the QRS complex unidentifiable, and these data were not included. For Experiment $^{\#}$1, heart rate variability (HRV) was calculated as the standard deviation of the time between successive R wave peaks (in ms).

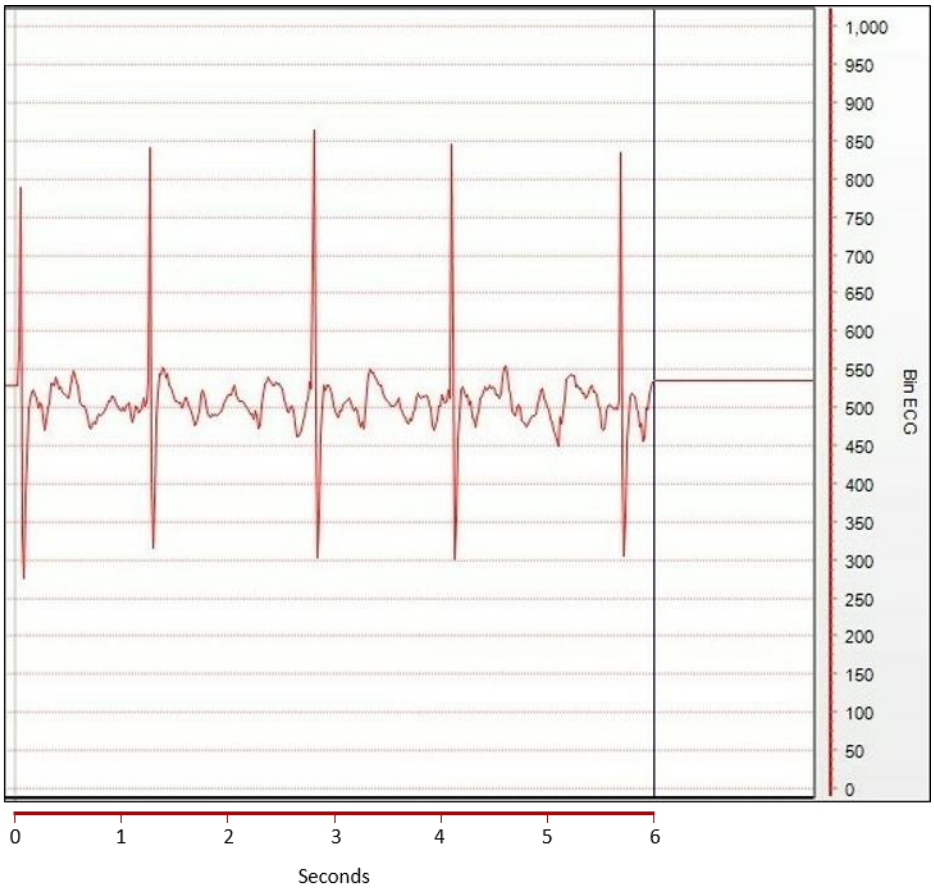

Figure 2.4. An electrocardiogram recorded in a salmon during one week of recovery in a large tank in JBARB. The ECG was randomly chosen to represent the typical recording from a salmon, where Bin ECG represents the amplitude of the QRS waveform and ranges from 0 to 1000 mV. Heart rate was calculated from the ECGs as the time between R wave peaks (measured in seconds). These values were then averaged, and 60 was divided by the average to obtain the fish's f_H in bpm. Heart rate variability (HRV) was calculated as the standard deviation of the time between successive R wave peaks (in ms).

Accelerometry Parameters

The centi-HRT ACT tag measures acceleration in three dimensions in reference to the earth's gravity. The Mercury software records and stores the raw data calibrated for gravitational acceleration. From these raw data, the software calculates external acceleration (EA), the variation in EA (VAR) and activity (ACT). Values of EA indicate when the sensor is measuring acceleration above normal gravity (measured in m-g at 1 Hz for 60 seconds, where g is the acceleration of gravity or 9.8 m s^{-2}) and was averaged over 1 minute samples. VAR is the variation in external acceleration over a set sampling period (1 Hz for 60 seconds). These two parameters were found to provide meaningful data related to activity in Experiments #1, 2 and 3. The parameter ACT (a threshold value derived from VAR) is less useful as it is a discrete measurement between 1 and 3, and thus, can only provide limited qualitative information. For this reason, it was not reported.

Statistics

In Experiment #1, several types of equations were fitted to the data to determine the best relationship (based on the R^2 values) between EA and swimming speed and TBF, and between VAR and the latter two parameters (see Table 2.2.). In some instances, exponential growth (EG) equations were given priority over polynomial (2P and 3P) equations with similar R^2 values, because some 2P/3P equations had undefined confidence interval errors and EG equations are more comparable to the previous literature. Relationships were determined using Prism 7 (GraphPad Software, Inc., San Diego, CA, USA). Linear mixed-effects (LME) models in RStudio (v. 1.2.1335, RStudio Inc., Boston, MA; http://www.rstudio.com) were used to analyze the f_H, HRV and the

Table 2.2. Relationships between external acceleration (EA, m-g) and swimming speed (BL sec^{-1}) and tail beat frequency (tail beats min^{-1}), and between the latter two parameters and variation in EA (VAR, minutes). The following equations were tested for each relationship: linear regression (LR), exponential growth (EG), one phase decay (D), second order polynomial / quadratic (2P), and third order polynomial / cubic (3P). Relationships with the resulting best fit (highest R^2 value) are in bold. For data presentation (see Figure 2.5), EG was used for all relationships given that it had the best fit, or its fit was extremely similar to that obtained by 3P.

Dependent Variable	**Independent Variable**	**Equation Tested**	**R^2**
Tail Beat Frequency	Speed in BL s^{-1}	LR	0.7862
		EG	0.7094
		D	0.8385
		2P	0.8293
		3P	**0.8469**
External Acceleration	Speed in BL s^{-1}	LR	0.5986
		EG	0.6365
		2P	0.6387
		3P	**0.6397**
External Acceleration	Tail Beat Frequency	LR	0.4998
		EG	0.6033
		2P	0.6347
		3P	**0.6406**

Variation in EA	Speed in BL s^{-1}	LR	0.4677
		EG	**0.7971**
		2P	0.6921
		3P	0.7840
Variation in EA	Tail Beat Frequency	LR	0.3518
		EG	**0.8305**
		2P	0.6488
		3P	0.8063

percentage of QI values = 0 data, with swimming speed (BL sec^{-1}) as a fixed-effect and fish as a random factor.

For Experiments #2 and #3, LME models in RStudio were also used to analyze f_H, EA, and the percentage of QI values = 0 data, with photoperiod and photoperiod order as fixed-effects, an interaction term for the two parameters, and fish as a random factor, included in the model. A photoperiod of 12h light:12h dark was maintained for both experiments, therefore photoperiod was assigned as 'day-time' between 8:00 AM and 7:59 PM or 'night-time' between 8:00 PM and 7:59 AM. Photoperiod order was used to described the daily values during each photoperiod, where N1 was the first night following surgery, and D1 through D6 or D42 represent the subsequent day-time and night-time values. In the second trial of Experiment #3 (n = 5 per trial), the temperature in the tank fluctuated between 8 and 12°C due to issues with facility temperature control, and thus, data for each trial was analyzed separately.

For data analyzed in RStudio, assumptions of normality, homogeneity and independence were analyzed by visual inspection of Q-Q plots and histograms of the residuals, residual-fit plots and residual lag plots, respectively. The estimated marginal means, or emmeans, package (Singmann et al. 2019) was used to perform Bonferroni's post-hoc tests on the LME models. The level of statistical significance was $P < 0.05$. All values presented in the text, and in figures and tables, are means ± standard errors of the mean (S.E.M).

Results

Relationship Between Accelerometry Parameters and Swimming Speed

Parameters of acceleration recorded in Atlantic salmon increased during the U_{crit} swimming test. EA and VAR increased exponentially with both swimming speed and TBF (EA: $y = 3.528e^{0.954x}$; $R^2 = 0.637$; $y = 3.340e^{0.009x}$; $R^2 = 0.603$, respectively; and VAR: $y = 0.364^{3.911x}$; $R^2 = 0.797$; $y = 0.261^{0.040x}$; $R^2 = 0.831$, respectively; Figure 2.5; Table 2.2). EA ranged from 6.77 ± 1.45 at 0.6 BL sec^{-1} / ~ 24 tail beats min^{-1} to 16.77 ± 1.86 at 1.6 BL sec^{-1} / ~ 180 tail beats min^{-1} (fold-change = 2.48), while VAR ranged from 22.15 ± 4.88 to 416.49 ± 157.49 minutes (fold-change = 18.80). Tail beat frequency increased with swimming speed (data not presented; $y = 297.6 + 802.1x – 503.7x^2 + 114.5x^3$; $R^2 = 0.847$).

During the U_{crit} test, f_H increased significantly from 61.1 ± 1.0 bpm at rest to 77.1 ± 0.7 bpm at 1.6 BL sec^{-1} ($P < 0.0001$), while HRV decreased significantly from 62.4 ± 3.62 to 30.1 ± 4.30 ms ($P < 0.0001$; Figure 2.6; Table 2.3). The percentage of good quality ECGs (i.e., QI = 0) did not change significantly with swimming speed but decreased marginally from 83% at rest to ~ 60% while swimming ($P = 0.074$). An average of 68% of measurements were QI = 0 and only 0.1% of these data could not be calculated post-analysis (Table 2.4). The absolute difference in f_H between manually calculated values and those calculated by the Mercury software increased with the reported QI value (i.e., 2.3 ± 8.0 bpm for QI = 0, 10.8 ± 27.2 bpm for QI = 1, 31.0 ± 63.0 bpm for QI = 2, 39.5 ± 41.2 bpm for QI = 3; Figure 2.7. A). Further, when I visually inspected the ECGs, I would have only considered 73.3 ± 8.1% of QI = 0 f_H recordings to be good quality, and recordings of QI = 1 and 2 to be of good quality 23.4 ± 9.1 and 19.6

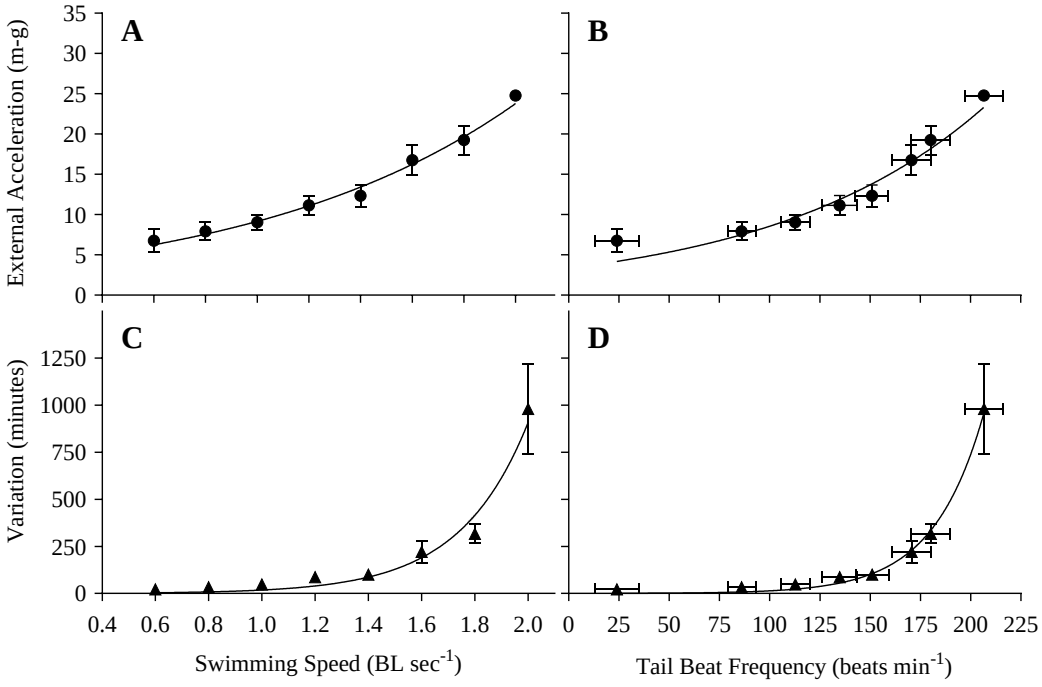

Figure 2.5. External acceleration (EA) and variation in EA (VAR) measured in Atlantic salmon during a critical swim speed (U_{crit}) test (speed increments of 0.2 BL sec^{-1}). Tail beat frequency (beats min^{-1}) was determined from 30 second video clips recorded during each swimming speed. The data for all fish were fitted with a number of types of equations (see Table 2.2), and the exponential equations that fit each relationship (based on the data for individual fish) were as follows: (A) $y = 3.528e^{0.954x}$, (B) $y = 3.340e^{0.009x}$, (C) $y = 0.364e^{3.911x}$ and (D) $y = 0.261^{0.040x}$. Data are means ± S.E.M.; n = 6 to 8.

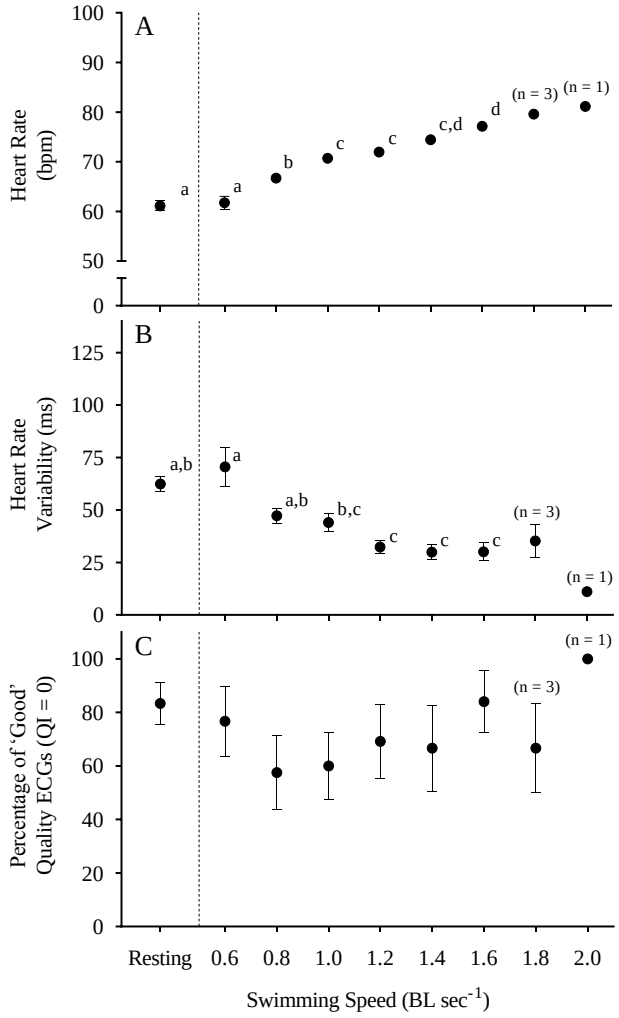

Figure 2.6. Changes in heart rate (f_H) and related parameters when Atlantic salmon were given a critical swim (U_{crit}) test (speed increment of 0.2 BL sec^{-1}). A) f_H (in bpm) was measured every 2 minutes in salmon immediately prior to (resting values) and during the U_{crit} test. Heart rate variability (HRV in ms; B) was manually calculated from the electrocardiograms and the percentage of 'good' quality index ECGs (QI = 0; C) was provided by the Star-Oddi Mercury software. Dissimilar lower-case letters indicate a significant difference between values, as determined by LME models. These models did not include the swimming speeds of 1.8 and 2.0 BL sec^{-1} due to low sample sizes. The percentage of good quality ECGs did not change significantly with swim speed (P = 0.074). Data are means ± S.E.M.; n = 6 to 8 except where indicated.

Table 2.3. Summary of the statistical outputs from linear mixed-effects models that were used to examine the effects of swimming speed on heart rate parameters in Atlantic salmon. Linear mixed-effects models were used to assess the effects of swimming speed (body lengths sec^{-1}) on heart rate, heart rate variability and the percentage of quality index values equal to zero (QI = 0).

Independent Factor	Dependent Factor	NumDF	DenDF	F	P
Heart Rate	(Intercept)	1	230	1915.2032	**<0.0001**
	Body Lengths Sec^{-1}	6	230	45.8207	**<0.0001**
Heart Rate Variability	(Intercept)	1	228	49.84475	**<0.0001**
	Body Lengths Sec^{-1}	6	228	4.45037	**3e-04**
Percentage of Quality Index Values = 0	(Intercept)	1	35	49.96008	**<0.0001**
	Body Lengths Sec^{-1}	6	35	1.69760	0.1508

NumDF and DenDF are the degrees of freedom of the numerator and denominator of the F distribution ratio.

Table 2.4. The percentage of ECGs of each quality index value (QI = 0 - 3), and percentage of heart rate (f_H) values that could not be manually calculated due to ECG artefacts during the U_{crit} test (n = 8), during one week of recovery (n = 10) and during six weeks of recovery (n = 10).

	Experiment	QI = 0	QI = 1	QI = 2	QI = 3
Percentage of f_H Data	U_{crit}	68.5 ± 9.2	13.1 ± 4.2	14.4 ± 4.4	3.9 ± 1.8
	Recovery (1 week)	88.8 ± 2.8	5.4 ± 2.2	5.0 ± 0.9	0.9 ± 0.3
	Recovery (6 weeks)	86.9 ± 4.7	3.5 ± 2.1	4.0 ± 1.5	5.6 ± 3.7
Percentage Unable to Calculate f_H	U_{crit}	0	0	1.7 ± 0.9	0.3 ± 0.3
	Recovery (1 week)	0	0.1 ± 0.1	0.3 ± 0.1	0.1 ± 0.0
	Recovery (6 weeks)	0.1 ± 0.0	0.4 ± 0.4	0.7 ± 0.6	4.7 ± 3.5

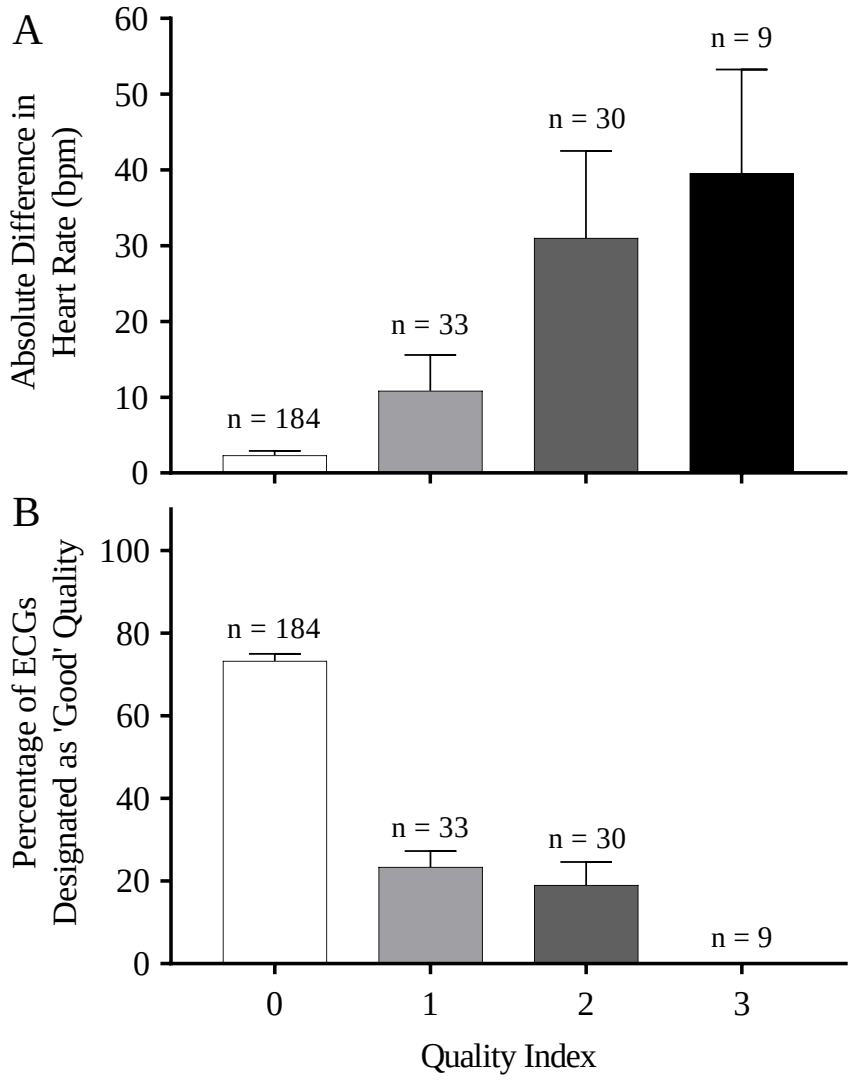

Figure 2.7. Electrocardiograms (ECGs) recorded by the Star-Oddi centi-HRT ACT tag in Atlantic salmon during a critical swim (U_{crit}) test. Heart rate (f_H) was calculated by the Mercury software from the ECGs, which also provided a value to indicate the quality of the data (QI = 0 means great quality, 1 and 2 have decreasing quality and 3 means there is no R-R interval). A) Absolute differences in f_H (bpm) between those recorded by the Mercury software and those same values calculated manually from raw ECGs. B) The percentage of ECGs designated as 'good' quality based on manual inspection as compared to the QI values provided by the Mercury software. Data are means ± S.E.M.

± 11.5% of the time. On the other hand, I did not find any QI = 3 recordings to be good of quality (Figure 2.7. B).

