Usage of Internal Heart Rate Bio-Loggers in Arctic Fish

MASTER THESIS

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN MARINE BIOLOGY

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Date of submission: 14th of December 2021

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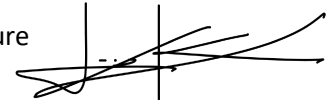Name: Luis Kuchenmüller   Enrolment number: 3 2 1 0 4 0 8

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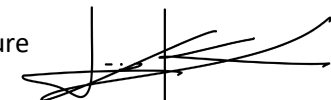Date: 1 4 . 1 2 . 2 0 2 1   Signature

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List of Abbreviations

α	O_2 solubility coefficient
A	Mass exponent describing the relationship of oxygen consumption and body weight
ANOVA	Analysis of Variance
AWI	Alfred-Wegener-Institute Helmholtz Center for Polar and Marine Research
BL	Body length

BW	Body weight
CLMM	Cumulative Link Mixed Model
C_aO_2	Oxygen content of arterial blood
C_vO_2	Oxygen content of mixed venous blood
DST	Data Storage Tag
ECG	Electrocardiography/ Electrocardiogram
EMG	Electromyography/ Electromyogram
EPOC	Excess Post-exercise Oxygen Consumption
f_H	Heart rate
Δf_H	Difference between manually calculated and on-board processed heart rates
GEEGLM	Generalized Estimation Equation Generalized Linear Model
GLMM	Generalized Linear Mixed-effects Model
HRT	Heart Rate and Temperature
IPCC	Intergovernmental Panel on Climate Change
MMR	Maximum Metabolic Rate
M_{O2}	Oxygen consumption rate (mass-related)
MS222	Tricaine mesylate
OCLTT	Oxygen and Capacity Limited Thermal Tolerance
pO_2	Oxygen partial pressure
QI	Logger-assigned Quality Index
QRS	Sequence of Q-wave, R-wave, and S-wave
RAM	Random-Access Memory
RCP	Representative Concentration Pathway
RMR	Routine Metabolic Rate
SMR	Standard Metabolic Rate
SSP	Shared Socioeconomic Pathway
T_{crit}	Critical temperature
T_p	Pejus temperature
U_{crit}	Critical swimming velocity
V_{O2}	Oxygen consumption rate (volume-related)
V_s	Stroke volume of the heart

Summary

By anthropogenic cause, even the most optimistic climate models (i.e. SSP1–RCP2.6) predict the Arctic system to heat up by more than 4°C until the year 2100, relative to the present. For ectothermic fishes, energy demand is fundamentally determined by temperature. As energy is physiologically limiting, their means to cope with climate change are limited. Therefore, understanding the impact of environmental changes on bioenergetics is imperative for the management of marine ecosystems. In recent years, the species-specific relevance of heart rate (f_H) as a proxy for energy expenditure has been highlighted by the scientific community. The advent of bio-logging sciences has enabled f_H observation in free swimming individuals. For Arctic fishes, however, harsh environmental conditions have restricted the pursue of f_H bio-logging so far. To bridge this knowledge gap, we partnered with Star-Oddi, who developed a novel, internal f_H and temperature bio-logger, calibrated for temperatures down to −5°C. In the present study, this bio-logger was implanted in the cold-adapted Arctic specialist polar cod (*Boreogadus saida*) and the f_H bio-logging methodology was progressed in simulation of the ecologically relevant temperature range (i.e. 0 to 8°C) and free-roaming exercise (i.e. critical swimming speed (U_{crit}) tests).

Bio-logger positioning with exterior-facing electrodes and increase in sampling frequency from 100 Hz to 125 Hz improved electrocardiogram (ECG) quality significantly ($p < 0.0001$ and $p = 0.02$, respectively), due to decreased electromyogram (EMG) noise penetration and more distinct mapping of processed ECG characteristics. Under these settings, in the range of 0 to 4°C, in relation to 1180 manually calculated ECG traces, $80 \pm 1.5\%$ of on-board processed f_H measurements displayed highest quality (i.e. QI = 0) with a confidence of $\Delta f_H = 0.45 \pm 0.56$ bpm. Furthermore, $53 \pm 5.5\%$ of measurements displayed highest quality homogenously across swimming velocities up to U_{crit}. Hence, present f_H bio-logging methodology was validated to be highly robust in response to simulated Arctic conditions.

Species-specifically for polar cod, standard metabolic rate (SMR) of bio-logged individuals at 0 ± 0.5°C amounted to 0.38 μmol/g/h. Therewith, it was lower than the previously determined 0.44 μmol/g/h for untagged conspecifics at 2.5 ± 1°C, indicating that present measurements were representative, especially given expected deviation from Q_{10} rules due to oxygen demands of cold adaptations. Polar cod f_H was highly sensitive to, and consequently significantly impacted by, both temperature and swimming velocity (each with $p < 0.0001$). Remarkably, f_H at U_{crit} mirrored f_{Hmax} values previously obtained at the same temperatures by humoral injections, supporting causal relationship of f_{Hmax} and consequent performance limitations. Further, incremental f_H Q_{10} values decreased from 2.54 ± 0.76 at 0–4°C to 2.00 ± 0.50 at 6°C

and 1.73 ± 0.74 at 8°C. Hence, polar cod f_H started failing to scale with temperatures past 4–6°C, which in accordance with previously described temperature ranges and susceptibilities, potentially indicated the transition to passive thermal tolerance. Overall, oxygen consumption was significantly correlated to f_H with a spearman rank correlation coefficient rho = 0.42. Lastly, the interaction of swimming velocity and temperature did not significantly impact M_{O2} (p = 0.71) and the relationship's slopes displayed high similarity between 0, 2, and 8°C. In conclusion, the contribution of f_H in regulation, and ultimately limitation, of oxygen supply in response to temperature- and performance-related energy demand, was determined as highly probable. Therefore, the potential of f_H as a proxy for energy expenditure in polar cod was highlighted over the course of the present research.

Zusammenfassung

Selbst die optimistischsten Klimamodelle (SSP1–RCP2.6) sagen, bezogen auf die Gegenwart, eine Erwärmung des arktischen Systems um mehr als 4°C bis zum Jahr 2100 voraus. Bei ektothermen Fischen wird der Energiebedarf im Wesentlichen durch die Temperatur bestimmt. Da die Energie physiologisch limitierend ist, sind ihre Möglichkeiten, mit dem Klimawandel fertig zu werden, begrenzt. Daher ist das Verständnis der Auswirkungen von Umweltveränderungen auf die Bioenergetik für das Management mariner Ökosysteme von entscheidender Bedeutung. In den letzten Jahren wurde die artspezifische Bedeutung der Herzfrequenz (f_H) als Indikator für den Energieverbrauch von der wissenschaftlichen Gemeinschaft hervorgehoben. Die Fortschritte der Bio-Logging-Wissenschaften haben die Erfassung von f_H in freischwimmenden Individuen ermöglicht. Bezüglich arktischer Fische haben jedoch raue Umweltbedingungen die Verfolgung von f_H-Bio-Logging bisher eingeschränkt. Deshalb haben wir uns mit Star-Oddi zusammengetan, die einen neuartigen, internen f_H- und Temperatur-Biologger entwickelt haben, welcher für Temperaturen bis zu –5°C kalibriert ist. In der vorliegenden Studie wurde dieser Bio-Logger in den auf die Arktis spezialisierten. käteangepassten Polardorsch (*Boreogadus saida*) implantiert. Ziel war es, die f_H-Bio-Logging-Methode mittels Simulation des ökologisch relevanten Temperaturbereichs (d. h. 0 bis 8°C) bei kritischen Schwimmgeschwindigkeiten (U_{crit}), zu validieren und weiterzuentwickeln.

Die Positionierung des Bio-Loggers mit nach außen gerichteten Elektroden und die Erhöhung der Messfrequenz von 100 Hz auf 125 Hz verbesserten die Elektrokardiogram(EKG)-Qualität

erheblich (p < 0,0001 bzw. p = 0,02), was auf eine geringere Elektromyogram(EMG)-Rauscheinwirkung und eine deutlichere Abbildung der prozessierten EKG-Merkmale zurückzuführen ist. Unter diesen Einstellungen zeigten 80 ± 1,5 % der im Bereich von 0 bis 4°C prozessierten f_H die höchste Qualität (d.h. QI = 0). Die Konfidenz betrug Δf_H = 0,45 ± 0,56 bpm, im Vergleich zu über 1000 manuell berechneten f_H. Darüber hinaus wiesen 53 ± 5,5 % der Messungen. homogen über alle Schwimmgeschwindigkeiten bis hin zu U_{crit}, die höchste Qualität auf. Die vorliegende f_H-Bio-Logging-Methode erwies sich somit als äußerst robust gegenüber den simulierten arktischen Bedingungen.

Speziesspezifisch für den Polardorsch betrug die Standard-Stoffwechselrate (SMR) der Individuen mit Bio-Loggern 0,38 µmol/g/h bei 0 ± 0,5°C. Damit war sie niedriger als die zuvor ermittelten 0,44 µmol/g/h für Artgenossen bei 2,5 ± 1°C, was darauf hindeutet, dass die vorliegenden Messungen repräsentativ für Individuen ohne Bio-Logger sind, insbesondere angesichts der erwarteten Abweichung von Q_{10}-Regeln aufgrund des Sauerstoffbedarfs der Kälteanpassung. Polardorsch-f_H zeigte sich sehr sensitiv gegenüber Temperatur und Schwimmgeschwindigkeit, und wurde dementsprechend signifikant von diesen beeinflusst (jeweils p < 0,0001). Bemerkenswerterweise spiegelte f_H bei U_{crit} f_{Hmax}-Werte wider, welche zuvor bei denselben Temperaturen durch humorale Injektionen erhalten wurden. Dies bestärkt den kausalen Zusammenhang zwischen f_{Hmax} und daraus resultierenden Leistungseinschränkungen. Außerdem sanken die inkrementellen Q_{10}-Werte der f_H von 2,54 ± 0,76 bei 0–4°C auf 2,00 ± 0,50 bei 6°C und 1,73 ± 0,74 bei 8°C. Somit begann f_H des Polardorschs bei Temperaturen über 4–6°C nicht mehr ausreichend zu skalieren, was in Übereinstimmung mit den zuvor beschriebenen Temperaturbereichen und Empfindlichkeiten möglicherweise auf den Übergang zu passiver Wärmetoleranz hindeutet. Insgesamt war der Sauerstoffverbrauch (M_{O2}) mit einem Korrelationskoeffizienten von rho = 0,42 signifikant mit f_H korreliert. Zusätzlich wirkte sich die Interaktion von Schwimmgeschwindigkeit und Temperatur nicht signifikant (p = 0,71) auf M_{O2} aus, und die Steigungen der Beziehung wiesen ausgeprägte Ähnlichkeit bei 0, 2 und 8°C auf. Zusammenfassend lässt sich sagen, dass der Beitrag von f_H zur Regulierung, und letztendlich zur Begrenzung, der systemischen Sauerstoffversorgung als Reaktion auf den temperatur- und leistungsabhängigen Energiebedarf als sehr wahrscheinlich einzustufen ist. Damit wurde folglich das Potenzial von f_H als Indikator für den Energieverbrauch in Polardorsch im Rahmen der vorliegenden Untersuchung hervorgehoben.

1. Introduction

1.1. The Importance of Heart Rate in Fish Ecophysiology

Energy is the currency of life (Kleiber, 1975) and all physiological processes depend on it. For ectotherms (i.e. animals whose regulation of body temperature depends on external sources) such as fishes, energy demand is fundamentally determined by temperature. In the modern era, by anthropogenic cause, global temperatures are rising at a pace out of order from any other climate reconstructions of Earth's history (IPCC, 2014, 2019). It is virtually certain that the ocean will continue to warm throughout the 21st century and marine heatwaves are projected to further increase both in intensity and frequency. Since energy is a physiologically limiting resource, means to cope with climate change are limited. Therefore, understanding the impact of environmental changes on bioenergetics and physiological responses is crucial for the management of marine ecosystems (Hansen et al., 1993). Measurements of energy turnover and allocation to specific activities are of central importance to understand the physiological, behavioral and evolutionary ecology of organisms (McNamara and Houston, 1996). Energy budgets do not only determine fish performances such as reproductive success (Calow, 1985), but also ecosystem-level processes by the means of energy transfer (i.e. foraging and excretion) and ultimately by changing geographic distribution ranges in response to changing food-web structure.

Energy budgets of fish are generally determined by measuring metabolic rates via rates of oxygen consumption ($\dot{V}_{O2}$ per volume, or M_{O2} per mass). But how to measure energy budgets if *in situ* studies are to be performed in order to monitor ecological variability and rule out laboratory-induced stressors? M_{O2} measurements require defined volumetric set-ups, and therefore, intermediate proxies have to be drawn upon. As the main player in temperature-dependent regulation of blood flow, heart rate (f_H) is directly linked to the organismal oxygen transport cascade and hence to M_{O2}. Therewith, it strongly correlates with metabolic rates and with rates of energy expenditure in fish in general (Butler et al., 2004; Green, 2011). Furthermore, the central physiological role of f_H as a major factor explaining thresholds in aerobic capacity and subsequent performance declines needs to be highlighted. In light of the hypothesis of 'Oxygen- and Capacity-Limited Thermal Tolerance' (OCLTT), the onset of thermal performance limitations is determined by constraints to supply oxygen to the tissues (Pörtner, 2010; Pörtner et al., 2017). Together with the oxygen-carrying capacity, primary

causality of those constraints is attributed to f_H. Hence, f_H is a key factor in the leveling off in metabolic rates at increasing temperature. In consequence, it indicates performance breakpoint temperatures such as the pejus temperature T_p (i.e. transition to passive thermal tolerance) and critical temperature T_{crit} (i.e. onset of anaerobiosis; beyond the frequently experienced thermal range).

