UTILIZING BIOLOGGERS TO INFER BEHAVIOUR AND PHYSIOLOGY OF COMMERCIAL ATLANTIC SALMON (SALMO SALAR) IN A CHANGING CLIMATE

by

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Dalhousie University is located in Mi'kma'ki,
the ancestral and unceded territory of the Mi'kmaq.
We are all Treaty people.

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ABSTRACT

There is a growing global market for Atlantic salmon (*Salmo salar*). Right-holders and stakeholders, including consumers, producers, and regulating bodies, have a vested interest in the future of commercial operations considering the ongoing and future impacts of ocean warming as it relates to fish stress and welfare. The challenges faced by Atlantic salmon farms due to climate change, characterized by rising sea temperatures and increased weather variability, emphasize the need for a comprehensive understanding of thermal stress and confounding variables specific to commercial farming to inform farm management practices. This study explores the physiological and behavioral responses of Atlantic salmon under the influence of seasonal thermal extremes during a standard production cycle. Utilizing two types of surgically implanted biologgers, measuring external acceleration, depth, heart rate, and temperature, observations were made over 245 days, providing a time series of direct observations of fish near harvest size. Apparent heart rate scope and Arrhenius breakpoint temperature were used to estimate optimal temperature and both methods resulted in an estimate of 12.7 °C. A reduction in heart rate scope was observed at temperatures less than 2 °C and greater than 19 °C, indicating proximity to limits of thermal stress. In addition, two extreme thermal events of different duration, short- and long-term, were investigated alongside farm operations and feeding regimes to explore the interaction of farming practices with potential thermal stress. Fish showed distinct responses to each event and displayed signs of both secondary (i.e. heart rate) and tertiary stress (i.e. activity). Heart rate and acceleration increased in response to feeding while the effect of operations was more nuanced. This research enhances the understanding of factors influencing Atlantic salmon on aquaculture farms, bridging the gap between controlled laboratory studies and real-world commercial operations. It focuses on the specific challenges faced by farmed fish, arising from the interaction between extreme temperatures and farm operations. This thesis contributes to the advancement of technology in precision fish farming applications, using biologgers to promote increased monitoring and observation of fish, enabling timely farm management decisions that can enhance fish welfare and overall production efficiency in commercial aquaculture.

LIST OF ABBREVIATIONS USED

ADST	Acoustic Data Storage Tags
DST	Data Storage Tags
ECG	Electrocardiogram
FFT	Fast Fourier Transform
K	Condition Factor
OWI	Operational Welfare Indicator
RMS	Root Mean Square
SOP	Standard Operating Procedure
T_{AB}	Arrhenius breakpoint temperature
T_{crit}	Critical temperature where aerobic scope is zero
T_{opt}	Optimal temperature where aerobic scope is at a maximum
UCLA	University Committee on Laboratory Animals

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CHAPTER 1: GENERAL INTRODUCTION

Fisheries and aquaculture production reached an all-time high of 179 million tonnes in 2018 after a substantial annual growth rate of 3.3% (FAO, 2022). However, since 2018, there has been a marginal decline in total production primarily driven by a decline in capture fisheries. Aquaculture production has been the driving force behind the trend in growth of global seafood production and as of 2020, aquaculture represents 49% of total production, a major increase compared to the 4% share it had in the 1950s (FAO, 2022). Total wild fisheries production fluctuates year over year but has generally maintained stable production since the late 1980s (FAO, 2022). This fact, combined with an exponentially growing population has allowed aquaculture to emerge as a crucial strategy to meet the increasing demand for seafood.

Atlantic salmon (*Salmo salar*) represents approximately one third of marine finfish aquaculture and its production is dominated by a few countries including Norway, Chile, Scotland, and Canada (FAO, 2022). In Canada, Atlantic salmon is a commercially important species, and currently represents approximately 62% of the total Canadian aquaculture production volume and 74% of its total value (Statistics Canada, 2022). Despite its importance, the aquaculture industry, and specifically Atlantic salmon production face challenges to overcome, one of which is climate change (Reid et al., 2019). Atlantic Canada is warming faster than the average rate of climate change, and this has had and will continue to have significant impact on aquaculture operations in these regions (Saba et al., 2016). Climate change is causing shifts in oceanographic conditions such as temperature, salinity and oxygen concentrations and the areas that support suitable salmon farming are projected to decline, including in Atlantic Canada (Froehlich et al., 2018). Shifting environmental conditions may eventually lead to different regions being considered for operations, but in the short-term, it presents challenges to existing infrastructure and leases that cannot be easily moved due to political, logistical, and economic barriers (Klinger et al. 2017).

As ectotherms, salmon do not have the ability to regulate their own internal temperature and so their environment plays a crucial role controlling their internal processes. Temperature is a critical factor that regulates both physiology (Farrell et al., 2009), and behaviour (Johansson, 2006). It controls metabolic rates and the energy available for everyday functions such as swimming activity, feeding and other processes that maintain functional integrity of the fish (Pörtner & Farrell, 2008). Accordingly, it also impacts the available energy for many life cycle processes such as growth and reproduction. Energy consumption is directly proportional to oxygen consumption (Nelson, 2016), and aerobic scope is the difference between the average and maximum consumption rates over a range of temperatures. The temperature at which the difference is maximised is used to estimate species optimal temperature (T_{opt}). At sub-optimal temperatures, there are trade-offs in energy allocation to cardiorespiratory function, swimming activity, feeding and growth (DFO, 2012). Unlike their wild counterparts that can avoid deleterious conditions, farmed fish are subject to the environment at the farm and extreme events like heatwaves can cause sudden, large mortality events, posing substantial economic and ecological challenges. In 2019, a mass mortality event of over 2.6 million farmed salmon occurred in Newfoundland when a warm water mass moved inshore resulting in prolonged warm water temperatures, which co-occurred with a sea lice infestation (Burke et al., 2020). Ocean warming threatens environmental stability and results in many other downstream effects, including changing plankton ecosystems, disease and pathogen presence and proliferation (Klinger et al., 2017). Increased ocean temperatures also have direct effects on fish, impacting their behaviour, physiology, and health in commercial operations (Calado et al., 2021).

Understanding the behavioural and physiological responses of aquaculture species to ocean warming is essential for effective management practices. Behavioural responses, such as shifts in feeding, depth patterns, and swimming behaviour, can indicate how species cope with challenging conditions (Hvas et al., 2017; Oppedal et al., 2011; Stockwell et al., 2021). Similarly, physiological responses, for example heart rate, to changing temperature regimes, provide insights into how these organisms are impacted at a metabolic level (Farrell, 2009). Fish health teams are responsible for developing fish health plans which include maintaining high water quality, managing disease, controlling parasites, and they are required to observe and report on fish stress and welfare (BAP, 2016). When a fish experiences or perceives a potential threat, the animal's homeostasis is disturbed and this elicits a response to overcome the perceived threat, this is known as the biological stress response (Wendelaar Bonga, 1997). The primary stress response is triggered by the release of hormones including cortisol and catecholamines by the adrenal glands into the bloodstream (Barton, 2002). Hormone release leads to many secondary responses like increased cardiorespiratory activity, increase in heat-shock protein synthesis, and increases in blood glucose, lactic acid, and major ion production within the body (Barton, 2022). Those responses then have impacts on the whole animal, including effects such as reduced growth rates, appetite levels, changes in swimming behaviour and in severe cases, mortality rates (Ashley, 2007). These are part of the tertiary stress response, and they are commonly used as indicators of fish welfare because can be observed and measured (Brijs et al., 2018). However, tertiary responses are often delayed and do not allow managers to identify the initial cause of stress, preventing mitigation actions from being implemented prior to their occurrence.

Advancements in technology have led to the development of bio-loggers that can measure physiological and behavioural responses with as little disturbance as possible. They can be surgically implanted to monitor variables such as heart rate, temperature, acceleration, and depth in free swimming fish, without human interaction or a laboratory setting (Axelsson et al., 2007, Svendsen et al., 2021, Zrini & Gamperl, 2021). Laboratory studies are a valuable way to improve the understanding of how fish respond in certain environments because there is an inherent ability to maintain a high degree of control over the conditions and any stressors the fish may perceive. Commercially operating open ocean farms inherently lack control over the environment and can be subject to extreme weather events, naturally occurring disease or parasites and other environmental challenges such as harmful algae blooms. Under these conditions, bio-loggers provide a unique ability to relate laboratory results to realistic farm conditions. There are current gaps in the literature when it comes to measuring behavioural and physiological responses in conjunction with environmental data to observe how the environment interacts with farm operations in a commercial aquaculture setting. Accordingly, the objective of this research is to measure heart rate, temperature, acceleration, and swimming depth as well as environmental data such as dissolved oxygen and temperature to study the physiological and behavioural response of salmon to natural environmental conditions and farm operations common to commercial aquaculture settings.

In this thesis, the following chapters make use of the same methods and data that was collected via biologgers and environmental monitoring equipment in conjunction with a log of farm operations. Chapter 2 examines fish physiology and behaviour over a 7-month period, capturing two opposing and extreme thermal regimes when environmental stress is known to occur. Fish response was captured using surgically implanted bio-loggers measuring heart rate, acceleration, temperature, and depth. The role of temperature was explored via heart rate (f_H) scope and Arrhenius plots to observe how it affects fish physiology during regular aquaculture operations. Chapter 3 uses the same data as Chapter 2 but focuses specifically on the summer period and examines the effect of two heat events, one short term (2.5 days) and one long term (34 days), on fish behaviour and physiology. Operational decisions are made to reduce fish stress as much as possible and so the effect of feeding events and operations (including net cleaning, mortality dives, predator net installations and other site maintenance) were examined comparatively under normal conditions and during heat events. This research is an important link between laboratory studies and the reality fish experience on commercial farms. The overarching goal is to better understand how fish respond to adverse conditions to help farmers respond appropriately and help inform the industry on the challenges they face in a changing climate.

CHAPTER 2: INFLUENCE OF TEMPERATURE ON THE BEHAVIOUR AND PHYSIOLOGY OF ATLANTIC SALMON (SALMO SALAR) ON A COMMERCIAL FARM[1]

2.1 Abstract

Commercial Atlantic salmon (*Salmo salar*) farms face challenges under climate change, as rising sea temperatures and higher variability in weather patterns can lead to thermal stress events, compromising fish health, and resulting in lower production efficiency. Temperature plays a critical role in influencing the physiological and behavioural response of salmon under stressful conditions and effective farm management requires a nuanced comprehension of its function to adjust farming practices and minimize further stress. Two types of biologgers, measuring external acceleration, depth, heart rate, and temperature were surgically implanted for 245 days to explore the effects of the thermal response of Atlantic salmon during a standard production cycle. Apparent heart rate scope and Arrhenius breakpoint temperature were used to estimate the optimal temperature and both methods resulted in an estimate of 12.7 °C. There was a reduction in apparent heart rate scope at temperatures below 2 °C and greater than 19 °C suggesting proximity to limits of thermal stress. The use of biologgers facilitates direct observations in commercial operations, providing essential information for aquaculture management. These findings contribute to a holistic understanding of the effect of temperature influencing Atlantic salmon physiology and behaviour on aquaculture farms, bridging the gap between controlled laboratory studies and real-world commercial operations.

2.2 Introduction

As ectotherms, temperature plays a critical role in regulating different aspects of salmon physiology and behaviour. It controls all regulatory processes such as metabolic rates, oxygen

[1] A version of this chapter has been submitted for publication and is currently in review.

consumption, and heart rate, as well as activity levels, appetite, and growth (Anttila et al., 2014; Farrell 2009; Steinhausen et al., 2008; Volkoff & Rønnestad, 2020). Atlantic salmon (*Salmo salar)* are eurythermal and can tolerate wide temperature ranges (Corey et al., 2017; Gerber et al., 2020; Hvas et al., 2017). However, temperature extremes and prolonged periods of high temperature can have a varying degree of negative effects on fish health, depending on fish condition, the intensity and duration of temperature extremes and the rate of change leading up to extreme temperatures (Calado et al., 2021). Understanding the role of temperature on fish physiology and behaviour is necessary for good aquaculture management, and critical to the future of the industry under a changing climate (D'Abramo and Slater, 2019; Hargrave et al., 2005; Reid et al., 2019; Thyholdt, 2014)

Standard metabolic rate (SMR) is a measure of the required energy to sustain base level body functions and can be measured when an organism is at rest (Brett, 1982; Fry, 1971). Maximum metabolic rate (MMR) refers to the capacity of fish to perform activity above base levels and the difference between the two measures is aerobic scope (Fry, 1947). Within the range of thermal tolerance, metabolic rates fluctuate in response to changes in temperature, displaying an optimal temperature threshold (T_{opt}) above which SMR increases faster than MMR, reducing aerobic scope. T_{opt} is the temperature at which aerobic scope is maximized and it has been reported to be between 13-18 °C for Atlantic salmon (Dwyer & Piper, 1987; Handeland et al., 2008; Hevrøy et al., 2012; Koskela et al., 1997). The temperature at which aerobic scope is reduced to zero, is known as the critical temperature (T_{crit}) (Pörtner, 2001). When aerobic scope is compromised, trade-offs in energy allocation between activity, digestive processes, growth, and reproduction emerge and at temperatures beyond T_{crit}, anaerobic metabolism begins, which is very costly from an energetic standpoint and not sustainable over long periods of time. (DFO, 2012).

The relationship between temperature and biochemical rates (i.e., metabolic rate) is often described by the Arrhenius equation and it has been widely applied in aquatic ecology to investigate the role of temperature on ectotherms (Regier et al., 1990). If a rate follows Arrhenius principles, there is a linear relationship between the logged rate and the reciprocal of temperature, indicating an exponential increase of the rate with temperature. However, biological rates cannot increase exponentially indefinitely due to physical limitations (Anttila et al., 2014b; Crozier, 1926; Kalinin et al., 2009). The temperature at which there is a break in the slope of an Arrhenius plot is known as the Arrhenius break temperature (T_{AB}) and it has been demonstrated using simultaneous measurements of aerobic scope and heart rate scope, that this temperature can be used as a proxy for determining T_{opt} with maximum heart rate (Casselman et al., 2012). The ability of the cardiovascular system to deliver oxygenated blood efficiently at high temperatures, is one of the limiting factors determining the upper thermal tolerance in fish (Anttila et al., 2014a; Farrell et al., 2009). The ability of fish to meet metabolic demands at high temperatures depends on the relative contribution of both stroke volume and f_H on cardiac output and it has been established that cardiac control favours increase in f_H to meet elevated metabolic demands (Gamperl et al., 2011; Steinhausen et al., 2008). In effect, heart rate is a key variable used to examine the role of temperature in fish physiology, and it is ideal for field studies because it is more easily measured in free swimming fish using biologgers without the need for swim tunnels, laboratory equipment and interference with fish during observations (Lucas et al., 1993; Morgenroth et al., 2024).

Temperature is a critical physiological driver but also relevant for behavioural responses, especially in farmed salmon that are limited in the space they have access to. It has been demonstrated that when there is thermal stratification in the water column, salmon display signs of active behavioural thermoregulation, seeking optimal temperatures along the vertical gradient (Johansson et al., 2009), but avoiding temperatures greater than 18 °C (Johansson et al., 2006). Other than a general avoidance of low temperatures (Oppedal et al., 2001), much less is known about the behavioural response of salmon to cold temperatures as most research is focused on the impacts of increasing temperature due to ocean warming (Szekeres et al., 2016). The physiological effects of high and low temperatures on Atlantic salmon include decreased growth performance, stress, reduced appetite, and mortality (Gamperl et al., 2020; Hvas et al., 2017; Vadboncoeur et al., 2023). In farming locations such as Eastern Canada and Iceland, a cold temperature event known as super-chill can occur when seawater temperature reaches -0.7 °C, the temperature at which fish blood freezes causing mass mortalities (Undercurrent News, 2020; The Fish Site, 2022); however, due to its irregular occurrence, there is limited research published on this phenomenon (Hargrave et al., 2005). Climate change is not only going to cause global sea surface temperatures to increase but it will affect the frequency of winter storms and upwelling events that bring cold, deep waters to the surface which can lead to cold shock, acute temperature changes, and super-chill events (Szekeres et al., 2016). It is critical for good farm management to understand the role of both temperature extremes on their livestock for both real time operational decision making and long-term production planning.