Heart Rate and Activity During Post-Surgical Recovery

Following tag implantation, f_H, EA, VAR and temperature were recorded in salmon recovering in holding tanks with conspecifics for one week (Figure 2.8) and six weeks (Figure 2.9). Heart rate decreased ($P < 0.0001$; Figure 2.10; Table 2.5), and EA increased ($P < 0.0001$), over the one week recovery period; from 58.8 ± 0.8 and 47.4 ± 0.9 bpm (day-time and night-time values, respectively) initially to 43.9 ± 0.7 and 36.9 ± 2.7 bpm by the final day of recovery. In contrast, EA increased from 9.3 ± 0.3 and 8.5 ± 0.4 m-g (~ 1.02 and 0.92 BL sec^{-1} as estimated from swim tunnel calibrations) to 10.5 ± 0.4 m-g and 9.6 ± 0.4 m-g (~ 1.14 and 1.04 BL sec^{-1}). Based on a visual inspection of this data, it appeared that the measured parameters began stabilizing after approx. 4 days of recovery (Figure 2.8). However, significant differences were observed until the final day of recovery (Day 7)(Figure 2.10).

Data from the six week period of recovery are presented in Figures 2.9 and 2.11, and summarized in Table 2.6. In the first trial at 8°C, f_H decreased significantly from 43.6 ± 1.4 and 31.2 ± 1.4 bpm to 29.1 ± 1.1 and 24.4 ± 0.9 bpm at 21 days post-surgery ($P < 0.0001$). By 42 days, f_H was ~ 30 bpm during the day and ~ 26 bpm during the night. EA increased slightly by 140% during the recovery period ($P < 0.0001$). Overall, salmon in this trial were less active (average EA = ~ 6.4 m-g; or 0.62 BL sec^{-1}) than salmon held for one week of recovery (average EA = ~ 9.5 m-g; or 1.04 BL sec^{-1}). Heart rate decreased and EA increased similarly in the second trial ($P < 0.0001$ and $P = 0.0095$, respectively).

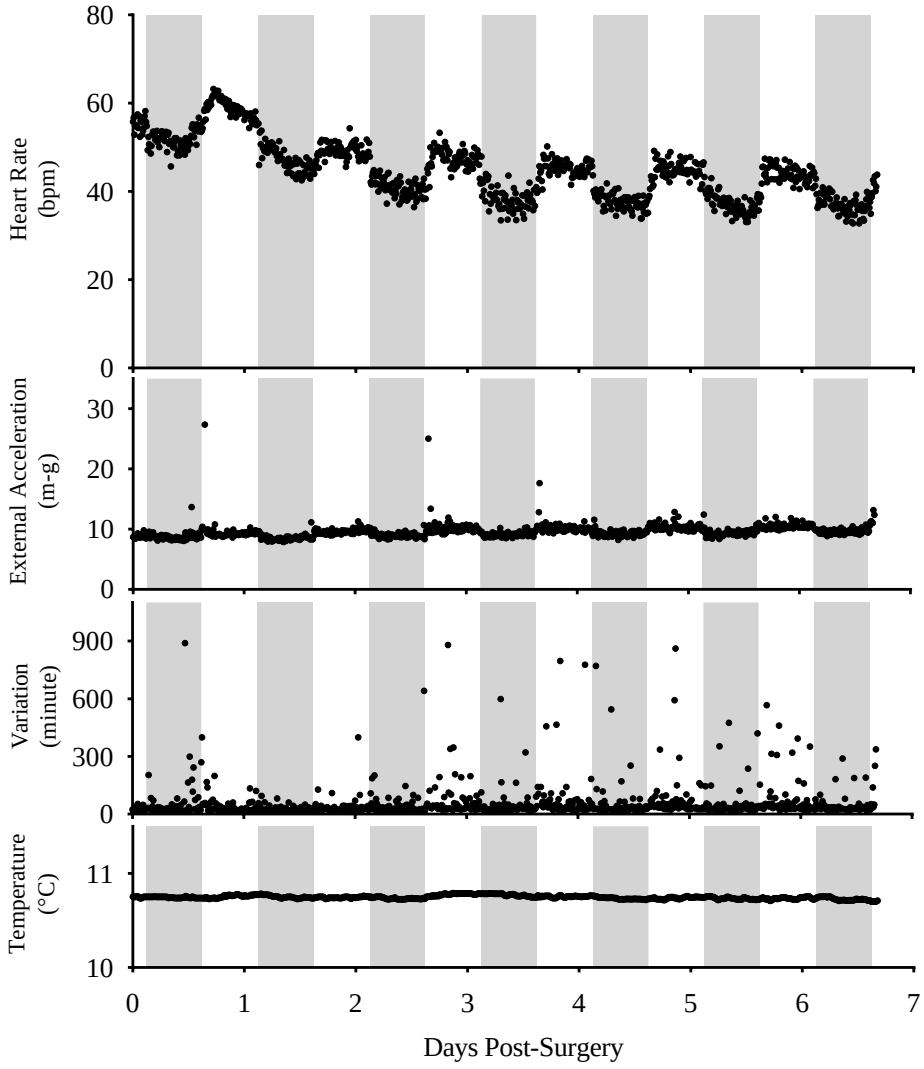

Figure 2.8. Heart rate (f_H, bpm), external acceleration (EA, m-g), variation in EA (VAR, minutes) and temperature (°C) values in free-swimming Atlantic salmon (n = 10) for 1 week following surgical implantation of the Star-Oddi centi-HRT ACT tag or the milli-HRT tag. Salmon were held in a large tank following surgical implantation of the tags. Fish were on a 12-hour light: 12-hour dark photoperiod (grey bars represent periods of darkness / night-time), and data were collected at 10 minute intervals.

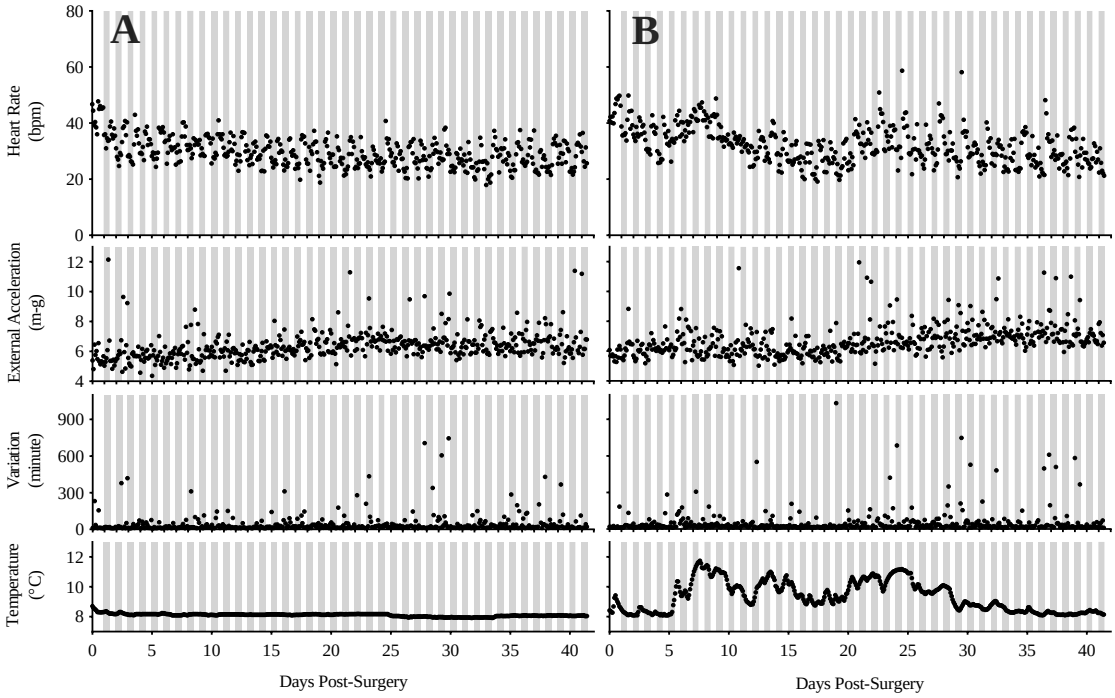

Figure 2.9. Heart rate (f_H, bpm), external acceleration (EA, m-g), variation in EA (VAR, minutes) and temperature (°C) values in free-swimming Atlantic salmon recorded for 6 weeks following surgical implantation of the Star-Oddi centi-HRT ACT tag or the milli-HRT tag. Salmon were maintained at a constant temperature of 8°C in the first tagging trial (A; n = 5). In the 2nd trial (B; n = 5), temperature ranged between 8 and 12°C due to issues with facility temperature control. Fish were on a 12-hour light: 12-hour dark photoperiod (grey bars represent periods of darkness / night-time), and data were collected every 2 hours.

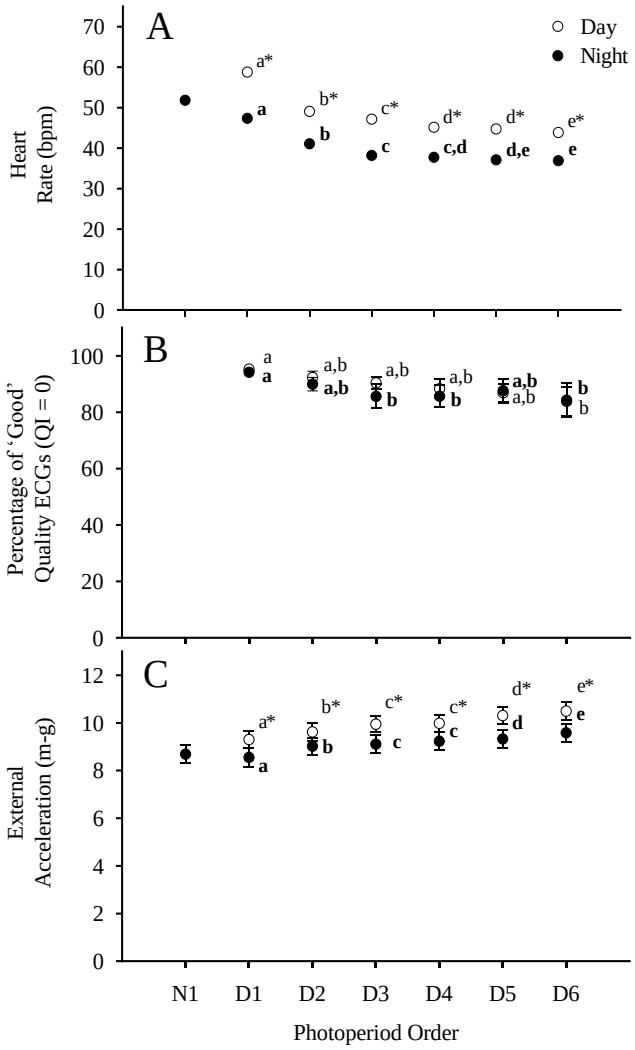

Figure 2.10. Average day-time and night-time heart rate (f_H in bpm; A), percentage of 'good' quality index ECG values (i.e., QI = 0; B) and external acceleration (EA in m-g; C) values in free-swimming Atlantic salmon (n = 10) recorded for 7 days following surgical implantation of the Star-Oddi centi-HRT ACT tag. Open symbols represent day-time, whereas dark symbols represent periods of darkness / night-time. Dissimilar lower-case letters indicate a significant difference within a photoperiod group (for night-time values the letters are bolded), while an asterisk (*) represents a significance difference between day-time and night-time values at each measurement period. Data are means ± S.E.M, with each value representing the average of n = 72 data points per fish.

Table 2.5. Summary of the statistical outputs from the linear mixed-effects models that examined the effects of night/day (photoperiod), days post-implantation (i.e., photoperiod order), and their interaction, on f_H parameters [f_H and percentage of quality index values equal to zero (QI = 0)] and external acceleration (EA) in salmon for 7 days post-surgery.

Independent Factor	Dependent Factor	Num DF	Den DF	F	P
Heart Rate	(Intercept)	1	8560	510.753	**<0.0001**
	Photoperiod	1	8560	3712.111	**<0.0001**
	Photoperiod Order	5	8560	805.027	**<0.0001**
	Interaction	5	8560	23.073	**<0.0001**
Percentage of Quality Index Values = 0	(Intercept)	1	98	923.827	**<0.0001**
	Photoperiod	1	98	1.944	0.1664
	Photoperiod Order	5	98	5.476	**0.0002**
	Interaction	5	98	0.631	0.6762
External Acceleration	(Intercept)	1	8586	95.752	**<0.0001**
	Photoperiod	1	8586	860.051	**<0.0001**
	Photoperiod Order	5	8586	131.749	**<0.0001**
	Interaction	5	8586	3.500	**0.0018**

NumDF and DenDF are the degrees of freedom of the numerator and denominator of the F distribution ratio.

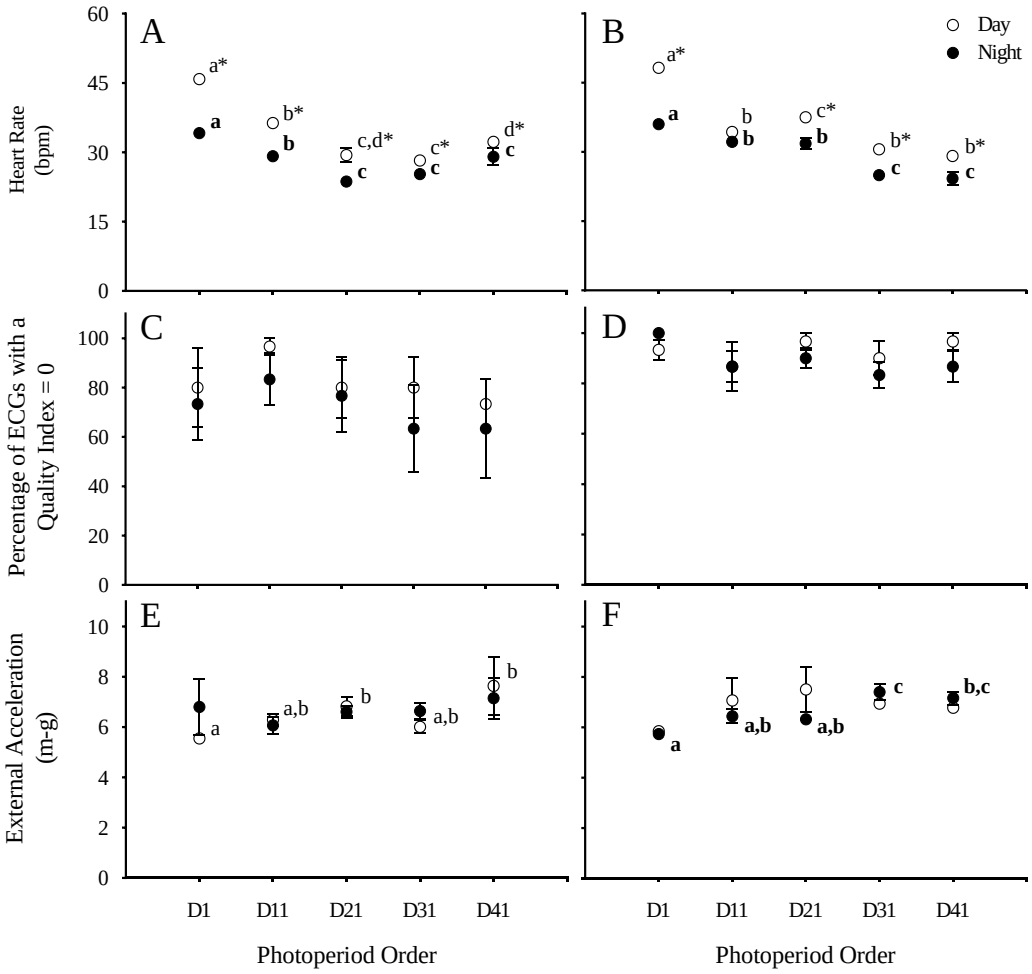

Figure 2.11. Average heart rate (f_H in bpm; A, B), percentage of 'good' ECGs (QI = 0; C, D) and external acceleration (EA in m-g; E, F) values in free-swimming Atlantic salmon held for 6 weeks following surgical implantation of the Star-Oddi centi-HRT ACT tag or milli-HRT tag. Salmon were maintained at a constant temperature of 8°C in the first tagging trial (A, C, E; n = 5), whereas temperature varied between 8 and 12°C in the second trial (B, D, F; n = 5) due to issues with facility temperature control. Open symbols represent day-time measurements, whereas dark symbols represent periods of night-time. Dissimilar lower-case letters indicate a significant difference within a photoperiod group (for night-time values the letters are in bold), while an asterisk (*) indicates a significant difference between day-time and night-time values. Data are means ± S.E.M, with each value representing the average of n = 6 data points per fish.

Table 2.6. Summary of the statistical output from the linear mixed-effects models that examined the effects of night/day (photoperiod), days post-implantation (i.e., photoperiod order), and their interaction, on f_H parameters [f_H and percentage of quality index values equal to zero (QI = 0)] and external acceleration (EA) in Atlantic salmon for 6 weeks post-implantation. Due to issues with facility temperature control, the data is separated into two trials with a tank temperature of 8°C in trial 1 (A) and 8-12°C in trial 2 (B).

Independent Factor	Dependent Factor	Num DF	Den DF	F	P
A. Trial 1					
Heart Rate	(Intercept)	1	252	116.8220	**<0.0001**
	Photoperiod	1	252	121.1120	**<0.0001**
	Photoperiod Order	4	252	79.46901	**<0.0001**
	Interaction	4	252	3.89345	**0.0044**
Percentage of Quality Index Values = 0	(Intercept)	1	35	67.76189	**<0.0001**
	Photoperiod	1	35	1.54935	0.2215
	Photoperiod Order	4	35	1.34151	0.2741
	Interaction	4	35	0.09792	0.9824
External Acceleration	(Intercept)	1	225	57.24601	**<0.0001**
	Photoperiod	1	225	0.70989	0.4004
	Photoperiod Order	4	225	7.70921	**<0.0001**

	Interaction	4	225	0.65493	0.6240
B. Trial 2					
Heart Rate	(Intercept)	1	282	414.2493	**<0.0001**
	Photoperiod	1	282	52.5477	**<0.0001**
	Photoperiod Order	4	282	45.7455	**<0.0001**
	Interaction	4	282	4.3768	**0.0019**
Percentage of Quality Index Values = 0	(Intercept)	1	35	3731.347	**<0.0001**
	Photoperiod	1	35	0.317	0.5767
	Photoperiod Order	4	35	1.210	0.3241
	Interaction	4	35	1.654	0.1826
External Acceleration	(Intercept)	1	458	104.4373	**<0.0001**
	Photoperiod	1	458	1.29215	0.2562
	Photoperiod Order	4	458	3.38926	**0.0095**
	Interaction	4	458	0.96735	0.4251

NumDF and DenDF are the degrees of freedom of the numerator and denominator of the F distribution ratio.

However, fluctuations in temperature altered the daily averages as compared to the first trial. For example, day-time f_H and EA on D21 were ~ 8 bpm and ~ 1 m-g higher in the second trial (at 9.3 ± 0.1°C) than in the first trial (at 8.1 ± 0.0°C). Indeed, when the f_H data from the second trial (excluding data from the first week of recovery) were plotted against temperature, there was a significant relationship (data not presented; $Y = 3.01x + 2.64$; $R^2 = 0.23$; $P < 0.0001$). When temperature was 8.5°C, f_H was on average 28.2 bpm whereas, at 12°C, f_H averaged 38.8 bpm.

Diel Heart Rate and Activity Patterns Post-Surgery

The effects of photoperiod on f_H (i.e., diel variation) were consistent and significant in all recovery trials ($P < 0.0001$; Figures 2.10 and 2.11; Table 2.5, 2.6). Salmon held at 10-11°C had day-time f_H values ~ 7 bpm higher than night-time values by one week post-surgery (Figure 2.10), while at 8°C, diel variation was ~ 4 bpm at 42 days. In salmon held at 10-11°C for one week, photoperiod also had a significant effect on EA (a difference of ~ 1 m-g, 0.09 BL sec^{-1} by one week post-surgery). However, there were no significant effects of photoperiod on EA during Trial 1 (8°C) or Trial 2 (8-12°C) when the salmon were recovered for 6 weeks ($P = 0.2562$ and 0.4004, respectively).

Measurement Quality and Tag Retention Post-Surgery

The percentage of good quality ECG values decreased significantly (by ~ 11%) over one week of recovery ($P = 0.0002$; Figure 2.10; Table 2.5), but did not change significantly over the six weeks of recovery (Trial 1: $P = 0.2741$; Trial 1: $P = 0.3241$; Figure 2.11; Table 2.6). There were also no significant diel patterns in ECG quality.

Throughout the experiments, the average percentage of good quality ECGs (i.e., QI = 0) was ~ 86 and 88%, and only a very small percentage of this f_H data could not be manually calculated from the ECGs (~ 2 to 6%; Table 2.4). Following 6 weeks of holding, all tags remained within 0.7 cm of the pericardium, 80% of the incisions were fully healed, and 80% of anterior suture knots and 70% of posterior suture knots were intact (see Figure 2.1 for a review of tag preparation). However, only 10% of incision sutures remained. All milli-F tags remained securely attached to the dorsal muscle of the salmon.

Discussion

Relationship Between Accelerometry Parameters, Heart Rate and Swimming Speed

A primary goal of this chapter was to establish relationships between the swimming speed of Atlantic salmon and the parameters of acceleration calculated by the Star-Oddi Mercury software. EA and VAR increased exponentially with swimming speed and TBF (Figure 2.5, Table 2.2), and the EA data were subsequently used to estimate the swimming speed of salmon in large tanks at the JBARB. Previous researchers have also recorded relationships between swimming speed and / or TBF and either acceleration (Kawabe et al. 2003; Clark et al. 2010; Wilson et al. 2013; Martos-Sitcha et al. 2019) or overall dynamic body acceleration (ODBA; Wright et al. 2014). For example, Clark et al. (2010) reported an exponential relationship between acceleration activity (AA; calculated as the sum of the X and Y acceleration values with no specific units) and TBF in sockeye salmon, and that there was a 7.2-fold change in AA from 40 to 170 tail beats min^{-1} (Clark et al. 2010). Similarly, Wright et al. (2014) reported that an exponential equation was the best fit between ODBA and swimming speed in European sea bass, *Dicentrarchus labrax*,

with a 6.1-fold increase in vectorial ODBA between 0.4 and 1.7 BL sec^{-1}. ODBA is the sum of the absolute values of acceleration from all three spatial axes measured in gravitational acceleration (g; Wilson et al. 2006; Gleiss et al. 2011; Qasem et al. 2012; Wright et al. 2014). According to the Star-Oddi ACT manual and representatives (pers. comm.), EA is calculated in a similar way to ODBA, and thus, it is not surprising that it also provides a relationship with swimming speed and TBF. In contrast to the above studies, there was only a 2.5-fold change in EA in my U_{crit} test between salmon at rest and those swimming at 1.6 BL sec^{-1}. Despite this, the acceleration parameters as calculated by the Star-Oddi software (i.e., EA and VAR), were good predictors of salmon activity / swimming speed.