The relationship between f_H and the rate of oxygen consumption ($\dot{V}_{O2}$) is summarized by Fick's convection equation for the cardiovascular system (Figure 1). Fick's equation also highlights that the potential for variability in both cardiac stroke volume (V_s) and the extraction of oxygen by the body tissues (C_aO_2–C_vO_2) must not be neglected in holistic metabolic rate modelling approaches. Nevertheless, as long as the so called 'oxygen pulse' $V_s(C_aO_2$–$C_vO_2)$ varies in a predictable and repeatable fashion, quantification of the relationship between f_H and $\dot{V}_{O2}$ is highly valuable. In fact, recent studies have underlined the importance of f_H as a proxy for energy expenditure in fish (Altimiras and Larsen, 2000; Schreer et al., 2001; Cooke et al., 2003; Clark et al., 2005, 2008; Clark and Seymour, 2006).

$$\dot{V}_{O_2} = f_H \times V_s \left(C_a O_2 - C_v O_2 \right)$$

$\dot{V}_{O_2}$ = Rate of oxygen consumption

f_H = Heart rate

V_s = Stroke Volume of the heart

C_aO_2 = Oxygen content of arterial blood

C_vO_2 = Oxygen content of mixed venous blood

Figure 1 *Fick's (1870) convection equation for the cardiovascular system.*

f_H is not the only well-established proxy for energy expenditure in fish. Locomotory activity proxies, such as acceleration activity, have increasingly been worked with in the past decades. The most commonly employed devices are tri-axial accelerometers, which measure acceleration in surge (forward, y), heave (vertical, x), and sway (horizontal, z) axes. They further possess the benefit of enabling monitoring of fine-scale behavior (Cooke et al., 2016). Nevertheless, f_H displays some significant advantages over acceleration activity when it comes to the estimation of metabolic rates. Temperature is considered to be the most important abiotic driver for distributional patterns of aquatic animals (Magnuson et al., 1979; Fossheim et al., 2015). Due to the strong physiological temperature-dependency of ectothermic animals, f_H

often provides better bioenergetic correlation than the temperature-independent acceleration activity (Clark et al., 2010). Additionally, correlates of fish movement tend to fail to represent of energy expenditure anaerobic metabolic pathways. 'Excess Post-exercise Oxygen Consumption' (EPOC) is often not represented, as low activity is resumed after vigorous exercise. In contrast, f_H typically increases to 'pay back' oxygen debt resulting from anaerobiosis (Wood, 1991; Lee et al., 2003a).

Furthermore, monitoring temporal variation in f_H is a valuable indicator for specific activities in fish. Pinpointing specific events strongly complements energy expenditure studies, which further highlights the importance of f_H for fish ecophysiology. Fish feeding events, for example, can be detected due to unique statistical properties of the f_H response (Shen et al., 2020). In the context of energy expenditure, feeding represents the other side of the generalized energy budget for fish: Energy consumed = Metabolism + Growth + Waste (Cooke et al., 2016). On top of that, events of physiological stress can be indicated, and subsequent recovery can be evaluated through changes in f_H. In the past, a wide variety of stressors have been addressed in such a way, some examples include handling stress (Laitinen and Valtonen, 1994), heat shock events (Heath and Hughes, 1973), low water qualities (Milligan and Wood, 1982), and surgical procedures (Lucas et al., 1991).

1.2. Heart Rate Bio-Logging Under Arctic Restrictions

f_H of fish is mostly determined by the use of electrocardiography. An electrocardiogram (ECG) records changes in electrical activity of the heart over time, induced by propagation of action potentials during each cardiac cycle (Figure 2). That is, however, not a direct measurement of cellular depolarization and repolarization, but rather the relative, cumulative magnitude of cells, eliciting changes in their membrane potential (Dupre et al., 2005). The resulting electrical field is detected by using electrodes (connected by wire to the device) placed in the ventral muscles of the heart (Claireaux et al., 1995; Cooke et al., 2001), in the stomach (Lucas, 1992; Armstrong et al.,1989) or within the coelom near the pericardial cavity (Clark et al., 2008). Further, some devices, including the Star Oddi bio-logger used in the present study, possess non-wired electrodes on their surface. Correct directional placement and positional fixation of electrodes throughout long deployment periods of ECG devices are central requirements for the optimization of data-output (Cooke et al., 2004).

Presently, internally-borne ECG devices designated for the field are archival tags, so called bio-loggers (i.e. Data Storage Tags, DSTs). Bio-loggers are attached or implanted electronic tags

which are used to integrate data about an animal's physiology, behavior, movement and/ or environment (Rutz and Hays, 2009). Although bio-loggers have been used for over 50 years, progress in this discipline has been outstanding within the last decade (Hussey et al., 2015; Wilmers et al., 2015; Tibbetts, 2017). Rapid advancements in modern technology, namely the development of more powerful microprocessors, increased battery life-times and progressively sophisticated sensors, have helped to overcome short-lived, low-resolution sampling of the past (Block, 2005; Cooke et al., 2005).
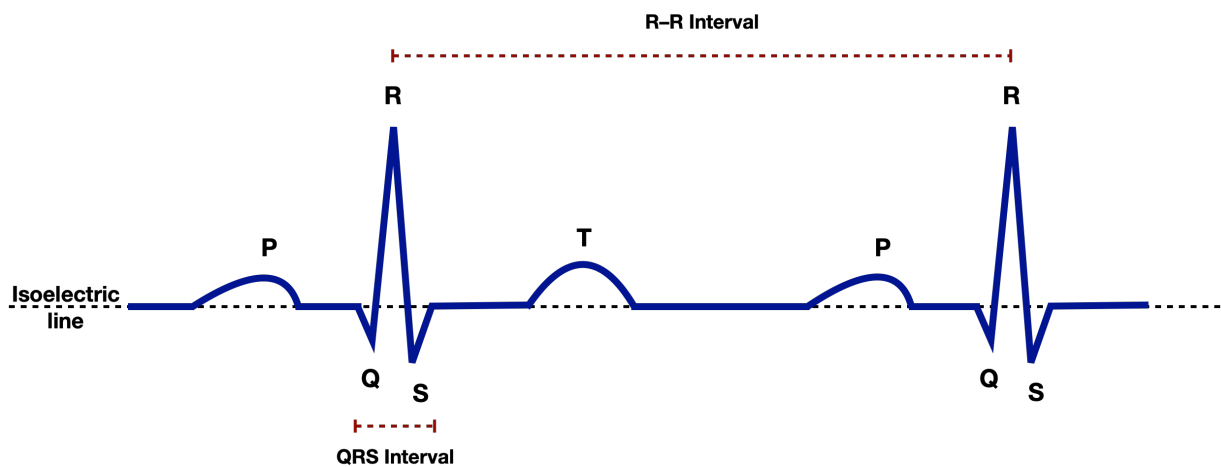
Figure 2 *Scheme of typical ECG trace properties. The P-wave indicates atrial depolarization, the QRS interval indicates ventricular depolarization, and the T-wave indicates ventricular repolarization. The R–R interval is generally used for heart rate (f_H) processing, denoting two consecutive heart beats.*

Up till now, bio-logging is responsible for the majority of f_H measurements in free-swimming, untethered fish (Cooke et al., 2016). The ECG waveform is either recorded in its entirety or immediately processed on-board of the logger, resulting in mean f_H calculations over defined durations. The latter approach significantly conserves memory capacity of the logger, however, morphology of the ECG waveform (e.g. duration and amplitude of the QRS interval), homogeneity of the ECG (e.g. f_H variability; Altimiras et al., 1996) and the potential for manual recalculation are precluded. Signal processing algorithms are used to determine f_H by the use of characteristic, cyclic ECG properties of each heart beat (Figure 2). Over the past 20 years, technological innovations and consequent miniaturization have led to bio-logging being applicable for increasingly smaller animals (Ropert-Coudert et al., 2009). Size-reduction of electronic circuitry has been achieved by surface mounting of components and integration of circuit technology. The need to miniaturize bio-loggers for the use in fish, however, is tied to

some of the most impactful restrictions on technology, as constraints in transducer size and battery volume are still strongly decreasing deployment periods (Rutz and Hays, 2009).

The cold Arctic pelagic presents itself additionally impeding on bio-logging. Temperatures around and under the freezing point do not only demand adaptations of present biological inhabitants, but also of the usage of technologies. First of all, low temperature regimes are generally challenging for electronic circuitry, but they are especially challenging on battery-lifetimes. Furthermore, electrical conduction is not only distinctly reduced on the technological side, but the biological electron flow and hence electrical signal recognition are also affected. Electrical conductivity of tissue is positively correlated to temperature. Consequently, the electrical conductivity of cooled tissues is substantially lowered (Daniels and Rubinsky, 2011). Peyraud-Waitzenegger and his team (1980) found that atrioventricular conduction in fish can be seriously compromised at low temperatures.

1.3. Polar Cod and It's Restrictions on Heart Rate Bio-Logging

The chosen study animal was polar cod, *Boreogadus saida* (Lepechin, 1744; Gadidae; also "Arctic cod" in the north American literature), a cold-adapted fish of circumpolar distribution in the Arctic Ocean. With an average maximum length of 300 mm (Scott and Scott, 1988, Hop and Gjøsæter, 2013) it is generally a small marine fish. Furthermore, polar cod is relatively short-lived with maximum age of seven years (Hop et al., 1997b), however, rarely exceeding five years of age (Bradstreet, 1986; Ajiad et al. 2011). Early maturation (males at two and females at three years) and high fecundity (~10000 eggs) further characterize the fish as an r-selected species (Hop and Gjøsæter, 2013).

The effectively inhabited core temperature range of polar cod is dependent on its life stage. Juveniles prefer surface-waters with thermal habitats of 2.0–5.5°C (Eriksen et al. 2015), while adult fish inhabit variable depths, including temperature regimes down to −1.8°C (Crawford and Jorgenson 1996; Crawford et al. 2012; Mark, 2018). Highest food conversion efficiency was found at 0.0°C (Kunz et al., 2016), highest swimming activity at 2.8–4.4°C (Schurmann and Christiansen, 1994), and optimum growth performance at 6.0°C (Leo et al., 2017). As to the upper thermal limitations, cardiac arrhythmia was shown to set in at 12.4°C (Drost et al., 2014). The onset of fitness-decrease, however, was described substantially earlier, due to impaired cardiac mitochondrial function (Leo et al. 2017) and swimming behavior (Schurmann

and Christiansen 1994). Leo et al. (2017) showed that temperatures >8°C caused decreased mitochondrial efficiency in long-term acclimated fish. Conclusively, the above-mentioned research indicates that polar cod has an overall limited ability of acclimation to rising temperatures.

Giving that currently the World's oceans are experiencing historically rapid warming by anthropogenic cause (IPCC, 2014, 2019), the future of polar cod could be severely endangered. In global comparison, warming is predicted to be fastest in the Arctic system (Figure 3). There, highest anthropogenic CO_2 emissions on the "Shared Socioeconomic Pathway 5" (SSP5) and "Representative Concentration Pathway 8.5" (RCP 8.5) are projected to lead to a rise in temperature of more than 10°C by the year 2100 compared to present-day. Even for the lowest SSP1–RCP2.6 scenario, high-warming models show a temperature increase of more than 4°C in the Arctic relative to the present (IPCC, 2021). The disproportional temperature increase, termed Arctic amplification, is mainly caused by heating Atlantic water inflow, leading to sea-ice cover declines and consequent positive feedback mechanisms regarding atmospheric radiation (Polyakov et al., 2010; Serreze et al., 2011).
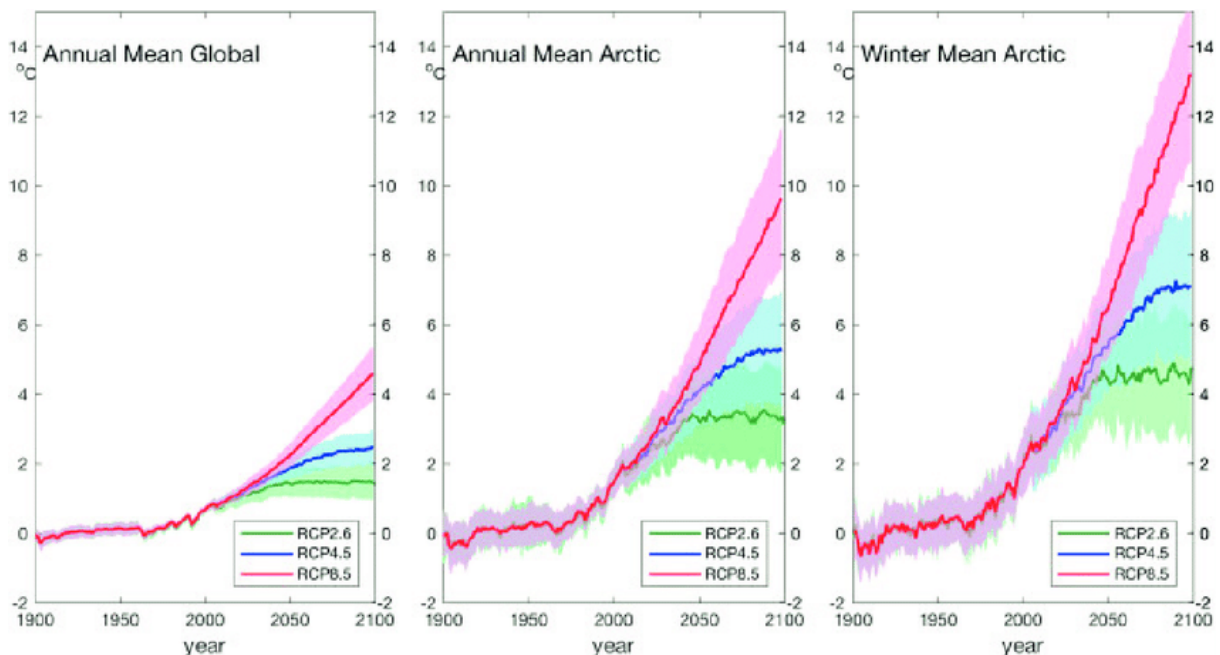
Figure 3 *Projections of mean global, mean Arctic, and mean Arctic winter temperatures until 2100; graph by Overland et al. (2019).*

The necessity to monitor polar cod and understand its physiology is further amplified by its upmost ecological relevancy. Polar cod is one of the most abundant fish species in the Arctic (Moskalenko, 1964; Ponomarenko, 1968; Hop and Gjøsæter, 2013) and occupies a highly centralized space within the food web. Therewith, it plays a key role in trophic transfer from lower to higher trophic levels. In the extreme, estimations go as far as to approximate that 75% of zooplankton production in the high-Arctic are channeled to predatory marine mammals, bigger fish and sea birds by polar cod (Benoit et al., 2014; Hop and Gjøsæter, 2013; Welch et al., 1992). In Arctic shelf areas, polar cod has recently been described to be the most abundant fish species, dominating fish assemblage of the demersal-pelagic zones (Aune et al. 2021; Geoffroy et al., 2016; Orlov et al., 2020*)*.

The Barents Sea stock of polar cod, although generally fluctuating, has been at considerably low levels in recent years. A reduction to about half of the historical stock size was witnessed (Gjøsæter et al., 2020). Since only small catches have been taken from this stock over the last four decades, the observed shift in abundance is considered to be due to environmental and/ or biological changes in the ecosystem (Fossheim et al., 2015). Gjøsæter et al. (2020) performed a modelling study and concluded that any perturbations from typical Arctic climate, i.e. rising maximum temperature and loss of sea-ice cover, appear to be detrimental to polar cod stock recruitment. Additionally, a displacement of the stock towards the northern and eastern parts of the Barents Sea has been observed and attributed to climate change as well as to the borealization of the habitat (Eriksen et al., 2017).