Biologgers are a novel technology development that provide unprecedented insights into fish behaviour and physiology in free-swimming fish (Macaulay et al., 2021). They are a critical link between results found in laboratory studies and the validation of these findings under field conditions (Stehfast et al., 2017). Studies on commercially operating farms are limited due to the difficulty of controlling the environment and the resulting challenge to design and carry out traditional experiments, but they provide direct observations and data that do not require further inference or extrapolation (Svendsen et al., 2021). In addition, some biologgers can transmit wirelessly in real time, enabling their potential use as welfare indicator tools on farms (Føre et al., 2017; Hvas et al., 2020). Biologgers have been used to examine stress response and fish welfare (Brijs et al., 2018; Hvas et al., 2020; Yousaf et al., 2022), and the behavioural response of salmon to their environment (Johansson et al., 2006; Johansson et al., 2009; Oppedal et al., 2001; Stockwell et al., 2021). However, much of this research has been carried out in laboratory facilities using tanks or in non-commercial net pens designed specifically for research, which limits the ability to assess interactions between the environment and the operational needs of a farm and their compounding effects on farmed fish.

The objectives of this work are to establish baseline observations of fish physiology and behaviour of Atlantic salmon during two high-risk periods of thermal stress in summer and winter under commercial operations. Atlantic salmon grown on commercial farms in Nova Scotia experience a large fluctuation of seasonal temperature and farmers must account for these environmental stressors when planning maintenance, feeding regimes and diver operations. Biologgers were used alongside an environmental monitoring system including temperature and oxygen sensors, and a log of all farm operations was kept throughout the study period to elucidate the interplay between temperature and farm management practices on the physiological and behavioural responses of Atlantic salmon. This research contributes valuable knowledge that helps bridge the gap between laboratory and in situ studies on thermal biology of salmonids and it promotes the optimization of commercial salmon farming practices, offering a holistic understanding of the factors influencing Atlantic salmon physiology and behaviour in commercial scale aquaculture.

2.3 Methods

2.3.1 Study Site

The study site is located at a commercial Atlantic Salmon farm in Liverpool Bay, owned and operated by Cooke Aquaculture, along the southern shore of Nova Scotia in Eastern Canada (Figure 2.1). The farm is located at the mouth of Liverpool Bay (44.520° N, -64.639587° W) and is sheltered by the presence of a small island. The sea surface temperature in this area is generally coldest between February and March and warmest between August and September (Seatemperature.info, 2023). During the study period, there were fourteen stocked cages with a circumference of 100m and about 10m deep. The depth of the site ranges between 12 and 15 m. The study cage had a lower stocking density with approximately ~8,000 fish compared to other pens on site, which had approximately ~25,000 fish at the start of the study period. Fish were mixed-sex, diploids, originating from the Sain John River strain, stocked in May 2020 with an average weight of 78 g in pen 14. The study started on July 30th, 2021, and ended on March 31st, 2022, for a total of 245 days. No disease or treatments were recorded in the pen throughout the study period.

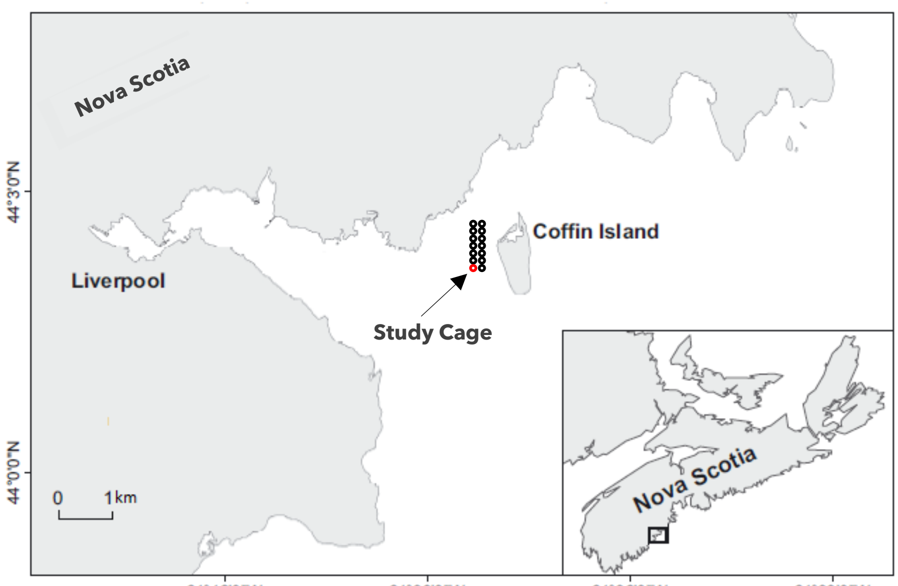

Figure 2. 1 Regional map of Liverpool Bay with farm location. Study pen is marked in red, and the inset shows the location in reference to Nova Scotia

2.3.2 Programming and Implantation of Biologgers

Two types of tags were surgically implanted into each fish: Acoustic Data Storage Tags (ADST) (V13AP, Innovasea Systems Inc, Bedford, Nova Scotia, Canada) and centi-HRT ACT Data Storage Tags (DST) (DST centi-HRT ACT G2, STAR-ODDI, Gardabaer, Iceland). The ADST tags (diameter: 13 mm, length: 43 mm, weight in air: 11.5 g) measure tri-axis acceleration and depth, log them internally and acoustically transmit (at 69 kHz) activity and depth at alternating intervals; however, the logged raw acceleration and depth data have higher temporal resolution and so no acoustic data was used in this analysis. Activity was derived from an algorithm that filters out gravity and tag orientation (tilt) on each individual axis to give a root mean square (RMS) value that represents generalized activity via the raw acceleration measurements. Raw acceleration data was sampled at 12.5 Hz for 20 s every 20 minutes and depth data were logged every minute for the entire duration of the study. The centi-HRT ACT DST tags (diameter: 15 mm, length: 46 mm, weight in air: 19 g) measure and log heart rate, acceleration, and temperature. Heart rate is calculated using an electrocardiogram (ECG) amplifier that uses an algorithm to calculate beats per minute (bpm) from the mean RR-interval (time between two R waves in the ECG) within a sample. Heart rate was sampled at 100 Hz over a short or long sampling interval (6 s or 15 s respectively). At the recommendation of the tag manufacturer, long sampling was used during the winter season to capture a high-quality ECG that can be analyzed by the heart rate algorithm when fish's heart rates are low due to cold temperatures. The centi-HRT ACT DST tag has a tri-axial accelerometer that measures raw acceleration at 10 Hz over a one-minute sampling period and stores the average, maximum, minimum, variance, skewness, and kurtosis of the sample set. External acceleration data is calibrated and normalized for static acceleration to remove the effects of tilt and standard gravity on the logger. The output is external acceleration in milli *g* (*mg*), which is the acceleration affecting the logger above standard gravity. External acceleration was used as a proxy for swimming activity as it has been shown to positively increase with swimming speed (Zrini & Gamperl, 2021). Due to memory and battery constraints of the loggers, an irregular sampling schedule was programmed for the centi-HRT ACT tags to target higher sampling intervals during the periods of interest in the study (Table 2.1).

Table 2. 1 Sampling schedule for centi-HRT ACT DST. Raw ECG data was stored for one week during both high frequency sampling periods (The 2nd and 4th period below).

Start Date	**End Date**	**Sampling Interval**	**Heart Rate (at 100 Hz)**	**Acceleration (at 10 Hz)**
July 30th 9:00AM	August 16th 9:00AM	1 hr	6 s sample	1 min sample
August 16th 9:00AM	September 16th 9:00AM	20 min	6 s sample	1 min sample
September 16th 9:00AM	January 1st 9:00AM	6 hr	6 s sample	1 min sample
January 1st 9:00AM	March 24th 1:00AM	20 min	15 s sample	1 min sample
March 24th 1:00AM	March 31st 11:00pm	1 hr	15 s sample	1 min sample

To surgically implant both tags, salmon were opportunistically sampled from pen 14 as they approached the vessel and using a dip net, placed into a holding tank on board one at a time. Each fish was tagged with both types of tags, which resulted in a total tag weight of 30.5 g. The producer's veterinarian performed all surgeries, and all experimental protocols were approved by the Dalhousie University Committee on Laboratory Animals (protocol number 20-119). Prior to surgery, all instruments and tags were disinfected in Virkon S® 1% solution for a minimum of 30 minutes. Each individual fish was moved from the holding tank to an induction bath of seawater and 200 mg L^{-1} tricaine methanesulfonate (TMS). Once fish were visually sedated and no longer responded to physical stimuli, they were moved to a scale to record their length and weight. Fish ranged from 2.7 to 5 kg in weight, (3.76 ± 0.67 kg) and between 55 and 70 cm in length (61.06 ± 4.25 cm), all means are reported as mean ± standard deviation. They were then transferred to a surgical v-trough and had a tube inserted into their mouths so that oxygenated water with a lighter dose of TMS (100 mg L^{-1}) could be irrigated over their gills throughout the procedure. A 2 cm, mid-ventral incision was made posterior to the pectoral fins using a scalpel. The ADST tag was inserted caudally, with the ceramic end in first and the pressure sensor facing cranially. The tag was left to be free moving within the body cavity. The centi-HRT ACT tag was then inserted with the flat-end first, cranial to the incision. The logger was gently pushed toward the pericardium and the tag was orientated so that the electrodes were facing the abdominal wall for optimal ECG signals (Brijs et al., 2018). The channel through the round-end of the ceramic of the tag was used to suture it in place using a surgeon's knot. The wound was closed with interrupted sutures using 3-0 sterile, non-absorbable monofilament. Surgery time ranged from 7 to 11 minutes (8 ± 1 min). A fluorescent T-Bar tag (Floy Tag Inc, Seattle, WA, USA) was injected through the dorsal fin of each fish for identification purposes. After that, each fish was transferred to the recovery tank, which had a continuous supply of fresh seawater and was regularly checked for adequate oxygen and temperature levels. Recovery time in the tank averaged 55 ± 37 min. The fish were returned to the pen only once they showed signs of normal locomotory and opercular movements. 17 fish were tagged in total; among those, the final three fish were unable to recover fully in the recovery tank prior to being returned to the net pen because the tank was emptied due to an on-board safety issue. From the real time acoustic data, it was assumed these fish subsequently died in the days following the tagging and their tags were not found.

2.3.3 Environmental and Farm Operations Data

In the study pen, 5 real time environmental monitoring sensors measuring dissolved oxygen, temperature, and depth were deployed 1.5 m apart on a profile line at approximately 2, 3.5, 5, 6.5 and 8m depth for the duration of the study (aquaMeasure DOTD, Innovasea, Halifax, Canada). This data was transmitted acoustically every 2-3 minutes and logged internally every five minutes. Sensors were deployed using a clothesline method and hung from the middle of the pen. Farm data was provided by Cooke Aquaculture and included operational activities that took place throughout the study including in situ net cleaning, diver timesheets, daily maintenance reports, feeding routines and mortalities. Periods of feeding and/or operations occurred during a small percentage of time relative to the whole dataset and were not removed from this analysis and the effect of all farm operations were analyzed in Chapter 3.

2.3.4 Data Processing and Statistical Analysis

Fish were harvested on July 26th, 2022, and seven out of the expected 14 tags were retrieved on the processing line overnight between July 26th and 27th. It is unknown what happened to the remaining seven tagged fish. The average weight at tag collection was 7.46 ± 0.40 kg and the average length was 75.34 ± 2.91 cm, representing approximately a 98% weight increase and a 23% length increase, respectively. Condition factors (K) were used as a metric to determine the girth of fish based on the relationship between their length and weight (Equation 1)

$$K = \frac{W \times 10^5}{L^3} \qquad \text{(Eq. 1)}$$

where *W* and *L* are weight (g) and length (mm), respectively. Condition factors were calculated at the time of tagging and after tags were retrieved. At the time of tagging, $K > 1.4$ for F2, F3 and F8 indicating a class of good and F4, F11, F12 and F14 had a $K > 1.6$, which classified them as excellent. At the time of harvest, all fish had a $K > 1.6$, classifying them as excellent except for F14 which had a poor condition factor ($1.0 > K > 1.2$). All weight, length and condition factors for individual fish at the time of tagging and recovery are available in Table D1 in Appendix D. Fish were sexed at the time of harvest, three were female (F2, F8 and F11), three were male (F3, F4 and F12) and one was unknown (F14). ADST tags were sent to Innovasea for data recovery and processing. Centi-HRT ACT tag data were retrieved using the application software Mercury (v6.14) and the associated Communication Box (STAR-ODDI, Gardabaer, Iceland). Due to the different processing algorithms on each tag, acceleration data from both tags were unable to be validated against each other, as a result, all data from the centi-HRT ACT tag were used (external acceleration, heart rate and temperature) and only depth data from the ADST tags were included in the analysis. The heart rate algorithm automatically assigns a quality index (QI) to each heart rate calculation ranging from QI_0 = great, QI_1= good, QI_2 = fair and QI_3= poor. All data with QI < 2 were used in the analysis. Tag datasets were processed using the tsclean() function in the R forecast package (Hyndman et al., 2023), which fits a robust trend using locally estimated scatterplot smoothing (loess) and removes outliers less than the 10^{th} and larger than the 90^{th} percentiles and replaces them using linear interpolation. To account for the different sampling frequencies, a 1-hour rolling average was used and all tag data were averaged hourly or daily for each fish and then combined for subsequent analyses.

To detect the presence of circadian rhythms in heart rate and activity, a spectral analysis was carried out seasonally (in summer and winter independently) as only data with at least an hourly sampling interval was used and data must be continuous. Heart rate and acceleration time series were transformed from the time domain into the frequency domain using a fast Fourier transform to determine the dominant frequencies governing the respective time series. The frequencies with the largest power spectra are converted back to the time domain by dividing by the sampling interval to derive the cycles per unit time. To analyze the apparent heart rate (f_H) scope, minimum, maximum, and average heart rates were calculated at each temperature recorded throughout the study, rounded to the nearest 0.1 °C. Apparent f_H scope was determined to be the difference between the maximum and the average heart rate at each temperature and was only calculated over a continuous range of temperatures where the thermal optimal temperature (T_{opt}) was expected to be observed. T_{opt} was determined as the temperature at which f_H scope was maximized. Critical temperature (T_{crit}) was defined as the temperature at which there was zero apparent f_H scope (Casselman et al., 2012). Arrhenius curves were plotted using the same methods as f_H scope, with average heart rate being calculated for each fish at each temperature (rounded to the nearest 0.1 °C). Arrhenius breakpoint temperature (T_{AB}) was defined as the temperature at which fish can no longer maintain a steady increase in heart rate and was assumed to be an estimate of T_{opt} by calculating the breakpoint in an Arrhenius plot via regression analysis (Casselman et al., 2012). This $T_{opt,}$ estimated with the Arrhenius plot was compared to T_{opt} derived from f_H scope. The slope under T_{AB} was considered to represent the Arrhenius temperature (T_A) which was used to temperature correct heart rate. P-values obtained from any statistical analysis were considered statistically significant if $P < 0.05$. All statistical analyses were performed in RStudio v. 4.2.2, RStudio Inc., Boston, MA, USA; https://www.rstudio.com/).

2.4 Results

2.4.1 Fish Recovery and Survival

After tagging, three fish died within two days of the surgical procedure likely because they were immediately returned to the pen without a recovery period due to an unexpected tank issue. This denotes a mortality rate of approximately 18%, which is comparatively similar to mortality rates documented in other field studies wherein salmon were promptly returned to net pens following biologger implantation (Føre et al., 2017), and it is plausible that a longer recovery period could have increased their survival. Recovery time following biologger implantation has varied in the literature ranging from 4 days to 3 weeks post surgery (Fore et al., 2021; Yousaf et al., 2022). All fish showed elevated heart rates following surgery and took four days to recover based on a segmented regression analysis (Figure A.1).