Acceleration and ODBA have most often been related to metabolism, and used as a proxy for energy expenditure in freely swimming fish (Clark et al. 2010; Gleiss et al. 2010, 2011; Wilson et al. 2013, 2014; Wright et al. 2014, Mori et al. 2015; Bouyoucos et al. 2017; Lear et al. 2017; Martos-Sitcha et al. 2019). Movement makes up a large portion of an animal's energy budget because the lateral undulations of the tail that provide the propulsive force for swimming are fueled by muscular contraction. Therefore, acceleration and ODBA provide important information about a fish's overall energy use (Kawabe et al. 2003; Wilson et al. 2006; Gleiss et al. 2010, 2011; Wright et al. 2014; Metcalfe et al. 2016). In this study, oxygen consumption ($\dot{M}O_2$) was not directly measured, however, f_H is highly correlated metabolic rate in fish under most circumstances (Armstrong 1986; Lucas 1994; Claireaux et al. 1995; but see Thorarensen et al.1996). The f_H of Atlantic salmon increased 1.26-fold during the swimming trial (i.e., from a resting value of 61 bpm to 77 bpm at 1.6 BL sec^{-1}; Figure 2.6). This is within the

range of fold increases reported by other authors for adult salmonids during swimming trials (1.05 to 2.42; Altimiras and Larsen 2000; Gallaugher et al. 2001; Dunmall and Schreer 2003; Claireaux et al. 2005; Dussault et al. 2008; Steinhausen et al. 2008; Eliason et al. 2013a,b). Given the increase in f_H in this study, and the well-established relationship between metabolism and acceleration, it is likely that EA would correlate with $\dot{M}O_2$ and that the centi-HRT ACT tags could be used as a tool to estimate the energy use of salmon in future studies. This, however, would require the establishment of a direct relationship between these parameters.

Further, the centi-HRT ACT tag provides the opportunity to save and analyze ECGs, and thus, investigate additional aspects of cardiac physiology. HRV (i.e., the variation in time between heart beats) is influenced by the autonomic nervous system and contains valuable information on functioning of the heart and fish physiology (Campbell et al. 2004; Jeanne et al. 2009; Gräns et al. 2014). For example, HRV measured in short-horned sculpin (*Myoxocephalus scorpius*) was shown to be a more sensitive predictor of $\dot{M}O_2$ than f_H (Campbell et al. 2004). However, it is not known whether this is true for other fish species. Studies quantifying HRV in fish are rare, probably due to the difficulty of recording ECGs for prolonged periods (Gräns et al. 2014). Although the f_H of salmon increased during the swim test, HRV decreased by approx. 52% (from resting values of 62 ms to 30 ms at 1.6 BL sec^{-1}; Figure 2.6). Since beat-to-beat variability is known to be predominantly regulated by cholinergic innervation of the cardiac pacemaker (Campbell et al. 2004), the decrease in HRV suggests enhanced parasympathetic control of f_H during the exhaustive swimming test.

U_{crit} tests conducted in a swim tunnel or flume are commonly used to calibrate acceleration values from fish implanted with loggers / transmitters. Often, the objective of a U_{crit} test is to determine the maximum swimming speed and aerobic performance of fishes (Brett 1964; Plaut 2001). In calibration studies, swimming fish at known speeds allows researchers to compare acceleration values to a given value of swimming speed, TBF or metabolism. However, it is important to recognize that some papers suggest that swim tunnels do not reflect the natural swimming behaviour or maximum swimming speed of fish (Nelson et al. 2002; Peake and Farrell 2006; Tudorache et al. 2007, 2010; Castro-Santos et al. 2013; Metcalfe et al. 2016). This is for two reasons: 1) the new environment (i.e., confinement in a swim tunnel) may lead to abnormal behaviour; and 2) short swim tunnels can limit the glide distance during burst-and-coast swimming which facilitates metabolic recovery. However, Wright et al. (2014) found that the range of activity levels of European sea bass in a swim tunnel were similar to those recorded in their holding tank. Likewise, the range of average EA values recorded from salmon in the swim tunnel (~ 6.0 to 22.5 m-g) were similar to those recorded in fish held for 1 week at 11°C (~ 5.8 to 62.2 m-g) and 6 weeks at 8-12°C (~ 3.7 to 47.6 m-g). Only 0.22 and 0.43% of EA values recorded over 1 and 6 weeks of recovery were greater than the maximum EA value recorded in the swim tunnel. This indicates that the majority of the salmon's range of swimming speeds were represented during the swim trial.

A gait transition typically occurs in fish, from steady swimming at low speeds to burst-and-coast swimming at higher speeds approaching U_{crit}, and signifies a switch from aerobic to anaerobic energy metabolism (Peake and Farrell 2004; Brown et al. 2013). This transition is meant to provide energy savings and increase endurance at higher

swimming speeds (Videler and Weihs 1982). In adult salmonids, the transition to burst-and-coast swimming occurs a few minutes before U_{crit}, typically between 1.6 and 2.2 BL sec^{-1} (e.g., see Tang and Wardle 1992; Booth et al. 1997; Lee et al. 2003b; MacNutt et al. 2006; Wilson et al. 2013; Hvas et al. 2017). I found that extremely high values of VAR were recorded during periods of burst-and-coast swimming, which began after ~ 1.6 BL sec^{-1} in this group of salmon. Interestingly, Wilson et al. (2013) reported that high values of acceleration were not linearly related to swimming speed, which may provide further evidence to suggest that swimming behaviour alters the relationship between acceleration and swimming speed. Clearly, it would be valuable to include continuous video recordings in order to establish the impact of behaviour (i.e., steady or burst-and-coast swimming) when determining the relationships between acceleration and swimming speed.

Heart Rate and Activity During Post-Surgical Recovery

The implantation of tags requires that procedures such as netting, handling and anaesthesia be used, and that the fish undergo surgery, which are all known to be stressful on the fish being tagged (Altimiras and Larsen 2000; Hill and Forster 2004; Rothwell et al. 2005; Gräns et al. 2014; Raby et al. 2015). Conveniently, the parameters being recorded by the centi-HRT ACT tag (i.e., f_H and activity) provide information about the impact of surgery and can help to establish recommendations for recovery time. For instance, it is well known that f_H indicates the level of stress experienced by fish, and thus, it is used to determine recovery time following a variety of stressors (e.g., see Anderson et al. 1998; Cooke et al. 2004b; Donaldson et al. 2010; Raby et al. 2015;

Prystay et al. 2017). In Experiment #2, it appeared that the salmon's f_H began to stabilize within the first 4 days following surgical implantation (Figure 2.10). This time-frame is similar to that reported by Brijs et al. (2018, 2019) for the f_H of rainbow trout tagged with Star-Oddi milli-HRT tags with a 15 minute surgical time, whereas f_H took between 8 and 10 days to stabilize in Atlantic cod (*Gadus morhua*) implanted with ultrasonic flow-probes placed on their ventral aorta by a 30 to 60 minute surgery (Webber et al. 1998). Further, it is consistent with Jepsen et al. (2001) found that cortisol in Chinook salmon smolts implanted with dummy tags took 7 days to return to normal, while Wagner et al. (2014) found that in juveniles implanted with micro-transmitters, it was still elevated above resting values by 14 days post-surgery. However, when holding salmon for extended periods during Experiment #3, it was revealed that the f_H of the salmon actually continued to decline for up to 21 days (Figure 2.11). The reason(s) for this prolonged recovery period is / are not known. However, recent data suggests that the presence of tags inside the body cavity induces a long-term immune response (Semple et al. 2018), and this may provide an explanation for the lengthy period required for f_H to achieve stable values.

Despite repeated recommendations to allow fish several days to recover following surgery and prior to experimentation (Altimiras and Larsen 2000; Gräns et al. 2014; Brijs et al. 2019), research recording f_H continues to rely on, and often fall short of, a 48 to 72 hours of recovery 'rule' (e.g., see Steinhausen et al. 2008; Gräns et al. 2009; Ekström et al. 2016; Prystay et al. 2017; Cheng et al. 2017; Joyce et al. 2018). Logistical, financial and temporal restrictions are likely driving researchers to ignore the importance of recovery time. The lowered cost and simplistic use of f_H biologgers can help to mediate

these constraints, and allow researchers to provide extended recovery periods for fish which have undergone surgery. As a recent example, rainbow trout implanted with Star-Oddi milli-HRT tags were allowed 7 and 8 full days of recovery before experimental protocols were performed [Ekström et al. (2018) and Wallerius et al. (2019), respectively].

It has also been suggested that the presence or absence of diel variations in f_H can provide important information about the welfare and recovery of fish (Brijs et al. 2018). Increased activity and $\dot{M}O_2$ stimulated by daylight results in altered circadian rhythms of cardiac output (Priede and Young 1977; Borch et al. 1993; Aissaoui et al. 2000). Diel patterns in f_H have also been recorded in a variety of fish including carp (*Cyprinus carpio*; Kneis and Siegmund 1976; Williams et al. 1997), rainbow trout (De Vera and Priede 1991; Borch et al. 1993; Brijs et al. 2018), brown trout (*S. trutta*; Priede and Young 1977), gilthead seabream (*Sparus aurata*; Aissaoui et al. 2000) and zebrafish (*Danio rerio*; Zhang et al. 2015). In this study, diel variations in f_H were recorded in salmon immediately following surgery and were maintained for the 7 day holding period in Experiment $^{\#}2$ (Figures 2.10 and 2.11; Table 2.5, 2.6); the mean difference in f_H between day and night was ~ 7 bpm and the range of values over 24 hours was ~ 14 bpm (Figure 2.8). Conversely, Brijs et al. (2018) reported that diel variations in f_H were not apparent until 3 days post-surgery in rainbow trout implanted with Star-Oddi milli-HRT loggers, and that the average circadian fluctuations in f_H was ~ 27 bpm. The reason for the delayed appearance of circadian rhythms in f_H in Brijs et al. (2018) and the ~ 2-fold greater daily variation in f_H in their study is not known. However, the fish in Brijs et al. (2018) underwent a prolonged fast prior to surgery (1 week) and their studies were conducted at

a warmer temperature (15 - 16°C vs. 10 - 11°C in the present study) and in a sea-cage. It is possible that these differences between the two studies influenced post-surgical recovery and the magnitude of the diel fluctuations in f_H. Nonetheless, based on the above studies, it appears that biologgers which can record f_H and EA continuously could be used to advance our understanding of circadian rhythms, which have major implications for the fitness of wild animals (Yerushalmi and Green 2009). For example, Payne et al. (2013) used depth and acceleration tags to study the activity of yellowfin bream, *Acenthopagrus australis*, and found that the fish switched from diurnal to nocturnal activity following a heavy rainfall.

During one week of holding at 10-11°C (Experiment #2), diel patterns in EA were present immediately following surgery and were maintained throughout holding (Figure 2.10; Table 2.5). In contrast, there were no significant diel variations in EA when salmon were held for 6 weeks at 8-12°C in Experiment #3 (Figure 2.11; Table 2.6). There are two possible explanations for the discrepancy in diel patterns of activity. First, during Experiment #2 the tags were programmed to record at a sampling frequency of 10 minutes (144 measurements per day for each fish). In contrast, the sampling rate was changed to every 2 hours (12 measurements per day for each fish) in order to save memory and battery life during the 6 week holding period in Experiment #3. This reduction in the amount (frequency) of data collected likely limited the ability to detect diel variations in EA. If the goal of future research is to study such fine scale patterns in swimming / behaviour, such as diel variations of activity in fish, it is imperative that researchers optimize their sampling rate given the length of experiment they intend to perform. Second, the two trials of Experiment #3 were conducted at colder temperatures, and the

salmon were less active overall. For example, average EA was 9.5 m-g (1.04 BL sec^{-1}) in Experiment #2, whereas it was only ~ 6.4 m-g (0.62 BL sec^{-1}) in the longer experiments. It is possible that this lower baseline activity contributed to the lack of detection of diurnal changes in EA.

Interestingly, salmon at both temperatures had a faster average swimming speed in tanks than Atlantic cod and rainbow trout with speeds between 0.33 to 0.35 and 0.62 to 0.68 BL sec^{-1}, respectively (Kawabe et al. 2003, 2004; Gollock et al. 2009). However, the average TBFs of Atlantic salmon in Experiments #2 and 3 (9.5 m-g / 116.15 tail beats min^{-1} and 6.4 m-g / 72.26 tail beats min^{-1}) were within the preferred swimming speed range of brown trout released to the wild (1 to 2 tail beats sec^{-1} or 60 to 120 tail beats min^{-1}; Ross et al. 1981). The minimum net cost of transport (COT) of cod is 0.3 BL sec^{-1}, and therefore, it makes sense that they spent the majority of their time swimming at that speed (Gollock et al. 2009; Syme et al. 2009). In contrast, salmonids are reported to have a minimum net COT at 0.4 to 0.6 BL sec^{-1} (Lee et al. 2003 a, b; Hvas et al. 2017). Thus, it appears that the Atlantic salmon in this study were also swimming at an average speed in Experiments #2 and 3 within the range of their minimum net COT.

Specific acceleration values or patterns can be used to estimate the amount of time fish spend exhibiting certain behaviours (Ropert-Coudert and Wilson 2005). For example, Tsuda et al. (2006) were able to determine that chum salmon could swim without tail beating in typical river flow conditions, and Kawabe et al. (2003) were able to distinguish between active swimming and inactive swimming / gliding in rainbow trout. In the swim tunnel, salmon began burst-and-coast swimming after ~ 1.6 BL sec^{-1}, when VAR was greater than ~ 220 minutes (Figure 2.5). Based on this information, I estimate that the

salmon in the tanks only exhibited burst-and-coast swimming 2.8 ± 0.5% of the time (26 / 962 VAR measurements were greater than 220 minutes; n = 10) during Experiment #2, and 3.6 ± 0.5% of the time (18 / 498 VAR measurements; n = 4) and 4.0 ± 0.6% of the time (20 / 498 VAR measurements; n = 4) during the two trials in Experiment #3. These data suggest that salmon spend the vast majority of their time swimming steadily in the tank environment, with few periods of rapid / burst swimming. Similarly, free-swimming Atlantic cod in tanks spend very little time burst swimming (Gollock et al. 2009).

Considerations for Tag Use and Future Feasibility Studies

The modified implantation method used in this study was effective for recording f_H and acceleration in Atlantic salmon. In order to effectively record f_H, the electrodes of f_H loggers and transmitters must remain close to the pericardium throughout deployment (Cooke et al. 2016). Therefore, I chose to suture the centi-HRT ACT tag to the body wall before closing the incision. This resulted in good quality ECG recordings during both the exhaustive exercise (U_{crit}) protocol and the 1 and 6 weeks that the salmon were held in the large tank; the average percentage of good quality ECGs (i.e., QI = 0) approximately 68, 86 and 88%, respectively (Table 2.4). It has been reported that increased activity can interfere with ECG recordings due to potentials produced by the aerobic muscles (Altimiras and Larsen 2000). However, this was very rare in these studies. There were two instances when f_H data was lost due to noisy signals and these corresponded with feeding activity. The positioning of the tag was also consistent with suggestions for implanting accelerometers, e.g. aligning the tag with the major plane of movement (i.e., the lateral movement of the tail) and placing the tags close to the animal's center of

gravity (Gleiss et al. 2010, 2011). Further, suturing the tags to the body wall would have reduced the potential for variation in logger position between individuals, which can impact the interpretation of accelerometry data (Halsey et al. 2009).

While previous research has reported issues with the retention of internally implanted tags and the survival of tagged salmonids (e.g., see Treasurer 1996; Welch et al. 2007; Ivasauskas et al. 2012; Smircich and Kelly 2014; Føre et al. 2017), all tags were found in their original position and survival was 100% for the Atlantic salmon tagged in this study. It is important to acknowledge that tag retention, healing and survival inside a tank or hatchery setting may not be representative of fish tagged in the wild or in the sea-cage environment, and often varies between studies. For example, Føre et al. (2017) experienced problems with tag ejection and mortality in sea-caged Atlantic salmon that were implanted with Star-Oddi micro-TD tags and released back into their cages on the same day as surgery. On the other hand, sea-caged rainbow trout tagged with Star-Oddi milli-HRT tags and recovered in a facility for 2 days before re-entering the sea-cage had zero mortality over 21 days (Brijs et al. 2018). While the tagging method used in the present study was effective for salmon held in a tank, and it may hold true for other adult salmonids, experiments using different species or tag types / sizes may have varying results as effective tagging depends on a range of factors (Cooke et al. 2011). Therefore, it is strongly suggested that feasibility studies investigating tag retention and survival be performed prior to the extended use of biologgers.

Overall, the ECGs recorded in the Atlantic salmon were of good quality, and the f_H values recorded by the HRT ACT tags were quite sensitive (responsive) to biotic and abiotic changes. For example, the tags were able to detect increases in f_H with swimming

speed (Figure 2.6), small diurnal changes in f_H (Figure 2.10) and those associated with changes in temperature. However, there were some issues with the quality of the recorded ECGs. During the critical swim speed test, f_H was sometimes miscalculated by as much as 39 bpm by the Star-Oddi Mercury software when ECGs were of poor quality (QI ≥ 1; Figure 2.7). Due to this concern, Prystay et al. (2017, 2019), Brijs et al. (2018, 2019) and Wallerius et al. (2019) chose a highly conservative approach, and removed all poor quality f_H values (QI ≥ 1). When manually examining the ECGs, I found that only approx. 73% of those designated as QI = 0 were 'good' quality and that 23 and 19% of those determined to be QI = 1 and 2, respectively, were not of poor quality. Therefore, I chose to either manually calculate f_H from all of the ECGs, or just those with poor quality, and as a result, very few data points had to be removed from each experiment (less than 1%). While the QI value does help identify ECGs with missing R-R peaks (QI = 3), and provides a rough estimate of ECG quality, I highly recommend that users of Star-Oddi f_H loggers prioritize saving and inspecting ECGs prior to data analysis and interpretation. However, a centi-HRT ACT tag with a full battery can record f_H, acceleration and temperature with a 2-hour sampling frequency for 230 days when all ECGs are saved, but the potential length of sampling is extended to 455 and 1838 days when 50% or no ECGs are saved.

Star-Oddi f_H loggers do not require external wires connected to recording devices, and thus, tagged fish are free to interact with conspecifics rather than be confined to a respirometer chamber. Research using non-invasive and un-tethered f_H recording methods, including this paper, have recorded the lowest resting f_H values in salmonids (~ 20 to 37 bpm: Altimiras and Larsen 2000; Donaldson et al. 2010; Clark et al. 2010; Gräns

et al. 2014). This research highlights the importance of using such technologies when trying to determine what cardiovascular parameters are in fish in the field (wild), but also some of the challenges when using these devices. Brijs et al. (2019) reported that the Star-Oddi f_H loggers were limited in their ability to record low resting f_H values in rainbow trout due to the maximum sampling and recording periods permitted by the manufacturer (i.e., 600 measurements per sampling period or 100 Hz for 6 seconds). For example, in a trout with a mean resting f_H of 23 bpm, these authors found that the milli-HRT logger only recorded 29% of f_H measurements as QI = 0. This is because at f_H values < 20 bpm the R-R interval is longer than the 6 sec. recording period. I observed a similar issue in salmon with low resting f_H over the 6 week holding period at 8°C. With a long recovery period and the low temperature, f_H was routinely reaching values lower than 20 bpm, especially during the night. For example, 75% of the f_H data in one fish during the last week of holding was lost because of low f_Hs; i.e., QI was equal to 3 because a full R-R interval could not be recorded. As mentioned in Brijs et al. (2019), Star-Oddi has now released tags that have the option to record ECGs for longer periods and up to 1500 measurements per sampling (i.e., 80 Hz for 18.8 seconds, 100 Hz for 15 seconds, or 125 Hz for 12.5 seconds), and this should allow research to be conducted on cold water species and throughout the winter months. However, users should be aware that the ability to sample f_H over prolonged periods is a tradeoff with increased battery consumption and memory usage when choosing to save ECGs.

Debate exists over the sampling frequency required when using acceleration to determine the behaviour of animals (Broell et al. 2013; Wilson et al. 2013). I used a lower sampling frequency (1 Hz for 60 seconds every 2 minutes, 10 minutes or 2 hours) which

has been referred to as discrete or burst / epoch sampling (Brown et al. 2013). In this study, this lower frequency was useful in estimating swimming speed in Atlantic salmon and can indicate when salmon are burst-and-coast swimming. Similarly, Wilson et al. (2013) found that a frequency of 10 Hz for 10 seconds could be used to effectively estimate swimming speed and $\dot{M}O_2$ in sockeye salmon. However, in this research, acceleration was averaged over one minute and thus, maximum swimming speeds could be underestimated using this method. While low frequencies allow for the characterization of one behaviour type at a certain point in time, and can utilize fixed-threshold manual behaviour referencing as previously described (e.g., Kawabe et al. 2003; Tsuda et al. 2006), they do not continuously record all micro-behaviours (i.e., define detailed animal behaviours). For such purposes, acceleration data recorded with a high sampling frequency can be used to assign acceleration waveforms to complicated behaviours using unsupervised or supervised machine learning algorithms (Brown et al. 2013). However, such recording requires significant memory and battery life, and thus once again, a user's choice of sampling frequency should reflect their research objectives.