Besides its ecological relevancy, polar cod was chosen as the study animal because it outstandingly represents the challenges of f_H bio-logging in Arctic fish. With its relatively small size, in addition to the Arctic low temperature implications, the demand for highly miniaturized devices further restricts bio-logging technologies. Furthermore, polar cod displays exceptionally low f_H values typical for polar specimens. Generally, low f_H is encountered in fish inhabiting low temperature regimes due to kinetic constraints of several biochemical processes (Rodnick and Gesser, 2017). This is best described by the temperature coefficient Q_{10}, which defines the rate of change of a biochemical process over a 10°C change in temperature. While a Q_{10} value of 1 implies that a biochemical process is independent of temperature change, a value of 2 implies that the process (e.g. diffusion or enzymatic reaction) increases two-fold for each 10°C increase. In the range between 0.5 and 5.5°C, the maximum heart rate (f_{Hmax}) of polar cod follows a mean Q_{10} of 2.4 ± 0.5 (Drost et al., 2014). Thus, both

adenosine triphosphate (ATP) demand and supply, as well as consequent contractile activity, are enhanced as temperature increases within the physiological range. With decreasing temperature (i.e. on the cold side of the thermal window), however, adaptations may compensate thermal constraints, leaving only kinetic constraints on physiological processes (Wittmann et al., 2012; Pörtner et al., 2013). In bio-logging, low f_H values are particularly challenging on signal recognition and processing, as sampling duration and sampling frequency need to be harmonized. On the one hand, as the minimum requirement to calculate mean f_H, sampling duration has to be long enough to record at least two consecutive heart beats. On the other hand, high sampling frequencies are needed to capture ECG characteristics, in particular the R-wave peak, to a high enough extent to distinguish them from ambient noise. Those two parameters, however, 'compete' against each other, as increasing sampling frequencies lead to sampling duration decline due to data-size restrictions in the microprocessor's RAM (i.e. short-term working memory).

Drost and colleagues (2014) measured f_{Hmax} of polar cod to average 26.4 bpm at 0.5°C and 54.5 bpm at 10.4°C. To the best of my knowledge, those are the only recordings of f_H in polar cod so far. f_H measurements, however, were conducted by the placement of electrodes on top of the skin in close proximity to the heart. Consequently, fish had to be immobile (i.e. anaesthetized) during the measurements. The present study will further expand the methods of f_H measurements in polar cod by the research of bio-logging methodology for free-swimming specimens.

1.4. Research Objectives and Hypotheses

This research aims to progress and validate the utility of novel Star-Oddi f_H bio-loggers for free-swimming Arctic fish. Hence, the overarching objective is to obtain processable, high-quality ECG traces in simulation of Arctic temperature and free-roaming exercise. Therefore, f_H bio-loggers will be implanted in polar cod and after recovering one month from surgery, fish will perform in swim tunnel U_{crit} tests. Over the course of five days, temperature will be adjusted daily in 2°C increments within the ecologically relevant range of 0–8°C.

To improve ECG quality, data and feedback will be continuously exchanged with Star-Oddi, enabling direct implementation of technological improvements in parallel to ongoing research. Within this study, multiple signal processing algorithms with varying frequency responses, different sampling frequencies, as well as adjustments in bio-logger placement within the fish,

will be compared in order to achieve this goal. Consequently, the main research questions are how the quality of ECG traces changes in response to variable (1) swimming velocities, (2) temperatures, and (3) above-mentioned adjustments. Increasing swimming velocities are hypothesized to cause an increase in electrical noise due to higher interference of EMG and potential logger movement. Further, decreasing temperatures will potentially lower ECG amplitudes due to decreased electrical conductivity (Daniels and Rubinsky, 2011).

While this study primarily focuses on progressing the bio-logging methodology, there is an array of additional opportunities for physiological observations to drive f_H bio-logging of polar cod towards field research. Most importantly, we aim to collect further insight on the potential of bio-logged f_H as a proxy for energy expenditure in polar cod. Hence, the relationship of f_H and M_{O2} will be examined across established temperature and swimming velocity regimes. Therefore, within the swim tunnel, M_{O2} will be measured in parallel to f_H over each temperature and each velocity step. Associated research questions are: (1) to which degree f_H and M_{O2}, are correlated, and (2) whether their relationship is constant across temperatures and swimming velocities. It is hypothesized that this study will reveal a strong correlation between f_H and M_{O2} due to the fundamental interdependency of both oxygen demand and oxygen supply to cardiac output. Due to potential cardiac output regulation via stroke volume, however, a complex and temperature-dependent relationship of f_H and M_{O2} at increasing oxygen demand is hypothesized.

Furthermore, M_{O2} measurements will be used to draw conclusions on the welfare of polar cod with implanted micro-HRT bio-loggers. Therefore, besides high-performance swim tunnel M_{O2}, fish will be transferred into respiration chambers and SMR will be monitored after one month of post-implantation recovery. The associated research questions is whether polar cod are fully recovered with SMR and MMR resembling previous studies. I hypothesize that both SMR and MMR will be resembling literature values, due to the micro-invasive nature of the DST implantation and the fairly long recovery period.

Lastly, to the best of my knowledge, this is the first study to examine f_H of polar cod in free-roaming individuals. Consequently, there is a descriptive objective in the exploration of f_H physiology and limitations in response to increasing temperature. Therefore, incremental Q10 of f_H will be observed across the physiologically relevant temperatures of 0 to 8°C. In accordance with general findings in aquatic ectotherms (Wang et al., 2014; Pörtner et al., 2017), I hypothesize that f_H patterns will indicate thermal limitations and Q_{10} values will decrease past the optimal performance temperatures of polar cod (2.8–4.4°C; Schurmann and Christiansen, 1994).

2. Material and Methods

2.1. Polar Cod Sampling

Polar cod were caught in Billefjord, Svalbard, Norway in October 2018 and August 2020, over the course of research cruises *HE519* and *HE560*, conducted by the Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research (AWI). A fish lift connected to a pelagic trawl was used at 150 m depth to capture fish alive and unscathed. Ambient environmental parameters were monitored, with water temperature at $-1.5°C$ and oxygen concentration at 5.75 ml/l. Subsequently, polar cod were directly transported to the AWIs holding facilities in Bremerhaven, where they were maintained at $0.0 \pm 0.5°C$ in flow-through tanks and fed *ad libitum*. To address for size requirements regarding the DST implantation, prior to their surgery, polar cod were transferred and acclimated to $4.0 \pm 0.5°C$ holding conditions. In the present study, n = 11 polar cod were sampled and fish weight ranged from 50 to 111 g (Table 1).

2.2. Star-Oddi Heart Rate and Temperature Bio-Loggers

We partnered with Star-Oddi, an Islandic manufacturer who offers miniaturized data storage tags (DSTs) for aquatic animals (Star-Oddi, Garðabær, Iceland; see https://www.star-oddi.com). The bio-logger used was the micro-HRT (G2), a long term f_H and temperature DST which records leadless single-channel ECG via internal electrodes. Therewith, no external wires were required, which made the logger implantation micro-invasive and fairly simple. With a weight of 11.8 g, 8.3 mm in diameter and 25.4 mm in length, the micro-HRT (G2) DST is the smallest f_H logger of its kind. While previous Star-Oddi f_H DSTs were designated for the usage in 5 to >45°C, bio-loggers of the present study were calibrated for temperatures down to $-5°C$. ECG burst measurements were taken in 5-minute intervals during swim tunnel trials, and once per hour during respiration chamber experiments as well as in between experimental runs. Mean f_H was processed on-board of the logger for each recording. For general quality assessment, ECG traces were stored during swim tunnel trials.

Within this study, multiple factorial variations in bio-logger methods were examined for their effect on resulting ECG quality (Table 1). Bio-logger positioning varied, as n = 1 logger possessed interior-facing (i.e. towards the intestine) electrodes, and n = 10 loggers possessed exterior-facing (i.e. towards the pelvic fins) electrodes. Sampling frequency was varied with

100 Hz (i.e. sampling duration = 15 s) for n = 5 loggers and 125 Hz (i.e. sampling duration = 12 s) for n = 6 loggers. Lastly, signal processing algorithms (i.e. frequency responses) were variable with either relatively low amplification of relatively narrow frequencies (n = 5) or high amplification of broad frequencies (n = 6).

Table 1 Polar cod weights [g] and respective bio-logger specifications in terms of electrode orientation, sampling frequency [Hz], and frequency response (either relatively low amplification of narrow frequencies or high amplification of broad frequencies).

Fish ID	Weight [g]	Electrode Orientation	Sampling Frequency [Hz]	Frequency Response
1	111	Interior	100	Low, narrow
2	104	Exterior	100	Low, narrow
3	63	Exterior	100	High, broad
4	62	Exterior	100	High, broad
5	81	Exterior	100	Low, narrow
6	83	Exterior	125	Low, narrow
7	69	Exterior	125	Low, narrow
8	68	Exterior	125	High, broad
9	53	Exterior	125	High, broad
10	50	Exterior	125	High, broad
11	62	Exterior	125	High, broad

Furthermore, Star-Oddi bio-loggers possess an inherent, algorithm assigned value for the quality of ECG traces: The Quality Index (QI). Each f_H calculation is paired with a QI value ranging between 0 and 3. If the recording displays more than one R-R interval (i.e. at least three consecutive heart beats) and the variability of the interval duration is less than 20%, it is assigned QI = 0. Further, the algorithm grades various morphological properties such as amplitude and "sharpness". Variation in those grades leads to decreasing quality (QI = 2 instead of QI = 1). Lastly, QI = 3 measurements display no consecutive R-R-interval (i.e. no

consecutive recognizable heart beats over the sampling duration), which disallows f_H calculation (Figure 4).
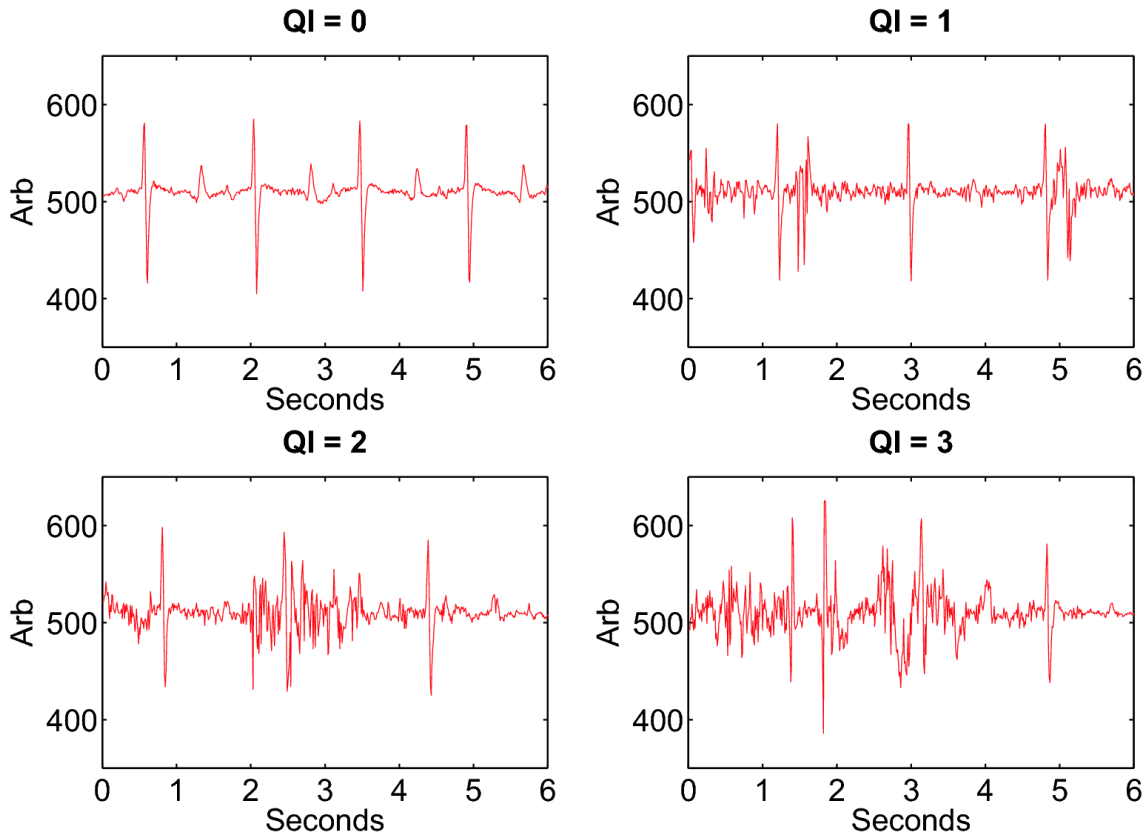
Figure 4 Exemplary ECG traces and their respective QI values; graph from Bjarnason et al. (2019).

2.3. Bio-Logger Implantation

Polar cod were carefully caught from their holding tanks and immediately anaesthetized in 0.125 g/L tricaine mesylate (MS222). In quick succession to the loss of caudal muscular reflexes, after approximately three minutes in the anesthetic agent, fish were marked with Passive Integrated Transponder (PIT) tags. Afterwards, they were measured (total length, standard length, width, depth) and weighted. For the surgery, fish were bedded on cotton wool wrapped with cloth in order to achieve fixation and maintain humidity. Here, they received 0.06 g/L MS222 directly over their gills. First, a laparotomy (i.e. an abdominal incision) of 15 mm length was conducted 15 mm posterior of the pelvic fin base. The bio-logger was pushed into the peritoneal cavity and positioned in close proximity to the pericardium. The smooth end of the bio-logger was positioned to face the pericardium and logger electrodes were directed to face abdominally (n = 1 logger faced the intestines). A pre-attached sewing thread was used to

fixate the bio-logger by performing an anchor stitch central and immediately on top of the incision. Afterwards, the wound was closed by performing three standard chirurgical sutures. To end the surgery, iodine solution and an analgesic were applied onto the wound. Polar cod were then transferred and the gills were flushed with circulating water to prevent suffocation.

2.4. Respiration Chamber

Approaching one month of recovery from DST implantation, n = 11 polar cod were transferred to a separate experimental set-up for SMR measurements. For each trial, individual M_{O2} of two fish was measured at $0.0 \pm 0.5°C$ via flow-through respirometry. Feeding was ceased three days prior to the transfer, to ensure standard metabolism.

The experimental set-up consisted of two chamber respirometry systems (2250 ml), which were constructed in-house (Figure 5). Perspex respiration chambers, PVC tubes and aquarium pumps (~6 l/min output) were used for the general construction. Pumps may be further divided into continuously running recirculation pumps and intermittent flush pumps. The connection of respective sockets to a simple timer was used to establish intermittent flushing phases. P_{O2} was determined by using interposed fiber-optic mini sensors (optodes), connected to an oxygen meter (FireSting®-O2, 4 Channels, PyroScience, Aachen, Germany). Calibration of the optodes took place with nitrogen (0 % oxygen saturation) and fully oxygenated seawater. The Pyro Oxygen Logger software (PyroScience, Aachen, Germany) was used to monitor oxygen saturation and temperature. Together with a temperature sensor and an oxygen supply source, chambers were submerged in a shared but comparted water basin (~50 litres), which prohibited visual contact between conspecifics.