2.4.2 Fish Tag Observations

The maximum daily average water temperature occurred on August 29th, with an average of 18.3 °C (across all depths), and the lowest was observed on February 16th, with an average of 2.0 °C (Figure 2.2a). Water temperature was highest from August to the end of October with higher interdaily variation compared to the rest of the study period. Throughout November and December, a gradual decline in temperature occurred until it stabilized at colder, winter temperatures from January to March. As ectotherms, the environmental temperature drives the internal temperature of fish, and both fluctuated seasonally (Figure 2.2a). Average fish temperature was more similar to surface temperatures in the summer period, which was warmer than at depth, and fish were predominantly observed in the surface layer. The maximum daily average fish temperature was 18.5 °C and occurred on September 26th, and the lowest daily average fish temperature was 1.9 °C and occurred on February 1st. Maximum daily average oxygen saturation occurred on August 27th with an average of 118.0 % (across all depths), and the lowest was 88.5 % on November 2nd. Less than 0.1 % of oxygen measurements across all depths were under 70 % saturation resulting in no further analysis of the oxygen data.

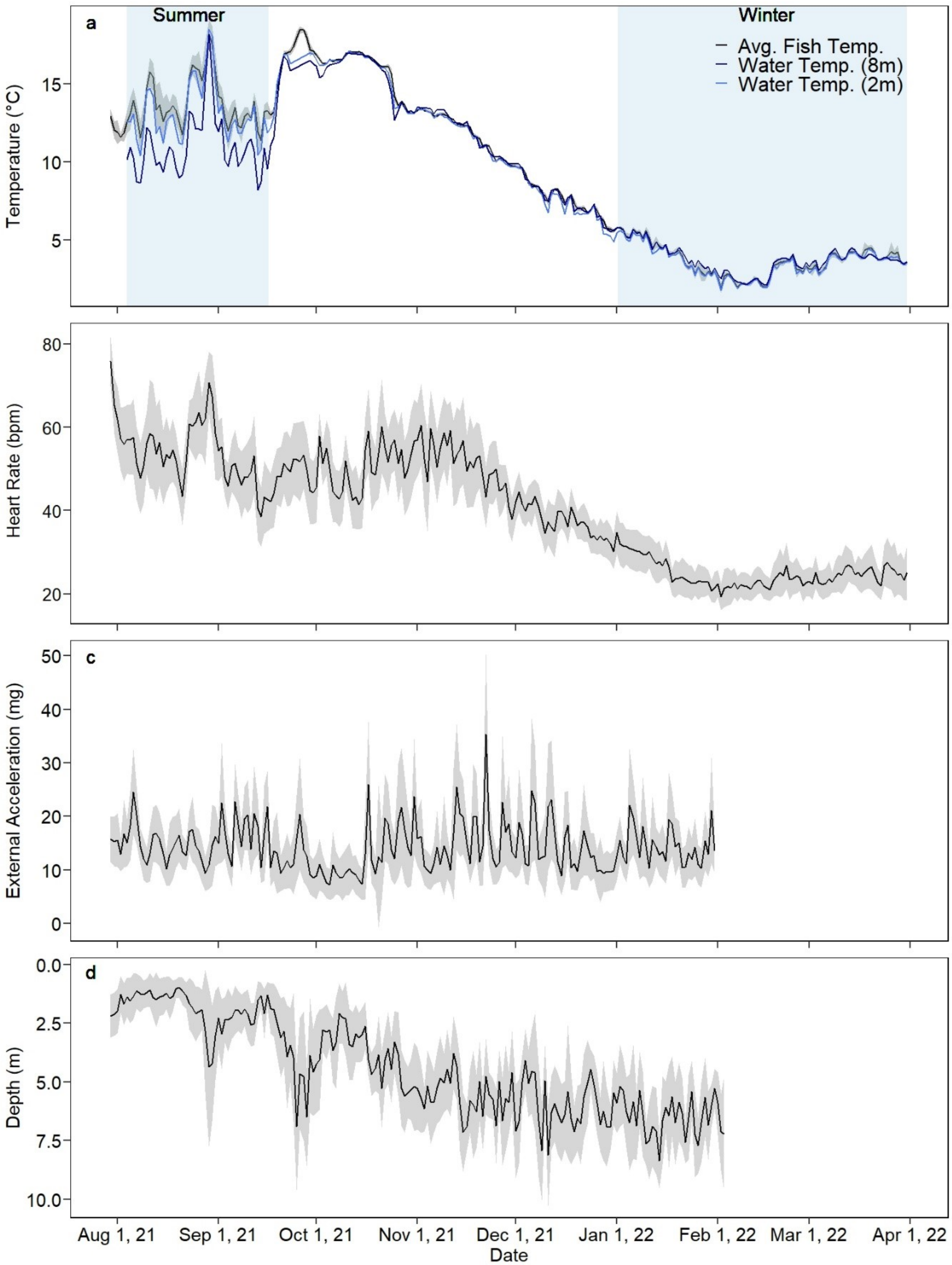

Figure 2. 2 Daily averaged time series for the full study duration, shaded grey areas represent ± standard deviation. a) Water temperature measured at 2 and 8m and fish temperature [°C]. Light blue panels represent the two periods of seasonal high frequency sampling. b) Raw heart rate [bpm]. c) External acceleration [mg]. d) Depth [m].

Heart rate followed a very similar seasonal structure to temperature but overall had more variation (Figure 2.2b). The maximum daily average heart rate was 71 bpm and occurred on August 29th, the same day as the maximum daily average water temperature. The minimum daily average heart rate was 19 bpm and occurred on February 1st, the same day as the minimum daily average fish temperature. There were no discernible seasonal patterns in the external acceleration of fish (Figure 2.2c). Spikes in acceleration occurred throughout the time series at irregular intervals and did not correlate with any other variables in the analysis. Fish depth was observed to be shallower on average during the summer months, and deeper during winter months. During both short- and long-term periods of high-water temperatures, in both August and September, fish were temporarily observed deeper in the water column and returned to the surface once temperatures decreased (Figure 2.2d). Due to technical issues, data after February 1st was unable to be used for both depth and external acceleration.

There was little variation in all recorded variables between individual fish (Figure 2.3). Both temperature and heart rate displayed a bimodal distribution with the largest density of observations occurring at ~4 and ~14 °C (Figure 2.3a) and ~20 and ~55 bpm (Figure 2.3b). This distribution in the data was a function of the seasonality over the sampling period but it was enhanced by the irregular sampling regime. There was increased sampling frequency during the summer (July 30th - September 16th, 2021) and winter (January 1st – March 31st, 2022) periods, which resulted in less than 5% of data collected between September 16th, 2021 - December 31st, 2021, when intermediate temperature and heart rates were observed. External

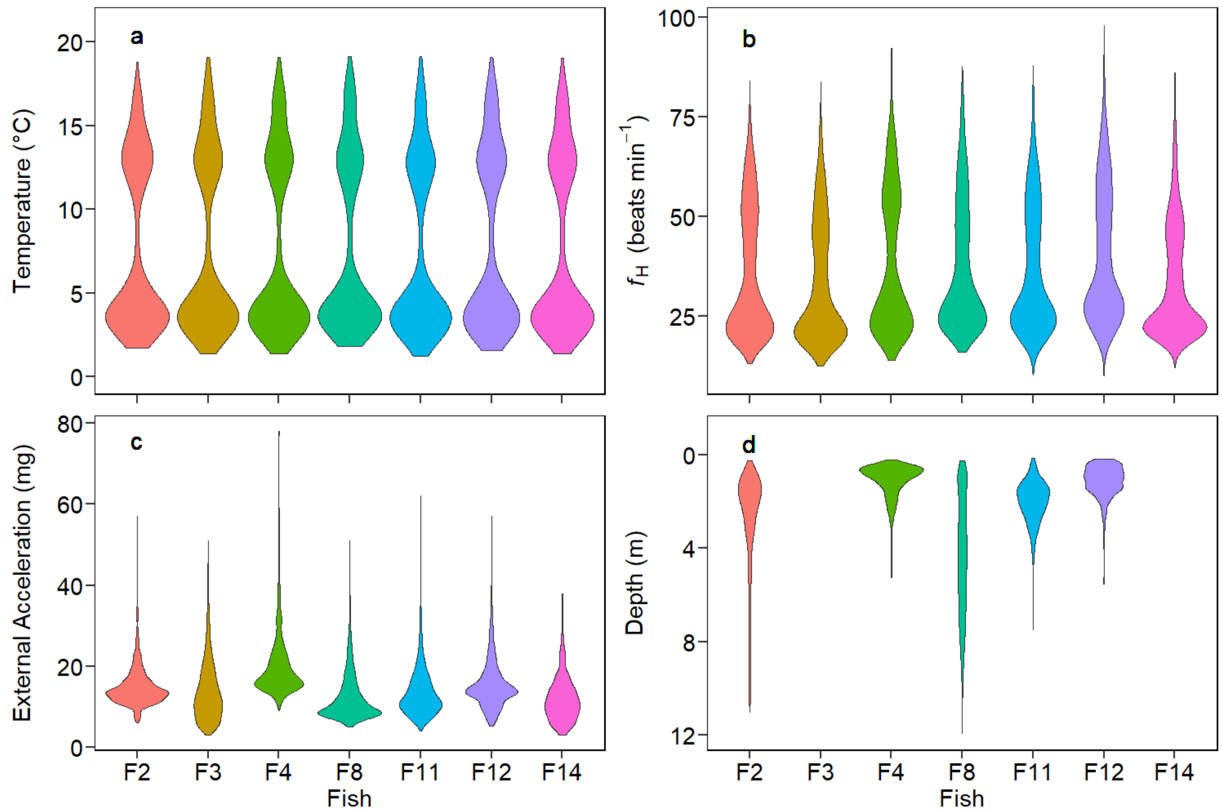

Figure 2. 3 Density of biologger measurements for all individuals throughout the study. a) Fish temperature [°C]. b) Heart rate [bpm]. c) External acceleration [mg]. d) Depth [m]. No depth data was available for F3 and F14 due to pressure sensor malfunction.

acceleration and depth both displayed a unimodal, skewed distribution with the largest density of data between 0 - 20 mg for acceleration (Figure 2.3c) and between 0 - 4 m for depth (Figure 2.3d). Fish exhibited circadian rhythms in both seasons for heart rate (Figure 2.4a and 2.4b) and only during summer for acceleration (Figure 2.4c). The circadian rhythm for heart rate was consistent among all individuals; however, there was more complexity in the acceleration time series between individuals, with different fish showing influence of different frequencies in their acceleration patterns. The dominant frequency observed in winter for acceleration was 3.9 days, which is not associated with any known tidal or weather signals in this region (Figure 2.4d).

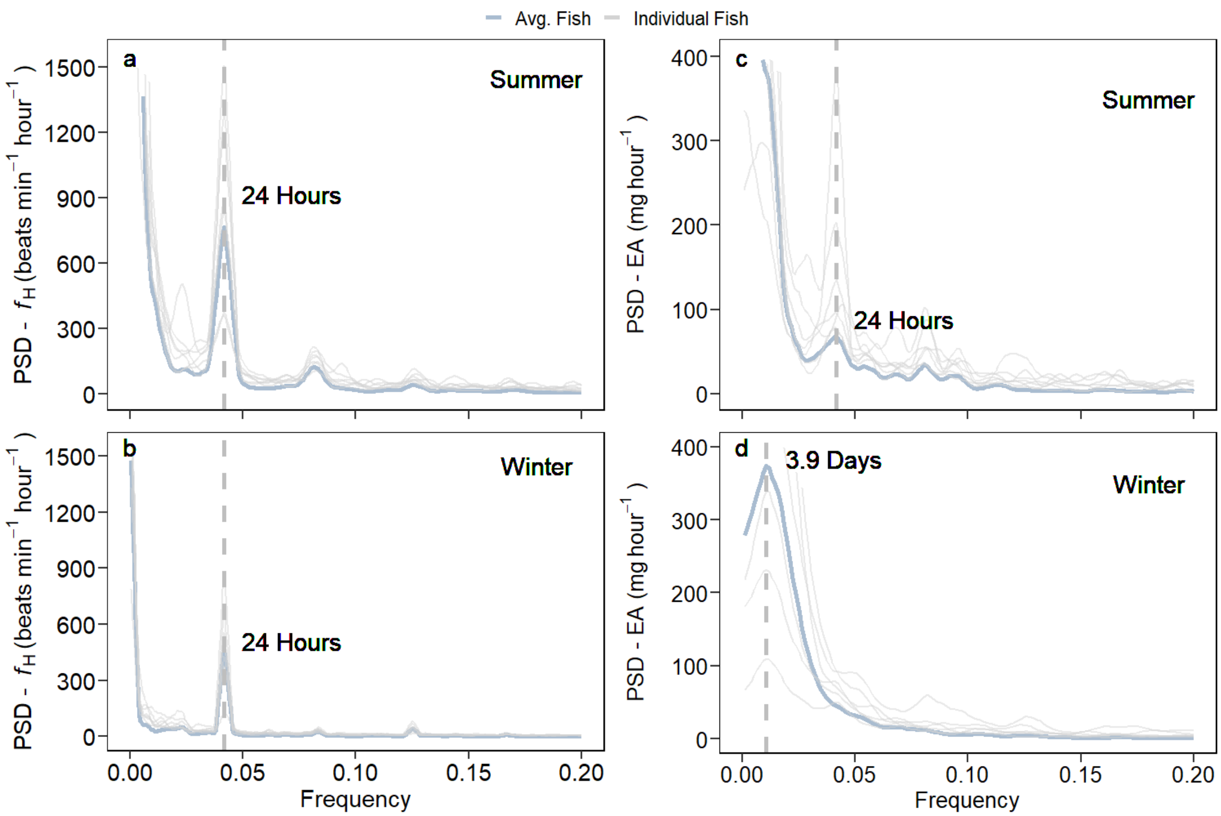

Figure 2. 4 Seasonal periodograms of the power spectral density (PSD) after a fast Fourier transform of hourly averaged time series for all fish (bold grey line) and individual fish (light grey line). The frequency with the highest power spectra was converted back to the time domain to determine the period which was 24 hours in all cases except winter acceleration. Grey dotted lines mark dominant frequency a) Summer heart rate [bpm]. b) Winter heart rate [bpm]. c) Summer external acceleration [mg]. d) Winter external acceleration [mg].