Conclusions

There is a growing need for validation / feasibility studies prior to the use of biologgers (Wilson et al. 2015), especially those that record multiple parameters or when they are being used in previously untagged or rarely tagged species. Currently, physiological sensors are underutilized, but could be an extremely useful tool for advancing fish welfare and conservation. For example, the f_H loggers in this paper effectively recorded good quality ECGs and measurements of f_H in free-swimming

salmon and could record small changes in f_H associated with exercise, diel variation and changes in temperature. With these tags, I found that salmon require a minimum of 4 days, and up to 21 days, for f_H to stabilize following the surgical procedures and implantation required for the use of the tag. Additionally, the loggers allowed for the quantification of HRV of salmon during a critical swim test, and thus, provide a new avenue of research for cardiac physiology. Lastly, the tags recorded values of acceleration that can be used to estimate salmon's swimming speed and TBF, and indicated whether salmon were swimming steadily or burst swimming. It is my hope that future users of biologgers follow the recommendations highlighted in this chapter. To further advance the fields of biologging, and fish ecology and conservation, it is imperative that rigorous feasibility studies, with robust sample sizes, are completed prior to the use of tags in the field.

Chapter 3: Using Data Storage Tags to Study the Effects of Hydrostatic Pressure on the Heart Rate of Lumpfish (*Cyclopterus lumpus*)

Abstract

Data on the effects of hydrostatic pressure on fish physiology are limited to a narrow range of species, and rarely consider the impact of this parameter on the effects of other environmental variables. In this study, lumpfish (*Cyclopterus lumpus*, 200 to 400 g), which can exhibit vertical migrations over 100 m daily and be found at depths up to 500 m, were recovered for 6 days after surgically implanting them with Star-Oddi micro-HRT tags. Then, their heart rate (f_H) response was measured in an IPOCAMP pressure chamber when exposed to: 1) increasing pressure (up to 80 bar; 800 m in depth) alone at 10°C; or 2) increasing temperature (12 to 20°C), decreasing temperature (12 to 4°C) or decreasing oxygen levels (to 50% air saturation at 12°C) in the absence and presence of 80 bar of pressure. In addition, I investigated the effect of prior exposure to 80 bar of pressure on post-chase f_H and determined the lumpfish's f_H response to increasing temperature up to their critical thermal maximum (CT_{MAX}, 22°C) at atmospheric pressure. Hydrostatic pressure increased f_H from 48 to 61 bpm, and increased the magnitude of the rise in f_H with temperature (i.e., f_H increased in control fish by ~ 30 bpm between 5 and 20°C vs. 45 bpm when under pressure). However, it did not increase the Q_{10} value or the slope of the relationship between temperature and f_H. In contrast, hydrostatic pressure eliminated the 5 bpm increase in f_H when control fish were exposed to hypoxia. Further, increasing temperature to CT_{MAX} or exhaustive exercise resulted in a maximum f_H of 81 and 77 bpm, respectively. My research suggests that pressure influences the f_H response to

environmental challenges, and provides the first evidence that lumpfish have a limited capacity to increase f_H.

Introduction

During vertical migrations to deeper waters, animals experience large changes in environmental conditions such as increases in hydrostatic pressure, and reductions in temperature, oxygen and light (Gross and Jaenicke 1994; Andrzejaczek et al. 2019). Data storage tags (DSTs) and other time-depth recording devices are improving our understanding of the vertical range of fishes (e.g., see De Pontual et al. 2012; Boje et al. 2014; Thorrold et al. 2014; Einarsson et al. 2018). However, due to the difficulty and high costs of gaining biological information while animals are under pressure (Guerrero et al. 2000; Shillito et al. 2014), there is still very little known about the physiological responses of fish to changing environmental conditions at depth (Andrzejaczek et al. 2019).

Early research on the effects of hydrostatic pressure focused on the pressure tolerance and behavioural response of a narrow range of fish species to acute increases in pressure (Brauer et al. 1974; Macdonald et al. 1987). Since then, the field has focused on the effects of acute and chronic pressure increases on the metabolic response of various fish species (e.g., see Sébert and Barthélémy 1985a,b; Simon et al. 1989; Sébert and Macdonald 1993; Sébert 2002; Speers-Roesch et al. 2004; and Vettier et al. 2005, 2006). However, to my knowledge, no studies have measured the physiological response of fish to hypoxia in combination with pressure, and very few studies have examined the

combined effects of temperature and pressure (Sébert et al. 1995a,b; Scaion et al. 2008a,b).

Further, even less is known about its effects on the cardiovascular system, and the published information is quite variable. For example, while compression to ~ 101 bar (≈ 1000 m) caused bradycardia in European eels (*Anguilla anguilla*) when water temperatures were greater than 24.5°C, it resulted in tachycardia at temperatures < 24.5°C (Belaud et al. 1976; Sébert and Barthélémy 1985b). Sudden compression to 50 bar (≈ 500 m) caused tachycardia, whereas acute exposure to pressures equal to 200 bar (≈ 2000 m) caused bradycardia in this species (reviewed in Sébert 2002). Finally, eels acutely exposed to 100 bar (≈ 1000 m) of pressure experienced hypotension in the dorsal aorta and hypertension in the mesenteric vein (reviewed in Sébert 2002), whereas ventral aortic relaxation was reduced in eels acclimated to this pressure for 21 days (Guerrero et al. 2000). Heart rate (f_H) and cardiac output are factors that limit the depth range of ecologically and economically important species such as tuna and billfishes (Brill et al. 1998), and the physiological capacity of the cardiovascular system to respond to changes in pressure, temperature and oxygen could have implications for future shifts in the bathymetric distribution of fish faced with global ocean warming and oxygen minimum zone expansion (Morris et al. 2015a,b; Andrzejaczek et al. 2019). Thus, it is critical that we learn more about the effects of these interacting abiotic variables on fish cardiovascular function.

The common lumpfish (*Cyclopterus lumpus*) is an ecologically important marine species that it is widely distributed on both sides of the Atlantic Ocean; e.g., it is found around Portugal, Greenland and Iceland, and in the Barents, Baltic and North Seas in the

eastern Atlantic, as well as around Hudson Bay and Baffin Island, and in Newfoundland and Labrador, New Brunswick, Nova Scotia, the Gulf of St. Lawrence and Maine, and on Georges Bank in the western Atlantic (Blacker 1983; Davenport 1985; Simpson et al. 2016; Powell et al. 2017). Further, it is a commercially important species due to its wide geographical range (Powell et al. 2017), the demand for their roe as a substitute for sturgeon caviar, and their use as a 'cleaner fish' in the Atlantic salmon (*Salmo salar*) aquaculture industry (Imsland et al. 2014; Powell et al. 2018). However, due to overfishing / harvesting, lumpfish are also considered to be 'Near Threatened' on the IUCN Red List (Lorance et al. 2015) and, more recently, were designated as Threatened by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC 2017). Clearly, information on the physiological limits of the species will be important for its future conservation and proper management in the roe fishery and in the aquaculture industry. However, there is a limited understanding of the basic physiology of lumpfish and its tolerance to different environmental conditions (Ern et al. 2016; Jørgensen et al. 2017; Hvas et al. 2018).

As solitary (mature) adults, lumpfish migrate into shallow, coastal, waters in the spring and summer to reproduce (Davenport 1985). However, once the young become juveniles (> 1 year of age), they migrate out to the open ocean (Davenport 1985). Most pelagic trawl records and video images suggest that lumpfish reside in the upper 60 m of the ocean, but that they can also frequently be found at deeper depths (Blacker 1983; Rosen and Holst 2013; Rosen et al. 2013). In order to better characterize their vertical distribution, researchers recently tagged Icelandic lumpfish with data storage tags (DSTs) which record depth and temperature (Kennedy et al. 2016). Their maximum recorded

depth and maximum extrapolated depth (from temperature data) were 309 and 498 m, respectively, and this species regularly engaged in daily vertical migrations greater than 100 m (Kennedy et al. 2016). This led the authors to suggest that lumpfish should be considered a semi-pelagic / semi-demersal fish species (Kennedy et al. 2016).

Given that lumpfish can be found at a variety of depths, and the need to better understand how depth (hydrostatic pressure) affects the response of the fish's cardiovascular system to other abiotic factors, I used two unique pieces of equipment (Star-Oddi micro-HRT DSTs, and the IPOCAMP pressure chamber; formally named "Incubateur Pressurisé pour l'Observation et la Culture d'Animaux Marins Profonds") to examine: 1) the f_H response of lumpfish to increasing hydrostatic pressure up to 80 bar (800 m in depth); 2) the effect of prior exposure to 80 bar of pressure on the post-chase f_H of lumpfish; and 3) the f_H response to increasing temperature (12 to 20°C), decreasing temperature (12 to 4°C) or decreasing oxygen levels (to 50% air saturation at 12°C) in the absence and presence of 80 bar of pressure. In addition, I measured the lumpfish's f_H response during an acute temperature increase (2 °C h^{-1}) up to their critical thermal maximum (CT_{MAX}) at atmospheric pressure. This latter experiment was performed to determine if the limited maximum f_H observed for this species in the IPOCAMP was similar to that measured by a more standard protocol (the CT_{MAX} test).

Methods

Animal Husbandry

All experimental work described was approved by the Institutional Animal Care Committee of Memorial University (Protocol #17-95-KG), and followed the standards

and guidelines outlined by the Canadian Council on Animal Care. The lumpfish used in these studies were originally held in the Dr. Joe Brown Aquatic Research Building (JBARB) at the Ocean Science Centre (OSC) in 3 m^3 tanks supplied with seawater at 6°C and 100-120% of air saturation and a 12h light:12h dark photoperiod, and fed at a ration of 1.0% body mass per day with 2.00 mm marine pellets (Skretting, Canada). The lumpfish were transferred to a 0.5 m^3 tank in the Laboratory for Atlantic Salmon and Climate Change Research (LASCCR) on April 23rd, 2018 (n = 56), July 23rd, 2018 (n = 22), and January 16th, 2019 (n = 20) that was supplied with seawater at ~ 7.5°C and 100-120% of air saturation with a 14h light:10h dark photoperiod (Figure 3.1 A). The temperature in these tanks was raised to 10 or 12°C at a rate of 0.5°C per day. These lumpfish were then held in the LASCCR for a minimum of 14 days before use in experiments, and fed a ration of 0.75% body mass per day of 3.00 mm marine pellets.

Data Storage Tag Implantation

The following experiments used the Star-Oddi micro-HRT tag (25.4 mm in length, 8.3 mm in diameter, and 3.3 g in air) which records f_H, electrocardiograms (ECGs) and temperature. In all experiments, the tags did not exceed 2% of the fish's body mass. DSTs were inserted into the tag-computer interface (COM-BOX) provided by Star-Oddi prior to implantation, and the start time, start date and sampling intervals were set using their Mercury software.

To prepare the tags, one piece of black, braided, non-absorbable and non-sterile 3-O silk suture was cut to a length of 30 cm, then tied around the DST (Figure 3.2). All tags and surgical equipment were clean and sterilized in 70% ethanol between uses. The

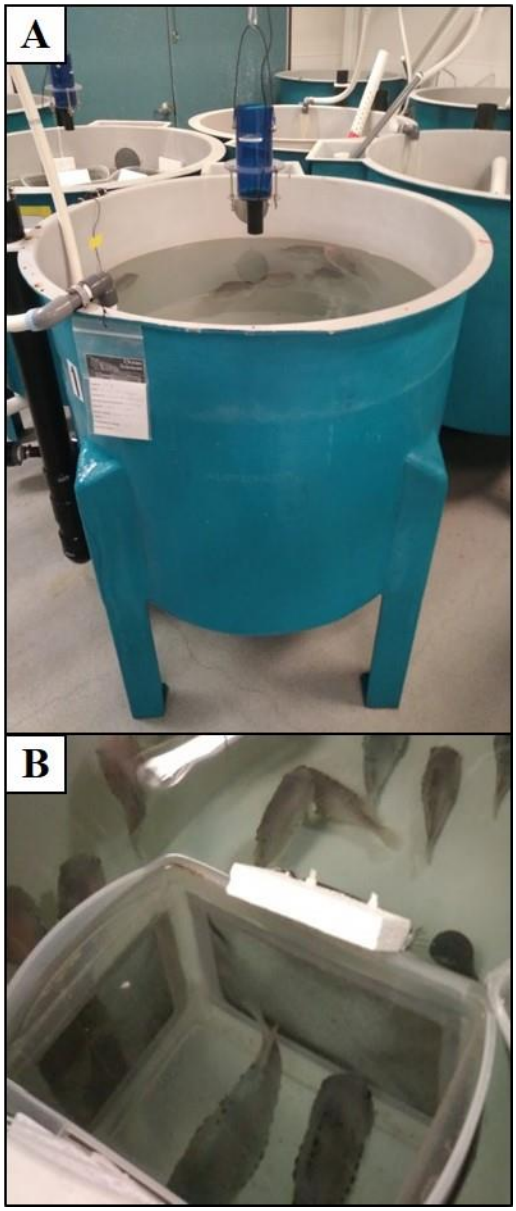

Figure 3.1. A) A 0.5 m^3 tank in the Laboratory for Atlantic Salmon and Climate Change Research (LASCCR) at the OSC, Logy Bay, Newfoundland. B) After being implanted with micro-HRT tags and recovering in their tank for 48 hours, two tagged lumpfish were moved to fasting 'baskets' for 66 hours before being moved to the IPOCAMP pressure chamber.

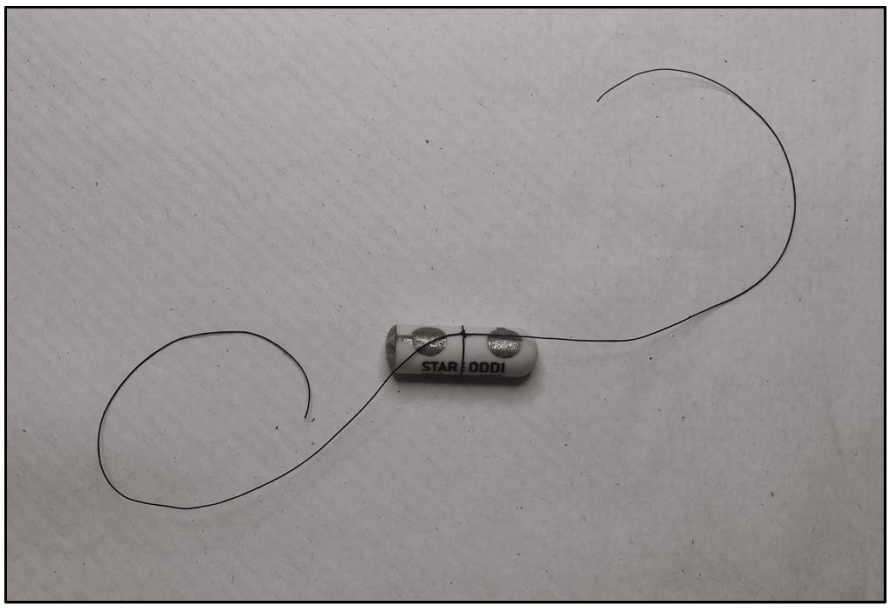

Figure 3.2. The Star-Oddi micro-HRT tag was prepared for implantation by tying one piece of black, braided, non-absorbable and non-sterile, 3-0 silk suture around the tag, and it was implanted into the lumpfish in a “sensors up” orientation.

lumpfish were anaesthetized in seawater containing 0.15 g L^{-1} tricaine methanesulfonate (MS-222), a suitable surgical dose given recent data (Skar et al. 2017). After losing equilibrium, the fish were moved to a wetted surgical sponge where their gills were irrigated with flowing and aerated ~ 10°C seawater containing 0.075 g L^{-1} MS-222. A 1.5 to 2.0 cm mid-ventral incision was made in the fish's body wall beginning immediately posterior to the sucker. The tag was then inserted (blunt end first) in a posterior direction and pulled anteriorly to within 0.5 cm of the pericardium using the attached suture. A cutting-edge needle was used to pass the suture threads through the skin to secure the tag to the body wall and begin closing the incision. Finally, the incision was closed with continuous stitches. One or two suture knots were attached to the dorsal muscle to allow for the identification of the fish once it was recovered and returned to its holding tank.

Following all experiments, the fish were euthanized in 0.6 g L^{-1} MS-222 in order to perform post-mortem dissections and recover the data. Post-mortem dissections were conducted to record the distance from the front of the tag to the pericardium, the tag's final position, to look for any signs of inflammation or infection, and to determine sex based on the absence (i.e., immature fish) or presence of eggs or testes. Data were retrieved using the COM-BOX and Star-Oddi's Mercury software.

IPOCAMP Set-Up

Experiments #1 and 2 used the IPOCAMP chamber (Autoclave, France; 19 L vessel, 60 cm high by 20 cm in diameter) in the Cold-Ocean and Deep-Sea Research Facility (CDRF) at the OSC (Figure 3.3 A). The temperature in the chamber of the IPOCAMP was controlled by a heater / chiller that regulates the temperature of both the

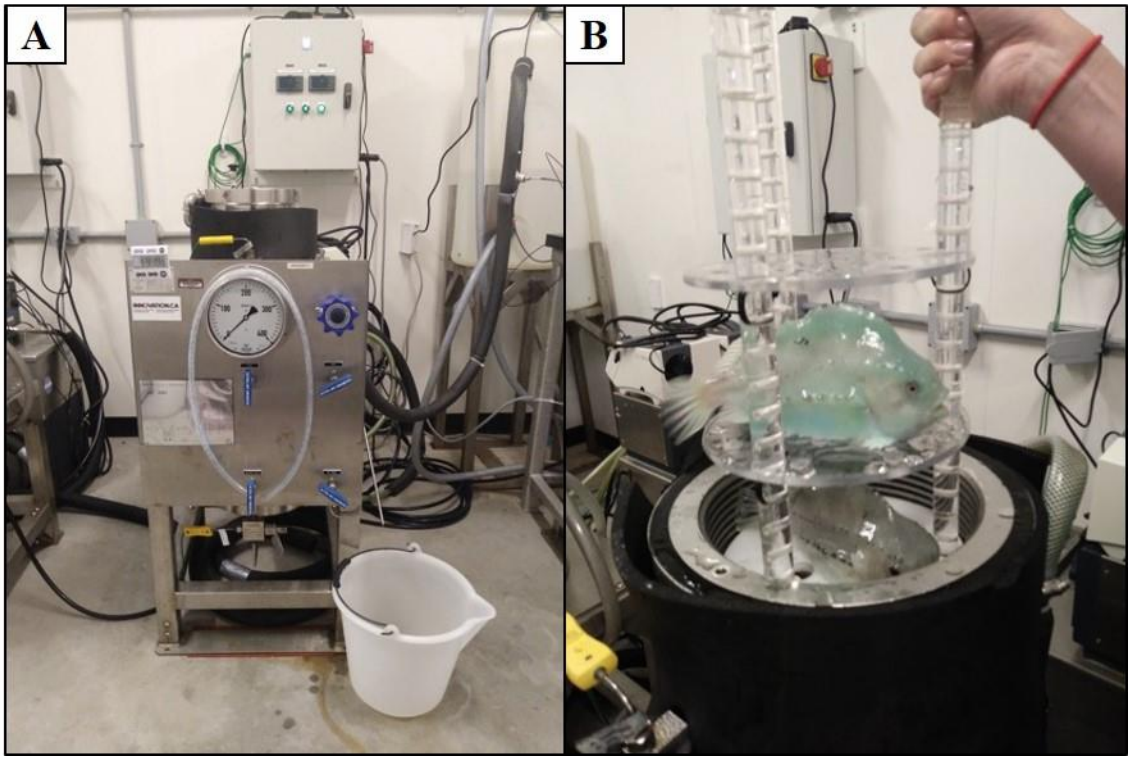

Figure 3.3. A) The IPOCAMP pressure chamber (19 L vessel, 60 cm high by 20 cm in diameter) in the Cold-Ocean and Deep-Sea Research Facility at the OSC. B) Lumpfish tagged with micro-HRT tags were placed, two at a time, onto platforms before being lowered into the IPOCAMP. The fish were acclimated to the chamber overnight at 10ºC and at 0 bar of pressure (i.e., equivalent to atmospheric pressure at the sea level).

in-flowing water and the glycol jacket surrounding the chamber. The water flowing into the IPOCAMP came from a 50 L reservoir in which the oxygen level was controlled by a fibre-optic oxygen probe connected to a Witrox 1 oxygen system equipped with WitroxCTRL software (Loligo Systems, Denmark). This system regulated the reservoir's water oxygen content within relatively narrow limits (± 2% air saturation) by bubbling air or nitrogen into the reservoir when water oxygen levels reached lower and upper set points, respectively. These set points were determined by monitoring the oxygen content in the water leaving the chamber, as recorded by a Fibox 3 LCD oxygen meter (PreSens, Germany). A pipe inspection camera was inserted into one of the viewports in the lid of the IPOCAMP to record the behaviour of the fish during all experiments, and red filters and adjustable lighting were inserted into the other two viewpoints in order to provide adequate light and to maintain photoperiod.

Experiment #1: Heart Rate Response to Hydrostatic Pressure and the Fish's Maximum Post-Exercise Heart Rate

After implantation of the micro-HRT tag, lumpfish (n = 14, 237.8 ± 5.3 g, 18.1 ± 0.3 cm) were returned to their tank in the LASCCR to recover. At approximately 48 hours post-surgery, two tagged fish were transferred to a 'basket' (38.7 cm in length x 24.8 cm in width x 29.2 cm in height) placed in the tank to be fasted for ~ 66 hours before being transferred to the IPOCAMP (Figure 3.1 B). This was necessary as water supplying the IPOCAMP passed through a fine filter that was easily clogged by fecal matter. Fish, two at a time, were placed on platforms of an insert that was lowered into the IPOCAMP (Figure 3.3 B) and acclimated to the chamber overnight at 10ºC and at 0 bar of pressure

(i.e., equivalent to atmospheric pressure at sea level).