Polar cod were kept in the respiratory system for 48 to 72 hours, however, SMR calculations were only derived from a 12-hour period over the last night of fish residence. The flush pump timer was set to 15-minute intervals, resulting in 15 minutes of measurement, followed by 15 minutes of flushing. Oxygen saturation measurements were taken continuously with 10 s sampling intervals, resulting in total of 24 measurement periods with 90 data points each, from which mean M_{O2} was calculated.
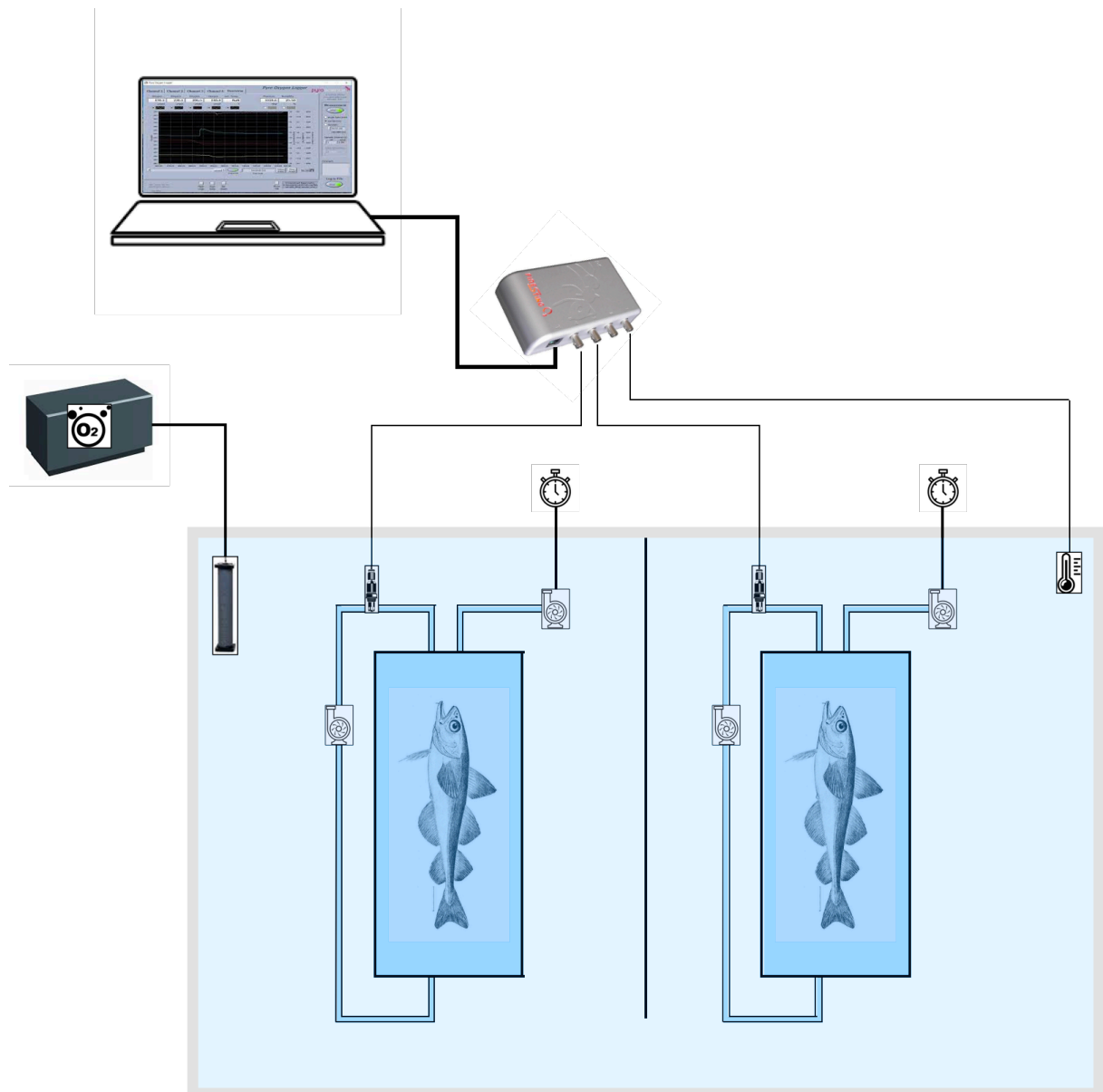
Figure 5 *Scheme of the respiration chamber system. Respiration chambers are connected to a recirculation pump and to a flush pump, which is connected to a timer. In between fish chamber and recirculation pump, flow probe vessels are installed, carrying the oxygen sensors (i.e. optodes). A four-channel oxygen meter (PyroScience, Germany) records oxygen in the respiration chamber system, as well as ambient oxygen and temperature in the water basin. The oxygen meter transfers data to a computer with the software "AutoResp" (version 2.3.0, Loligo Systems ApS, Denmark). Compressed air (O_2) is supplied to the water basin.*

2.5. Swim Tunnel

After one full month of recovery from surgery, n = 11 polar cod performed in temperature-controlled U_{crit} tests with simultaneous M_{O2} monitoring. Each fish swam on five consecutive days at exactly the same times. Similar to surgery and SMR measurements, each experimental iteration was conducted with a batch size of two fish, resulting in morning (11:00 am) and evening (04:30 pm) swimming individuals. Temperature ranged from 0 to 8°C with daily

increments of 2°C. Starting temperature on day one of each U_{crit} test alternated between 0 and 8°C to account for potential effects of temperature and exhaustion.

A Brett-type swim tunnel respirometer (30 x 7.5 x 7.5 cm, Loligo Systems ApS, Denmark) was submerged within a water basin to maintain stable abiotic conditions (Figure 6). Water velocity and circulation were regulated by a control unit linked to a propeller within the swim tunnel chamber (Loligo Systems ApS, Denmark). Calibration of water velocity to voltage output from the control system was achieved by using a flow sensor. Intermittent flushing phases were established via a flushing pump, controlled by a data acquisition instrument (DAQ-M) (Loligo Systems ApS, Denmark). M_{O2} was monitored with an interposed optode, connected to an oxygen meter (Loligo Systems ApS, Denmark, Witrox 4 oxygen meter for mini sensors). Both the DAQ-M instrument and the oxygen meter were linked to the "AutoResp" software (version 2.3.0, Loligo Systems ApS, Denmark), which was used to monitor and control the entire U_{crit} test. Optodes were calibrated with nitrogen (0 % oxygen saturation) and fully oxygenated seawater. Additionally, an ambient oxygen optode, a temperature sensor and an oxygen supply source were placed into the reservoir tank.

The swim tunnel experiment followed a two-hour U_{crit} ramp protocol with 10-minute velocity steps. For acclimation, transfer from the holding tanks to the swim tunnel was set two hours prior to the experimental start. Further, 30 minutes prior to the start, swimming commenced at 1.2 bodylength/second (BL/s) water velocity. The U_{crit} ramp started with fish swimming at 1.4 BL/s and the water velocity increment was 0.15 BL/s. U_{crit} was defined as the swimming velocity at which fish ceased swimming for longer than 60 s. The intermittent-flow respirometer system was established by subdividing every 10-minute velocity step into phases of 50 s flushing, 70 s waiting, and 540 s measuring. P_{O2} was determined once per second.
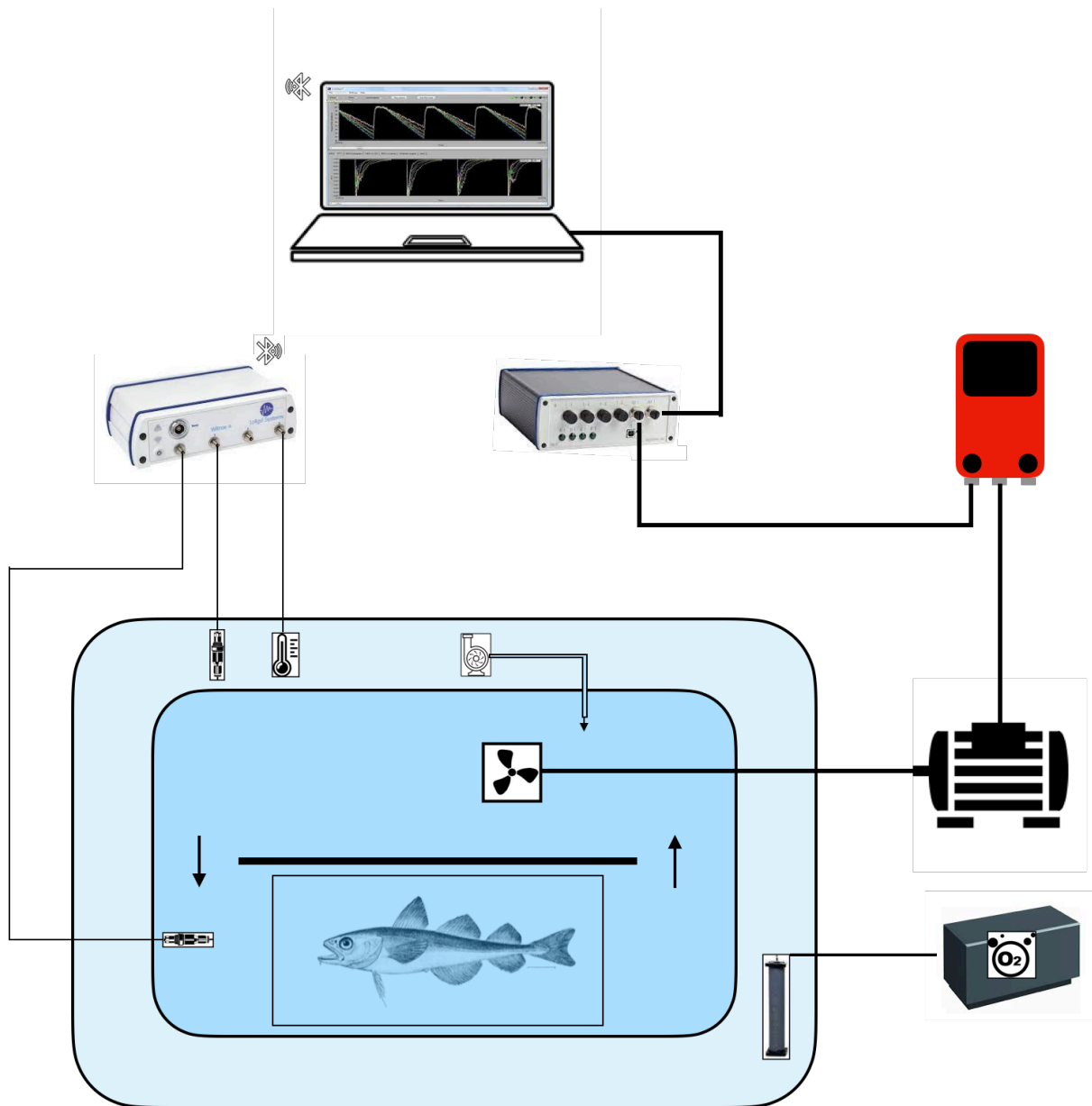
Figure 6 *Scheme of the swim tunnel system. The fish swims in a Brett-type respirometer (30 x 7.5 x 7.5 cm, Loligo Systems ApS, Denmark), which is submerged in, and water flushed by, a temperature-controlled water basin. A four-channel oxygen meter (Loligo systems ApS, Denmark, Witrox 4) records oxygen in the respirometer, as well as ambient oxygen and ambient emperature in the water basin. The oxygen meter transfers data via Bluetooth to a computer with the software "AutoResp" (version 2.2.0, Loligo systems Aps, Denmark). Water flow is regulated via a propeller, connected to a controller, which itself is connected to a data acquisition instrument (DAQ-M). The DAQ-M is connected to, and controlled by, a computer with the software "AutoResp". Compressed air (O_2) is supplied to the water basin.*

2.6. Data Handling

Statistical analyses were performed with R 3.6.1, RStudio (version 1.2.5001). In consequence to multiple swimming trials of the same fishes, measurements were statistically treated as repeated and dependent. Similar for all of the collected data, normality was tested with Shapiro-Wilk tests and homoscedasticity was tested with Levene tests. Models were chosen based on

the outcome of those parametric assumption tests. A backward model selection procedure, starting with the most complex model (including all interactive effects among fixed factors) and ending with only significant factors was used.

2.6.1. Electrocardiogram and Heart Rate

Initially, bio-logger stored data was transferred to, and visualized in, Star-Oddi's in-house software Mercury (version 5.98). Mercury provided graphical and tabular view of f_H, temperature, and QI values over the whole experimental time-span. To enable in-depth analysis of ECG traces, data was fed into the novel Star-Oddi software "HRT Analyzer" (beta version 1.0). For manual calculation, amplitude and duration of characteristic heart beat electric potential differences were analyzed. The main heart beat characteristics are: The P-wave (atrial depolarization), the QRS-complex (ventricular depolarization), and the T-wave (ventricular repolarization). Here, the QRS-inherent R-wave was of upmost importance, as it showed highest amplitude and confined duration. Hence, the R-wave was labelled throughout the whole ECG trace to obtain average f_H.

The mean difference of on-board processed and manually calculated measurements $\Delta f_H \pm$ SD was determined for each QI value to determine measurement confidences. As a result, only f_H measurements with QI ≤ 1 were used in subsequent physiological analyses. Furthermore, in addition to QI ≥ 2 measurements, $f_H > 55$ bpm were discarded as statistical outliers.

The effect of electrode orientation, sampling frequency and frequency response on resulting QI values was assessed via cumulative link mixed models (CLMMs) and ordinal logistic regressions by the R package "ordinal" (version 2019.12-10). Polar cod individuals were set as random effect. After this assessment, f_H measurements of interior-facing electrodes (n = 1 bio-logger) were excluded in all further modelling approaches and physiological analyses. After exclusion of qualitatively inferior bio-logging methods, robustness of the resulting method was assessed by comparison of QI ratios across simulated Arctic temperatures and swimming velocities.

For determination of temperature and swimming velocity impact on f_H, generalized linear mixed-effects models (GLMMs) and multiple linear regressions by the R package "lme4" (version 1.1-27.1) were used. Individual fishes were set as random effect. Further, incremental

Q_{10} values of f_H in response to 2°C steps from 0 to 8°C were calculated after Drost et al. (2014) using following equation:

$$Q_{10} = \frac{f_{H2}}{f_{H1}}^{\left(\frac{10}{T_2 - T_1}\right)} \qquad (1)$$

where f_{H1} and f_{H2} are heart rates at first temperature (T_1) and second temperature (T_2).