2.4.2 f_H Scope and Arrhenius analysis

Average heart rate was calculated at all temperatures observed throughout the study, but maximum and minimum heart rate were only calculated at temperatures observed during the high frequency sampling periods in summer and winter (Figure 2.5a). Heart rate steadily increased with temperature and there was a discernible difference in the minimum and maximum heart rates between 2 and 19 °C. The maximum average heart rate was observed at 17.9 °C (80.78 ± 6.56 bpm) and the minimum average heart rate was observed at 4 °C (14.89 ± 1.89 bpm). Extreme temperatures (T < 2 °C and T > 19 °C) and intermediate temperatures (6 °C < T < 10 °C) were underrepresented in the data set and therefore removed from f_H scope and scope was only calculated over the largest continuous range of data where T_{opt} was expected to occur (Figure 2.5b). The maximum f_H scope occurred at 12.7 °C and

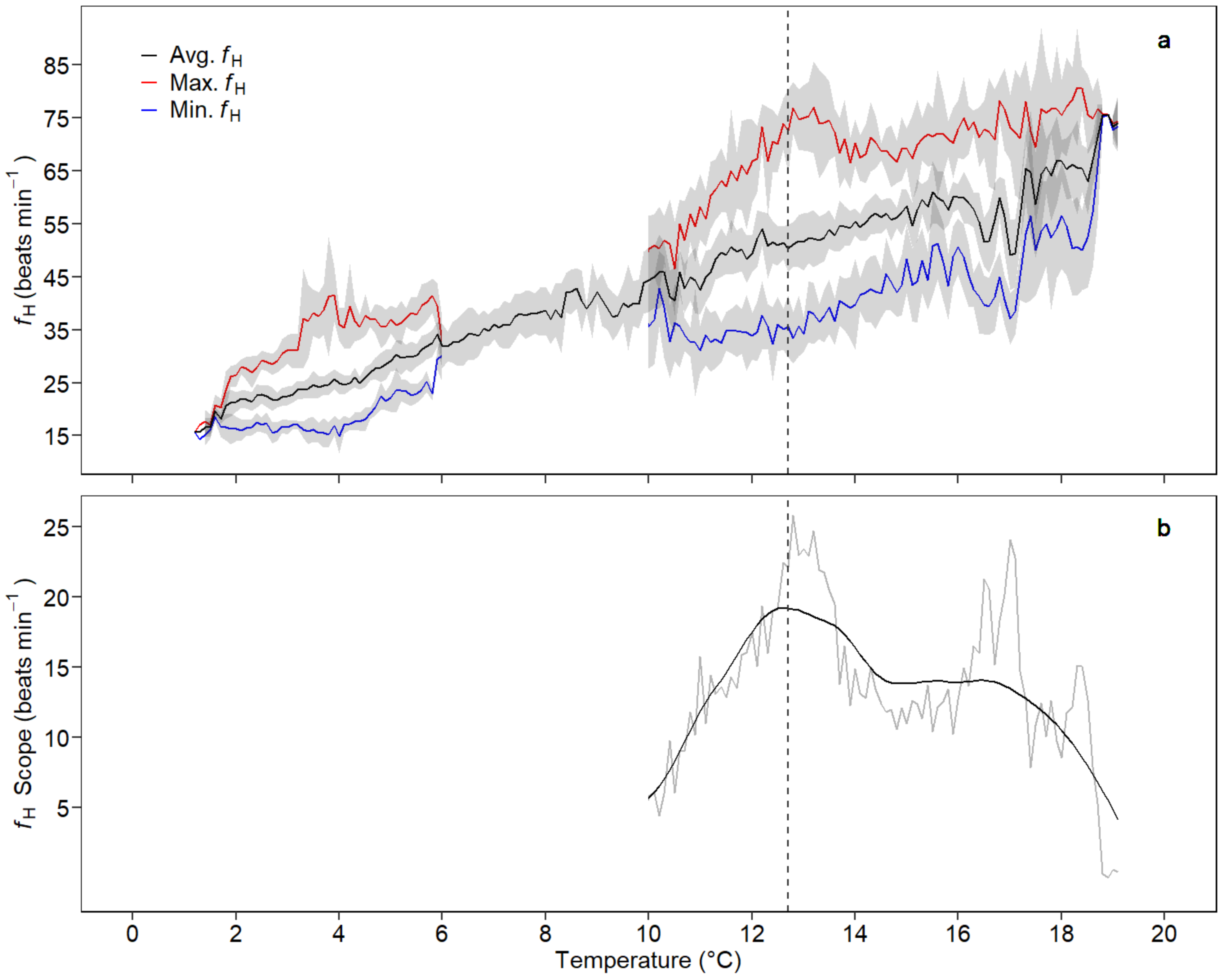

Figure 2. 5 a) Maximum, minimum, and average heart rate over the full range of observed temperatures. Values between 6 °C and 10 °C were removed for minimum and maximum heart rate due to limited data over intermediate temperature range. b) Raw (grey) and smoothed (black) difference between average and maximum heart rate at given temperatures presented as apparent f_H scope. f_H scope was only calculated where sufficient continuous data was available ($T > 10$ °C). Dashed lines are drawn where f_H scope is maximized and T_{opt} was estimated at 12.7 °C.

was assumed to be the optimal temperature for these fish. Maximum and average heart rate started to converge after 12.7 °C and began to overlap above 19 °C, suggesting this temperature approaches T_{crit}. The Arrhenius breakpoint temperature was calculated as an additional estimate of T_{opt} using a breakpoint regression analysis, which estimated the break in the Arrhenius plot at 12.7 °C (Figure 2.6).

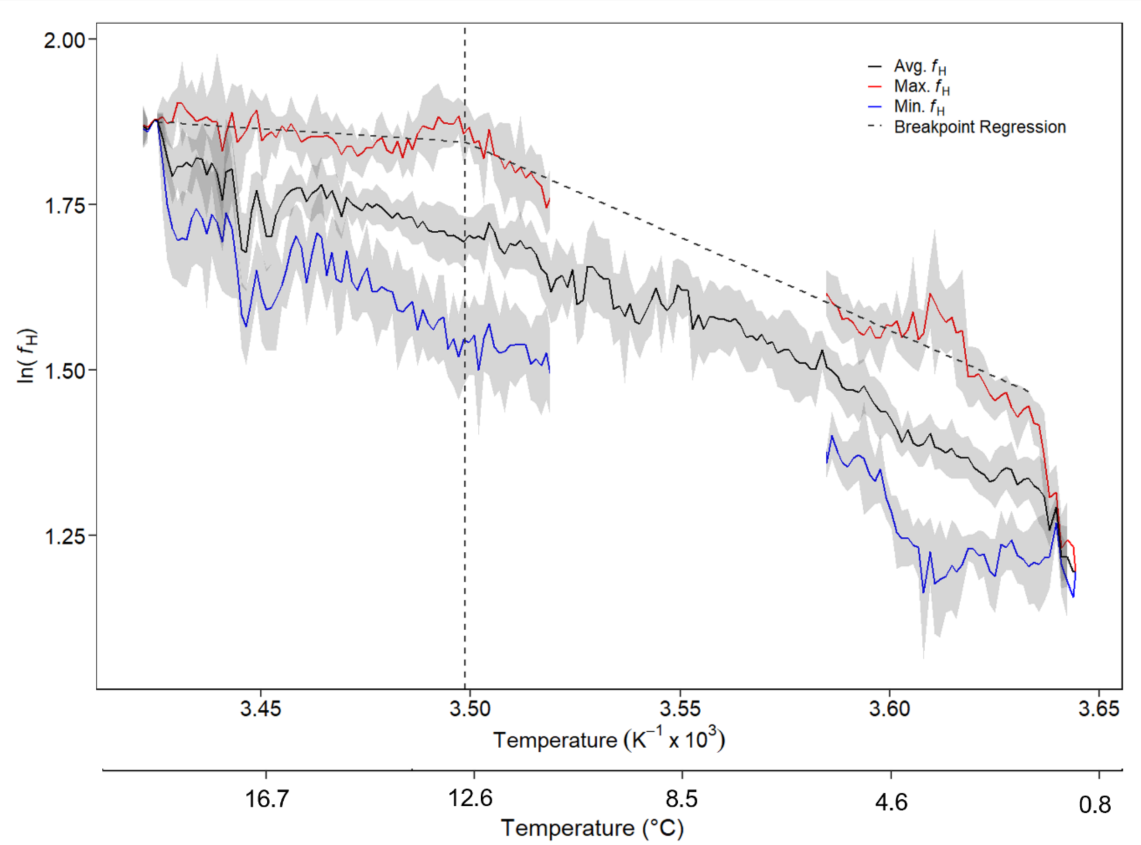

Figure 2. 6 Arrhenius plot for maximum, minimum and average heart rate over the full range of observed temperatures. Values between 6 °C and 10 °C were removed for minimum and maximum heart rate due to limited data over intermediate temperature range. Dashed lines are the piecewise regression analysis and mark the estimated breakpoint at 12.7 °C, indicating $T_{opt.}$

2.5 Discussion

The heart rate, body temperature, swimming activity and depth of Atlantic salmon near harvest size during a standard production cycle allowed the characterization of in situ physiological and behavioural responses under a broad thermal range, enabling the cross-validation of controlled laboratory experiments. Heart rate exhibited a strong signal linked to water temperature, which co-occurred with a steady circadian rhythm. Maximum heart rate (81 bpm) occurred at 18.3 °C and f_H scope was reduced at both temperature extremes > 19 °C and < 2.0 °C, indicating thermal stress and proximity to T_{crit}. Using f_H scope as a proxy for aerobic scope suggested that the optimal temperature for these fish was 12.7 °C, which was corroborated using T_{AB}, as it was also estimated at 12.7 °C using a breakpoint regression analysis.

Salmon swimming depth was observed predominantly at the surface during the summer period; however, during periods of high temperature (≥16 °C), they occupied a broader range of the water column depth, likely attempting to find colder water. The net pen extended approximately 10m in the water column and there was evidence of a thermocline during the summer period with surface water temperatures ranging between 1 - 3 °C warmer than at depth, except when temperature reached at least 16 °C and warmed the entire water column and fish were observed deeper. It has been demonstrated that fish in aquaculture cages exhibit behavioural thermoregulation by searching for optimal water temperatures when the water column is stratified but avoid temperatures greater than 18 °C (Johansson et al., 2006; Oppedal et al., 2001). In colder months, temperature varied between 2 and 5 °C and fish were observed deeper in the water column despite there being almost no differences in temperature at the surface compared to the bottom of the pen. At low temperatures, salmon have a reduced appetite and as a result, they occupy a larger volume of the pen instead of residing near the surface in anticipation of feed (Juell et al., 1994), which has been observed in other studies monitoring depth across seasons (Oppedal et al., 2001).

Swimming activity did not have any long-term seasonal trends. It was hypothesized that fish would be more active and have a higher average activity level during summer months because of the effect of 1) temperature (Martin et al., 2012) and 2) the difference in feeding regimes during summer vs. winter periods described above (Oppedal et al., 2001). However, there were no long-term trends in swimming activity, and peaks in external acceleration were not correlated with any variable collected in this study. On shorter timescales (hours to days), there was a distinguishable circadian rhythm in swimming activity that emerged during the summer months, although this pattern was not observed during the winter months. It is possible the winter signal was depressed compared to the summer signal, which was observed in the circadian rhythm of heart rate where the strength of the signal was much higher in summer compared to winter; although for heart rate, a defined signal was apparent in both seasons. The lack of circadian rhythm in swimming activity at colder temperatures is comparable to other studies that were able to detect diel patterns in activity in some experiments but not in others, which was suggested to be caused by lower temperatures (Zrini & Gamperl, 2021). The differential response across seasons was also observed in salmon smolts, which showed a distinct difference in nocturnal and diurnal swimming speeds in summer vs. winter months (Martin et al., 2012). This suggests that temperature serves as a pivotal regulatory mechanism influencing swimming activity, but further research should be undertaken at cold temperatures.

The role of temperature in regulating heart rate is well defined; however, using f_H scope and maximum f_H to identify T_{opt} has only been carried out in controlled laboratory settings (Antilla et al., 2014b; Casselman et al., 2012; Farrell et al., 2009; Gamperl et al., 2020). This study extended over both low and high thermal extremes, capturing heart rate over a broad range of temperatures from 1.3 to 19.2 °C. The f_H scope reached a maximum at 12.7 °C, which is along the low end of the T_{opt} range that has been identified for Atlantic salmon (13 – 18 °C) (Dwyer & Piper, 1987; Handeland et al., 2008; Hevrøy et al., 2012; Koskela et al., 1997). The large range of thermal optimums is likely caused by using alternative methods to determine T_{opt} and by the use of different salmon populations that could be thermally adapted to different thermal regimes (Antilla et al., 2014a). Salmon in this region originated from the St. John River stock and have been modified for aquaculture production under the Atlantic Salmon Broodstock Development Program, therefore it is possible that their optimal temperature is lower than salmon in other regions that experience a higher average temperature range (Chang, 1998). The agreement of T_{opt} estimated with f_H scope and T_{AB} from the Arrhenius plot (12.7 °C) is encouraging given the nature of this observational approach.

The use of f_H scope further allowed the identification of thermal stress at temperatures above 19 °C and below 2.0 °C resulted in the reduction of f_H scope. Physiologically, salmon can tolerate these temperature extremes, but energetic trade-offs emerge, causing changes in swimming activity, a reduction in appetite, increased metabolic rates and the synthesis of heat shock proteins (Barton, 2002). Estimates of T_{crit} have not been estimated using f_H scope previously and although the reduction in scope leading up to the extreme temperatures suggests proximity to T_{crit}, the number of observations at extreme temperatures were insufficient to derive an estimate. In addition, due to the energy requirement of swimming activity within the cage, using average and maximum heart rate to derive f_H scope, may underestimate the true f_H scope and should be taken into consideration when interpreting results. The slope under T_{AB} was used to estimate the Arrhenius temperature (T_A = 1663). This estimate was within the reported range (T_A = 1018 – 2032), for this species (Antilla et al., 2014a; Penney et al., 2014). Arrhenius temperature can be used to correct biological rates to remove the effects of temperature, and this is relevant for future field studies to observe how other factors on an aquaculture farm affect heart rate after removing the influence of temperature.

To observe fish response during thermal extremes, sampling frequency targeted data collection during two 4-week periods in summer and winter enabling observations of heart rate near reported thermal stress limits (1 & 20 °C as per Vadeboncoeur et al., 2023 & DFO, 2012 respectively). This approach limited the ability to calculate f_H scope across the full temperature range, however the sampling schedule was a direct result of balancing biologger battery and memory constraints. As biologgers continue to improve, there will be more potential for long-term studies measuring data at higher sampling frequencies over the full grow-out period. Salmon displayed signs of active behavioural thermoregulation in response to elevated temperatures and swimming activity displayed a circadian rhythm during summer. There were no long-term trends in external acceleration which was used as a proxy for swimming activity (Morgenroth et al., 2024). Acceleration is a challenging variable to interpret without context, and it is possible that the measurement frequency used in this study, 20 minutes, was too low to observe long-term activity trends due to the quick-burst nature of acceleration, and the inability to maintain bursts of acceleration resulting in a high likelihood of missing a behavioural response. There are also challenges associated with research in commercial settings, including lack of experimental control and the resolution of farm operations data is very coarse compared to sensor data making it difficult to explain patterns or anomalies in the biologger data. In addition, the 7 tagged fish that were not recovered represent half of the potential data in the study and further highlight the complexity of using biologgers in commercial operations. Regardless of their fate, the recovery of their tags would have either strengthened the analysis or provided insights into behaviour and physiology leading up to death and represent a limitation of field studies.

Knowing and understanding the implications of T_{opt} and thermal stress limits for Atlantic salmon in commercial aquaculture is essential for selecting suitable farming locations and optimizing production cycles. In an aquaculture setting, husbandry decisions, such as withholding feed, are made when temperatures approach stressful conditions. Providing estimates of T_{opt} and thermal stress limits for Nova Scotia salmon aquaculture can help advise government regulators about site locations and inform how current sites will perform under climate change. The results from this study are a good baseline for how commercial fish respond to the thermally challenging conditions prevalent in this region. Future work should consider long-term monitoring of salmon throughout the entire grow-out cycle to further the understanding of thermal stress limits found in laboratory experiments and their application in commercial aquaculture settings and biologgers offer a unique ability to monitor fish physiology and behaviour of free-swimming fish as they experience challenging environmental conditions and farm operations.

2.6 Conclusions

Temperature plays a critical role influencing the physiological and behavioral response of Atlantic salmon on commercial farms. The optimal temperature that was estimated at 12.7 °C provides insight to the aquaculture industry in Nova Scotia that optimal temperatures may be lower than expected when compared with optimal temperatures of Atlantic salmon originating from other stocks. The estimate of T_{opt} was on the lower end of the range that has been previously identified for Atlantic salmon, which provides novel information to producers who can implement this when planning feeding regimes over a range of temperatures. Knowledge of optimal temperatures helps producers target favourable growing conditions and temperatures to maximize feed delivery, consumption, and ultimately, growth.

In addition, thermal stress limits for Atlantic salmon, observed by a reduction in heart rate scope at temperatures below 2 °C and above 19 °C, provides temperature limits, above or below which farms should avoid feeding or undertaking non-essential operations. These findings link thermal stress limits that have been identified in the literature for Atlantic salmon in this region to the conditions experienced by farmed salmon during a production cycle and demonstrates their impact on fish behaviour and physiology.

The use of biologgers allowed for direct and repeated observations of individuals, which is rare in a standard commercial farm setting, and it provides valuable information for aquaculture management. Advances in sensor technology and increased data collection in aquaculture settings will continue to improve our understanding between environment and commercial operations and how they impact fish stress. This enables regulators to make informed decisions and supports growth of the aquaculture industry in Nova Scotia with region-specific insights.

CHAPTER 3: EFFECT OF AQUACULTURE OPERATIONS ON THE PHYSIOLOGY AND BEHAVIOUR OF ATLANTIC SALMON (SALMO SALAR) DURING TWO HEAT EVENTS ON A COMMERCIAL FARM.