Immediately following surgery, the pre-programmed micro-HRT tags saved ECGS and recorded f_H (100 Hz for 6 seconds) and temperature at a sampling frequency of 4 hours during the recovery period. However, the morning following acclimation to the IPOCAMP, the tags began to save ECGs and record f_H (100 Hz for 6 seconds) and temperature at a frequency of 2 minutes. After acclimation overnight, some lumpfish (n = 8) were exposed to increasing levels of hydrostatic pressure. Hydrostatic pressure was initially increased to 20 bar over 2 minutes, then held at this pressure for 8 minutes. Thereafter, pressure was increased to 35, 50, 65 and finally 80 bar using the same protocol. This series of pressure changes equivalent to a fish descending from the ocean's surface to 800 meters in 50 minutes. The lumpfish were then decompressed in the opposite sequence. Six lumpfish were held at 0 bar over the same timeframe to serve as time-matched controls.

The lumpfish were allowed to recover for one hour after exposure to changes in pressure before being removed from the IPOCAMP. Some of these fish were immediately euthanized. However, a subset of the fish (4 from each treatment) were placed into a large, aerated bucket with 30 L of 10°C seawater for 30 minutes. These lumpfish were subsequently chased with a net for 1.5 minutes. This allowed for an estimation of the fish's maximum f_H in response to exercise at 10°C. After 30 minutes of recovery from being chased, these lumpfish were also euthanized, and data was retrieved using the COM-BOX and Mercury software.

Experiment #2: Influence of Hydrostatic Pressure on Heart Rate and Its Response to Changes in Temperature and Hypoxia

Lumpfish were recovered from surgery, fasted and transferred to the IPOCAMP chamber as described in Experiment #1. The micro-HRT tags were set to save ECGs and record f_H (100 Hz for 6 seconds) and temperature at a sampling frequency of 4 hours on the day of being transferred to the IPOCAMP, every 2 minutes during the increase in hydrostatic pressure and when initially held at 80 bar (i.e. between 8:00 and 10:20 AM), and every 5 minutes for the rest of the duration of the experiment to save tag memory and battery.

On the morning following acclimation to the IPOCAMP, lumpfish were exposed to increasing hydrostatic pressure to 80 bar (see protocol in Experiment #1) or maintained at atmospheric pressure (0 bar, control fish) for 1 hour. Upon acclimation to 80 bar for 1 hour, the lumpfish were exposed to one of the following treatments. In the first trials, lumpfish were exposed to decreasing temperature from 12 to 4°C (n = 14, 350.1 ± 12.4 g, 21.1 ± 0.2 cm). In the second set of trials, lumpfish (n = 15, 404.9 ± 14.1 g, 21.2 ± 0.3 cm) were exposed to increasing temperature from 12 to 20°C (~ 2°C lower than the previously calculated CT_{MAX} for lumpfish; Ern et al. 2016). The rate of temperature change in both trials was ~ 2°C h^{-1}. Lastly, lumpfish (n = 16, 435.8 ± 23.9 g, 21.9 ± 0.4 cm) were exposed to decreasing oxygen from 100% to 50% air saturation (~ 15% air saturation above their P_{crit} calculated at 10°C; Ern et al. 2016). Temperature and oxygen were not brought close to the CT_{MAX} or P_{crit} of the lumpfish because they can attach to the platforms with their suckers even when unconscious, making loss of equilibrium difficult to determine (Ern et al. 2016). In addition, decompression and removal of fish from the

pressure chamber takes a considerable amount of time which posed ethical concerns for the welfare of the fish. A time-matched control (i.e., fish without exposure to pressure or changes in environmental parameters) was not included in these experiments as f_H did not significantly change in lumpfish held at 0 bar for the duration of Experiment #1. Following the experiments, the fish were removed from the IPOCAMP and euthanized.

Experiment #3: The Normobaric Heart Rate Response to Increased Temperature

Lumpfish (n = 12, 544.5 ± 19.1 g, 23.7 ± 0.4 cm) were tagged, four at a time, then returned to their holding tank in the LASCCR to recover for 72 hours. After recovery, the fish were transferred into individual buckets (26.5 cm in diameter x 23.5 cm in height, 8 L) in a water bath with flowing seawater and sufficient aeration to maintain water oxygen levels near 100% saturation. Seawater was supplied to the water bath from a temperature-controlled aerated 80L reservoir. Lumpfish were given 24 hours at 12°C to acclimate to the buckets. Photoperiod was maintained at 14h light: 10h dark during this period.

On the morning following acclimation, the pre-programmed micro-HRT tags began saving ECGs and recording f_H (100 Hz for 6 seconds) and temperature at a sampling frequency of 5 minutes at 8:00 AM. At 9:00 AM, water temperature was increased by 2°C h^{-1} to a maximum of 22°C (n = 8). Some lumpfish were held at 12°C to serve as a time-matched controls (n = 4). Following the experiments, the lumpfish were euthanized.

Calculation of Heart Rate Parameters

All reported measurements of f_H were calculated manually using the method described for Atlantic salmon. Briefly, the average time between R wave peaks was measured (in sec), and then 60 was divided by this number to obtain the fish's f_H in beats per minute (bpm). Quality index (QI) measurements were provided by the Mercury software (0 means great quality, 1 and 2 have decreasing quality and 3 means there is no R-R interval). When ECG artefacts made the QRS complex unidentifiable, manual calculation was not possible, and the data were not included. Percentage change in f_H was calculated for each fish based on initial f_H values in each experiment (e.g., as a percentage of 0 bar values during exposure to hydrostatic pressure). Heart rate variability (HRV) was calculated as the standard deviation of time between each R wave peak (in ms).

Lumpfish Activity

Video was recorded during all experiments by connecting the pipe inspection camera in the viewports in the lid of the IPOCAMP to a laptop running VideoVelocity (CandyLabs, Vancouver, Canada). From these videos, the activity of all individuals was scored by assigning fish with a rank for each 10 minute period during exposure to pressure: 0 represented fish that were completely inactive; 1 represented fish that were mostly inactive but had some spontaneous activity; 2 represented fish that were mostly active, but had some periods of rest; and 3 represented fish that were active for the entire 10 minute period. Activity in Experiment $^{\#}$1 was ranked for all steps of compression and decompression, while activity in Experiment $^{\#}$2 was only ranked at 0 bar, when pressure first reached 80 bar, and at 30 and 50 minutes after pressure reached 80 bar.

Statistics

To assess the effects of surgical recovery on f_H, linear mixed-effects (LME) models were used with photoperiod (day-time between 6:00 AM – 7:59 PM; night-time between 8:00 PM – 5:59 AM) and photoperiod order (daily values during each photoperiod, where N1 was the first night following surgery and D1-D5 represent the subsequent days) as the fixed-effects, an interaction term for the two parameters, and fish as a random factor.

The effects of hydrostatic pressure on f_H, the percentage change in f_H, HRV and the percentage of 'good' quality index ECGs (QI = 0) were assessed using LME models with pressure step (0, 20, 35, 50, 65 and 80 bar, and either the reverse during decompression or 10 minute increments when pressure was held at 80 bar) and treatment (control fish at 0 bar or pressure-exposed fish) as the fixed-effects, an interaction term for the two parameters, and fish as a random factor. The effect of sex (immature, female, male) and temperature (10 or 12°C) on resting f_H, and the increase in f_H or the percentage increase in f_H at 0 vs. 80 bar in all pressure-exposed fish of all experiments, was determined using one-way ANOVAs and unpaired t-tests, while the relationship between weight / length was assessed using a linear regression.

Linear regression analysis was also used to examine the relationships between f_H and the percentage change in f_H for each environmental parameter (decreased temperature, increased temperature, and decreased oxygen) for each treatment group; including determining whether the slopes and intercepts were different between treatment groups. Note: Prism would only compare intercepts when slopes were not significantly different. An LME model with temperature / oxygen and treatment as fixed-effects, an

interaction term for the two, and fish as a random factor was used to assess changes in the percentage of 'good' quality ECGs (QI = 0). Linear regression analysis (applied on the data up to 20.8°C) and an LME model were used to assess the same parameters for the CT_{MAX} experiment, where the control group was held at 12ºC and the CT_{MAX} group represented fish exposed to increasing temperatures up to 22°C.

The LME models were performed using RStudio (v. 1.2.1335, RStudio Inc., Boston, MA; http://www.rstudio.com), while the one-way ANOVAs, t-tests and the linear regression analyses were performed using Prism 7 (GraphPad Software, Inc., San Diego, CA, USA). Assumptions of normality, homogeneity and independence were analyzed by visual inspection of Q-Q plots and histograms of the residuals, residual-fit plots and residual lag plots, respectively, for data analyzed in RStudio. The estimated marginal means, or emmeans, package (Singman et al. 2019) was used to perform Bonferroni's post-hoc tests on all LME models and Tukey's multiple comparison tests were performed on one-way ANOVAs in Prism. The level of statistical significance was $P < 0.05$. All values presented in the text are means ± standard errors of the mean (S.E.M).

Results

Heart Rate Recovery and Diel Patterns Post-Surgery

Following surgical implantation of the micro-HRT tags, the f_H of lumpfish recovering in a holding tank at 10ºC was recorded for five days (Figure 3.4). Average daily f_H decreased significantly during the recovery period ($P < 0.0001$; Figure 3.5; Table 3.1), from 61.8 ± 0.9 and 59.1 ± 1.1 bpm (day-time and night-time values) on the first day to 54.4 ± 0.9 and 51.9 ± 1.0 bpm by the final day of recovery. There was also a significant

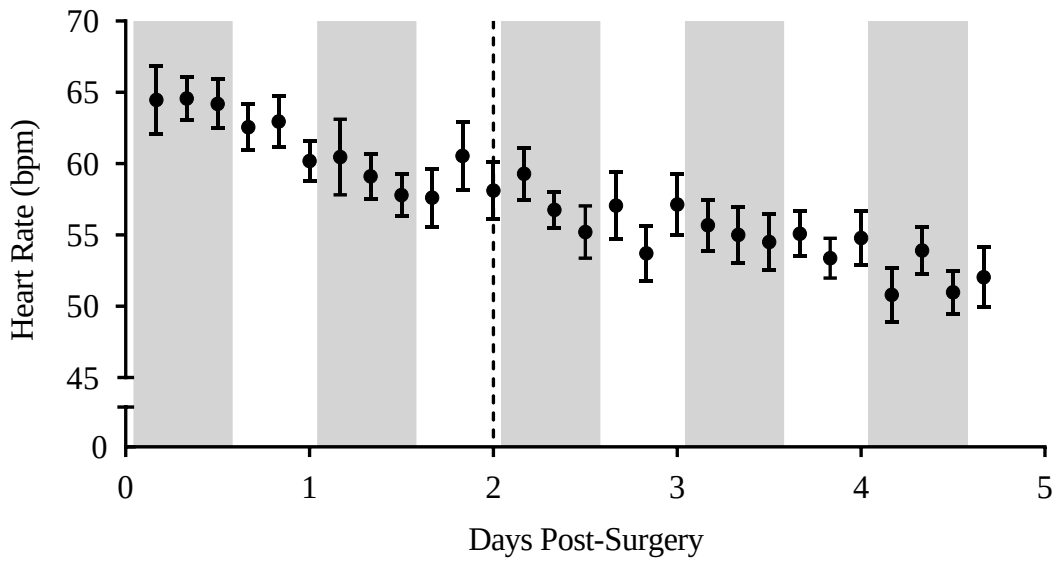

Figure 3.4. Average heart rate (f_H, bpm) values in free-swimming lumpfish (n = 14) recorded every 4 hours for 5 days post-surgery. Lumpfish were recovered in a tank in the Laboratory for Atlantic Salmon and Climate Change Research (LASCCR) following surgical implantation of the Star-Oddi micro-HRT tag. After 48 hours (dotted line), two lumpfish were transferred to 'baskets' inside the tank to be fasted for an additional ~ 66 hours. Fish were on a 14-hour light: 10-hour dark photoperiod (grey bars represent periods of darkness / night-time).

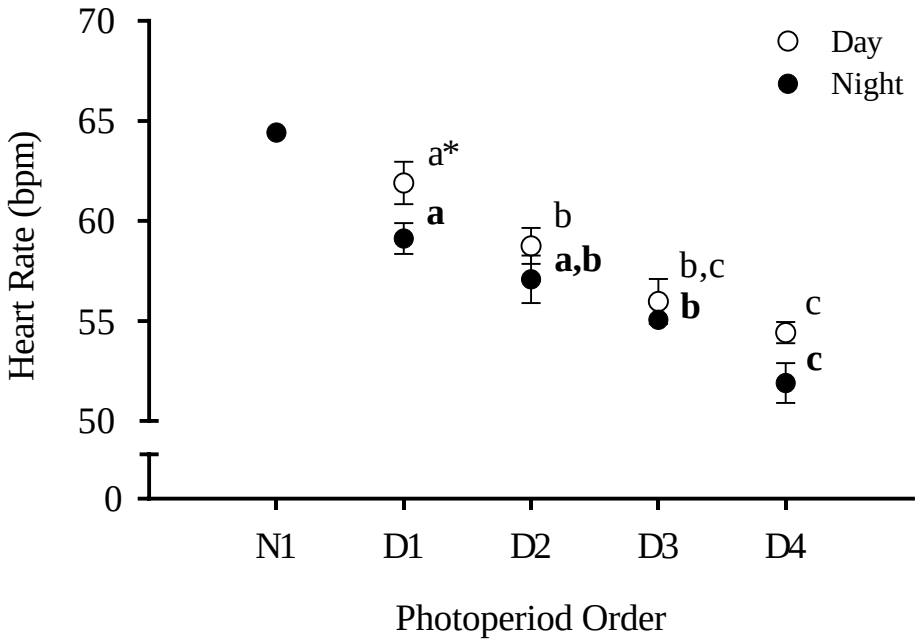

Figure 3.5. Average day-time and night-time heart rate (f_H, bpm) values in free-swimming lumpfish (n = 14) for 5 days after being implanted with Star-Oddi micro-HRT tags. Open symbols represent day-time, while dark symbols represent night-time. Dissimilar lower-case letters indicate a significant difference within a photoperiod group (for night-time values the letters are bolded), while an asterisk (*) represents a significance difference between day-time and night-time values at each measurement point. The data were recorded every 4 hours and the symbols represent means ± S.E.M (with each value representing the average of n = 3 data points per fish). Linear mixed-effects models, followed by Bonferroni's post-hoc tests performed using emmeans, were used to identify significant differences ($P < 0.05$) in f_H during surgical recovery. Note: N1 was not included in the analysis.

Table 3.1. Summary of the statistical outputs from the LME models which examined the effects of photoperiod (day-time / night-time), photoperiod order (days post-implantation), and their interaction on the f_H of lumpfish over 5 days of post-surgical recovery.

Independent Factor	Dependent Factor	Num DF	Den DF	F-value	P
Heart Rate	(Intercept)	1	285	2851.6346	**<0.0001**
	Photoperiod Order	3	285	24.5178	**<0.0001**
	Photoperiod	1	285	10.2754	**0.0015**
	Interaction	3	285	0.5724	0.6336

NumDF and DenDF are the degrees of freedom of the numerator and denominator of the F distribution ratio.

effect of photoperiod (P = 0.0015; Table 3.1). However, there was only a significant difference between day and night-time values on the first day following surgery and diel variation was relatively small (~ 2 - 4 bpm; Figure 3.5).

The Heart Rate Response to Hydrostatic Pressure and the Fish's Maximum Post-Exercise Heart Rate

Heart rate, the percentage change in f_H, HRV and percentage of 'good' quality ECGs remained constant in the control fish (Figure 3.6). At 10ºC, hydrostatic pressure had a significant effect on the f_H and percentage change in f_H of the lumpfish (P = 0.0025; P = 0.0012; Table 3.2). Heart rate began to increase between 35 and 50 bar, and while further increases were limited, f_H reached 61.5 ± 1.7 bpm (129.1 ± 3.8 % of initial values) by 80 bar as compared to 48.1 ± 1.4 bpm in the control fish at that time point (Figure 3.6). The f_H and percentage change in f_H remained elevated in lumpfish during decompression. Following removal from the IPOCAMP, maximum post-exercise f_H was 73.2 ± 1.4 and 76.8 ± 1.2 bpm (in control and pressure-exposed fish, respectively), suggesting that the pressure-induced increase in f_H was only ~ 47% of the fish's scope for increases in f_H (Figure 3.6). Statistical analysis was not performed due to the low sample sizes (n = 4), but the pressure-exposed group only had a ~ 3 bpm higher post-exercise f_H.

Overall, treatment did not significantly affect HRV or the quality of ECGs (P = 0.2932 and P = 0.0519, respectively; Table 3.2). However, there was a significant interaction between treatment and 'pressure step' for HRV. Post-hoc testing revealed that HRV in the pressure-exposed group decreased throughout the experiment from 100.9 ± 14.5 ms at the beginning to 71.4 ± 13.5 ms at 80 bar, and to 57.6 ± 8.5 ms after

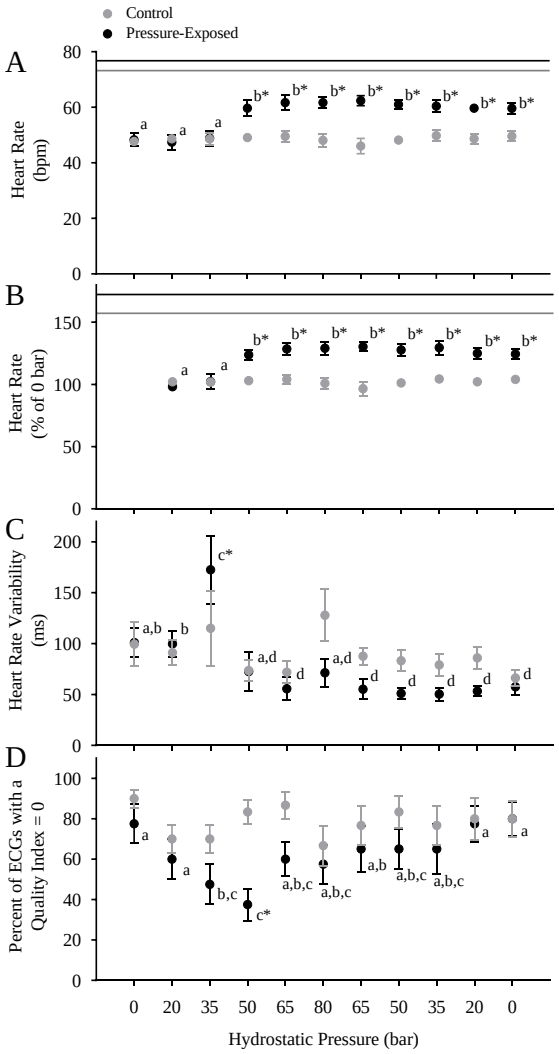

Figure 3.6. A) Heart rate (f_H, in bpm) in lumpfish held in the IPOCAMP at atmospheric pressure (0 bar; grey symbols; n = 6) or exposed to hydrostatic pressure in a step-wise protocol (black symbols; n = 8). Pressure was initially increased to 20 bar over 2 minutes, then held for 8 minutes. Pressure was then increased in a similar manner to 35, 50, 65 and 80 bar, followed by decompression in the opposite sequence. B) The percentage change in f_H (as a % of initial values at 0 bar) and C) heart rate variability (HRV in ms) were manually calculated from the electrocardiograms. D) the percentage of 'good' quality index ECGs (QI = 0) provided by the Star-Oddi Mercury software. Dissimilar lower-case letters indicate a significant difference within the pressure-exposed group (no differences existed in the control group), while an asterisk (*) indicates a significance difference ($P < 0.05$) between the pressure-exposed and control groups at a particular pressure step. Data were recorded every 2 minutes, and the symbols represent means ± S.E.M (n = 5 per fish).

Table 3.2. Summary of the statistical outputs from the LME models which examined the effects of treatment (control vs. pressure-exposed), pressure (0, 20, 35, 50, 65, 80 bar and decompression in the opposite sequence) and their interaction, on f_H, the percentage change in f_H (% of initial 0 bar values), heart rate variability (HRV) and the percentage of f_H values that were of 'good' quality (i.e., QI = 0).

Independent Factor	Dependent Factor	Num DF	Den DF	F	P
Heart Rate	(Intercept)	1	683	2007.1524	**<0.0001**
	Treatment	1	12	14.4433	**0.0025**
	Pressure Step	10	683	23.9438	**<0.0001**
	Interaction	10	683	19.0484	**<0.0001**
Percentage Change in Heart Rate	(Intercept)	1	617	2209.5170	**<0.0001**
	Treatment	1	12	17.8351	**0.0012**
	Pressure Step	9	617	15.6858	**<0.0001**
	Interaction	9	617	14.4017	**<0.0001**
Heart Rate Variability	(Intercept)	1	679	291.05371	**<0.0001**
	Treatment	1	12	1.20865	0.2932
	Pressure Step	10	679	5.09640	**<0.0001**
	Interaction	10	679	3.71900	**<0.0001**
Percentage of Quality Index Values = 0	(Intercept)	1	119	409.5971	**<0.0001**
	Treatment	1	12	4.6563	0.0519

Pressure Step	10	119	2.4598	**0.0104**
Interaction	10	119	1.2864	0.2458

NumDF and DenDF are the degrees of freedom of the numerator and denominator of the F distribution ratio.

decompression. Interestingly, HRV spiked to 172.5 ± 33.4 ms at 35 bar in this group, this value significantly higher than measured in the control group at the same point. The percentage of 'good' quality ECGs fell from 0 – 50 bar, and gradually returned to initial values (i.e., at ambient pressure) as pressure was increased to 80 bar and then the steps in pressure were reversed; the quality significantly different from initial values at 35 and 50 bar, and from that of the control group at 50 bar (Figure 3.6. D). Interestingly, 35-50 bar of pressure corresponded with the beginning of f_H increases, and the relationship between QI and pressure was a mirror image of that for activity (Figure 3.7. A).