2.6.2. Swimming Performance

The impact of (1) temperature regime and (2) fish weight on responding U_{crit} [BL/s] was assessed via one-way ANOVA and Tukey's HSD tests. Further, absolute U_{crit} [cm/s] was similarly tested for differences across individual fishes.

2.6.3. Respiratory Measurements

The Metabolic rates (M_{O2}) were calculated with the R package "FishResp" (version 1.0.3), based on following equation:

$$M_{O_2} = \Delta P_{O_2} \cdot V \cdot \alpha \cdot \frac{1}{M} \cdot \frac{1}{\Delta t} \qquad (2)$$

Where ΔP_{O2} is the change in oxygen partial pressure (P_{O2}) [kPA], V is the respirometer volume minus the fish volume [l], α is the oxygen solubility coefficient after Boutilier et al. (1984), M is fish mass [kg], and Δt is the elapsed time [h].

To account for variable fish weight throughout this study, M_{O2} data was normalized after Steffensen et al. (1994) with the R package "FishResp" (version 1.0.3), using the equation:

$$M_{O_2} = M_{O_{2(I)}} \cdot \left(\frac{BW}{100}\right)^{(1-A)} \qquad (3)$$

Where M_{O2} is the oxygen consumption for a 100g fish, $M_{O2}(I)$ is the oxygen consumption of an individual fish with the body weight (BW) [g], and A is the mass exponent describing the relationship between M_{O2} and BW. After Steffensen et al. (1994) and Holeton (1974), A = 0.8 was chosen for the present study.

The volume of the Brett-type respirometer (20 L) was relatively large compared to polar cod volume (50 to 111g fishes). Therefore, swim tunnel M_{O2} measurements with $R^2 < 0.4$, as well as lowest and highest 5% of all recorded measurements, were discarded as outliers. To assess the effect of temperature and swimming velocity on f_H, generalized estimation equation generalized linear models (GEEGLMs) and multiple linear regressions by the R package "geepack" (version 1.3-2) were used. Individual fishes were set as random effect.

2.6.3.1. Standard Metabolic Rate

SMR was calculated with the 0°C respiration chamber data of n = 11 fish after Chabot et al. (2016). After removal of the lowest 5% of M_{O2} measurements as outliers, average of the remaining lower 15% quantile was determined as SMR. For the calculation, only data was used, which was recorded at night (18:00 to 06:00 hrs.) and at least 24 h after fish transfer.

2.6.3.1. Maximum Metabolic Rate

Temperature-specific MMR was calculated with the swim tunnel data of n = 10 fish. After removal of the highest 5% of all recorded metabolic rates, the remaining highest metabolic rate per individual and temperature was taken as MMR. The average MMR, and standard deviation across individuals, was calculated for each temperature treatment.

2.3.3. Metabolic Rate and Heart Rate Correlation

The relationship of f_H and M_{O2} was analyzed by performing a Spearman rank correlation test. Furthermore, the effects of f_H, temperature, and swimming velocity on responding M_{O2}, as well as the interactive effects of both temperature and swimming velocity with f_H, were assessed with generalized estimation equation generalized linear models (GEEGLMs) and multiple linear regressions by the R package "geepack" (version 1.3-2). Individual fishes were set as random effect.

3. Results

A total of n = 11 polar cod were successfully implanted Star-Oddi f_H bio-loggers and ultimately performed in temperature-controlled U_{crit} tests ranging from 0 to 8°C. Over the course of this study, logger-positioning, sampling frequency, and frequency response were modified in order to improve ECG quality. In consequence to these methodological explorations (and a case of swimming refusal), sample sizes vary and therefore are referenced in each of the physiological subsections 3.3 Swimming Performance, 3.4 Heart Rate measurements, and 3.5 Respiratory measurements.

3.1. Bio-Logger implantation

Overall, 15 polar cod underwent the surgical procedure and recovery from anesthesia was successful for all operated fish. After maximum two minutes, gill function was regained and after approximately 20 minutes, fish proceeded swimming. Insufficient wound-healing and consequent logger-exposure, however, manifested themselves as reoccurring problems, especially with decreasing fish sizes. Resulting was an overall mortality of 26.67% (n = 4), due to necessary euthanasia of fish with exposed bio-loggers.

3.2. Electrocardiogram Quality

For ~20% (n = 1180) of 6476 total f_H measurements, bio-loggers were set to additionally save the original ECG traces. Those raw ECG traces were used to manually calculate f_H and initially validate the assigned QI values for the following work with the respective on-board processed f_H calculations.

3.2.1. Manual Heart Rate Calculation

The distribution of manually calculated f_H was strongly focused on swim tunnel trials. Nevertheless, the highest proportion (64%) of on-board processed f_H calculations was assigned highest ECG quality (i.e. QI = 0). On-board processed f_H calculations showed increasing deviation from manual f_H calculations with decreasing ECG quality (i.e. with respective QI values as index: $\Delta f_{H,0} = 0.45 \pm 0.56$ bpm, $\Delta f_{H,1} = 2.82 \pm 3.94$ bpm, $\Delta f_{H,2} = 15.86 \pm 32.37$ bpm,

$\Delta f_{H,3}$ = 30.18 ± 9.13 bpm; Figure 7). Based on manual f_H calculations, on-board processed f_H measurements of QI ≤ 1 were methodologically validated with 2.82 ± 3.94 bpm confidence and therefore included in the following physiological analyses of the present study.
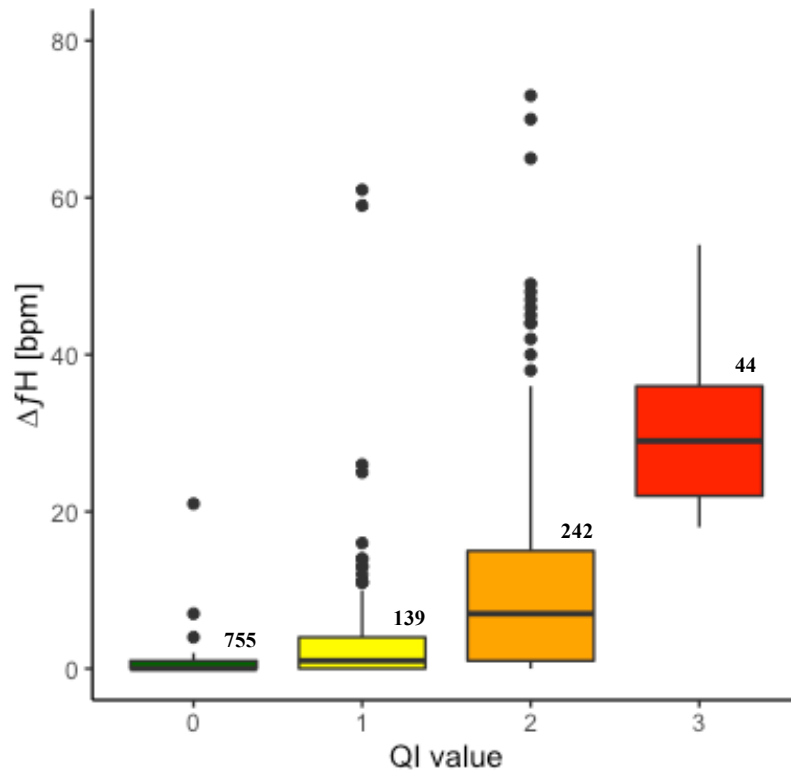
Figure 7 Mean heart rate (f_H) difference Δf_H [bpm] of on-board processed and manually calculated measurements per QI value. QI values are color-coded and measurement numbers (n) are displayed in bold on top of the boxplots.

3.2.2. Logger-Positioning, Sampling Frequency and Frequency Response

In the following step, the significances of adjustments in bio-logger positioning, sampling frequency and frequency response were analyzed for their impact on ECG quality (i.e. assigned QI value of f_H measurements).

Bio-logger positioning with interior-facing electrodes (n = 1 bio-logger) caused a high percentage of low quality f_H measurements (i.e. 82% QI ≥ 2; Figure 8). Associated ECG traces were not manually calculable as the criterium of short, ECG typical QRS duration was not met. In contrast, exterior-facing electrodes (n = 10) generated f_H measurements of significantly (p < 0.0001) higher quality (i.e. 79% QI ≤ 1) with a model-estimated decrease of –2.34 ordinal-ranked, relative QI values (Table 2).

By increasing sampling frequency of bio-loggers from 100 Hz (n = 5 bio-loggers; 15 s sampling duration) to 125 Hz (n = 6 bio-loggers; 10 s sampling duration), quality of f_H measurements increased significantly (p = 0.02) as model-estimated QI values decreased by −1.47 ordinal ranks. Since the QRS complex lasts approximately 100 ms, at 125 Hz it was recorded over ~12.5 data points, compared to ~10 data points at 100 Hz. Congruently, 100 Hz measurements displayed 70% QI ≤ 1 and sampling with 125 Hz caused a rise to 83% QI ≤ 1.

The variable usage of signal processing algorithms, however, showed no significant difference (p = 0.99) between applied frequency responses of the current study. The relatively low amplification of narrow frequencies (n = 5 bio-loggers) generated 80% QI ≤ 1 and the relatively high amplification of broader frequencies (n = 6 bio-loggers) caused 79% QI ≤ 1.
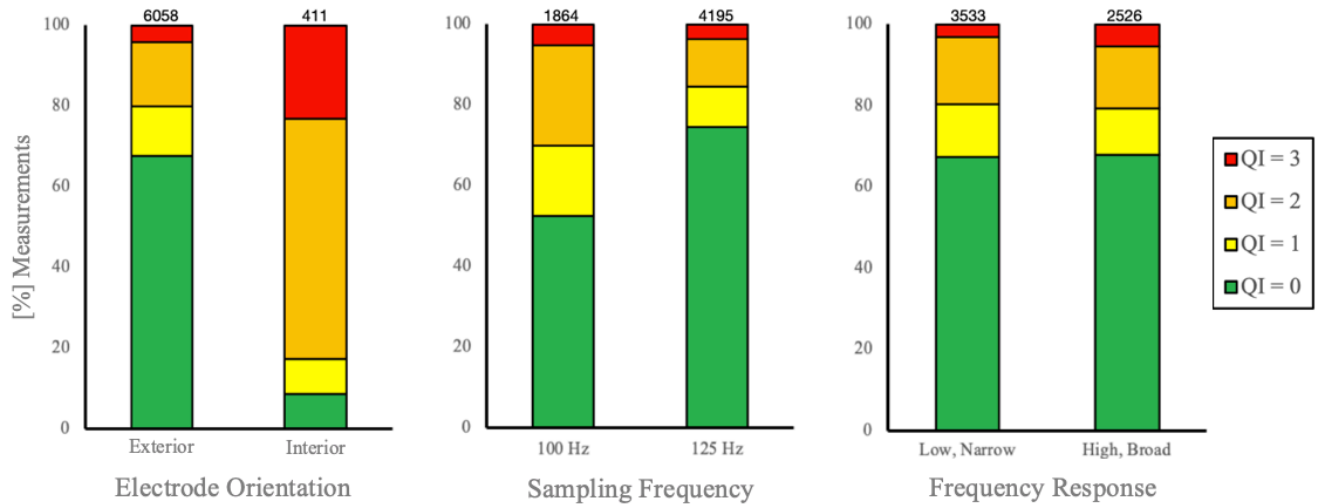
Figure 8 QI value percentages in response to variable electrode orientation, sampling frequency and frequency response. Measurement numbers (n) are on top of the bars.

Table 2 Cumulative Link Mixed Model (CLMM) and ordinal logistic regression – Number of adjusted bio-loggers (n); estimates, standard error (SE), and p-values of variable electrode orientation, sampling frequency and frequency response on QI values of respectively generated heart rate (f_H) measurements. Significant p-values are printed in bold.

Predictor Variable	n	Estimates	SE	p-value
Exterior Electrode Orientation	10	-2.34	0.52	**$7e^{-06}$**
125 Hz Sampling Frequency	6	-1.47	0.62	**0.02**
High, Broad Frequency Response	6	0.01	0.62	0.99

3.2.3. Swimming Velocity and Temperature

As a consequence of the results presented in the previous section, measurements with interior-facing electrodes, as well as 100 Hz sampled measurements, were excluded in order to assess the robustness of ECG quality to Arctic temperature regimes and high swimming activity.

At 0 BL/s water velocity, the quality of ECG traces was highest, with overall 90% QI $\leq$ 1 (Figure 9). The shift to imposed swimming activity (i.e. water velocities $\geq$ 1.4 BL/s) caused pronounced noise in the ECG. Morphology of the noise signals was generally highly variable, including unique events of multiple high-amplitude spikes, as well as overlong periodic signals that increased in frequency with increasing swimming velocity. Across the range of 1.4 to 2.6 BL/s, quality of f_H measurements was relatively constant over increasing swimming velocity with a mean of 59.3 $\pm$ 6.6% QI $\leq$ 1.
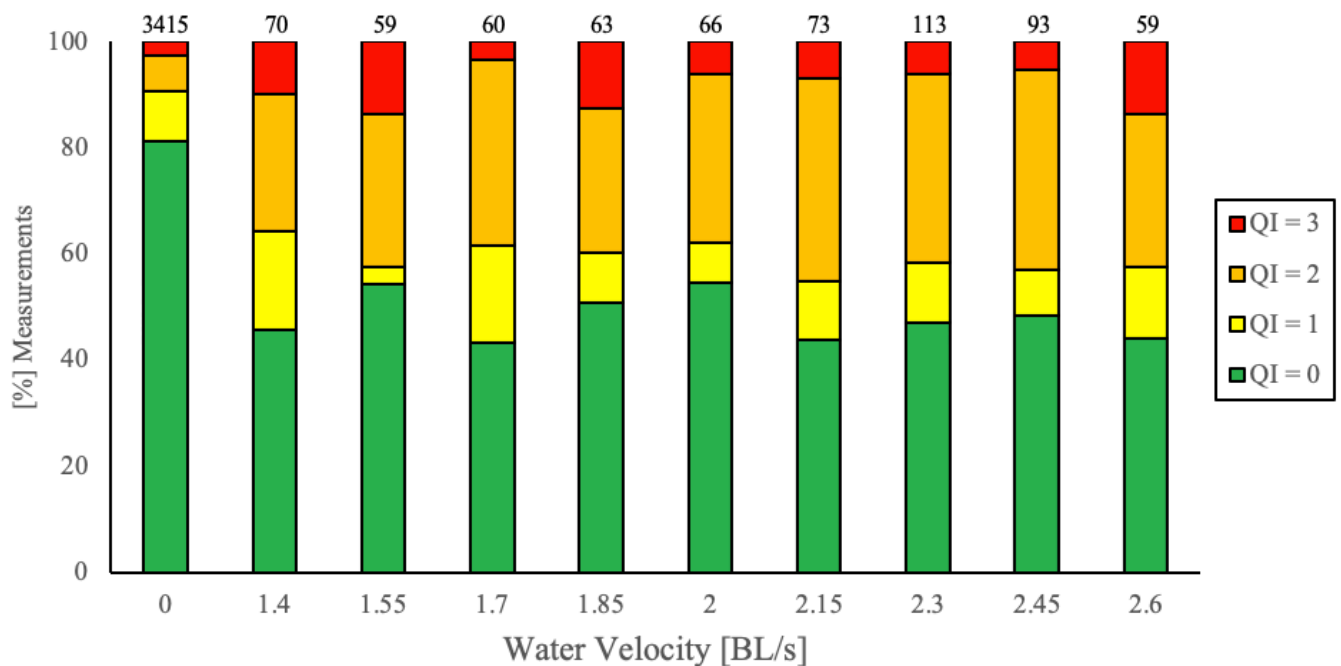
Figure 9 *QI value percentages in response to variable water velocity (i.e. swimming velocity at velocities > 0 BL/s). Measurement numbers (n) are on top of the bars.*