3.1 ABSTRACT

Aquaculture farms represent a complex 3D environment and face regular seasonal challenges such as acute and chronically elevated temperatures during summer. Further, fish are exposed to the interaction between their environment and farm operations, which can cause challenging conditions. In the context of modern net-pen aquaculture and ocean warming, there is therefore a need to understand the welfare of these commercially important species under the realistic conditions they encounter. Fish were tagged with two types of biologgers measuring temperature, heart rate, external acceleration, and depth of fish as they experienced standard aquaculture operations over two periods of thermal stress, one short-term and one long-term. The fish response during the thermal stress events was compared with the periods preceding and following both events, and an additional analysis was carried out to further explore the effects of feeding and farm operations. Fish displayed signs of both secondary and tertiary stress in response to the short- and long-term heat event and both heart rate and acceleration increased in response to feeding but displayed a more nuanced response to operations. As part of the broader concept of precision fish farming, this research contributed to advancing the use of biologgers as tools for recognizing early signs of stress by observing the secondary stress response, thereby demonstrating the potential for informed and timely stress identification to guide farm management decisions to enhance fish welfare and production efficiency in commercial aquaculture.

3.2 INTRODUCTION

In the dynamic landscape of modern net-pen aquaculture and ocean warming, there is a pressing need to understand the intricate interplay between aquaculture operations and environmental conditions on fish stress, physiology, and behaviour of cultivated species (Calado et al., 2021; Froehlich et al., 2018; Stehfast et al., 2017). Temperature is both a direct and indirect driver that modulates metabolic rates, appetite, immune response, and growth rates (Barton et al., 2002; Farrell, 2009; Reid et al., 2019). Atlantic salmon (*Salmo salar*) are susceptible to thermal stress (Dwyer & Piper 1987; Gallant et al., 2017; Gamperl et al., 2020), and production cycles regularly span two summers to maximize fast-growth periods, where, in addition to thermal stress, salmon can be exposed to other stressors (Burt et al., 2012; Jones & Price, 2022; Mardones et al., 2021). Farmers must be able to recognize granular changes in fish performance to respond with appropriate husbandry decisions to stressors, improving production cycles, growth, and fish welfare (Ashley, 2007). In Nova Scotia, maximum temperatures occur in late summer and early autumn, and this time of year is highly relevant for farmers in this region due to the likelihood of hypoxia and thermal stress events (Burke et al. 2020; Burt et al., 2012). Thermal stress can be acute or chronic in nature and it is difficult to determine absolute impacts on fish when additional sources of stress, e.g. farm operations (Iversen & Eliassen, 2014), co-occur and reduce fish ability to respond to stressors (Brijs et al., 2018; Gallant et al., 2017).

Fish stress on farms is inferred through operational welfare indicators (OWI), which serve as a straightforward and reproducible mechanism for farmers to promptly evaluate fish stress and react to suboptimal conditions to improve welfare (Noble et al., 2018). The stress response in fish is initiated by a disruption to the organism's homeostasis, the body's natural ability to regulate itself to maintain stability, in response to a real or perceived threat (Wendelaar Bonga, 1997). During the primary stress response, hormones are released by the adrenal glands into the blood stream, which results in secondary responses, including increased cardiorespiratory activity, increase in heat-shock protein synthesis and changes in plasma and metabolite levels

(Ackerman et al., 2000). These physiological changes can modify certain biological activities and reallocate the energy of an individual to enable them to try and overcome the stressor they are experiencing (Huntingford, 2006). During events of prolonged stress, e.g. disease state, chronic stress may lead to tertiary responses such as reduced appetite and growth, altered swimming behaviours and a poor immune response (Barton, 2002). However, if fish are showing signs of tertiary stress responses, it is difficult to identify the initial cause of the stress and it is often too late to take action to prevent this response from occurring in the first place (Brijs et al. 2018).

Measurements of heart rate have been used in previous studies as a secondary stress-indicator in response to netting, chasing, and crowding events (Boman, 2014; Brijs et al, 2018; Hvas et al., 2020) and they present a unique opportunity to identify early stress responses in cultured fish (Axelsson et al., 2006). Behavioural studies done in tanks on land have also shown that swimming activity increases in response to induced stress, such as simulated crowding (Svendsen et al., 2021), and similarly, increased swimming activity has been observed in response to crowding and delousing on commercial farms (Føre et al., 2018a). Additionally, low dissolved oxygen and elevated temperature decrease salmon swimming speed and feeding activity in net pens and recirculating aquaculture systems (Kolarevic et al., 2016; Oldham et al. 2018, Stockwell et al., 2021). Consequently, monitoring the physiology, i.e. heart rate, and behaviour, i.e. activity, of farmed fish during commercial operations offers a direct means to assess the impact of various stressors. These observations require less interpretation and provide a new potential method to assess fish welfare compared to more traditional indicators.

Modern, intensive salmon aquaculture requires monitoring and control of the farm system to ensure that fish are raised in an environmentally and ethically responsible way that prioritizes fish health and efficient production systems. Using concepts from traditional land-based farming, precision fish farming (PFF) is an approach to farming that promotes enhanced monitoring, control, and databasing of all available data streams to develop support systems for enhanced decision making (O'Donncha & Grant, 2019). This includes improving previously manual or labor-intensive procedures or creating new data streams using modern, innovative technology (O'Donncha et al., 2021). Despite the progress of machine vision algorithms learning to identify individual fish (Schraml et al., 2021), these tools are not widely used in commercial settings, which results in a limited ability to examine repeated observations of the same individuals throughout the grow out cycle. Other than intermittent health checks that do not provide repeated observations for individuals, most fish-based observations are conducted at the group level (i.e, tertiary stress responses). Biologgers are a potential avenue to overcome this data gap and use the secondary stress response as an input to automated decision support systems that have been proposed for precision farming (Føre et al., 2018b). Furthermore, they provide a new data stream that enables data driven decision making and promotes repeatability in operational decisions, which are key components of PFF. There are still challenges that must be overcome before biologgers are adopted into standard operating procedures, and research undertaken on commercially operating farms is an important step to achieve this.

The objective of this work is to explore the behavioural and physiological responses of farmed Atlantic salmon to 1) thermal stress, and 2) the compounding effects of general farm operations and feeding events. Salmon farms in Nova Scotia experience acute and chronic warm temperatures during the summer periods of production cycles and with the ongoing effects of climate change impacting the North Atlantic, it is important to understand how salmon respond to elevated temperatures (Klinger et al., 2017). Biologgers provide a unique avenue to observe free swimming fish as they experience standard aquaculture operations in commercial net pens and allow for direct observations of fish exposed to compounding stressors. In this study, biologgers were used alongside an environmental monitoring system with oxygen and temperature sensors. Further, a farm log was kept to record when environmental stress overlapped with routine and exceptional farm operations. This research helps further the use of biologgers as precision aquaculture tools in commercial aquaculture and provides a mechanism to recognize earlier signs of stress in farmed salmon, which can help to inform farm management to potentially alleviate sources of stress sooner.

3.3 Methods

3.3.1 Experimental animals and ethical statement

This study took place on a commercially operating Atlantic salmon farm in Nova Scotia, Canada. The data used in this study are a subset of thermal stress periods from a longer study that focused on the thermal physiology of salmon (Chapter 2). Accordingly, additional information can be found in Chapter 2, but in brief, two types of biologgers were implanted, an Acoustic Data Storage Tags (ADST, model V13AP, diameter: 13 mm, length: 43 mm, weight in air: 11.5 g; Innovasea Systems Inc, Bedford, Nova Scotia, Canada) that measures tri-axis acceleration and depth, logging them internally and acoustically transmitting (at 69 kHz) and a centi-HRT ACT Data Storage Tags (DST, DST centi-HRT ACT G2, diameter: 15 mm, length: 46 mm, weight in air: 19 g; STAR-ODDI, Gardabaer, Iceland), that records heart rate, temperature, and acceleration. The ADST tags, which were not used in this analysis due to a malfunction in some of the pressure sensors, limiting statistical power, and the centi-HRT ACT DST were surgically implanted (see below) into 17 fish on July 29th, 2021, on a vessel alongside the study pen. The external acceleration measured by the loggers was used as a proxy for general activity. All means are reported as mean ± standard deviation. The tagged fish weighed between 2.7 and 5 kg (3.76 ± 0.67 kg) and the combined tag weight was 30.5 g, resulting in a maximum tag contribution of 1.1% of body weight. Fish were kept in a pen of ~8,000 conspecifics that had a circumference of 100 m and the net extended 10 m deep. The depth surrounding the site was between 12 and 15 m. The surgeries were performed by the producer's veterinarian, and all experimental protocols were approved by the Dalhousie University Committee on Laboratory Animals (UCLA) (protocol number 20-119).

3.3.2 Surgical Procedure

Individual fish were dip-netted from an aquaculture pen one at a time and placed into an induction tank with seawater and 200 mg L^{-1} tricaine methanesulfonate (TMS). Once fish had lost all signs of consciousness and no longer responded to physical stimulation, they were transferred to a scale to record their weight and length. Fish were then placed on a surgical v-trough, and seawater containing a lighter dose of anesthetic (100 mg L^{-1}) was flushed over the gills for oxygenation and sedation. The surgical procedure is detailed in Chapter 2 but in brief, a 2 cm incision was made posterior to the pectoral fins to implant both the ADST tag and centi-HRT ACT tag. The ADST tag was positioned caudally, while the centi-HRT ACT tag was placed superior to the incision with electrodes oriented for optimal electrocardiogram (ECG) signals. The centi-HRT ACT tag was secured in place using the channel provided, and the incision was closed with interrupted sutures. A fluorescent T-Bar tag was injected through the dorsal fin for fish identification. Following surgery, the fish were moved to a recovery tank fitted with a hose, pumping oxygenated seawater, and monitored until they exhibited normal locomotory behavior. Seventeen fish were tagged, with three unable to recover fully due to an unforeseen tank issue. Real-time acoustic data suggested subsequent mortality for these three fish. Overall, the surgical procedure averaged 8 minutes, and recovery time in the tank averaged 55 minutes.

3.3.3 Tag Programming

Centi-HRT ACT tags were programmed to target periods of expected thermal extremes during a standard production cycle. To capture this, a 1-hour sampling rate was used immediately following surgery for 2 weeks to observe fish recovery from July 30th to August 16th, 2021. Following this, a 4-week period from August 16th – September 16th, 2021, of 20-minute samples was used to capture high frequency data during potential warm thermal events. From the 16th of September onwards, a reduced rate of 6-hours was used to conserve battery life and memory storage. The summer period was considered finished in mid-November, after the end of the long-term heat event (see below), equating to a study period of 124 days. Although data collection continued until March 31st, 2022, winter data was outside the scope of this work and only analyzed in Chapter 2.

3.3.4 Farm Data

To provide context to the biologger measurements, logs of farm operations data were collected throughout the study period. Anytime divers were on site for maintenance work or mortality assessments, a time sheet was logged, and this data was combined with other site reports recording net cleaning operations, general site maintenance and predator net removal to create an operational time series. Feeding times and amounts were also recorded and used as an hourly time series in the analysis. In addition to the farm logs, 5 real time environmental monitoring sensors (aquaMeasure DOTD, Innovasea, Halifax, Canada) measuring dissolved oxygen, temperature and depth were deployed on a vertical profile line 1.5 m apart at approximately 2, 3.5, 5, 6.5 and, 8m. Data was transmitted acoustically every 2-3 minutes and logged internally every 5 minutes for the duration of the study.

3.3.5 Data Processing and Analysis

Fish were harvested on July 26th, 2022, but only seven fish with tags were retrieved and used in the analysis. The remaining seven fish were not found, and it is unknown what happened to them, but from the acoustic data, five of the remaining seven were reporting normal data at the end of March 2022, so they were not considered mortalities. The average weight at harvest was 7.46 ± 0.40 kg and the average length was 75.34 ± 2.91 cm representing a 98% weight increase and a 23% length increase (see fish biometrics in Table D1). Fish condition was assessed using the condition factor (K):

$$K = \frac{W \times 10^5}{L^3} \qquad \text{(Eq. 1)}$$

where *W* and *L* are weight (g) and length (mm), respectively. The condition factor was recorded at the time of tagging and after harvest and all values of K (K > 1.4) indicated good or excellent condition in all fish except F14, which was classed as poor at the time of harvest only (1.0 < K < 1.2). For all analyses, data were processed using the tsclean() function in the R forecast package (Hyndman et al., 2022) and data were averaged hourly, daily or by event for each individual fish and then combined for subsequent analysis.

To explore periods of thermal stress, heat maps were generated by filling in missing data points at each depth over time and then upsampled over the water column using linear interpolation to smooth the transition across depths. In September and October, two distinct periods of elevated temperature were observed and classified by an acute increase followed by a swift decrease in temperature throughout the entire water column. The start and end points of these heat events were manually selected based on the sharpest rate of change between subsequent points. The average response in heart rate and acceleration during the heat events was compared to non-heat event periods for each fish using a type II regression to account for two independent variables. External acceleration of all individuals followed a skewed distribution, so data was log transformed and tested for normality using the Shapiro-Wilks test prior to averaging for all subsequent analyses. The slope and intercept of the regression were statistically compared to 1 and 0, respectively, to determine the effect of the heat event. Further statistical analyses were conducted to assess the impact of the individual heat events by comparing them to the time periods immediately before and after each thermal event. The before and after periods for the short-term heat event (STHE) were selected to be the same duration as the heat event (2.5 days), however due to the nature of the second, long-term heat event (LTHE), the before and after periods were determined to be half (17 days) of the duration of the heat event (34 days) to avoid overlap with the first short-term heat event. Comparisons of heart rate, log-transformed external acceleration, and temperature were made before, during, and after each heat event using the Friedman test and post-hoc analysis was done using the Nemenyi test. The Friedman test is a non-parametric rank-sum test between three of more paired groups and the Nemenyi post-hoc is used to test significance between paired groups. The number of times fish were fed per day was also averaged for each period.

To analyze the effects of feeding and farm operations on behaviour and physiology, daily averages of all biologger data were calculated using daytime data (subset using hourly rounded sunrise and sunset times) to remove the daytime bias of feeding and operations. To remove the confounding effect of temperature in this analysis, heart rate was corrected using the Arrhenius temperature (T_A) (Eq. 2):

$$f_H(T) = f_{H(obs)}\, exp\left(\frac{T_A}{T_{obs}} - \frac{T_A}{T}\right) \qquad \text{Eq. 2}$$

where f_H is heart rate, T_{obs} is the observed temperature in Kelvin, T_A is the Arrhenius temperature ($T_A = 1663$, Chapter 2) and T is the reference temperature that heart rate is being corrected to, in this case, 13.9 °C was used because it was the average temperature observed from August to mid-November, the full extent of the period considered in this analysis. To determine whether there were any statistically significant differences in heart rate and log-transformed acceleration during all combinations of feeding vs. non-feeding periods and during operations vs non-operations periods, a Friedman test and *post hoc* Nemenyi test were carried out. P-values obtained from any statistical analysis were considered statistically significant if $P < 0.05$. All statistical analyses were performed in RStudio v. 4.2.2, RStudio Inc., Boston, MA, USA; https://www.rstudio.com/).

3.4 Results

3.4.1 Environmental Conditions

Temperature had little variation throughout the water column and averaged 13.1 ± 2.8 °C from August to November, with a maximum temperature of 21.5 °C occurring on August 13th and a minimum temperature of 6.4 °C occurring on September 14th (Figure 3.1a). The maximum daily average was 18.3 °C on August 29th and the minimum daily average temperature was 9.0 °C on September 13th. Oxygen saturation ranged from 62.3 to 147.0 % throughout this period; values over 140% can be indicative of biofouling and these values were predominantly apparent at the surface where sensors are most affected (Figure 3.1b). Oxygen displayed high variability day to day but showed no distinct seasonal trends. When oxygen was stratified throughout the water column, oxygen was generally higher at the surface and there were only three days throughout the period where oxygen dropped below 70%. The average oxygen saturation throughout the water column was 102.7 ± 6.6% from August to November with a maximum daily average of 118.1 % on August 27th and a minimum daily average of 88.5 % on November 20th.