Influence of Hydrostatic Pressure on Heart Rate and Its Response to Changes in Temperature and Hypoxia

At 12ºC, the effects of hydrostatic pressure were similar to those at 10ºC. However, f_H began to increase at 35 bar instead of 50 bar (Figure 3.8). Heart rate was significantly elevated by hydrostatic pressure ($P = 0.0053$) throughout the period of exposure, and this was also reflected in values of f_H when expressed as a percentage of initial (i.e. 0 bar) values ($P < 0.0001$ for both parameters; Table 3.3). The f_H, and the percentage change in f_H, did not change over the experiment in control fish. When pressure reached 80 bar, f_H was 67.7 ± 1.6 bpm compared to 57.7 ± 1.7 bpm in control fish at the same sampling point (i.e., 121.1 ± 4.1 % of initial values), and f_H remained elevated above control values after the 1 hour of acclimation to 80 bar of pressure. Activity was also stable during exposure to 80 bar of pressure, with the average value for pressure-exposed lumpfish approx. 2-fold higher than that of control fish (Figure 3.7. B).

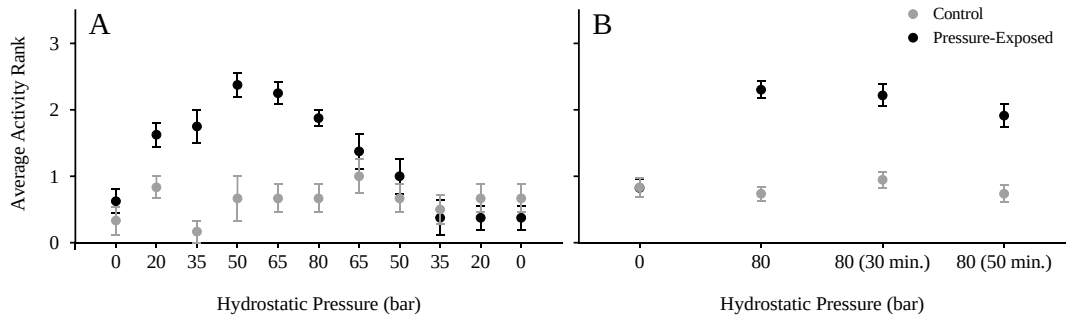

Figure 3.7. The average activity rank of lumpfish held in the IPOCAMP at atmospheric pressure (0 bar, grey symbols) or exposed to hydrostatic pressure in a step-wise protocol (black symbols). Pressure was initially increased to 20 bar over 2 minutes, then held for 8 minutes. Pressure was then increased in a similar manner to 35, 50, 65 and 80 bar, followed by decompression in the opposite sequence (A; n = 6 control and n = 8 pressure-exposed fish) or holding at 80 bar for 1 hour (B; n = 19 control and n = 23 pressure-exposed fish). The activity rank for each fish was determined from video recordings, where: 0 represents fish that were completely inactive; 1 represents fish that were mostly inactive but has some spontaneous activity; 2 represents fish that were mostly active but had some periods of inactivity; and 3 represents fish that were active for the entire 10 minute period. The symbols represent means ± S.E.M.

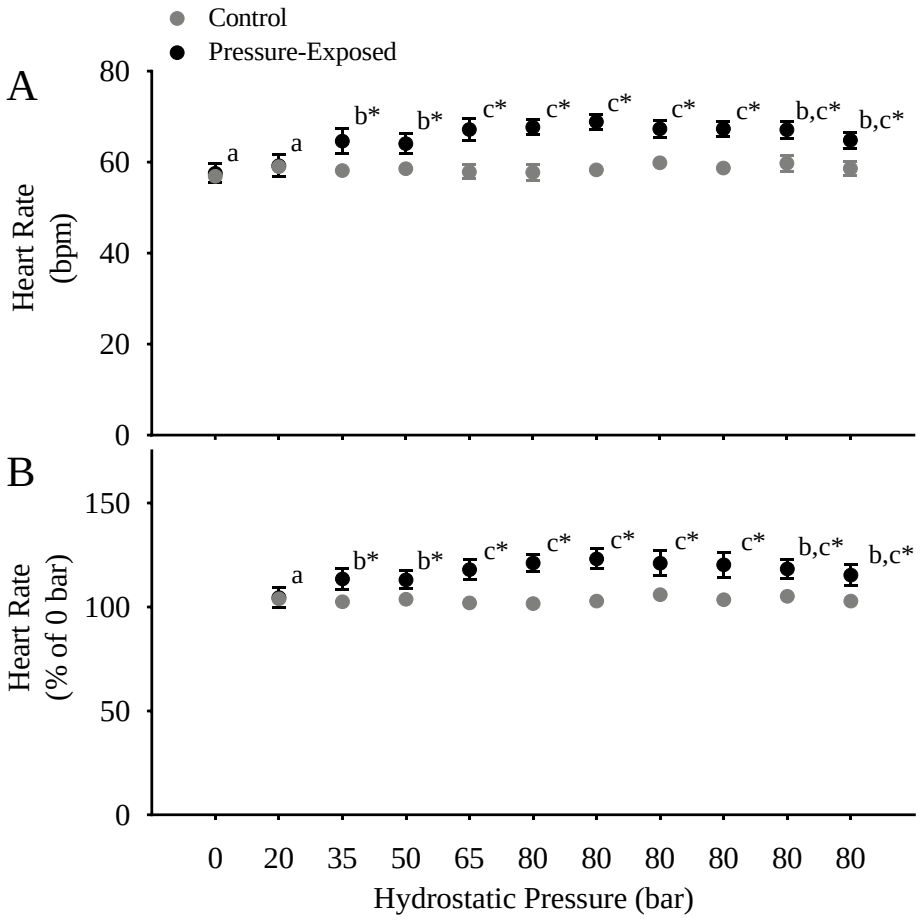

Figure 3.8. A) Heart rate (f_H in bpm) data recorded every 2 minutes in lumpfish held in the IPOCAMP at atmospheric pressure (0 bar; grey symbols; n = 22) or exposed to hydrostatic pressure in a step-wise protocol (black symbols; n = 23) in Experiment $^{\#}2$. Pressure was initially increased to 20 bar over 2 minutes, then held for 8 minutes. Pressure was then increased in a similar manner to 35, 50, 65 and 80 bar, followed by exposure to 80 bar of pressure for 1 hour. B) The percentage change in f_H (as a % of initial values at 0 bar) was manually calculated from the electrocardiograms. Dissimilar lower-case letters indicate a significant difference ($P < 0.05$) within the pressure-exposed group (no difference existed in the control group), while an asterisk (*) represents a significance difference between the pressure-exposed and control values within a pressure step. Data were recorded every 2 minutes, and the symbols represent means ± S.E.M (n = 5 per fish).

Table 3.3. Summary of the statistical outputs from the LME models which examined the effects of treatment (control vs. pressure-exposed), pressure (20, 35, 50, 65, 80 bar and time at 80 bar), and their interaction, on f_H and the percentage change in f_H [i.e., % of initial (0 bar) values].

Independent Factor	Dependent Factor	Num DF	Den DF	F	P
Heart Rate	(Intercept)	1	1923	3900.899	**<0.0001**
	Treatment	1	43	8.539	**0.0055**
	Pressure Step	10	1923	10.124	**<0.0001**
	Interaction	10	1923	8.904	**<0.0001**
Heart Rate (Percentage of Initial)	(Intercept)	1	1719	2956.4579	**<0.0001**
	Treatment	1	43	8.6105	**0.0053**
	Pressure Step	9	1719	4.4402	**<0.0001**
	Interaction	9	1719	6.8357	**<0.0001**

NumDF and DenDF are the degrees of freedom of the numerator and denominator of the F distribution ratio.

Temperature significantly influenced resting f_H, and at 0 bar the f_H of the fish in these experiments was ~ 9 bpm higher than that measured in fish held at 10°C (P = 0.018; Table 3.4). Weight and length had no effect on resting f_H (i.e., values at 0 bar), but sex significantly affected resting (initial) f_H (P = 0.015), with females having a 14.6 and 14.1 bpm higher f_H than immature or male fish, respectively (P = 0.014 and P = 0.092; Table 3.4). Neither sex, temperature, weight or length significantly affected the increase in f_H or the percentage increase in f_H at 80 bar (P > 0.05; Table 3.4). Further, it is unlikely sex impacted the overall results of the experiments. There was an equal distribution of known sexes between control and pressure-exposed groups (5 and 3 males, 5 and 5 females, respectively).

In control and pressure-exposed fish, f_H fell with decreasing temperature ($Y = 3.49x + 16.15$, $R^2 = 0.92$, $P < 0.0001$ and $Y = 4.08x + 16.04$, $R^2 = 0.94$, $P < 0.0001$, respectively; Table 3.5; Figure 3.9. A, C, E). The f_H of pressure-exposed fish was 7.0 bpm higher than that of control fish before the temperature began to decrease (i.e., at 80 bar), and the slopes of the relationship between f_H and temperature were significantly different between the two groups (P = 0.007; Table 3.5) as f_H reached a minimum of ~ 38 and 39 bpm (Q_{10} = 2.10 and 2.07; in control and pressure-exposed fish, respectively) at ~ 5.7°C. In contrast, the slopes of the relationships between f_H (as a percentage of the initial value) and temperature were not significantly different between the control and pressure-exposed groups (P = 0.846).

Heart rate was 16.0 bpm higher in the pressure-exposed group before temperature was increased, and increased in both control and pressure-exposed fish with rising temperature ($Y = 1.53x + 33.17$, $R^2 = 0.54$, $P < 0.0001$ and $Y = 2.81x + 28.07$, $R^2 = 0.49$,

Table 3.4. Summary of the statistical outputs from the ANOVA and Tukey's post-hoc tests, unpaired t-tests and linear regressions performed in Prism 7. These tests examined the effect of sex (I = immature, F = female, M = male), acclimation temperature (10 or 12°C), weight (g) and length (cm) on the initial f_H and change in f_H / percentage change in f_H at 0 vs. 80 bar in pressure-exposed lumpfish from the experiments conducted in the IPOCAMP. Significant differences are indicated in bold type.

		F	R^2	P	N1	N2
ANOVA and Tukey's Post Hoc Summary						
Sex vs. Initial f_H	ANOVA	5.38	0.39	**0.015**		
	I vs. F			**0.014**	12	5
	I vs. M			0.994	12	3
	F vs. M			0.092	5	3
Sex vs. Change in f_H	ANOVA	1.81	0.19	0.198		
	I vs. F			0.181	10	5
	I vs. M			0.979	10	3
	F vs. M			0.456	5	3
Sex vs. % Change in f_H	ANOVA	2.19	0.23	0.147		
	I vs. F			0.128	10	5
	I vs. M			0.935	10	3
	F vs. M			0.448	5	3
Unpaired T-Test						
Temperature vs. Initial f_H	10 vs. 12	2.16	0.18	**0.018**	22	8
Temperature vs. Change in f_H	10 vs. 12	2.13	0.03	0.421	20	8

Temperature vs. % Change in f_H	10 vs. 12	1.43	0.04	0.292	20	8

Linear Regression					
Weight vs. Initial f_H	1.45	0.05	0.239	27	
Weight vs. Change in f_H	0.003	0.0001	0.956	27	
Weight vs. % Change in f_H	0.05	0.002	0.826	27	
Length vs. Initial f_H	0.84	0.03	0.367	30	
Length vs. Change in f_H	0.15	0.006	0.702	30	
Length vs. % Change in f_H	0.18	0.007	0.678	30	

Table 3.5. Relationships between f_H and percentage change in f_H, and changes in environmental variables (decreased temperature, increased temperature, decreased oxygen, or increased temperature up to 20.8°C in a CT_{MAX} experiment), for control and pressure-exposed / CT_{MAX} groups. Significant relationships, and significant differences in slopes or intercepts, are indicated in bold type.

Relationship	Treatment	Equation	R^2	P
Decreased Temperature				
Heart Rate x Temperature	Control	Y = 3.49x + 16.15	0.92	**<0.0001**
	Pressure-Exposed	Y = 4.08x + 16.04	0.94	**<0.0001**
	Are the slopes equal?			**0.007**
	Are the intercepts equal?			NA
Percentage Change in Heart Rate x Temperature	Control	Y = 5.93x + 27.14	0.90	**<0.0001**
	Pressure-Exposed	Y = 6.01x + 25.32	0.93	**<0.0001**
	Are the slopes equal?			0.846
	Are the intercepts equal?			0.1319
Increased Temperature				
Heart Rate x Temperature	Control	Y = 1.53x + 33.17	0.54	**<0.0001**

	Pressure-Exposed	Y = 2.81x + 29.07	0.49	**<0.0001**
	Are the slopes equal?			**0.0078**
	Are the intercepts equal?			NA
Percentage Change in Heart Rate x Temperature	Control	Y = 3.34x + 58.73	0.53	**<0.0001**
	Pressure-Exposed	Y = 5.02x + 36.52	0.42	**<0.0001**
	Are the slopes equal?			0.1069
	Are the intercepts equal?			**0.0324**
Decreased Oxygen				
Heart Rate x Temperature	Control	Y = -1.31x + 71.31	0.39	**<0.0001**
	Pressure-Exposed	Y = 0.01x + 63.37	0.002	0.7859
	Are the slopes equal?			**0.0002**
	Are the intercepts equal?			NA
Percentage Change in Heart Rate x Temperature	Control	Y = -0.30x + 132.4	0.48	**<0.0001**
	Pressure-Exposed	Y = 0.01x + 100.6	0.002	0.7603

	Are the slopes equal?			**<0.0001**
	Are the intercepts equal?			NA
CT_{MAX}				
Heart Rate x Temperature	Control	Y = 0.29x + 52.83	0.02	**0.0496**
	CT_{MAX}	Y = 3.41x + 11.16	0.50	**<0.0001**
	Are the slopes equal?			**<0.0001**
	Are the intercepts equal?			NA
Percentage Change in Heart Rate x Temperature	Control	Y = 0.56x + 88.85	0.02	**0.037**
	CT_{MAX}	Y = 6.96x + 16.34	0.42	**<0.0001**
	Are the slopes equal?			**<0.0001**
	Are the intercepts equal?			NA

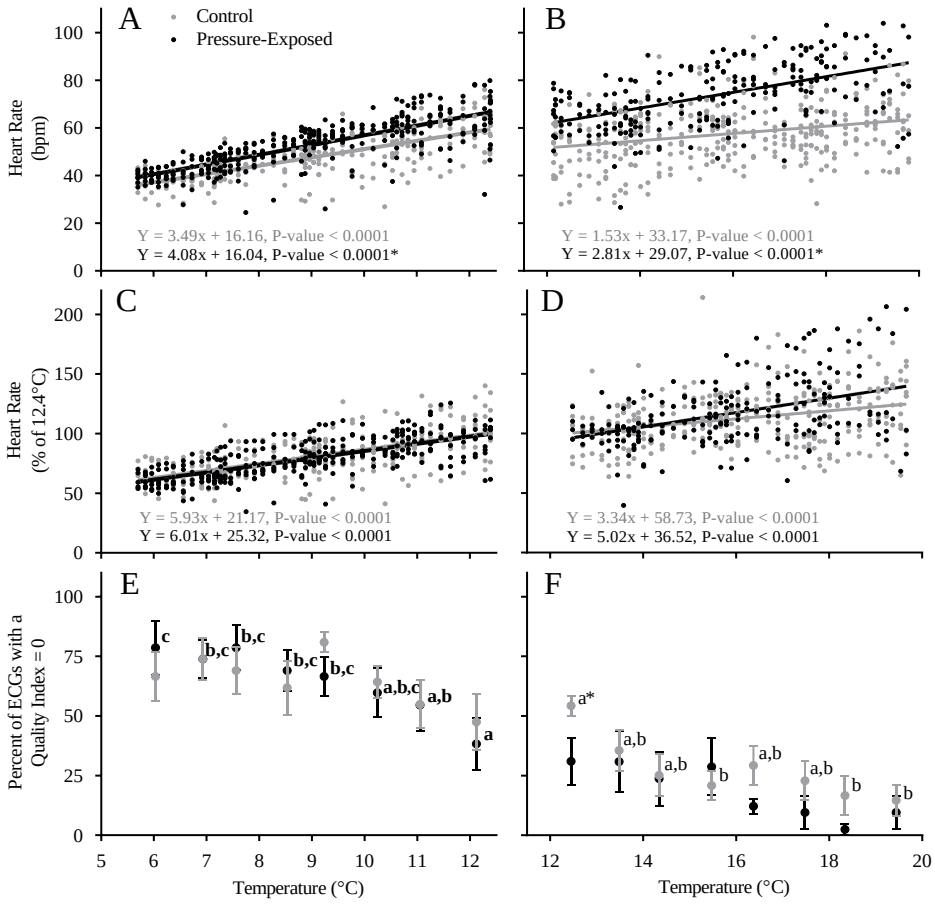

Figure 3.9. A) Heart rate (f_H, bpm) in lumpfish exposed to decreasing (at 2ºC h^{-1}; A, C, E) or increasing (at 2ºC h^{-1}; B, D, F) temperature in the IPOCAMP chamber. Prior to the decrease in temperature, lumpfish were held at atmospheric pressure (black symbols; n = 7) or exposed to 80 bar of pressure (grey symbols; n = 7). C, D) The percentage change in f_H (as a % of initial values at 0 or 80 bar) was manually calculated from the electrocardiograms. Relationships between f_H and the percentage change in f_H with temperature were determined by linear regression analysis, and an asterisk (*) indicates a significant difference between the slopes for relationships between control and pressure-exposed groups. E, F) The percentage of 'good' quality ECGs (i.e., with a QI = 0) were provided by the Star-Oddi Mercury software, and significant differences within the control (regular letters) or pressure-exposed groups (bold letters) are represented by dissimilar lower-case letters. No differences existed in the percentage of 'good' quality ECGs between pressure-exposed and control fish at any of the temperature steps. Data were recorded every 5 minutes, and the symbols represent means ± S.E.M (n = 6 values per fish).

P < 0.0001, respectively; Table 3.5; Figure 3.9. B, D, F); the slopes of this relationship significantly greater in the pressure-exposed group (P = 0.0078; Table 3.5). Maximum f_H was ~ 64 and 83 bpm (Q_{10} = 1.37 and 1.39; in the control and pressure-exposed groups, respectively) at ~ 19.6°C; the latter is a higher maximum f_H than was reached when lumpfish were exposed to exercise (73-76 bpm) after removal from the IPOCAMP. Although f_H (as a percentage of initial values) also increased with temperature (see Table 3.5), the relationships for the two treatments did not have significantly different slopes (P = 0.1069). When the two temperatures challenges are considered together, the f_H of control and pressure-exposed lumpfish increased from 38 to 64 bpm and 39 to 83 bpm over from ~ 5 to 20°C in the control and pressure-exposed fish, respectively.

When held at atmospheric pressure in the IPOCAMP, the f_H of lumpfish increased with decreasing oxygen levels ($Y = -1.31x + 71.31$, $R^2 = 0.39$, P < 0.0001; Table 3.5; Figure 3.10); i.e., from 58.7 ± 3.2 bpm at 106 % air sat. to 62.3 ± 2.9 bpm at 57 % air sat. However, exposure to 80 bar of hydrostatic pressure eliminated the effect of decreasing oxygen levels as f_H remained unchanged from 103 to 52 % air sat. ($Y = 0.01x + 63.37$; R^2 = 0.002, P = 0.7859; Table 3.5; Figure 3.10). The same relationships were evident when f_H data was calculated as a percentage of initial values (see Table 3.5).

Hydrostatic pressure did not have a significant effect on the percentage of 'good' quality ECGs in the decreasing or increasing temperature experiments (P = 0.9784 and P = 0.1939, respectively), but did significantly affect the quality of ECGs during the decreased oxygen experiments (P = 0.0292; Table 3.6; Figures 3.9 and 3.10). On average, ECGs with a QI = 0 were ~ 25% fewer in pressure-exposed fish compared to control fish in this experiment (48.4 vs. 23.6 %). Conversely, decreasing or increasing temperature,

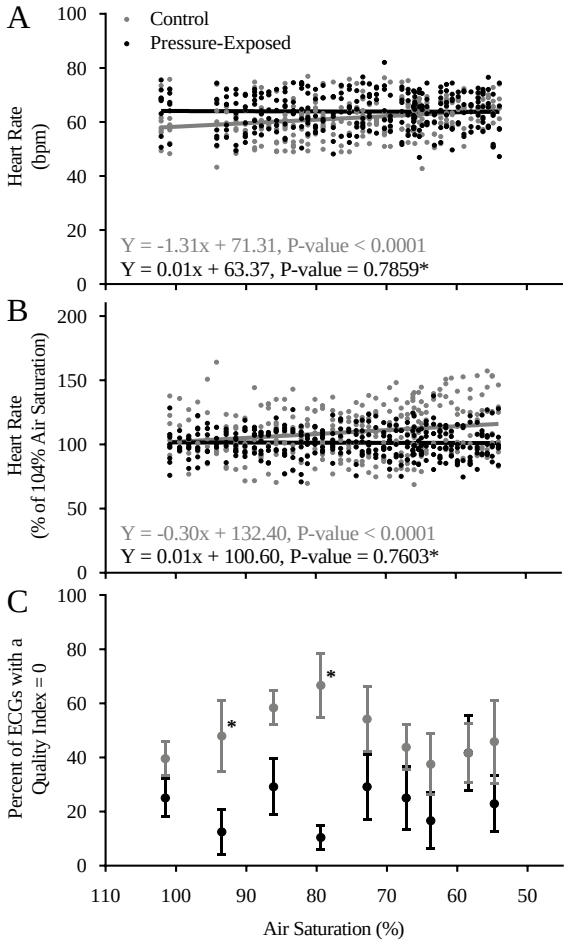

Figure 3.10. A) Heart rate (f_H, bpm) in lumpfish exposed to decreasing oxygen levels (air saturation; %) in the IPOCAMP chamber over 3 to 4 hours. Prior to the decrease in oxygen levels, lumpfish were held at atmospheric pressure (black circles; n = 8) or exposed to 80 bar of pressure (grey circles; n = 8). B) The percentage change in f_H (as a % of initial values at 0 or 80 bar) was manually calculated from the electrocardiograms. Relationships between f_H and percentage f_H with oxygen level were determined by linear regression analysis, and an asterisks represents a significant difference in the slopes of the relationships between control and pressure-exposed lumpfish. C) The percentage of 'good' quality index ECGs (QI = 0) were provided by the Star-Oddi Mercury software, and in this panel, an asterisks represents a significant difference between pressure-exposed and control groups at a particular oxygen level. Within each treatment group, there were no differences in percentage of 'good' quality index values. Data were recorded every 5 minutes, and the symbols represent means ± S.E.M (n = 6 values per fish).