Regarding the simulation of Arctic temperatures, ECG quality was constantly high at lower temperature regimes with a mean of 90.2 ± 3.5% QI ≤ 1 from 0 to 4°C (Figure 10). With further increase in temperature, however, a stepwise decrease in ECG quality was revealed, as 6°C f_H measurements were assigned with 70.8% QI ≤ 1 and at 8°C f_H measurements with 60.6% QI ≤ 1. Furthermore, morphology of the ECG traces displayed no remarkable trends in response to variable temperatures.
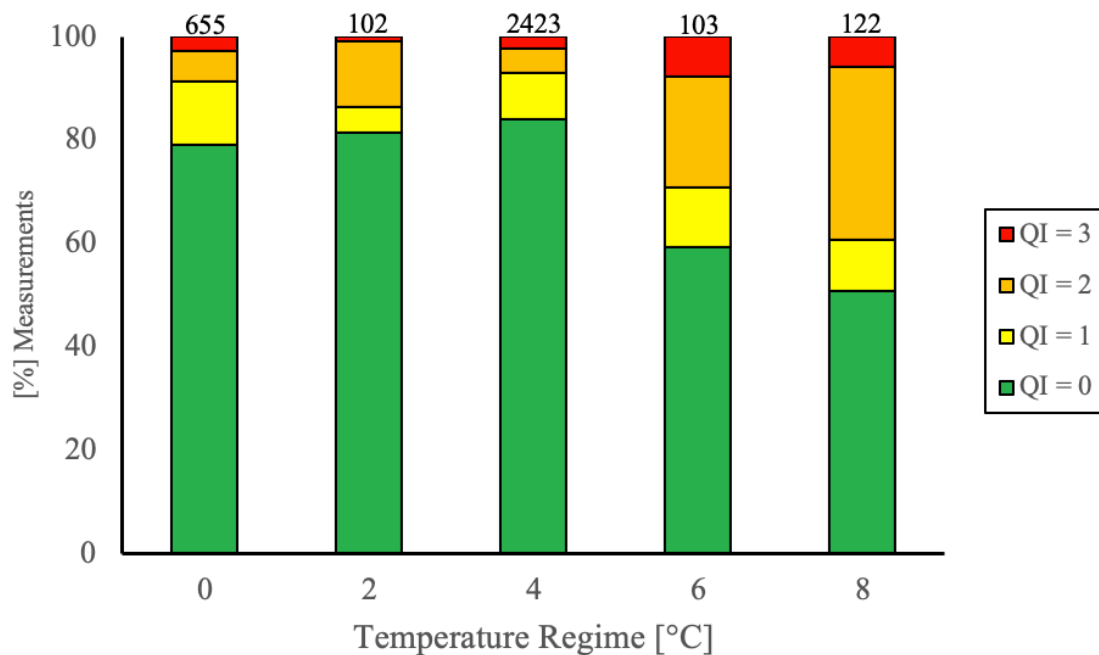
Figure 10 *QI value percentages in response to variable temperature treatment. Measurements were recorded at 0 BL/s water velocity. Measurement numbers (n) are on top of the bars. Variation in n is explained by SMR recording at 0°C and holding at 4°C.*

3.3. Swimming Performance

The swimming performance of polar cod was highly heterogenous. Multiple variable behaviors were observed across the 11 sampled individuals. Positioning within the swim tunnel ranged from full water column swimming to high proportions of dragging on the swim tunnel floor. All fishes displayed periods of resting at the back end of the swim tunnel, however, while most fish rested during critical velocities, n = 1 fish entirely refused to swim.

3.3.1. Critical Swimming Speed (U_{crit})

The individual which entirely refused to swim was excluded from all further activity-related analyses (resulting n = 10). Overall, U_{crit} was highly variable among polar cod and ranged from 1.55 to 2.75 BL/s, which corresponded to 40.00 to 70.13 cm/s. Neither U_{crit} [BL/s] nor U_{crit} [cm/s] were significantly (p > 0.7) affected by temperature (Figure 11). However, while U_{crit} [BL/s] was significantly impacted by fish weight (p < 0.0001) with 2.47 ± 0.22 BL/s for 50 to 83 g fish and 1.92 ± 0.20 BL/s for 98 to 111 g fish.
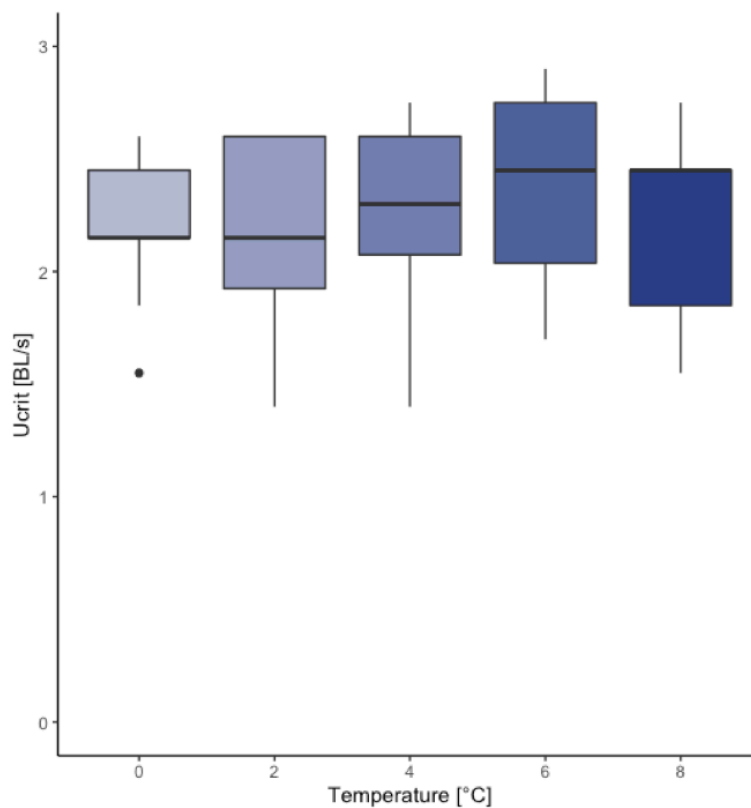
Figure 11 Critical swimming speed (U_{crit}) [BL/s] for n = 10 polar cod across temperatures ranging from 0 to 8°C. Temperature is color-coded.

3.4. Heart Rate Measurements

Across the entirety of this study, 5169 high quality (i.e. QI ≤ 1) f_H measurements were obtained for n = 10 polar cod (exclusion of n = 1 logger with interior-facing electrodes). Manually calculated f_H ranged from minimum 8 bpm in the respiration chamber to maximum 55 bpm during U_{crit} tests.

3.4.1. Swimming Velocity and Temperature Impact on Heart Rate

f_H was significantly (p < 0.0001) influenced by swimming velocity (Table 3). For an increase of 1 BL/s swimming velocity, f_H averaged an increase of 4.65 ± 0.43 bpm, which was relatively constant across temperatures (i.e. 5.10 bpm at 0°C, 4.47 at 2°C, 4.34 bpm at 4°C, 4.22 bpm at 6°C, and 5.12 bpm at 8°C; Figure 12 A and B). The temperature-specific relationships between f_H and swimming velocity, however, did neither significantly fit linear regression, nor exponential growth models (both $R^2 < 0.4$).

The increase in temperature also caused a significant increase in f_H (p < 0.0001), however, there was no interactive effect of swimming velocity and temperature (p = 0.3). Mean f_H at 0 BL/s water velocity was 21.64 ± 2.23 bpm at 0°C, 26.13 ± 3.34 bpm at 2°C, 31.67 ± 3.75 bpm at 4°C, 36.74 ± 4.43 bpm at 6°C, and 40.91 ± 5.18 bpm at 8°C. Incremental Q_{10} values (2°C increments from 0 to 8°C) of f_H displayed similar results across slow (1.4 to 1.7 BL/s), medium (1.85 to 2.15 BL/s), and fast (2.3 to 2.6 BL/s) water velocities (Figure 12 C). Average Q_{10} values were highest at 2 and 4°C with Q_{10} equal to 2.58 ± 0.69 and 2.50 ± 0.82 respectively. At 6°C, Q_{10} values declined to 2.00 ± 0.50 and at 8°C they declined below 2 with 1.73 ± 0.74. f_H increased linearly with temperature ($R^2 = 0.75$; exponential growth: $R^2 = 0.72$) with a spearman rank correlation coefficient rho = 0.87.

Table 3 *Generalized Linear Mixed-Effects Model (GLMM) and multiple regressions – Degrees of freedom (DF), Chi square values (X^2) and p-values of swimming velocity (v), temperature (T) and the interaction of the two (v:T) predicting heart rate (f_H) of n = 10 polar cod during critical swimming speed (U_{crit}) tests. Significant p-values are printed in bold.*

Predictor Variable	DF	X^2	p-value
v	1	1001.2	**< 2.2e^{-16}**
T	4	73.3	**< 2.2e^{-16}**
v:T	4	4.8	0.3
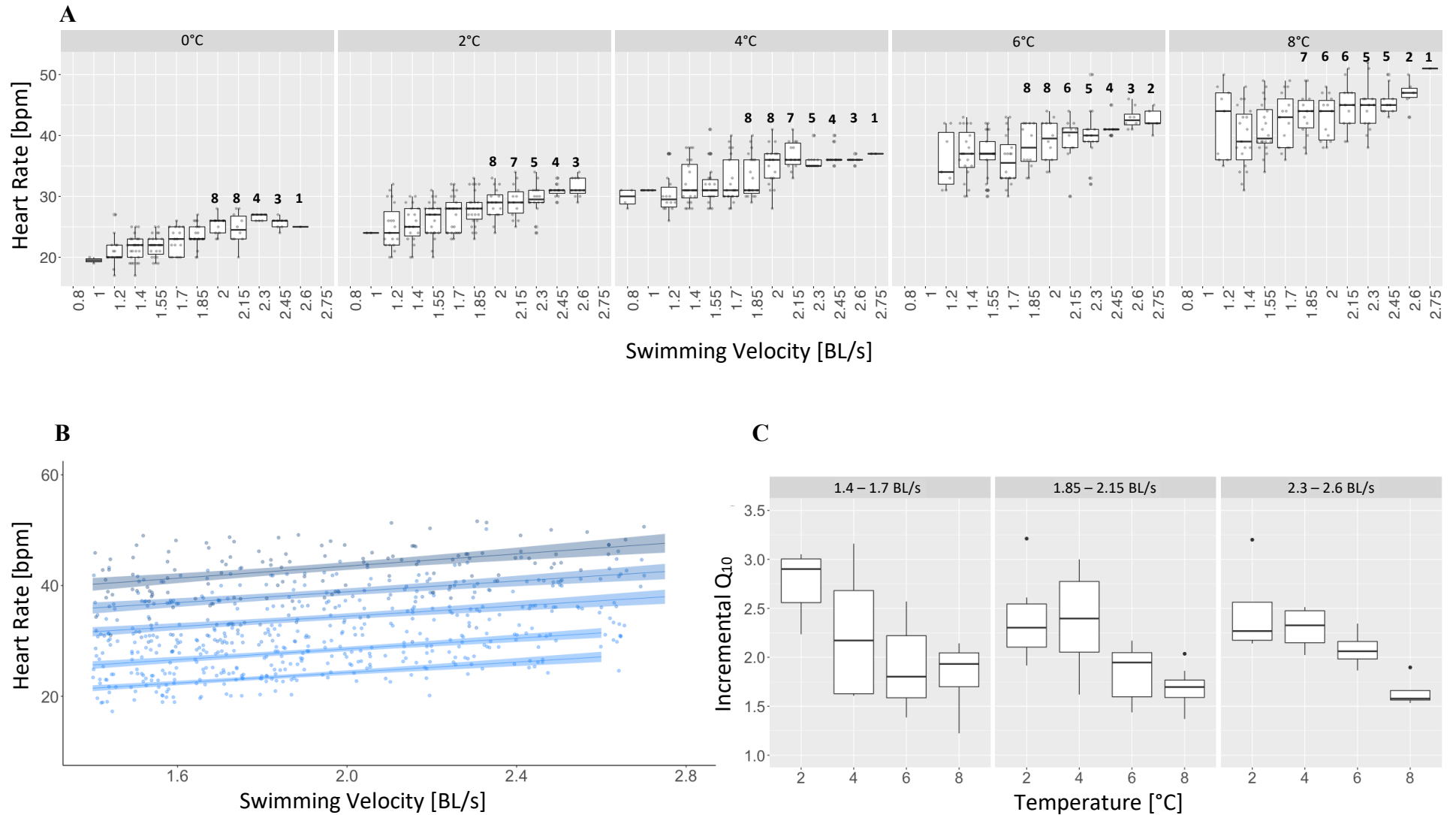
Figure 12 Heart rate (f_H) measurements of n = 10 polar cod, obtained by internal Star-Oddi bio-loggers, across increasing temperature and swimming velocity. **A** Temperature-specific f_H sequences across U_{crit} test 10-minute velocity intervals. Decreasing sample sizes are indicated by numbers on-top of boxplots. **B** Linear regressions of temperature-specific f_H increase with increasing swimming velocity. Temperature is color-coded and ribbons display standard error. **C** Incremental Q_{10} values of f_H across 2°C steps at slow, mid, and fast swimming velocities.

3.5. Respiratory Measurements

Over the course of this study, both flow-through respirometry in the respiration chamber and intermittent-flow respirometry in the swim tunnel were performed. Within the respiration chamber, 200 M_{O2} measurements of n = 11 fishes were recorded at 0°C. Those measurements were solely used for SMR calculations, while in the swim tunnel, 297 M_{O2} measurements of n = 10 fishes were recorded and used for all other respiratory analyses.