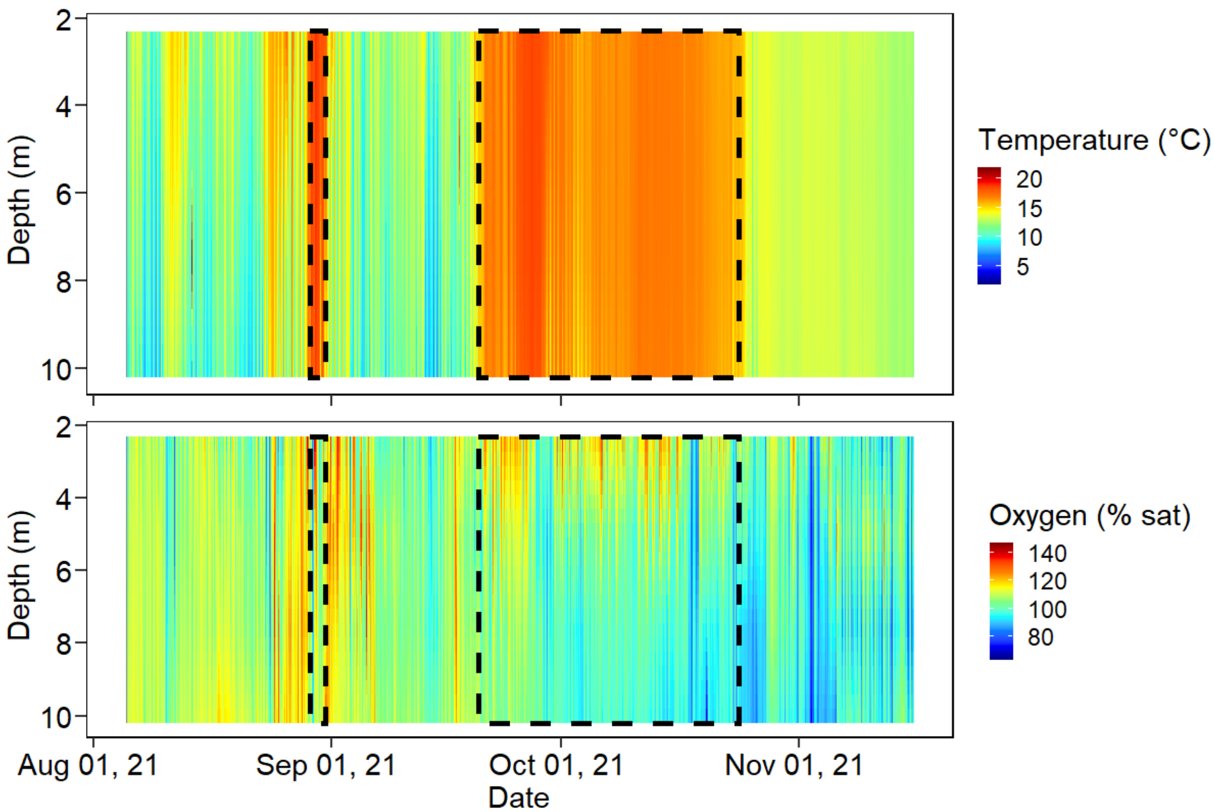

Figure 3. 1 Environmental data from cage 14 throughout the duration of the summer period from 5 oxygen, temperature and depth sensors on a profile line separated by 1.5m a) Water temperature [°C]. b) Oxygen [% Saturation]

3.4.2 Response to Heat Events

A general comparison between the average heart rate of individual fish during the periods with heat events and the rest of the study period revealed a positive linear relationship ($r^2 = 0.85$, $p < 0.01$, Figure 3.2a), suggesting that variation between individual responses persists under the effect of thermal events. The linear relationship between the heart rate of individuals under both conditions revealed that the slope was significantly lower than 1 ($p < 0.05$), but the intercept was not ($p = 0.08$). This suggests that individuals with naturally higher heart rate could be restricted in their capacity to proportionally increase heart rate in response to thermal stress, in contrast to those with naturally lower heart rates. The same comparison between log-transformed average external acceleration during heat events and the rest of the study period was carried out and revealed a similar positive relationship ($r^2 = 0.80$, Figure 3.2b). The slope of the linear relationship between the log-transformed acceleration of individuals was significantly lower than 1 ($p =< 0.001$) and, the intercept was also statistically different than 0 ($p < 0.01$), suggesting that fish activity was consistently higher under normal conditions than under thermal stress but the change in activity was not proportional to normal conditions across individuals.

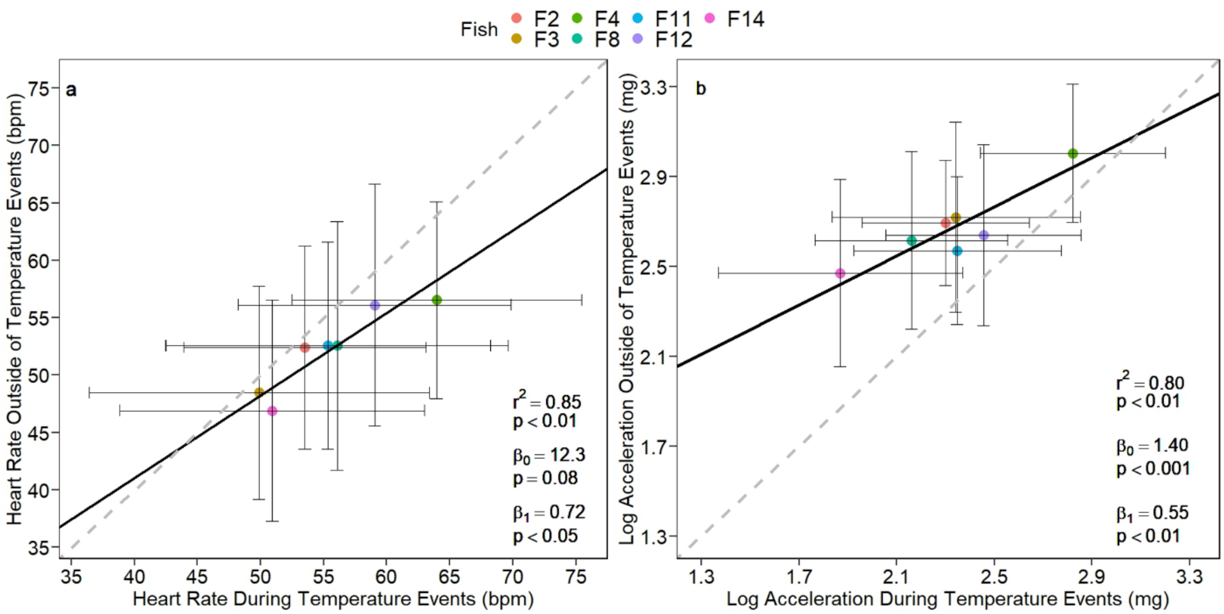

Figure 3. 2 Type II regression analysis of (a) f_H [bpm] and (b) log-transformed acceleration [mg] of individual fish during the heat events and not during the heat events. Dashed line shows 1:1 relationship. Regression slope and intercept were tested against 1 and 0, respectively, and estimates of the intercept (β_0) and slope (β_1) are presented on the plot

A detailed analysis of the heat events with the periods immediately before and after the event revealed statistical differences between heart rate for both the LTHE and the STHE event ($P < 0.001$, Table 3.1). The STHE occurred at the end of August over a period of 2.5 days starting August 28th, and it was defined by an acute temperature change (> 2 °C) compared to the 2.5 days before and after the event (during vs. after: $p <0.001$, Figure 3.3a). The LTHE started on September 20th and lasted until October 24th, totalling 34 days, and was similarly defined by a temperature difference > 2 °C during the event, which was significantly different to the temperature before the event ($p < 0.001$, Figure 3.3b). The severity of the LTHE was less

*Table 3. 1 Average temperature, heart rate and log-transformed acceleration during both the STHE and the LTHE. Test statistic is for Friedman's test comparing between the three groups and the number of stars represents the level of significance * < 0.05, ** < 0.01, *** < 0.001.*

Heat Event	**Fish Variable**	**Mean Before**	**Mean During**	**Mean After**	**Friedman Test Statistic**	**P-Value**
Short-Term Heat Event (STHE)	Temperature (°C)	15.5 ± 0.4	18.1 ± 0.6	14.9 ± 0.4	14	***
	Heart Rate (bpm)	59 ± 3.14	69 ± 4.66	54 ± 5.17	14	***
	Log Acceleration (*mg*)	2.4 ± 0.2	2.4 ± 0.3	2.7 ± 0.2	10.6	**
Long-Term Heat Event (LTHE)	Temperature (°C)	12.7 ± 0.1	16.9 ± 0.1	13.5 ± 0.0	14	***
	Heart Rate (bpm)	48 ± 4.4	49 ± 5.5	55 ± 8.4	6	*
	Log Acceleration (*mg*)	2.7 ± 0.2	2.3 ± 0.3	2.6 ± 0.3	12.3	**

than the STHE (16.9 °C vs 18.1 °C); however, the 34-day duration made it noteworthy. Heart rate increased during the STHE from 59 to 69 bpm and dropped to 53 bpm following the event ($p < 0.001$, Table 3.1) and *post hoc* analysis revealed that the decrease following the event was significant ($p < 0.001$, Figure 3.3c). Similarly, there were significant differences in heart rate over the LTHE ($P < 0.05$, Table 3.1); however, in contrast to the response to the STHE, *post hoc* analysis showed heart rate increased significantly following the LTHE from 49 to 55 bpm ($p < 0.05$, Figure 3.3d). Regarding log-transformed external acceleration, there were significant differences throughout both the STHE and the LTHE ($p < 0.01$, Table 3.1). *Post hoc* analysis revealed that it significantly increased from 2.4 mg before and during the STHE to 2.7 mg after the event ($p < 0.05$ and $p < 0.01$, Figure 3.4.3e) and significantly decreased from 2.7 mg before the LTHE to 2.3 mg during the event ($p < 0.01$, Figure 3.3f). Daily feed regimens were recorded throughout the duration of both events and were reported as an average for each period, and in both cases, the daily rate was lower during the events compared to the periods before and after (Figure 3.3g, 3.3h).

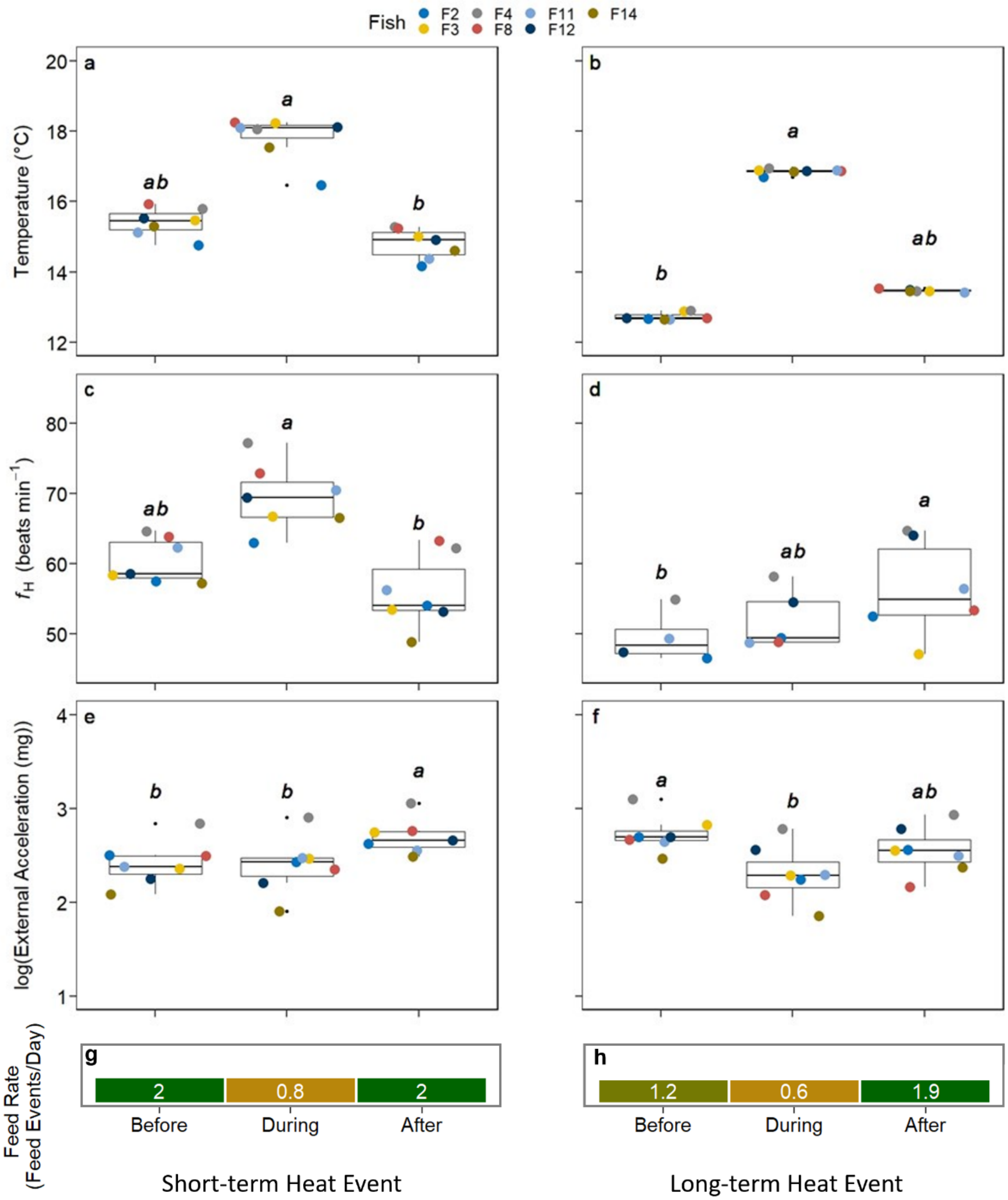

Figure 3. 3 Comparison of fish observations before, during and after a short and long term heat event. Colored points indicate the individual fish average. All comparisons were made with the Friedman test and post-hoc analysis was done using the Nemenyi test. Significant differences are indicated by lettering. All comparisons were made between the three periods before, during, and after a) STHE tempertaure [°C] b) LTHE tempertaure [°C]. c) STHE f_H [bpm] d) LTHE f_H [bpm]. e) STHE log-transformed external acceleration [mg] f) LTHE log-transformed external acceleration [mg] g) STHE feed rate [feed events/day] h) LTHE feed rate [feed events/day]

3.4.3 Response to Feeding and Operations

Temperature corrected heart rate was used to compare the effect of feeding and other farm operations on heart rate. Significant statistical differences emerged when comparing the four categories of feeding and operations, feeding and no operations, operations and no feeding and no feeding and no operations (Friedman chi-squared: 19.97, $p < 0.001$). The combined effect of feeding and operations made heart rate significantly higher than periods when no feeding was occurring regardless of operations ($p < 0.001$ when neither feeding nor operations were occurring and $p < 0.05$ for non feeding periods when operations were occurring, Figure 3.4a). When operations were not occurring, heart rate was significantly elevated during periods of feeding compared to non-feeding periods ($p < 0.05$). Similarly, significant statistical differences emerged when comparing the log-transformed external acceleration between the same four categories combining feeding and operations (Friedman chi-squared: 15.51, $p < 0.001$). Logged acceleration was higher during feeding periods only when there were no operations co-occurring compared to non feeding periods when operations were occurring ($p < 0.01$) and when no operations were occurring ($p < 0.05$, Figure 3.4b).