Table 3.6. Summary of the statistical outputs from the LME models which examined the effects of treatment (control vs. pressure-exposed), changes in environmental variables (decreased temperature, increased temperature, decreased oxygen or increased temperature in a CT_{MAX} experiment), and their interaction, on the percentage of ECGs that were that of 'good' quality (QI = 0).

Independent Factor	Dependent Factor	Num DF	Den DF	F-value	P
Decreased Temperature					
Percentage of Quality Index Values Equal to Zero	(Intercept)	1	83	253.00233	**<0.0001**
	Temperature Step	7	83	4.62633	**0.0002**
	Treatment	1	12	0.00076	0.9784
	Interaction	7	83	0.99657	0.4397
Increased Temperature					
Percentage of Quality Index Values Equal to Zero	(Intercept)	1	90	38.74583	**<0.0001**
	Temperature Step	7	90	5.54831	**<0.0001**
	Treatment	1	13	1.87718	0.1939
	Interaction	7	90	1.13536	0.3483

Decreased Oxygen					
Percentage of Quality Index Values Equal to Zero	(Intercept)	1	111	46.92705	**<0.0001**
	Oxygen Step	8	111	1.26361	0.2698
	Treatment	1	14	5.90138	**0.0292**
	Interaction	8	111	1.93140	0.0621
CT_{MAX} Experiment					
Percentage of Quality Index Values Equal to Zero	(Intercept)	1	89	42.41553	**<0.0001**
	Treatment	1	10	13.74208	**0.0041**
	Temperature Step	9	89	1.99405	**0.0491**
	Interaction	9	89	3.33289	**0.0015**

NumDF and DenDF are the degrees of freedom of the numerator and denominator of the F distribution ratio.

but not decreasing oxygen ($P < 0.0001$, $P < 0.0001$ and $P = 0.2698$, respectively; Table 3.6; Figures 3.9 and 3.10) strongly effected the quality of the ECGs. Overall, the percentage of 'good' quality ECGs (i.e., with QI = 0) fell from 72.6% at 6.0°C to 12.3% at 19.4°C. In general, the R wave of lumpfish has a small average voltage amplitude (avg. ~ 171 mV, range 430 to 600 mV; Figure 3.11), and this may have contributed to the decrease in the quality of ECGs that was observed.

The Normobaric Heart Rate Response to Increased Temperature

When lumpfish underwent a CT_{MAX} experiment (increasing temperature at 2°C h^{-1} up to 22°C) under normobaric conditions (atmospheric pressure in a water table), f_H was 52.4 ± 2.5 bpm at 12.3°C, peaked at 81.0 ± 3.6 bpm at 20.8°C (the f_H – temperature relationship between 12.3 and 20.8°C $Y = 3.41x + 11.16$, $R^2 = 0.50$, $P < 0.0001$), and then declined to 71.7 ± 3.6 bpm by 22.1°C (a scope of ~ 29 bpm between 12.3 and 20.8°C; $Q_{10} = 1.67$) (Table 3.5; Figure 3.12). This value for maximum f_H was ~ 17 bpm higher than the corresponding value reached in fish held at atmospheric pressure in the IPOCAMP (~ 64 bpm at 19.6°C), but comparable to the maximum f_H recorded for lumpfish at 80 bar (~ 83 bpm at 19.6°C). The f_H of the time-matched control fish (held at 12°C) also increased significantly, but only by ~ 2 bpm (see Table 3.5 and Figure 3.12). Increasing temperature resulted in a significant decrease in the percentage of 'good' quality ECGs, and values were significantly lower than the time-matched control group at temperatures > 15°C ($P = 0.0492$ and $P = 0.0041$; Table 3.6; Figure 3.12). Similar to the IPOCAMP experiment, the quality of the recorded ECGs decreased from 45.8 % at 12.3°C to 1.8 % at 21.6°C (compared to 41.7 and 67.9 % in the time-matched controls).

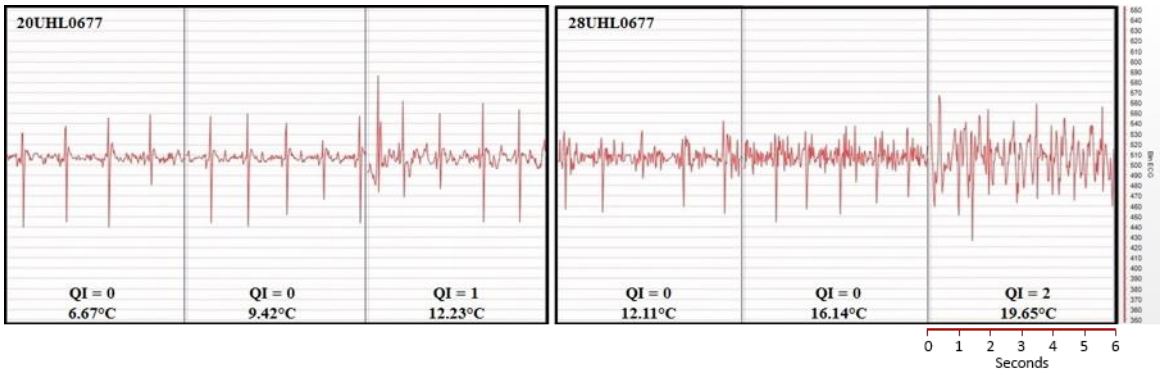

Figure 3.11. Electrocardiograms recorded in a lumpfish exposed to decreasing (at 2°C h^{-1}; left panel) or increasing (at 2°C h^{-1}; right panel) temperature in the IPOCAMP chamber at atmospheric pressure (0 bar). This fish was chosen as its ECG recordings, and their response to temperature, were typical of what was observed for this species. Bin ECG represents the amplitude of the QRS waveform and ranges from 0 to 1000 mV, but only the range from 350 to 650 mV is presented. The quality index (QI) was assigned to ECG recordings (where 0 means great quality, 1 and 2 have decreasing quality and 3 means there is no R-R interval) by the Star-Oddi Mercury software.

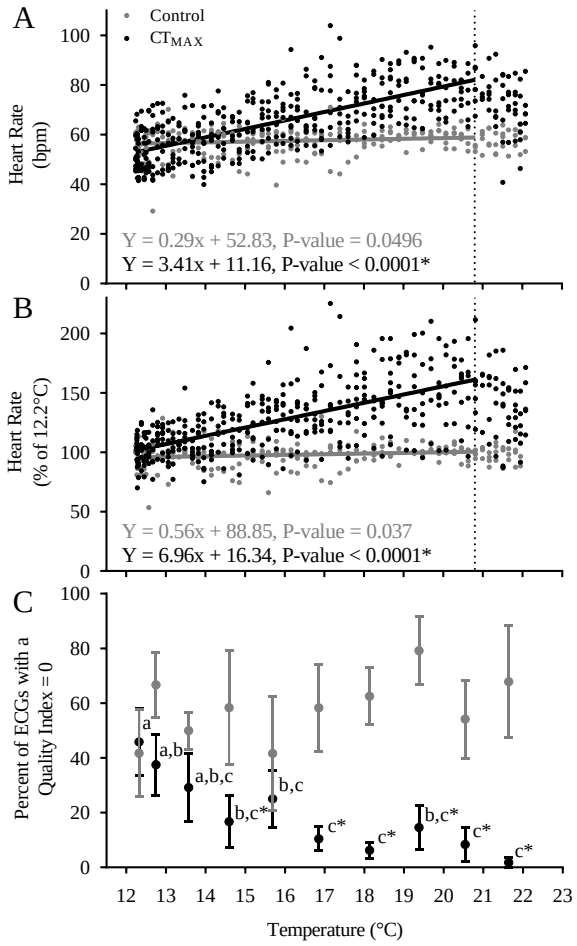

Figure 3.12. A) Heart rate (f_H, bpm) in lumpfish during a CT_{MAX} experiment (black symbols; n = 12) in a water table, where temperature was increased at 2 °C h^{-1} vs. when fish were held at a constant temperature of 12ºC (grey symbols; n = 4). B) The percentage change in f_H (as a % of initial values at 12.2°C) was manually calculated from the electrocardiograms. Relationships between f_H and the percentage change in f_H with temperature were determined by linear regression analysis for data up to 20.8°C, and an asterisk (*) indicates a significant difference ($P < 0.05$) in the slopes between control fish and those exposed to increasing temperature. C) The percentage of 'good' quality ECGs (i.e., with a QI = 0) were provided by the Star-Oddi Mercury software. Significant differences within the CT_{MAX} group (there were no differences in the control group) are represented by dissimilar lower-case letters, and an asterisk represents a difference between the CT_{MAX} and control group at a particular temperature. Data were recorded every 5 minutes, and the symbols represent means ± S.E.M (n = 6 per fish). The dotted line indicates the temperature of 20.8°C. Beyond this temperature the f_H of the lumpfish began to decrease, and thus these data was not included in the linear regression.

Discussion

Post-Surgical Recovery and Diel Patterns in Heart Rate

The methods used to surgically implant DSTs induce a physiological stress response in fish leading to increased f_H (Altimiras and Larsen 2000; Hill and Forster 2004; Rothwell et al. 2005; Gräns et al. 2014; Raby et al. 2015). While not a primary goal of this research, the f_H of lumpfish was recorded following the implantation of micro-HRT tags to monitor recovery. Initially, the day-time f_H of lumpfish was 62 bpm, but their f_H declined in the days following surgery indicating recovery / partial recovery from this stressor (Figures 3.4 and 3.5; Table 3.1). After 48 hours of recovery, f_H was approximately 60 bpm, and by 5 days post-surgery it was 54 bpm (at 10°C; Figure 3.5). While, to my knowledge, no other published values for resting f_H in lumpfish exist, these data can be compared with that measured for the salmon in Chapter 2 that underwent similar procedures. The day-time f_H of the salmon at 11°C was slightly lower post-surgery (~ 59 bpm), and decreased to about 45 bpm by day 5 (Figure 2.10). The higher f_H (and smaller decrease post-surgery) in lumpfish may reflect an intrinsically higher f_H in this species as compared to salmon, and is somewhat surprising given this species limited activity and that the fish were fasted for more than 2 days at the end of the recovery period. Digestion and other physiological processes associated with feeding increase the $\dot{M}O_2$ and f_H of fish (McCue 2006; Eliason et al. 2008), and thus, fasting would have been expected to decrease f_H. Further, given the limited maximum f_H in this species (~ 80 bpm, see below), it might be expected that they would have a low resting f_H so that they would have a scope for f_H comparable to other teleost species (1.8 to 2.6-fold increase in f_H during CT_{MAX} tests; Gollock et al. 2006; Clark et al. 2008; Vornanen et al. 2014; Penney

et al. 2014; Motyka et al.2017). Thus, these data may indicate that the lumpfish had not fully recovered from surgery, and that this explains their high resting f_H.

Overall, there was a significant effect of photoperiod on the f_H of lumpfish during the recovery period (Figures 3.4 and 3.5; Table 3.1). However, the day-time values were only significantly different than the night-time values on the first day post-surgery, and diel variations in f_H were relatively small (2 to 4 bpm). In this study, a lower sampling frequency was chosen (every 4 hours post-surgery) and less data were collected in order to save battery life and memory for the pressure experiments. This may have affected the magnitude of the diel variation recorded in f_H. Lumpfish are also not very active swimmers, and they were held in a relatively small tank (and baskets during fasting), and this may have limited activity-dependent changes in f_H. The salmon in Chapter 2 were held in much larger tanks and swimming activity (as measured by EA; Figure 2.10; Table 2.5) had a similar diel pattern as f_H. Thus, the larger diel variation in salmon was likely, at least partially, related to changes in activity over the day.

Heart Rate Response to Hydrostatic Pressure

The initial goal of this research was to investigate the effect of hydrostatic pressure on the f_H of lumpfish. In response to an acute exposure to 80 bar of pressure, the f_H of 10°C-acclimated lumpfish increased by ~ 14 bpm (20-30%) above resting values (Figure 3.6; Table 3.2). Further, this tachycardia was sustained during the 1 hour of pressure exposure at 80 bar (Figure 3.8; Table 3.3) and only diminished slightly during decompression (Figure 3.6). Previous research on this topic is extremely limited, possibly due to technical limitations (Guerrero et al. 2000). However, our results are generally

consistent with other studies that have measured the effect of hydrostatic pressure on f_H in fishes at temperatures within the middle of a species' thermal range. For example, Sébert and Barthélémy (1985b) reported that: 1) exposure to ~ 101 bar of pressure increased the f_H of freshwater eels acclimated to 15-20°C by ~ 30-80% and that this tachycardia was sustained during the 1 hour of pressure exposure; and 2) while f_H did fall to some degree during decompression, it was still not back to pre-exposure levels by 1 hour. Also, Naroska (1968) showed that abrupt compression to ~ 50 bar produced a transient tachycardia in 5°C eel pout (*Zoarces viviparous*), and Belaud et al. (1976) found that pressure induced a tachycardia below the temperature of 24.5°C in eels (c.f. Sébert and Macdonald 1993).

In addition, our results are in agreement with studies on the effects of pressure on f_H in other aquatic taxa, and the effects of pressure on oxygen consumption ($\dot{M}O_2$) in fishes; the latter data highly relevant as changes in f_H often reflect those in $\dot{M}O_2$ (Armstrong 1986; Lucas 1994). Heart rate increased by ~ 65% in the shallow-water spider crab (*Maja brachydactyla*) when hydrostatic pressure was raised from atmospheric pressure to ~ 100 and 150 bar at 20°C (Thatje and Robinson 2011). Similar to the change in the f_H of lumpfish, $\dot{M}O_2$ has been shown to increase in response to acute pressure exposure in European plaice (*Pleuronectes platessa*), great sand eel (*Hyperoplus lanceolatus*), sand goby (*Pomatoschistus minutus*), Kessler's sculpin (*Cottus kessleri*), European flounder (*Platichthys flesus*), rainbow trout (*Oncorhynchus mykiss*), goldfish (*Carassius auratus*) (reviewed in Sébert and Macdonald 1993), bloater (*Coregonus hoyi*; Speers-Roesch et al. 2004) and European eel (Sébert and Barthélémy 1985a; Simon et al. 1989; Sébert et al. 1995a,b; Vettier et al. 2003, 2005, 2006; Scaion et al. 2008a).

With regards to the pressure at which increases in f_H begin in fishes, the data are difficult to compare as the maximum hydrostatic pressure the fish is exposed, the rate of compression, and temperature, all influence the f_H response to increased pressure (Sébert and Macdonald 1993). In this study, f_H began to increase between 30 and 50 bar and this is consistent with Sébert and Barthélémy (1985b) where, after no change or a brief bradycardia in some eels, f_H began to increase at 40 to 50 bar. In contrast, the pressure at which the $\dot{M}O_2$ of male and female eels began to increase was between 50 and 80 bar (depending on pressure; Scaion et al. 2008a), and Speers-Roesch et al. (2004) demonstrated that pressures as low as 3 bar increased $\dot{M}O_2$ in the bloater. The latter data suggest that the life history of a given species (i.e., its' normal depth range) likely has a significant effect on the sensitivity of their responses to increasing pressure. In this study, the maximum pressure-induced f_H in lumpfish was ~ 62 bpm at 10°C, whereas the fish's maximum f_H at this temperature (as determined by chasing and increased temperature; Figures 3.6 and 3.12) was ~ 73 - 83 bpm. These data suggests that the increase in f_H induced by hydrostatic pressure was only ~ 47% of the available scope for f_H. Again, this is consistent with Sébert and Barthélémy (1985b) who reported that while the maximum temperature-induced f_H in eels is ~ 120 bpm, f_H when exposed to ~ 101 bar did not exceed 60 bpm. These data indicate that fish at high pressure (at least those whose life history includes excursions to the applied pressures) still have a considerable scope available for increases in f_H.

Many authors attribute the reported increases in $\dot{M}O_2$ to a simultaneous increase in motor activity during compression (Sébert and Barthélémy 1985a; Simon et al. 1989; Sébert et al. 1997; Vettier et al. 2003; Speers-Roesch et al. 2004), which Speers-Roesch et

al. (2004) suggested was partially related to compression of the swim bladder in bloater, and thus, a loss of buoyancy. Lumpfish do not possess a swim bladder (Powell et al. 2017) and were supported by platform in the IPOCAMP chamber. Nonetheless, they became agitated and more active during compression and this hyperactivity was maintained for the hour of compression at 80 bar (see Figure 3.7), and Sébert and Barthélémy (1985b) found that increases in motor activity during compression in eels were associated with tachycardia. These data strongly suggest that increased activity was largely responsible for the increase in f_H with pressure exposure. However, while activity decreased during decompression and was back to control levels by 35 bar (Figure 3.7), f_H remained elevated. It is possible that the tachycardia associated with increases in hydrostatic pressure was also related to alterations in the neurohormonal control of f_H. This conclusion is based on three lines of evidence. First, exposure of isolated eel hearts to increased pressure results in a pronounced bradycardia, not tachycardia (Pennec et al. 1988). Second, Belaud et al. (1976) and Sébert and Barthélémy (1985b) show that atropine, and adrenergic agonists and antagonists, markedly alter the magnitude of the tachycardic response when eels are exposed to increased hydrostatic pressure. Third, HRV was considerably lower in the pressure-exposed group near the end of the compression period at 80 bar and remained lower during decompression (Figure 3.6. C). The mechanisms involved have not been elucidated but could be related to alterations in cholinergic or adrenergic tone, or receptor function / affinity associated with changes in pacemaker cell membrane fluidity (Sébert and Barthélémy 1985b).

Several studies have also provided evidence that a pressure-induced decrease in membrane fluidity, or “rigidification”, results in “compression-induced histotoxic

hypoxia" in fish (Sébert and Barthélémy 1985a; Sébert et al. 1987; Sébert et al. 1993). However, most studies that have examined the acute effects of pressure on fish $\dot{M}O_2$ report an increase (not a decrease) in $\dot{M}O_2$ (see references above), and Scaion et al. (2008b) showed that the $\dot{M}O_2$ of permeabilized red muscle fibers increases or does not change with acute pressure exposure (depending on test temperature and pressure). Further, the hypothesized decrease in tissue oxygen consumption (extraction) would be predicted to increase venous PO_2 and oxygen content, and arterial PO_2 and content do not change in trout when exposed to ~ 101 bar of pressure (Sébert at al. 1997), and thus, there would be no chemoreceptor-mediated drive for increased f_H. These data call into question whether 'compression-induced histotoxic hypoxia' is a real phenomenon, versus related to changes in fish activity over time or the particular methods used in those experiments.

Interestingly, there is evidence that the duration of exposure also impacts the physiological response of fishes to hydrostatic pressure. It is known that $\dot{M}O_2$ increases above resting levels with acute pressure exposure, but when pressure is maintained and fish are allowed to acclimate, $\dot{M}O_2$ falls back to resting levels (Simon et al. 1989; Sébert and Macdonald 1993; Sébert et al. 1995a; Vettier et al. 2003). For example, Simon et al. (1989) reported that the $\dot{M}O_2$ of eels reached resting levels by 6 to 7 days at sustained pressure. Sébert et al. (1995a) showed that the $\dot{M}O_2$ of eels exposed to ~ 101 bar returned to resting values within 3 hours at pressure and decreased below control values by 4 days of acclimation. Finally, Sébert and Barthélémy (unpublished; c.f. Sébert and Macdonald 1993) indicated that the f_H of European eels returned to resting values within 3 days at ~ 100 bar. In this study, lumpfish were either acutely exposed to pressure or sustained at pressure for only one hour prior to environmental challenges. Therefore, based on the

available data, I hypothesize that the f_H of lumpfish would eventually fall to resting values with acclimation to increased hydrostatic pressure.

The increase in lumpfish f_H at 80 bar was quite variable (from -9 to 24 bpm) suggesting that other variables may have influenced the effect of pressure on f_H. Individual difference in activity were likely a primary influence on the variability of the f_H response to hydrostatic pressure (see above). However, by combining the data from all of the experiments in the IPOCAMP, it was possible to also investigate the influence of acclimation temperature, sex, and fish mass / length on the response of f_H to compression (Table 3.4). The resting f_H of females in the IPOCAMP (68 bpm, n = 5) was higher than immature lumpfish (53 bpm, n = 12), but not significantly higher than males despite a difference of 14 bpm (54 bpm; n = 3). Additionally, lumpfish acclimated to 12°C had a higher resting f_H (58 bpm) than those acclimated to 10°C (48 bpm). Despite this, it was found that temperature, sex, weight and length did not affect the change in f_H or the percentage change in f_H when lumpfish were compressed to 80 bar of pressure. However, there was an unequal distribution of immature fish in the experiments, and the sex of 22 fish were not assessed following the experiments. The lack of an effect of sex on pressure induced physiology in lumpfish at 10-12°C is consistent with the $\dot{M}O_2$ data that Scaion et al. (2008a) report for eels at temperatures below 15°C. However, these authors also report that the $\dot{M}O_2$ of female eels was much more sensitive at 22°C as compared to males, and thus, the effects of sex on hydrostatic pressure-related changes in the f_H of fishes cannot be excluded.