3.5.1. Standard Metabolic Rate (SMR)

M_{O2} of polar cod initially decreased after transfer to the respiration chamber and a distinct increase of M_{O2} during day-time was monitored. Therefore, measurements of the present study were recorded at night, at least 24 h after fish transfer. SMR was calculated after Chabot et al. (2016) as an average of the lower 15% quantile of M_{O2} measurements at 0°C in the respiration chamber. The derived SMR of bio-logged polar cod, approximately one-month post-surgery, amounted to 0.38 µmol/g/h (Figure 13). Within the respiration chambers, M_{O2} ranged over ~10 orders of magnitude from 0.23 µmol/g/h to 2.28 µmol/g/h.
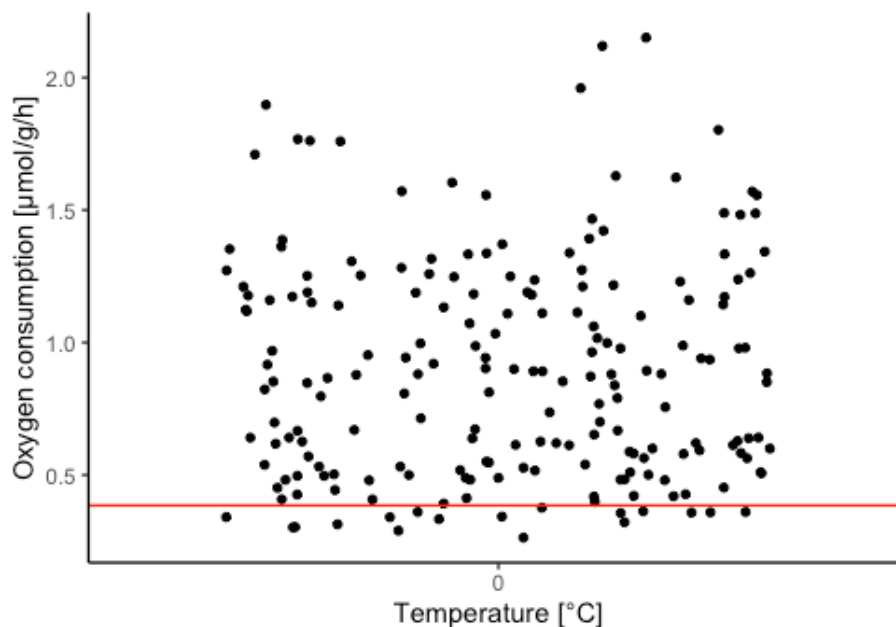
Figure 13 *Respiration chamber oxygen consumption (M_{O2}) measurements of n = 10 polar cod at 0°C. The red line indicates standard metabolic rate, calculated after Chabot et al. (2016).*

3.5.2. Metabolic Rate Across Temperature and Swimming Velocity

After a two-hour acclimatization period to the swim tunnel environment and basic water velocity, active M_{O2} was recorded in 50 U_{crit} tests of 10 polar cod (n = 1 fish refused to swim). M_{O2} was significantly (p < 0.0001) impacted by swimming velocity (Table 4). For an increase of 1 BL/s in water velocity, M_{O2} averaged an increase of 3.91 µmol/g/h at 0°C, 4.36 µmol/g/h at 2°C, 3.39 µmol/g/h at 4°C, 2.43 µmol/g/h at 6°C, and 4.67 µmol/g/h at 8°C (Figure 14). The temperature-specific relationships of M_{O2} and water velocity were best fit to a linear regression at 2°C (R^2 = 0.57), 4°C (R^2 = 0.47), and 8°C (R^2 = 0.49).

Further, M_{O2} significantly increased with rising temperature (p = 0.002). However, the combination of both swimming velocity and temperature did not assert an additional effect (p = 0.712). Mean M_{O2} at lowest water velocities (i.e. 1.45 to 1.55 BL/s) increased under rising temperatures (i.e. 0°C: 3.43 ± 1.84 µmol/g/h; 2°C: 3.80 ± 1.37 µmol/g/h; 4°C: 4.41 ± 1.58 µmol/g/h; 6°C: 4.70 ± 1.47 µmol/g/h; 8°C: 5.92 ± 1.78 µmol/g/h) with the distinctly largest incremental difference of 1.22 ± 2.31 µmol/g/h between 6 and 8°C.

Table 4 Generalized Estimation Equation Generalized Linear Model (GEEGLM) and multiple regressions – Degrees of freedom (DF), Chi square values (X^2) and p-values of swimming velocity (v), temperature (T) and the interaction of the two (v:T) predicting oxygen consumption (M_{O2}) of n = 10 polar cod during critical swimming speed (U_{crit}) tests. Significant p-values are printed in bold.

Predictor Variable	DF	X^2	p-value
v	1	24.14	**$7e^{-06}$**
T	4	16.88	**0.002**
v:T	4	2.13	0.712
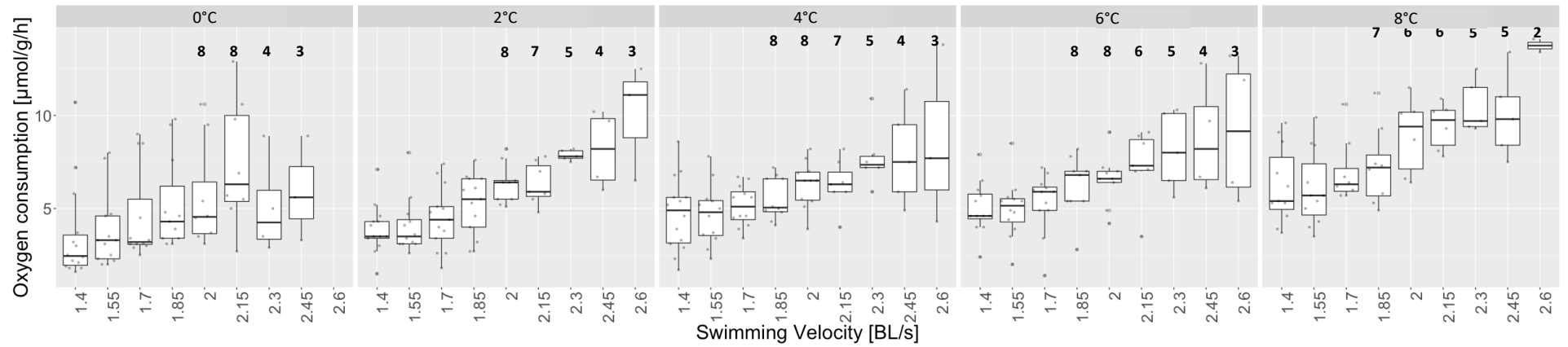
Figure 14 *Temperature-specific oxygen consumption (M$_{O2}$) of n = 10 polar cod across 10-minute velocity intervals of critical swimming speed (U$_{crit}$) tests. Bold numbers on top of boxplots indicate the decrease of sampled fishes due to ceased swimming activity.*

3.5.3. Maximum Metabolic Rate

After exclusion of the highest 5% of swim tunnel M_{O2} measurements, highest M_{O2} for each fish at each respective temperature was taken as individual MMR. Mean MMR displayed the trend to increase with increasing temperature, however, individual variability was high and the impact of temperature was not significant (p = 0.24) on MMR (0°C: 5.54 ± 2.24 µmol/g/h; 2°C: 6.76 ± 1.62 µmol/g/h; 4°C: 6.68 ± 1.11 µmol/g/h; 6°C: 7.20 ± 1.40 µmol/g/h; and 8°C: 8.26 ± 1.37 µmol/g/h; Figure 15).
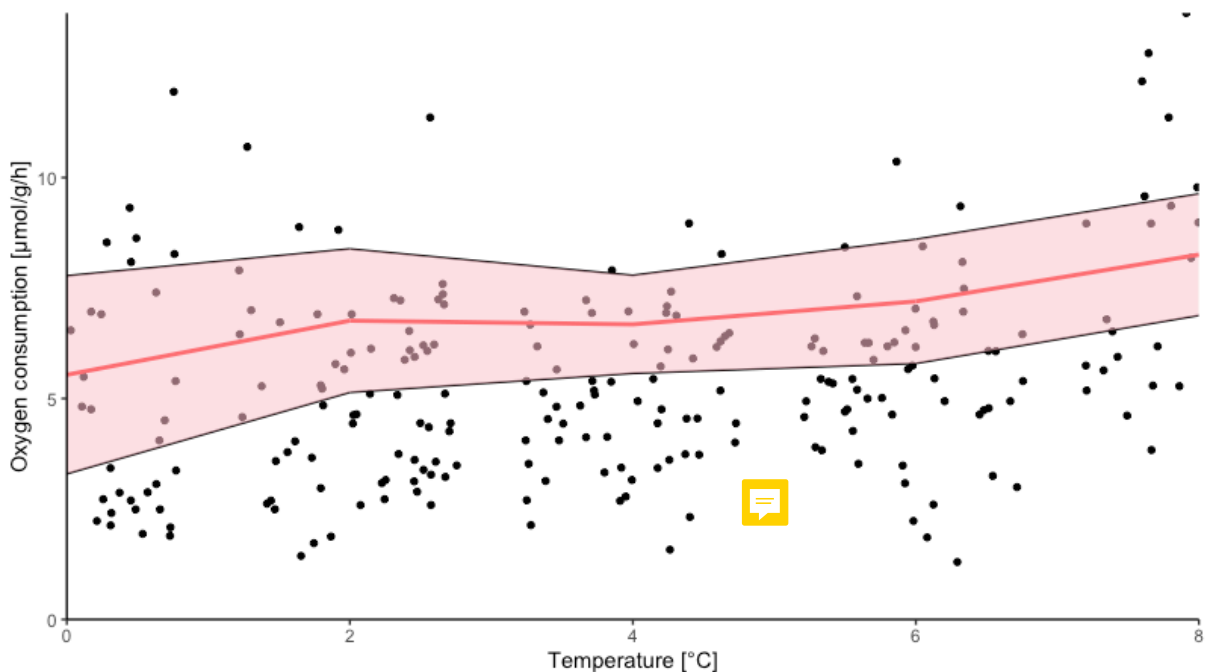
Figure 15 *Oxygen consumption (M_{O2}) of n = 10 polar cod across temperature ranging from 0 to 8°C during U_{crit} tests. The red line displays maximum metabolic rates (MMR) (i.e. respectively highest individual M_{O2}) at given temperatures. Ribbons indicate standard deviation.*

3.5.4. Heart Rate and Metabolic Rate Correlation

f_H and M_{O2} were recorded in parallel across 50 experimental swim tunnel runs with n = 9 polar cod. M_{O2} was significantly (p < 0.01) impacted by f_H (Table 5) with a positive correlation coefficient rho = 0.42. M_{O2} averaged an increase of 0.17 µmol/g/h for a f_H increase of 1 bpm (Figure 16), however, the algebraic relationship of M_{O2} and f_H did neither fit linear regression nor exponential growth models significantly (both with $R^2 < 0.4$). Furthermore, there was no significant interactive effect of f_H and temperature (p = 0.25) or f_H and swimming velocity (p = 0.41) on M_{O2}.

Table 5 *Generalized Estimation Equation Generalized Linear Model (GEEGLM) and multiple regressions – Estimates, standard error (SE) and p-values of heart rate (f_H), the interaction with temperature (f_H:T), and the interaction with swimming velocity (f_H:v) predicting oxygen consumption (M_{O2}) of n = 9 polar cod during critical swimming speed (U_{crit}) tests. Significant p-values are printed in bold.*

Predictor Variable	Estimate	SE	p-value
f_H	0.1701	0.0575	**0.0031**
f_H:T	0.0105	0.0092	0.2515
f_H:v	-0.0198	0.0241	0.4110
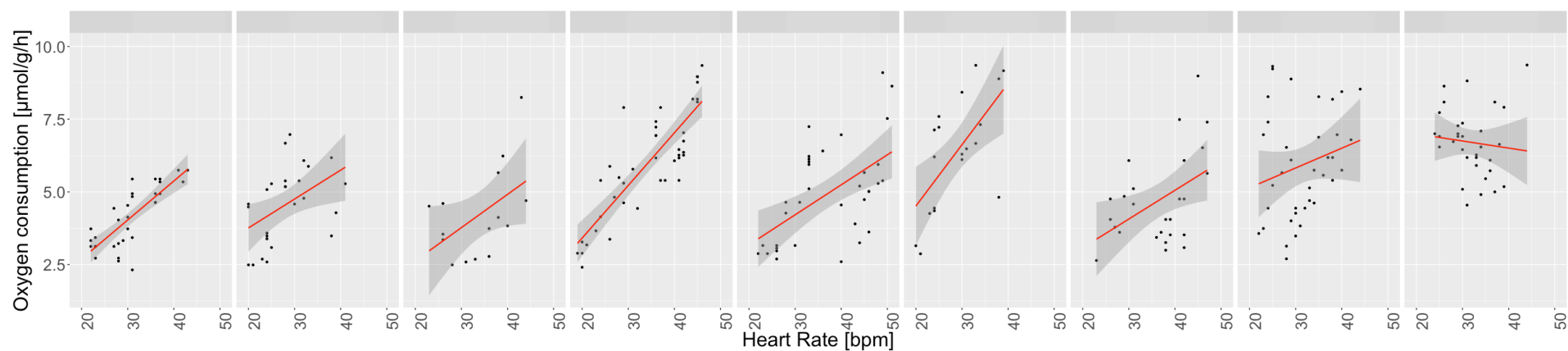
Figure 16 *Individual oxygen consumption (M_{O2}) of n = 9 polar cod in relation to simultaneously measured heart rates (f_H). The red lines visualize linear regressions. Ribbons display standard error.*

4. Discussion

4.1 Bio-Logger Implantation

Intracoelomic surgical implantation via laparotomy is generally considered most appropriate for long-term bio-logging applications (Jepsen et al., 2002; Bridger and Booth, 2003; Brown et al., 2009). Nevertheless, to the best of my knowledge, it has never been performed in polar cod. Scientific research on the methodology of surgical procedures in pelagic marine fishes is scarce (Cooke et al., 2011), which is why in this section, trial-and-error-based experiences and necessities for future research involving the implantation of electronic tags in polar cod will be discussed. The bio-logger implantation with a midline 15 mm laparotomy in close proximity to the pelvic fins was overall highly successful. All 16 operated fish resumed swimming after the implantation of 11.8 g foreign material and the period of approximately 30 min anesthesia. Furthermore, in the dissection after the experimental runs, polar cod displayed little inflammatory response to incision and sutures. While that is indicative of accurate surgical sterility, it also reflects the slow incision healing of polar cod, as the inflammatory process is the initial phase of tissue regeneration (Brookman et al., 1988). In fact, insufficient wound closure and consequent logger exposure was a reoccurring problem of the present study and associated euthanasia was the singular cause for fish mortality (n = 4). Slow incision healing is expected at 4°C holding conditions, due to decreasing cellular responses at colder temperatures (Finn and Nielsen, 2006) and generally slower biochemical reactions following Q_{10} rules. Therefore, recovery periods of the present study were set to minimally one month and it is advisable to follow that in future studies. Tag size, suture material, and techniques used for wound closure potentially impact incision healing (Cooke et al., 2011). Although Star-Oddi's most miniaturized f_H bio-logger was used, it is important to address that the logger weighed 10.8 to 24.0% of total fish weight. Even though the arbitrarily accepted "2%" rule proposed by Winter (1983) has been opposed by a growing body of literature showing no significant mortality, tag loss or sublethal impact with implanted devices up to 8–12% of body mass (e.g. Brown et al., 1999; Lacroix et al., 2004), the importance to work with polar cod individuals as large as possible must be highlighted. Besides potential sublethal limitations on physiology (see section 4.3), reduced intracoelomic volume in smaller fishes, leading to increased pressure of the bio-logger against the suture, possibly represents the largest obstruction on wound closure. The suture material of the present study changed several times between monofilament and braided silk. Both sutures were nonabsorbable as previous research hinted that absorbable

sutures were lost more quickly (Walsh et al., 2000; Cooke et al., 2011). While braided silk sutures were easier to tie, reducing surgery time, monofilament sutures appeared to persist longer. Since wound closure and suture loss were reoccurring problems, in future research within our working group, we aim to compare presently used parallel, single stitching sutures to diagonal stitching which reduces tensile stress due to four points of fixation instead of two. In conclusion, polar cod revealed itself to be highly robust towards the implantation, with diminutive inflammatory response and display of standard swimming behavior despite the fairly large weight proportion of the internally borne bio-logger. Striving for field applications, ongoing empirical studies, designed to enable higher powered analysis, will be needed to improve sutures and wound closure to ensure logger retention.