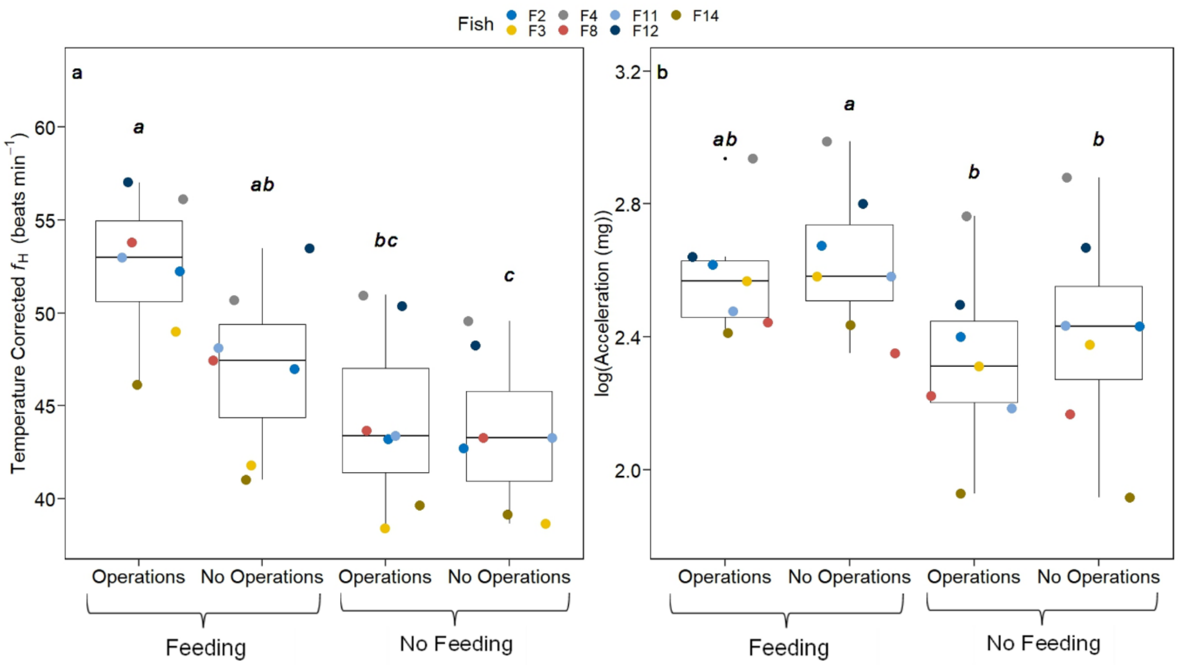

Figure 3. 4 Comparison of fish observations between all combinations of when operations and feeding events are and are not occurring. Colored points indicate the individual fish averages. All comparisons were made with the Friedman test and post-hoc analysis was done using the Nemenyi test. Significant differences among groups are presented by lettering. a) f_H [bpm] b) Log-transformed external acceleration [mg]

3.5 Discussion

The analysis of the physiology and behaviour of Atlantic salmon using biologgers enabled an examination of the complex interactions between thermal heat events and commercial farm operations. Fish exhibited distinct responses in heart rate and log-transformed external acceleration (hereafter activity) to a short-term heat event (STHE) compared to a long-term heat event (LTHE), and fish showed physiological and behavioural responses consistent with secondary stress response during the STHE (i.e. increased heart rate) and tertiary response during the LTHE (i.e. decreased activity). Generally, feeding had a stronger effect than operations and caused both heart rate and activity to increase, and operations increased heart rate but caused a decrease in activity; however, the interaction between feeding and operations on heart rate and activity was more complex, and caused varied responses.

During the STHE, temperature reached a maximum of 19.2 °C and averaged 18.1 ± 0.6 °C. From a physiological perspective, heart rate followed the expected response to the thermal fluctuations over the course of the STHE, increasing cardiac output via heart rate to meet increased metabolic demands at higher temperatures (Gamperl et al., 2011). In a separate analysis of these data over the full temperature range, i.e. including the winter months, heart rate scope was reduced substantially at temperatures greater than 18 °C, suggesting proximity to thresholds of thermal stress during the STHE (see Chapter 2). These temperatures are also comparable to those that have been cited as near the upper thermal tolerance for Atlantic salmon, specifically those used for commercial aquaculture in Canada, above which negative effects on their appetite, growth and stress have been observed (Gamperl et al., 2020). The efficiency of salmon growth at or above 19 °C varies across studies in different regions, underpinning the importance of research being carried out on specific stocks to inform aquaculture management in different regions (Hevrøy et al., 2015; Nuez-Ortin et al., 2018; Tromp et al., 2018). Therefore, the temperature regime observed during the STHE exposed the individuals to their upper thermal limit.

During the LTHE, temperature reached a maximum of 18.8 °C and averaged 16.9 ± 0.6 °C over a 34-day period. Contrary to STHE, heart rate did not follow the expected response with temperature and, instead, heart rate increased after the event, when temperatures dropped. Observations of heart rate under chronic thermal stress are limited in the literature; however, long-term exposure (over 1 month) to elevated temperatures (19-20 °C) has shown a reduction in energy body reserves, reduced feed intake and increased feed conversion ratio (Gamperl et al., 2020; Hevrøy et al., 2012; Koskela et al., 1997). The effects of chronic exposure to elevated temperature on growth vary depending on the length and severity of the exposure, size of fish and development stage and notably, larger, post-smolt salmon show reduced growth at chronically high temperatures compared to juveniles (Handeland et al., 2008, Hevrøy et al., 2012; Norambuena et al., 2016). During periods of long-term thermal stress, heart rate cannot be sustained at elevated levels, and the reallocation of energy can become detrimental to fish, resulting in these tertiary level stress responses (Handeland et al., 2008; Opinion et al., 2023). Accordingly, the chronic exposure to temperatures approaching stressful limits during the LTHE could cause the observed reduction in heart rate; however, this reduction could also be a consequence of the daily feed rate, which was lower throughout the LTHE compared to the periods before and after. During both the short-term and long-term heat events, management decisions were taken to reduce feeding frequency to reduce stress; a common response in salmon farming to heat stress (e.g. Oppedal et al., 2011). Feeding periods were associated with increased heart rate when direct comparisons were made between the different combinations of operations and feeding. Despite the increase in heart rate as a response to feeding, heart rate increased during the STHE when feed rates were reduced, suggesting that thermal response at temperatures close to their thermal limit had a stronger influence than the absence of feeding. Contrarily, at tolerable yet elevated temperatures during the LTHE, feeding rates had a greater impact on regulating heart rate. As sea surface temperatures continue to increase due to climate change, the severity and frequency of chronic exposure to elevated temperatures is also expected to increase (Klinger et al., 2017) and having a holistic understanding of the implications on stress and growth, particularly as they relate to confounding variables such as feeding, are necessary to inform farming practices.

Fish activity also displayed distinct responses to the STHE and the LTHE. Before and during the STHE, activity was consistent and significantly increased after the event. When exposed to acute temperature shock (greater than 28 °C for up to 5 minutes), Atlantic salmon exhibited intense swimming speed and activity, which was interpreted as a panic response

(Nilsson et al., 2019). Although swimming speed and activity are not the same measurement, many studies have shown a positive correlation between them (Kawabe et al., 2003; Zrini & Gamperl, 2021). Swimming activity has also exhibited an increased response to other stressors common to aquaculture such as crowding and delousing procedures (Svendsen et al., 2021; Føre et al., 2018a). Temperature is a unique stressor as it has both direct and indirect effects (Segner et al., 2012) and while critical swimming speed has been shown to increase when experiencing acute temperature shock, it is possible that the change in temperature was not intense enough to elicit that behavioural response, or other confounding factors such as the reduced feeding rate during the heat event, may have impacted their behavioural response. Further, operations occurred before the STHE, which also may have decreased their activity. Similarly, it is also possible that the increase in activity following the STHE was driven by an increase in the daily feed rate as activity was shown to be higher during feeding periods.

During the LTHE, activity was significantly lower than it was before the event suggesting that chronic thermal stress may have a dampening effect on the overall activity of salmon. When Atlantic salmon were acclimated to warmer temperatures over multiple weeks, swimming speed was shown to increase up to a thermal optimum of 18 °C but started to decrease at higher temperatures (Hvas et al., 2017). A long-term study that recorded swimming speed in response to seasonal changes in temperatures over multiple years, observed a similar swimming speed response curve, with maximum speeds occurring at 10.5 °C and an 80% reduction in swimming speed at temperatures lower than 4 °C and greater than 17 °C (Martin et al., 2012). Non-feeding periods were also associated with decreased activity and the reduction of feeding during the LTHE may have contributed further to lower activity. Under chronic stress, the tertiary stress response can impose a reduction in swimming capacity (Barton, 2002), caused by the increased energy allocation towards maintaining homeostasis and reducing the available energy for regular swimming activity. The effects of chronic stress can become cumulative and limit the ability of salmon to cope with additional stressors (Madaro et al., 2015, Brijs et al., 2019). Accordingly, long-term monitoring of swimming activity can provide insights into behavioural changes over time and identify when tertiary stress may be occurring.

The potential effects of temperature on Atlantic salmon behaviour and physiology have been examined above; however, it is important to recognize that thermal responses are usually confounded with farm operations such as feeding or other environmental conditions. Hypoxic conditions can occur due to oxygen consumption of fish (Solstorm et al 2018), reduced water exchange across the farm driven by tidal cycles (Burke et al., 2021) and temperature induced hypoxia due to the combined effect of increased metabolic demands and the reduction in oxygen solubility of seawater at elevated temperatures (Neubauer & Andersen, 2019). Hypoxic conditions were not observed in the pen during the study period, but they regularly occur in this region during the late summer and fall, and many laboratory studies have examined the combined negative effects of these stressors on growth and survival (Antilla et al., 2013; Gamperl et al., 2020; Remen et al., 2012; Remen et al., 2016). Farm operations is another variable, unique to aquaculture that is often overlooked as a factor to consider in studies exploring the behavioural and physiological response of farmed salmon. The effect of operations had a more nuanced effect on physiology and behaviour, in that it amplified the increase in heart rate when feeding was co-occurring, but there was no effect on acceleration. Operations is a broad term that was used to encapsulate diver activities, net cleaning, predator net removal and other general maintenance so it is reasonable to assume that fish have varied responses to each activity. This analysis suggests that operations are less relevant than feeding but a (non-significant) trend of reduced activity when operations were occurring seemed to emerge. Further, the comparisons between heat events, feeding and operations are limited by the small sample size (n=7). Unfortunately, 7 individuals were not recovered at the time of harvest, which resulted in a loss of half of the potential data. Despite observing significant differences across many comparisons, the extrapolation of conclusions to group-level patterns should be taken with caution, and further research should aim to increase the statistical power of the analyses.

This examination of Atlantic salmon physiology and behavior investigates the complex interplay between thermal conditions, feeding patterns, and farm operations, offering insights with broader implications for precision fish farming. The sensitivity of heart rate fluctuations is a useful indicator for potentially recognizing the secondary stress response in fish. However, it requires careful consideration within the proper context and in conjunction with other variables to accurately determine whether the observed increase in heart rate is due to stress or other drivers. Notably, the findings highlight that feeding and farm operations influence heart rate and acceleration, recognizing that there is nuance in how the irregularity and the type of operation can have various impacts on fish. The findings also demonstrate that there is potential in the use of biologgers as precision farming tools. Further research should focus on the development of industry tools using additional observations and monitoring in commercial net pens to mitigate potentially confounding effects such as land-based environments, stocking densities, and the interaction between environmental stressors and farm operations. This could lead to the development of new fish based OWI's that could promote better recognition of fish stress and welfare determinations in commercial aquaculture. As we better understand how fish respond to various and complex stressors, these insights pave the way for more informed and tailored approaches in aquaculture management, contributing to the ongoing development of efficient and responsible practices in commercial fish farming.

3.6 Conclusions

The analysis of Atlantic salmon physiology and behavior in the context of thermal heat events on commercial farms provides valuable insights that can inform industry practices. The study revealed distinct responses in heart rate and activity during short- and long-term heat events, representative of effects consistent with different phases of the stress response. A comprehensive understanding of conditions that lead to stress or behaviors that are indicative of stress is crucial for farm management and demonstrates the potential of individual-based measurements that could be incorporated as OWI's. Specifically, the study found that fish exhibited physiological responses consistent with secondary stress during a short-term heat event and behaviors that have been associated with tertiary stress during long-term heat events, however, average temperatures during both events were lower than previously reported limits of thermal stress. This highlights the importance of monitoring fish response in commercial settings in order to estimate thermally challenging conditions for local aquaculture operations.

The observed temperatures, particularly during the short-term heat event, approached thermal limits for Atlantic salmon, which also has detrimental effects on fish appetite and growth. As sea surface temperatures continue to rise due to climate change, the frequency and severity of thermal stress events are expected to increase, underscoring the need for proactive management strategies to mitigate these effects. Increased heart rate or water temperature above a certain threshold could inform operators to activate aeration or oxygenation systems that promote mixing within the water column to reduce surface temperatures. Furthermore, the study demonstrates the complex interplay between feeding patterns, farm operations, and fish physiology and behavior. The findings emphasize the importance of considering multiple variables in aquaculture management decisions to optimize fish welfare and performance. The results demonstrate the potential of biologgers as precision farming tools to monitor fish health and welfare in commercial net pens. Future research should focus on further developing these tools and integrating additional observations to improve stress recognition and welfare determinations in commercial aquaculture settings, ultimately contributing to the advancement of responsible and efficient fish farming practices.

CHAPTER 4: DISCUSSION

The use of biologgers, measuring tri-axis acceleration, depth, heart rate, and temperature, offers a direct and nuanced approach to monitor fish in a complex 3D environment. The biologgers enabled the study of the physiological and behavioral responses of Atlantic salmon in commercial aquaculture under the influence of seasonal thermal extremes, providing valuable insights into the intricate dynamics of fish health and production efficiency in the face of climate change. The ability to estimate optimal temperatures using apparent heart rate scope and Arrhenius breakpoint temperature presents the possibility of using commercially available tools to measure thermal stress limits on commercial farms. The estimate of optimal temperature derived from this work, 12.7 °C, is comparable to those that have been identified for Atlantic salmon, which range between 13 – 18 °C (Dwyer & Piper, 1987; Handeland et al., 2008; Hevrøy et al., 2012; Koskela et al., 1997). In addition, the reduction in heart rate scope at temperatures lower than 2 °C and greater than 19 °C were used to identify thermal stress limits and these values are comparable to other reported thresholds of thermal stress (1 and 20 °C from Vadeboncoeur et al., 2023 and DFO, 2012, respectively). During the STHE, fish showed signs of secondary stress, increasing cardiac output via heart rate to meet increased metabolic demands that occur at elevated temperatures (Gamperl et al., 2011). Alternatively, during the LTHE, heart rate did not increase in response to temperature. It was hypothesized that this was caused by the long-term nature of the event as fish tried to reallocate energy reserves (Handeland et al., 2008) or, because of the impact of the reduced feeding schedule that occurred throughout the event. Heart rate and activity have been shown to increase in response to feeding (Føre et al., 2011; Hvas et al., 2020) and this was also observed during the feeding periods throughout the study, and this underscores the importance of the consideration of confounding effects of temperature stress and feeding regimes on fish physiology and behaviour. The results suggest that at temperatures near thermal stress limits, like those observed during the STHE, temperature plays a larger role than feeding in regulating heart rate; however, at elevated but tolerable temperatures, feeding may have a stronger effect. Estimates of optimal temperature and thermal stress limits can help to inform regulators about current and prospective sites and their suitability in relation to the optimal and thermal stress thresholds of cultured fish in Canada. Understanding how feeding and farm operations impact the effects of thermally challenging water temperature can help to inform farm management decision-making during stressful conditions. Bridging the gap between controlled laboratory studies and real-world commercial operations, this research emphasizes the importance of considering the interplay between thermal stress and farm conditions. The findings contribute not only to the fundamental understanding of Atlantic salmon physiology and behavior but also to the broader concept of precision fish farming, where biologgers could emerge as indispensable tools for early stress detection, empowering timely and informed farm management decisions to enhance fish welfare and production efficiency in the evolving landscape of aquaculture.

4.1 Lessons Learned

The project was planned amidst the unique challenges of conducting research within a commercial setting, where control and experimental design are inherently constrained, and operational decisions wield a substantial influence. Navigating the dynamic environment of a salmon farm underlined the need for adaptability and resilience. The initial surgery and harvest dates were unpredictable due to the nature of daily operations, emphasizing the importance of flexibility to navigate unforeseen circumstances. This aspect of the project highlighted the balance between the rigor of controlled experiments and the pragmatic realities of commercial aquaculture, shedding light on the specific challenges faced when conducting scientific investigations within operational constraints; however, the results reflect the commercial environment and provide direct observations of fish behaviour and physiology on operational farms.