Influence of Hydrostatic Pressure on Heart Rate and Its Response to Changes in Temperature and Hypoxia

In my experiments, I exposed lumpfish to 80 bar of pressure for one hour and then raised / or lowered the temperature from 12°C at 2°C h^{-1}. This research showed that temperature resulted in a linear change in f_H until the experiments were stopped at ~ 6 and 20°C, and that while the relationship was steeper for absolute f_H in fish exposed to hydrostatic pressure, the relationship was similar to control fish (i.e., exposed to 0 bar) when the elevated f_H in pressure exposed fish at 12°C was taken into account (i.e., when the change in f_H was expressed as a % of initial values; Figure 3.9). These results suggest that while hydrostatic pressure does have an effect on resting (baseline) f_H, it does not influence the sensitivity of f_H to changes in temperature. This finding was quite surprising as Scaion et al. (2008a) showed that temperature had a significant effect on the sensitivity of $\dot{M}O_2$ to increases in hydrostatic pressure, and Sébert et al. (1995b) reported that exposing eels to a 5°C temperature increase (from 15 to 20°C) concomitantly with an increase in pressure to ~ 101 bar reduced the acute increase in $\dot{M}O_2$ by approx. 50%. Finally, while tachycardia is seen in pressure exposed eels at lower temperatures, this response changes to a bradycardia at temperatures near this species' CT_{MAX} (~ 31°C) (Belaud et al. 1976; Claësson et al. 2016). The difference in response to temperature between our study and these studies may be related to the species used (eels vs. lumpfish). However, it is also probable that differences in experimental methodologies contributed to the reported differences. Most importantly, I exposed the lumpfish to elevated pressure (80 bar) for 1 hour prior to any changes in temperature, whereas the eels

were exposed to changes in temperature either before, or in concert with, changes in hydrostatic pressure.

In this study, the Q_{10} for f_H with increasing temperature (from 12 to ~ 20°C) in fish held in the IPOCAMP was only ~ 1.38 (for both pressure-exposed and control fish), whereas it was 1.67 when fish were given a CT_{MAX} test in shallow containers in a water table (i.e., compare the f_H data in Figures 3.9 vs. 3.12). The reason for this is unknown as resting f_H was only slightly higher in control fish in the IPOCAMP as compared to those used in the CT_{MAX} experiment (i.e., 54.7 vs. 50.4 bpm), and oxygen levels were maintained close to air saturation in both experimental set-ups. However, it is possible / probable that this diminished temperature sensitivity of fish in the IPOCAMP influenced the effect of increased temperature on f_H. For example, if Q_{10} had been 1.67 in the IPOCAMP experiment, maximum f_H in pressure-exposed fish would have been reached at approx. ~ 17-18°C. Clearly, more research needs to be performed to understand how these two important parameters interact with respect to f_H in fishes, and future experiments investigating the physiological responses of fish to pressure should consider providing longer acclimation periods to the pressure (IPOCAMP) chamber.

In the lumpfish, decreasing water PO_2 at atmospheric pressure from ~ 100% saturation to ~ 55% saturation resulted in a slight increase in f_H (by ~ 5 bpm). This minor increase in f_H was somewhat surprising as f_H generally does not change as water PO_2 is lowered to the point of bradycardia. However, such a response has been seen in several other fish species including the Atlantic cod (*Gadus morhua*) (Gamperl and Driedzic 2009; Petersen and Gamperl 2011). Exposure to 80 bar of pressure eliminated the small increase in f_H that was observed in the control fish (Figure 3.10). This is an interesting

observation, and while the mechanism(s) mediating this difference are unknown, these data suggest that fish experiencing increased hydrostatic pressure and moderate hypoxia may have a very limited scope for increases in f_H. Bradycardia is typically recorded at oxygen levels similar or slightly higher than a species' critical oxygen tension, P_{crit} (e.g. see Marvin and Heath 1968; Gehrke et al. 1988; Speers-Roesch et al. 2010). Therefore, it is very likely that bradycardia was not recorded in this study because the lumpfish did not reach their P_{crit} (~ 40% air saturation at 12°C; Ern et al. 2016) due to ethical concerns when using the IPOCAMP during these initial experiments. First, it is difficult to determine loss of equilibrium in lumpfish because of their ability to attach to surfaces with their sucker, and it was not known whether pressure acclimation would increase the P_{crit} of lumpfish. In addition, fish cannot be removed from the chamber quickly because the vessel must be decompressed before opening. Future experiments are being planned to examine if hydrostatic pressure affects the oxygen level at which bradycardia is initiated and the magnitude of the decrease in f_H. Ultimately, the most relevant experimental scenario would be one which accurately reflects the environmental and behavioural challenges of a vertical migration: i.e., simultaneous increases in pressure, and decreases in temperature and water oxygen levels while the fish is actively swimming.

Maximum Exercised and Temperature-Induced Heart Rate of Lumpfish

Given the low maximum f_H recorded in the IPOCAMP at 20°C (63 bpm) and following exercise at 10°C (72 - 77 bpm), a CT_{MAX} experiment was performed under more typical experimental conditions in a water table. During the CT_{MAX} experiment, the

f_H of lumpfish increased up to approx. 20°C (Q_{10} = 1.67) and began falling as temperatures approached the lumpfish's CT_{MAX} of 22°C (Figure 3.12; Table 3.5; Ern et al. 2016). This response is typical of that seen in other fish species, where f_H increases (at Q_{10} values ranging from 1.5 to 2.5) up until approx. 2°C before the fish's CT_{MAX}, and the decrease in f_H is thought to be associated with a loss of ventricular excitability due to an imbalance in trans-sarcolemmal sodium and potassium currents (Gollock et al. 2006, Steinhausen et al. 2008; Clark et al. 2008, Vornanen et al. 2014; Motyka et al. 2017; Gilbert et al. 2019). The highest individual f_H recorded in lumpfish was 95 bpm while the highest average f_H at 20°C was 81 bpm. Thus, it appears that lumpfish have low maximum f_H relative to fish species such as the channel catfish *Ictalurus punctatus* (150 bpm; Burleson and Silva 2011) and salmonids which have a maximum f_H between 105 and 132 bpm (Clark et al. 2008; Steinhausen et al. 2008; Ekström et al. 2014; Vornanen et al. 2014; Motyka et al. 2017), and more typical of those recorded in species such as the Atlantic cod (72 bpm; Gollock et al. 2006), winter flounder, *Pseudopleuronectes americanus* (73 bpm; Mendonça and Gamperl 2010) and European perch, *Perca fluviatilis* (83 bpm; Jensen et al. 2017).

These results, combined with previous data, suggest that lumpfish are well-adapted to a passive, yet still pelagic, lifestyle. Hvas et al. (2018) reported that lumpfish have a low critical swimming speed and aerobic scope due to a limited maximum $\dot{M}O_2$. The results of this study agree with the findings of Hvas et al. (2018), as lumpfish were found to have a low scope for f_H and a low maximum f_H. Additionally, research shows that lumpfish have relatively low values of exercise-induced cortisol, glucose and lactate, which indicates that lumpfish have a limited capacity to perform exhaustive exercise

(Clow et al. 2017; Jørgensen et al. 2017; Hvas et al. 2018). These physiological features are in contrast to most pelagic fish which are built for strong swimming and aerobic performance, however, not surprising given the lumpfish's globiform shape, weak tail musculature and uniquely docile nature (Hvas et al. 2018).

Considerations When Using Data Loggers

Temperature had a strong effect on the quality of ECGs recorded by the micro-HRT tag, with the percentage of good quality (QI = 0) ECGs as low as 12% at 19.4°C inside the IPOCAMP (Figure 3.9; Table 3.6) and 2% at 22°C outside the IPOCAMP (Figure 3.12; Table 3.6). While most f_H values could still be calculated by manually examining the ECG recordings, this is a concern for research being conducted at high temperatures or close to the CT_{MAX} of the species being studied. It has been suggested that low quality ECGs are related to increased activity at higher temperatures because the potentials from aerobic muscles overlap with the ECG (Altimiras and Larsen 2000). Interestingly, the percentage quality of ECGs also transiently decreased during compression, which was also associated with an increase in activity (Figures 3.6 and 3.7; Table 3.2). However, I do not believe that this was the main factor impacting the 'quality' of the ECGs recorded in lumpfish using Star-Oddi data loggers. For example, the percentage of QI = 0 recordings in Atlantic salmon during the U_{crit} protocol never fell below 50%. Instead, I believe that it was the low amplitude of the signal received by the HRT-tag that was the primary issue. In the salmon, the QRS (R wave) amplitude was approx. 510 mV, but only approx. 170 mV in the lumpfish (see Figures 2.4 vs. 3.11). This low signal amplitude was not due to the size of the heart as the relative ventricular mass

of lumpfish reared at 9°C is 0.94 (Hvas et al. 2018), and within the range of that reported for Atlantic salmon (Deitch et al. 2008; Antilla et al. 2014, 2015). Further, it is not that the lumpfish has a particularly large liver (e.g., the hepatosomatic index is only 2.5%; Hvas et al. 2018). However, the heart is relatively deep within the body cavity in lumpfish, and the liver's position is such that it lies directly between the location of tag implantation and the heart. This may diminish the strength of the signal received by the data logger. It is possible that modification may be able to be made to the tag's design, or to the software / algorithms to used calculate f_H, to enhance the tag's usefulness for this species.

Conclusions

The effects of hydrostatic pressure on the cardiovascular system of fish are poorly understood, and this is often attributed to the difficulty of obtaining physiological data while fish are at pressure (Guerrero et al. 2000; Shillito et al. 2014). With the miniaturization and growing popularity of biologgers for use in fish (Ropert-Coudert et al. 2012; Wilson et al. 2015) we are learning about the vertical movement patterns of marine species, but this also leads to further questions such as: how physiological perturbations associated with pressure influence their capacity to deal with other environmental challenges; or how simultaneous changes in conditions such as temperature and oxygen levels affect the hearts response to pressure. Star-Oddi micro-HRT tags and the IPOCAMP chamber were successfully utilized in this research to show that acute exposure to hydrostatic pressure produced a tachycardia in the lumpfish (f_H increasing by 29%), but that this had no effect on the slope of the temperature - f_H

relationship when this pressure-induced increase was taken into account (i.e., by expressing the f_H data as a percentage of initial values). In contrast, the minor increase in the f_H of control fish to decreasing water PO_2 was eliminated by exposure to hydrostatic pressure. Lastly, lumpfish were found to have a low maximum f_H in response to exhaustive exercise or temperature increase to their CT_{MAX} (77 and 81 bpm, respectively), relative to other fish. My research suggests that pressure influences the f_H response to environmental challenges, and provides the first evidence that lumpfish have a limited capacity to increase f_H. I hope that the findings of this study, and that biologgers were successfully used to address such research questions in my thesis, will inspire a resurgence of research into the physiological responses of fish to vertical migrations.

Chapter 4: Summary and Perspectives

In this thesis, I set out to evaluate the suitability of commercially available data storage tags (DSTs) produced by Star-Oddi for studying the physiology of fishes. This ultimate goal was addressed in two data chapters, each focusing on a separate fish species and using different DST products. First, I investigated the effectiveness of using the recently developed centi-HRT ACT tag to record heart rate (f_H), electrocardiograms (ECGs), tri-axial acceleration and temperature in Atlantic salmon (*Salmo salar*). Secondly, I used micro-HRT tags to study the physiological response of lumpfish (*Cyclopterus lumpus*) to changes in hydrostatic pressure and other abiotic challenges (i.e., temperature and hypoxia). Here, I will briefly discuss the findings of each data chapter and their overlapping themes, as well as the implications of these results for the field of biologging and fish physiology.

In Chapter 2, I determined that the centi-HRT ACT tags were reliable and effective tools for recording f_H and activity in salmon. While a few previous studies have reported f_H measurements recorded by Star-Oddi's milli-HRT tag (Prystay et al. 2017, 2019; Ekström et al. 2018; Brijs et al. 2018, 2019; Wallerius et al. 2019), this was a novel objective because no research has been published using, or evaluating, the data recorded by the centi-HRT ACT tag in fish. In the first experiment, salmon implanted with these tags were swum in a swim tunnel at increasing speeds. The results showed that the acceleration parameters calculated by the tag software (EA and VAR) increased with both swimming speed and tail beat frequency (Figure 2.5). EA was found to be a reliable predictor of swimming speed, while VAR has the potential to provide information about the behaviour of free-swimming salmon. In two separate experiments, salmon were

surgically implanted with centi-HRT ACT tags and recovered in large tanks (Figure 2.8, 2.9). The tag reliably recorded diel changes in f_H and activity, as well as temperature-induced changes in f_H (Figures 2.10 and 2.11). Surprisingly, while f_H stabilized by 4 days post-surgery, it continued to decline up to 21 days following implantation, indicating that the presence of the tags and / or the effects of the surgery were having longer lasting effects than previously believed. Based on the above data, overall, I highly recommend that researchers seeking to understand the welfare and conservation of free-living fishes take advantage of the capabilities of the centi-HRT ACT tag.

In Chapter 3, I found that increasing hydrostatic pressure resulted in tachycardia, and altered the f_H response to decreasing oxygen levels in lumpfish. Research concerning the effects of hydrostatic pressure on fish physiology is extremely limited and has focused primarily on the European eel (*Anguilla anguilla*; e.g., Sébert and Barthélémy 1985a; Simon et al. 1989; Sébert and Macdonald 1993; Sébert 2002; Vettier et al. 2005, 2006). To the best of my knowledge, no previous studies have investigated whether pressure alters the f_H response to decreasing oxygen levels. In this chapter, I first established that the f_H of lumpfish increased during a step-wise exposure to 80 bar of pressure (Figure 3.6. A, B). In three follow-up experiments, I then found that: f_H remained elevated when pressure was maintained at 80 bar for one hour and hydrostatic pressure suppressed the increase in f_H of lumpfish in response to decreasing oxygen levels; but the hydrostatic pressure did not effect the sensitivity of the f_H response to increasing or decreasing temperature (Figures 3.8, 3.9 and 3.10). Further, lumpfish were found to have a relatively low maximum f_H in response to temperature increases inside the pressure chamber, following exhaustive exercise and during a CT_{MAX} test under normobaric conditions

(Figures 3.6, 3.9 B and 3.12 A). In summary, the micro-HRT tags were a convenient and effective tool which allowed me to study the f_H response of lumpfish to hydrostatic pressure and other concomitant environmental challenges.

When considering the findings from each data chapter, I have identified some overlapping challenges and insights to consider when using biologgers. First, it is important to understand the limitations of Star-Oddi's f_H loggers, including the possibility that potentials produced by muscles during increased activity can interfere with ECG recordings as first reported by Altimiras and Larsen (2000). In the U_{crit} test on tagged salmon, the percentage of 'good' quality ECGs (i.e., QI = 0) did not change significantly with increasing swimming speed (Figure 2.6 C). However, there was a trend for this parameter to decrease with swimming speed, and the percentage of QI = 0 values recorded in lumpfish decreased by 33% when pressure was increased to 50 bar and this was correlated with increased activity of the lumpfish during compression (Figures 3.6 D and 3.7). Further, I found that the Mercury software could miscalculate the f_H of salmon from ECGs recorded with a QI > 0 by up to 39 bpm (Figure 2.7). Based on these studies, I recommend that researchers using f_H loggers prioritize saving ECGs, and consider manually calculating f_H from both good and poor quality recordings in order to prevent the loss and / or misinterpretation of data.

It is also worth discussing the clear difference in the quality of ECGs recorded in salmon as compared to lumpfish. When the fish were recovering with conspecifics in their holdings tanks for one week, the average voltage amplitude of the R peaks and the average percentage of 'good' quality ECGs were much higher in salmon at 11°C than in lumpfish at 10°C (Figures 2.4 and 3.11; ~ 516 vs. ~ 171 mV; 89 vs. 73%, respectively).

The centi-HRT ACT tag (50 m length, 15 mm diam., 19 g in air) was used to record f_H in salmon, while the micro-HRT tag (25.4 mm length, 8.3 mm diam., 3.3 g in air) was used for lumpfish because smaller fish were required for use in the IPOCAMP. It is possible that the smaller surface area of the micro-HRT electrodes contributed to the lower voltage amplitude recorded, however it is more likely that the differences in amplitude and quality were related to the species-specific morphology of lumpfish. I observed in post-mortem dissections that the liver of the lumpfish prevented the tag from sitting closer to the pericardium (heart), which lies deeper within the body cavity of the lumpfish. It is very likely that researchers will have to alter their surgical procedures for species with anatomy that differs from salmonids, and that modification will be necessary to the tag's sensitivity or algorithms in order to accurately measure / calculate f_H. Nonetheless, it is highly recommended that species-specific preliminary testing be performed prior to experiments which release tagged fish into wild or aquaculture environments (Wilmers et al. 2015; Wilson et al. 2015).

In addition to the potential noise caused by activity, temperature had a very strong impact on the quality of ECG recordings. For example, the percentage of 'good' quality ECGs recorded in lumpfish was as low as 12% at 20°C in the IPOCAMP and 2% at 22°C under normobaric conditions in a water table (Figures 3.9 F and 3.12 C). In contrast, data recorded in salmon had quality issues when the fish's f_Hs were slow (i.e. below 30 bpm). For example, 75% of data for one salmon held for 6 weeks of recovery at 8°C was lost during their final week of recovery. All of this lost data was designated as QI = 3 by the Mercury software, indicating that a full R-R interval could not be recorded. This issue was previously mentioned by Brijs et al. (2019), who found that R-R intervals were

missed when the milli-HRT tags were set to record f_H for 6 seconds in rainbow trout (*Oncorhynchus mykiss*). The issues with recording good quality ECGs during activity or at high / low temperatures should be largely alleviated by the recent update to the tag settings by Star-Oddi. In the tag programming, there is now a "long ECG" option which allows the user to record f_H for up to 15 seconds. During short bursts of activity and the high f_H caused by elevated temperatures, a longer recording time could potentially mitigate the amount of data lost by increasing the number of R-R intervals used to calculate f_H. Similarly, and most notably, the longer ECGs will improve the accuracy of f_H measurements (compared to a 6 sec. recording period) and allow Star-Oddi users to record f_H in fish with lower resting f_H values, such as during overwintering (low temperature) periods and during physiological tests such as critical thermal minimum (CT_{MIN}) experiments.

However, there are still aspects of tag use that need to be addressed / considered. First, the accuracy of f_H recordings was not actually 'validated' in this study. Although many researchers have recorded values of f_H using Star-Oddi loggers in fish (e.g., Brijs et al. 2018, 2019; Wallerius et al. 2019) and other animals (e.g., elephant seals, *Mirounga leonina*; Chaise et al. 2017), it would be valuable to confirm the accuracy of the tags with regards to recording time, and thus f_H, by simultaneously fitting some fish with Doppler or Transonic® flow probes. Additionally, researchers need to be aware of situations where f_H is not a reliable proxy for $\dot{M}O_2$ / cardiac output (Brijs et al. 2019). The relationship between $\dot{M}O_2$ and f_H is influenced by physiological (i.e., feeding, stress), behavioural (i.e., activity, recovery) and environmental conditions (i.e., hypoxia, temperature) (Thorarensen et al. 1996; Cooke et al. 2016; Treberg et al. 2016), and a primary concern

is that many fish species can modulate their cardiac output through changes in stroke volume alone, or in addition to f_H (Cooke et al. 2016). For example, during hypoxia the f_H of rainbow trout changes without any effect on $\dot{M}O_2$ (Holeton and Randall 1967), and both f_H and stroke volume increase when fish are exercised / their swimming speed increases (Farrell and Smith 2017). Thus, researchers looking to use f_H to estimate energy use ($\dot{M}O_2$) or cardiac output should be careful when using f_H to calculate these parameters in situations where stroke volume is also modulated (Thorarensen et al. 1996; Treberg et al. 2016).

Despite the challenges encountered, the Star-Oddi tags have many valuable characteristics. For example, in both species I found the tags were easy to use and large amounts of data could be obtained (e.g., 962 data points per salmon during a one week recovery period). Due to their simplistic use, biologgers will improve our ability to collect data which was previously difficult to obtain. For example, there is a lack of data on the effects of hydrostatic pressure on the cardiovascular system of fish, which is often attributed to the technical difficulty of recording f_H at pressure (Guerrero et al. 2000; Shillito et al. 2014). By combining f_H loggers with the IPOCAMP pressure chamber, I was able to investigate many aspects of the f_H response during compression over a short experimental timeline (i.e., five experiments were completed in ~ 4 months). Furthermore, exciting new discoveries were made in both chapters of this thesis. Most notably, I found that the f_H of salmon continued to decline for 21 days following the surgical implantation of the centi-HRT ACT tag (Figure 2.11 A, B). This was a novel finding which has large implications for the welfare of fish being used in biologger research and the validity of "resting" f_H values previously recorded in fish which only

received 48 to 72 hours of recovery (e.g., see Steinhausen et al. 2008; Gräns et al. 2009; Ekström et al. 2016; Prystay et al. 2017; Cheng et al. 2017; Joyce et al. 2018). Additionally, I found that lumpfish have a low maximum f_H, which was not previously known, and which compliments previous research indicating that lumpfish have low aerobic capacities despite their semi-pelagic lifestyle (Figures 3.9 B and 3.12 A; Clow et al. 2017; Jørgensen et al. 2017; Hvas et al. 2018).

The reduced cost, miniaturization and growing popularity of biologgers will allow us to answer research questions that were difficult to address in the past (Cooke et al. 2004a; Ropert-Coudert and Wilson 2005; Chmura et al. 2018). For example, there is a growing desire to better understand heart rate variability (HRV) and it's use as an indicator for animal stress and welfare (Gräns et al. 2014; Gaidica and Dantzer 2019). In both salmon and lumpfish, saving ECGs allowed for the measurement of the R-R interval and calculation of the standard deviation of those intervals, also known as HRV. It will likely become more common for software that analyzes ECGs to provide these calculations, making it easier to measure and study HRV. I was able to determine that HRV decreased with increasing swimming speed in salmon (Figure 2.6 B) and decreased transiently during compression (i.e. exposure to high hydrostatic pressure) in lumpfish (Figure 3.6 C). A decrease in beat-to-beat variability suggests an increase in neurohormonal control of f_H, and therefore biologgers recording ECGs will be an important tool for understanding the control of f_H in fish under varied conditions (Campbell et al. 2004; Gräns et al. 2014; Gaidica and Dantzer 2019). In conclusion, biologgers will be an extremely beneficial tool and allow for new avenues of research, especially as we strive to understand how animals will respond to changes in their

environment such as those related to global climate change (Cooke et al. 2004a; Ropert-Coudert and Wilson 2005; Cooke 2008; Wilson et al. 2015; Chmura et al. 2018).

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