The project also underlined the importance of thorough documentation when collaborating with industry. Farm records can be complex and as most farms only make use of data for operational decisions, acquiring properly documented and annotated datasets for historical production cycles can be challenging. For example, feeding records were stored on a local server that malfunctioned, which delayed access to some aspects of project data for many months. It is in the farm's best interest to keep digital records of their production cycle data for historical analysis. Digitization of farm data is increasing, and good record-keeping strategies and organized databases are important for both research and industry applications. Future collaborations with industry should emphasize strong data management from the initial stages of projects to facilitate organized datasets, and while more cumbersome, it may be beneficial to amass farm data on a regular basis throughout the project rather than all at once at the culmination of the data collection period.

Finally, this research highlighted that collaboration amongst industry and academia is a valuable way to both provide new information to farm management and advance our understanding of the robustness of an important commercial industry. Producers that participate in research to better understand how their stocks might be affected by changes to their environment caused by climate change, are better equipped to anticipate and mitigate potential challenges preemptively. Research that can provide critical information such as optimal growth temperature and limits of thermal stress can help to determine the suitability of current sites and inform future site selection. Finally, understanding the impact of management decisions such as operations planning and feed regimes during thermally challenging conditions can help reduce fish stress and facilitate better operations scheduling.

4.2 Conclusions

In conclusion, this thesis underscores the importance of understanding the complex interactions between Atlantic salmon physiology and environmental factors, particularly in the face of climate change and its impact on commercial aquaculture. The established global market for Atlantic salmon necessitates a thorough examination of the challenges posed by rising sea temperatures and weather variability, as these factors significantly influence fish health and production efficiency. By delving into the physiological and behavioral responses of Atlantic salmon during a standard production cycle, this study helps to bridge the gap between thermal stress examinations in controlled laboratory studies and real-world commercial operations. The utilization of biologgers for direct observations proves to be a useful tool, providing insights into the effects of combined environmental stressors and farm operations.

The findings of this research contribute to the understanding of thermal conditions influencing Atlantic salmon in the North American maritime provinces. The estimation of optimal temperatures using apparent heart rate scope and Arrhenius breakpoint temperature offers practical application of commercial tools to determine optimal and thermal stress limits. Notably, the study identified optimal temperature at 12.7 °C and temperature thresholds < 2 °C and > 19 °C, below and above which, the apparent heart rate scope diminished, signifying proximity to critical temperature thresholds. The comparisons between periods with and without operations and feeding, along with an examination of both a short-term and long-term thermal stress event, provides a comprehensive analysis of fish responses under varying conditions common to commercial aquaculture settings. The findings emphasize, from a physiological and behavioral standpoint, that the decision to moderate feeding during periods of thermal stress can contribute to a reduction in heart rate and activity, thereby reducing the metabolic demand.

Despite the novelty of this data there are challenges associated with carrying out research on commercial farms and the application of the findings to operational decision making, notably the lack of experimental control and the access to biologger data as real time information. The intention was to capture environmentally challenging conditions on aquaculture farms, however there was no ability to schedule when stressful conditions would occur, and often research is interested in extreme conditions, which are more unpredictable and less frequent. In addition, there is little ability to set up controls for statistical comparisons, but this trade-off can be meaningful when there is no need to extrapolate results to real-world settings. The effort required to obtain data from biologgers is not insignificant despite the unique information they can provide to inform decision making, and more complex measurements such as heart rate and acceleration are currently only available when a tag is retrieved, not real time. As technology continues to advance, these limitations may be overcome, and carefully planned research on commercial farms will be instrumental in understanding the unique challenges of farmed fish. Future research should examine how this data could be included as new welfare indicators and examine their integration with other commercially available data to develop more sophisticated and direct indicators.

As part of the broader concept of precision fish farming, this research contributes to advancing the knowledge of fish physiology and behaviour of Atlantic salmon using novel biologgers. It demonstrates the potential thermal vulnerabilities of this ecologically and economically vital species, highlighting the reliance of optimal food production systems on environmental conditions that reflect closest optimal temperatures. This thesis emphasizes the need for strategic planning in research design, adaptability to operational constraints, and collaboration between laboratory and field-based research efforts. Ultimately, the insights gained from this research are instrumental in informing farm management and government regulators on the susceptibility of Atlantic salmon to environmental stressors on commercial farms and in the ever-evolving landscape of modern, commercial aquaculture, these findings promote mechanisms to improve fish welfare and production efficiency.

Appendix A

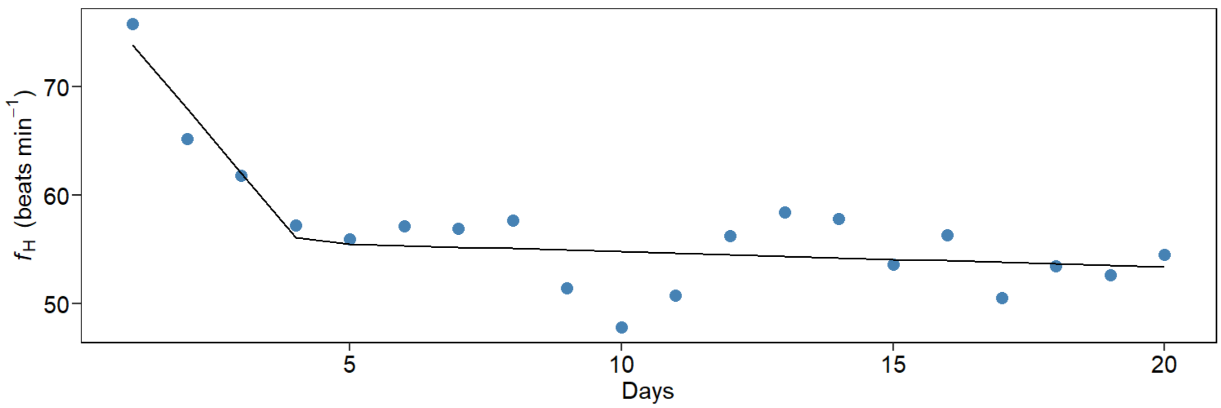

Figure A.1 Daily average heart rate following tag surgery with breakpoint regression analysis which estimated the break point to be on day 4 (August 2nd, 2021) (P < 0.001), where the slope changed from -5.9 to -0.14.

Appendix B

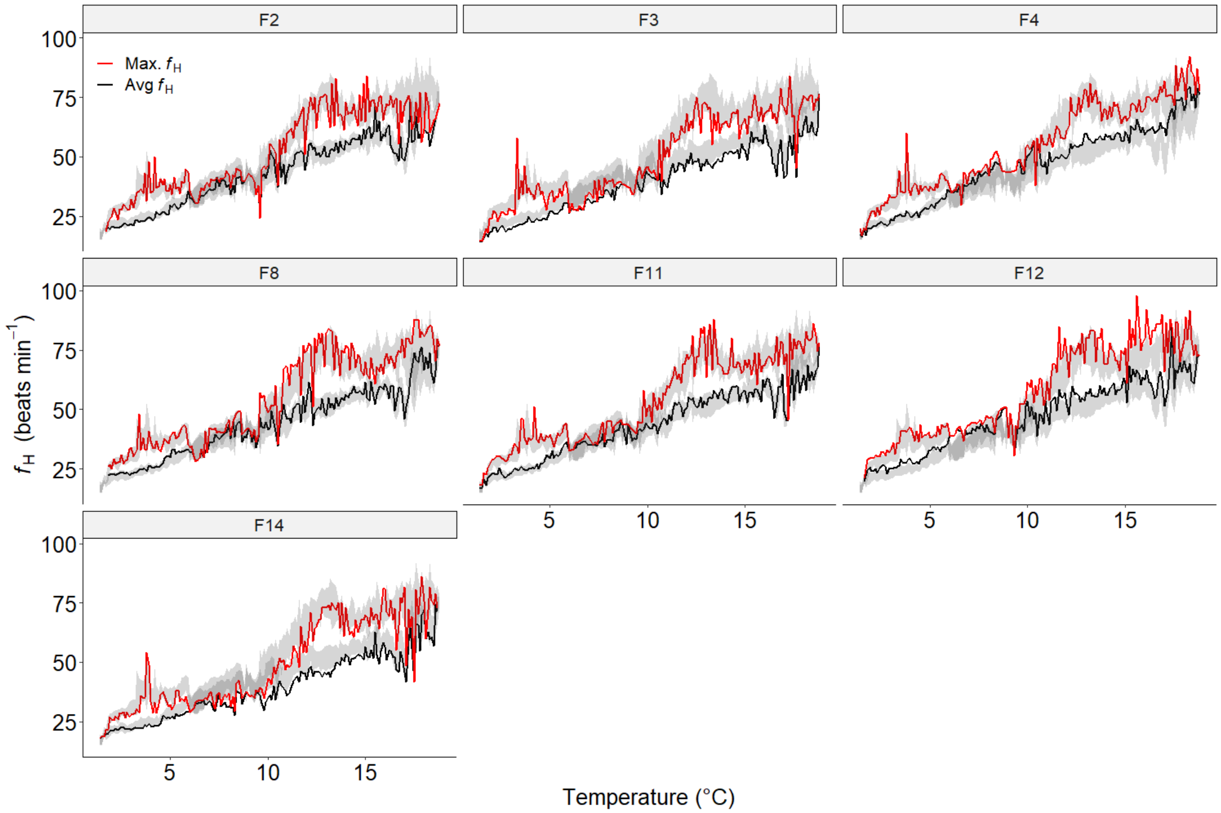

Figure B.1 Maximum and average heart rate over the full range of observed temperatures for each individual fish. Grey shaded area represents ± 1 standard deviation of the average of all fish.

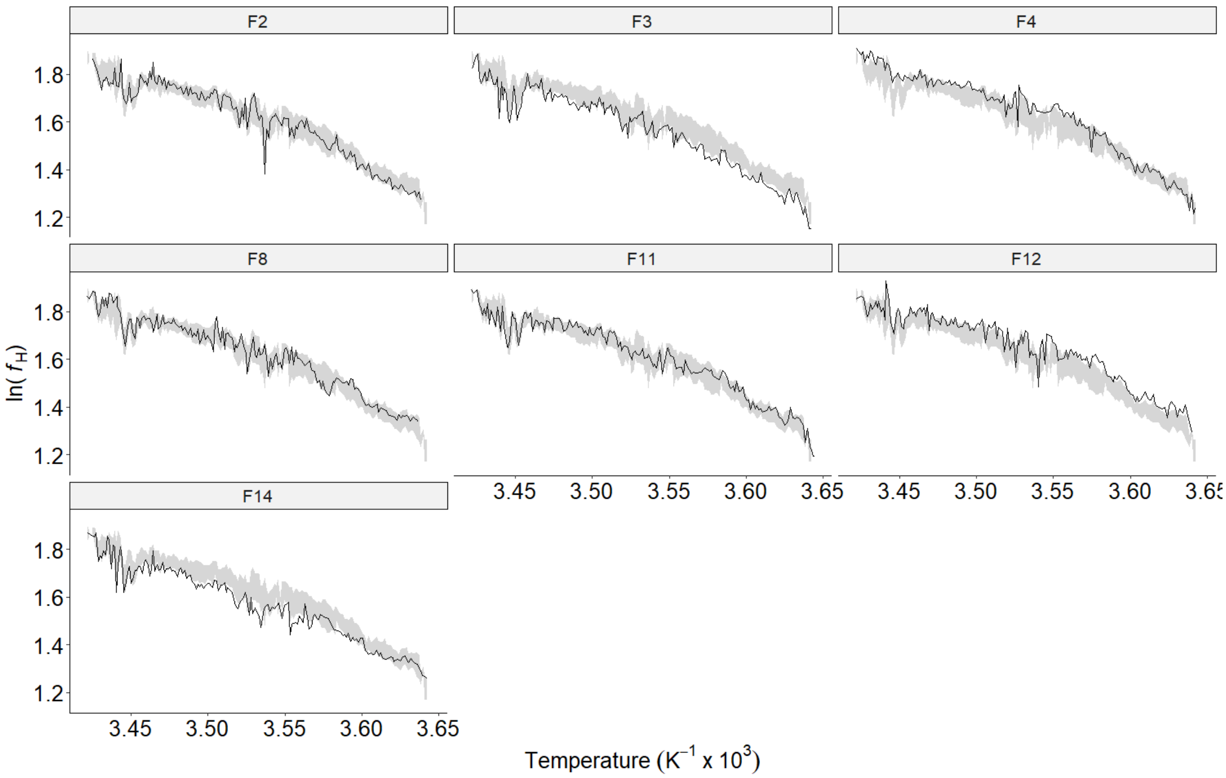

Figure B.2 Arrhenius plot for the average heart rate over the full range of observed temperatures for each individual fish. Grey shaded area represents ± 1 standard deviation of the average of all fish.

Appendix C

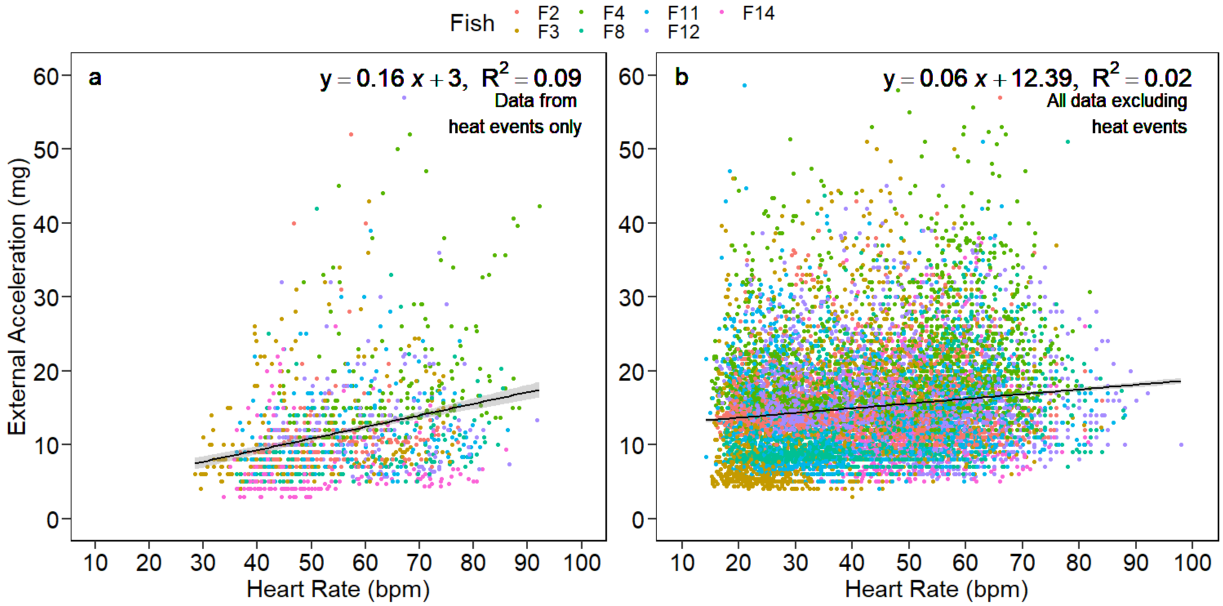

Figure C.1 Correlation between f_H [bpm] and acceleration [mg]. Colored points indicate individual fish a) Data from heat events only. b) All other data excluding heat events.

Appendix D

Table D.1 Weight (kg), length (cm) and condition factors for all fish at the time of tagging and for the individuals recovered when tags were retrieved at the time of harvest.

Fish	Weight (kg) (Tagging)	Length (cm) (Tagging)	K Factor (Tagging)	Weight (kg) (Recovery)	Length (cm) (Recovery)	K Factor (Recovery)
F1	2.70	70	0.79	-	-	-
F2	3.45	62	1.45	7.40	69	2.25
F3	3.70	62	1.55	8.10	79	1.64
F4	4.70	66	1.63	7.30	69	2.22
F5	3.80	58	1.95	-	-	-
F6	3.20	58	1.64	-	-	-
F7	3.50	60	1.62	-	-	-
F8	3.50	61	1.54	6.00	73	1.54
F9	4.70	65	1.71	-	-	-
F10	2.80	55	1.68	-	-	-
F11	3.20	57	1.73	6.40	68	2.04
F12	5.00	66	1.74	9.20	81	1.73
F13	4.20	61	1.85	-	-	-
F14	4.70	66	1.63	7.80	88	1.12
F15	4.00	55	2.40	-	-	-
F16	3.30	56	1.88	-	-	-
F17	3.50	60	1.62	-	-	-

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