4.2 Electrocardiogram Quality/ Methodological Validation

This is the first study to use f_H bio-loggers at Arctic temperature regimes or within free-roaming polar cod. Both technological restrictions due to lowered electron conductivity at decreasing temperature, and physiological restrictions due to generally low f_H of polar fish, challenged the implemented Star-Oddi f_H bio-loggers and left uncertainty regarding the ability to obtain high-quality ECG recordings. Bio-logging a previously untagged species requires initial feasibility analysis of the general methodology (Wilson et al., 2015). Hence, in the present study, manual f_H calculation via saved ECG traces determined confidence of on-board processed f_H measurements of respective QI values. Although ECG recordings were focused heavily on swim tunnel trials, overall 64% displayed the highest quality of QI = 0. Further, QI = 0 measurements were close to equal in comparison to on-board processed measurements with $\Delta f_{H,0} = 0.45 \pm 0.56$ bpm (see Figure 7). Consequently, both these results were strong initial indicators for the success of present Arctic f_H bio-logging methodology.

QI = 1 measurements were still within $\pm$ 10% of average manually calculated f_H, while on-board processed QI $\geq$ 2 measurements could not be included in subsequent physiological analyses (i.e. $\Delta f_{H,1} = 2.82 \pm 3.94$ bpm, $\Delta f_{H,2} = 15.86 \pm 32.37$ bpm, $\Delta f_{H,3} = 30.18 \pm 9.13$ bpm). Further literature comparison on Star-Oddi f_H bio-loggers revealed similar results throughout all studies drawn upon. For example, Zrini and Gamperl (2021) discussed f_H miscalculations up to 39 bpm for QI $\geq$ 1 measurements in *Salmo salar*. Therefore, similarly to most other studies, f_H measurements with QI $\geq$ 1 were removed for their analyses (e.g. Prystay et al., 2019; Brijs et al., 2019; Wallerius et al., 2019). As a general rule for future studies, if large-enough amounts of QI = 0 measurements are recorded, exclusion of QI $\geq$ 1 on-board processed

measurements is probably advisable. Limitations in memory and battery life, however, could impact the acceptance-range of measurement confidences. This particularly holds true for cold water species, as generally low f_H demands long-enough sampling duration and seldomly tagged species demand ECG savings for feasibility analysis. Lastly, it is important to conclude that for true validation of f_H recording accuracy, simultaneous experimental runs with Doppler or Transic® flow probe controls should be considered (Zrini and Gamperl, 2021).

After the initial validation of Star-Oddi f_H bio-loggers, further adjustments regarding implantation, sampling and processing were compared in order to improve the percentage of highest quality measurements further. Positioning bio-loggers with electrodes facing exterior (i.e. facing the incision) instead of interior, had the largest impact on improving ECG quality. Brijs and colleagues (2018) obtained similar results, as highest ECG quality was obtained when electrodes were oriented "ventrally" in *Oncorhynchus mykiss*. Zrini and Gamperl (2021) differentiated bio-logger positioning according to *Salmo salar* sizes, and individuals < 1.7 kg were implanted with electrodes facing the exterior (i.e. "label up") due to artifacts in the ECG recording when facing the interior. In the present study, examination of the ECG traces led to similar conclusions, and decreasing quality of interior-facing electrodes to an increase in noise penetration was observed. The repetitive nature of this noise and its increase in frequency with swimming velocity increase, indicates that it is electromyogram (EMG) noise. The interference of EMG is a well-known problem, as electric potentials of red muscles (i.e. aerobic muscles) overlap with the frequency range of the fish's electrocardiographic signals (Thakor et al., 1984). Secondly, sampling at 125 Hz was found to produce significantly higher amounts of QI = 0 measurements compared to 100 Hz sampling. Generally, sampling at higher frequency bears the potential to improve quality as a more close-meshed and distinct mapping of ECG properties (i.e. the QRS interval) is obtained. Since increased sampling frequency is accompanied by decreasing sampling duration, however, this adjustment has to be questioned in terms of capturing the lowest f_H range of Arctic fish. With lowest manually calculated f_H of 125 Hz measurements being 8 bpm, it was fairly close to the theoretical lower limit of 12 s sampling duration: ~6 bpm. 100 Hz measurements, however, displayed similar f_{Hmin} over 15 s records, validating the reduction to 12 s sampling duration for polar cod.

Lastly, the comparison of frequency responses displayed no significant differences. Since this information is typically under disclosure of the developer, future generations of Star-Oddi f_H bio-loggers will reveal whether measurement quality can further be refined by adjustments to the signal processing algorithms.

Building on above-mentioned findings, only 125 Hz measurements with exterior-facing electrodes were included in the final assessment of ECG quality response to Arctic temperature and swimming velocity.

Surprisingly, ECG quality displayed no effect of lowered electron conductivity in response to decreasing temperature. On the contrary, with an average of 80%, the percentage of QI = 0 measurements was highest at temperatures ranging from 0 to 4°C, and it decreased to 60% at 8°C. Zrini and Gamperl (2021) observed a similar trend in lumpfish (*Cyclopterus lumpus*), however, at temperatures increasing from 12 to 22°C. It must be stated that temperature-calibrated frequency responses, generally not accessible by the researcher, potentially have remarkable impact on resulting measurement quality. Nevertheless, decreasing ECG quality with increasing temperature could be explained by increasing EMG noise pollution, due to overall higher muscular activity.

While increasing swimming velocity within the range of 1.4 to 2.6 BL/s had no qualitative impact on corresponding ECG (53% QI = 0), measurement quality was distinctly higher without water circulation in the swim tunnel (80% QI = 0). Overall, ECG properties were clearly more distinguished for traces recorded outside of U_{crit} tests. Once more, this probably is to be attributed to activity of aerobic muscles during swimming performances (Altimiras and Larsen, 2000). Generally, considerable further explanations for noise interferences, determined by previous research, are double recordings of electric potentials (Skeeles et al., 2020) and logger movement in consequence to insufficient internal fixation (Cooke et al., 2016).

Concluding the assessment of present f_H bio-logging methodology, for 125 Hz measurements with exterior-facing electrodes, ECG quality was highly robust in response to Arctic temperature regimes. Generally, QI = 0 percentages of 80 to 90% are highly desirable. In *Salmo salar*, Zrini and Gamperl (2021) obtained 68 to 88% QI = 0 and Skeeles et al. (2020) determined a successful run when QI = 0 spanned across at least 80% in the small marine sparid *Chrysoblephus laticepus*. Furthermore, >50% of QI = 0 measurements during swimming activity are also to be assessed as fairly robust. Decreasing ECG quality during U_{crit} tests of the present study has to be set into perspective of future research objectives. Maximum performance levels within the swim tunnel are different to natural performances ranges of polar cod in the field, both in duration and in extent. Lastly, f_H measurements of the present study were highly sensitive in response to temperature and swimming velocity (see section 4.4), further indicating great promise for continued f_H bio-logging studies species-specifically with polar cod.

4.3. Metabolic Rate and Heart Rate as Proxies for Polar Cod Welfare

In the laboratory, metabolic rates have been commonly used as direct links to fitness and stress (Burton et al., 2011; Nelson and Chabot, 2011). A large proportion of bio-logging studies and responding fish welfare analyses, either as premise or as ultimate objective, come from aquacultural research. Existing tools to evaluate fish welfare are referred to as Operational Welfare Indicators (OWIs; Noble et al., 2018). f_H has increasingly been considered to be such an OWI, as it directly relates to metabolic rate, and consequently reflects the level of stress in fish (Heath and Hughes, 1973; Laitinen and Valtonen, 1994; Lucas, 1994; Donaldson et al., 2010). In recent years, f_H bio-logging has successfully reflected stress responses in farmed rainbow trout (*Oncorhynchus mykiss*) and farmed Atlantic cod (*Gadus morhua*) (Brijs et al., 2018, 2019; Bjarnason et al., 2019). For the present study, the term stress has to be further defined to avoid confusion. Increasing "demand for change" is often used in equivalent to stress. Given the organismal ability for compensation, however, such demand falls into the physiologically addressable performance scope. Only past this normal tolerance range, demand for change is to be termed "stress". Nevertheless, in evaluation of the present bio-logging methodology, generally increasing performance demands will be discussed. During the evaluation of whether obtained f_H measurements are representative for untagged fish, with the ultimate goal to derive metabolic rates (i.e. energy budgets), the welfare of polar cod with internally-borne bio-loggers was assessed by comparison of fish M_{O2} (SMR and MMR), f_H, and swimming behavior to untagged controls. A previous study within our working group, performed by Kempf (2020) was drawn to for controls. In a similar experimental design to that of the present study, polar cod were measured in respiration chambers and performed in U_{crit} tests. In Kempf's study, however, the experiments were carried out at 2.5 ± 1°C and under variable oxygen saturation levels ranging from 5 to 100%.

With the exception of one fish which entirely refused to swim, bio-logged polar cod showed standard swimming behavior, similar to control fish of the previous study. It must be commented, however, that polar cod generally displays variable swimming behavior within the swim tunnel, and trials are streaked with periods of resting as well as dragging on the swim chamber walls. Hence, in comparison to active fish species, which would display homogenous swimming behavior and positioning up to substantially higher velocities, polar cod is to be characterized as a "bad swimmer" (or a "sluggish" fish (Pörtner et al., 2017)).

SMR of the present study was calculated as the average of the lowest 15% of all recorded metabolic rates in the respiration chamber, after removal of the lowest 5% as outliers, and amounted to 0.38 µmol/g/h at 0 ± 0.5°C. Therewith, despite the additional logger carriage, it was lower than in control fish of Kempf (2020) with 0.44 µmol/g/h at 2.5 ± 1°C. Nevertheless, as Q_{10} values within the active tolerance range of stenotherm and "sluggish" fish are generally high (Pörtner et al., 2017; see section 4.4), one would expect a more than two-fold increase in M_{O2} from 0 to 2.5°C. The minor decrease, however, does not necessarily reflect SMR elevation in response to bio-logger bearing. Firstly, relatively large temperature variance of ± 1°C in the previous study might have caused a low SMR reading at 1.5°C. Furthermore, protection against lowest temperatures, such as the formation of antifreeze glycoproteins (Osuga and Feeney, 1978), could have led to an adaptive increase in SMR. In the past, Holeton (1974) raised a debate on whether metabolic cold adaption (i.e. elevated metabolic rates at lowest temperature regimes) holds true for Arctic fish, in relation to temperate species. While he generally disproved such metabolic elevation, *Boreogadus saida* was the sole example which displayed increased M_{O2} at 0°C. Later, Steffensen (2002) reanimated the debate, asking once again if metabolic cold adaption is "a fact or an artefact". His research contradicted metabolic cold adaption, species-specifically for polar cod, and contradictory results of the past were attributed to outdated respirometric methodology. Comparing SMR of the overall clearly cold-adapted *Boreogadus saida* (i.e. 0.38 µmol/g/h at 0 ± 0.5°C; normalized to a 100 g fish) to previous literature on eurythermal species at similar weight and temperature, 100 g *Gadus morhua* with ~ 0.5 – 1.0 µmol/g/h at 2°C (Tirsgaard et al., 2015), and 100 g *Zoarces viviparous* with ~ 0.3 µmol/g/h at 0°C (Pörtner, 2001), the present study supports the findings of Steffensen and contradicts large-scale metabolic cold adaption across the observed temperature range. Overall, low SMR despite internally-borne loggers is a strong indicator of fish welfare. One-month post-surgery, polar cod appear to be fully recovered from the bio-logger implantation and standard metabolism displays no detrimental effect of bearing the logger, which would consequently imply that measurements obtained are representative for untagged fish (Cooke et al., 2011; Jepsen et al., 2015). Furthermore, despite generally high variability in routine metabolic rates, the average of lowest individual M_{O2} was fairly homogenous across individuals with 0.48 ± 0.18 µmol/g/h after weight normalization. This was surprising, given that loggers weighed up to 24% of body weight in smallest individuals. Overall, those findings indicate exceptional robustness of polar cod and highlight the minimally invasive nature of the bio-logging implantation.

As polar cod f_H was not previously recorded in similar experimental design, f_{Hmax} measurements of Drost and colleagues (2014) were drawn to for controls. However, they measured f_{Hmax} of anesthetized fish via intraperitoneal injections of atropine and isoproterenol. While f_H at lowest swimming velocities within the present study was strictly and uniformly below Drost's f_{Hmax} measurements by ~5 bpm across all respective temperature regimes, f_H at U_{crit} was highly similar to f_{Hmax} of the previous study. Hence, there was no stress-related humoral or neural chronotropic regulation of pacemaker activity leading to an increase in f_{Hmax} above previously measured levels (Farrell et al., 2009). However, potential increase in stroke volume to meet oxygen demands (see section 4.5), and effects of the referenced methodology, such as effects of anesthesia, must not be neglected. Therefore, in the present study, the proxy of M_{O2} is weighted stronger for fish welfare analysis and consequent species-specific methodological assessment. With confidence, however, it can be stated that f_H measurements of the present study were highly sensitive to temperature and swimming velocity increase (see section 4.4). In counter conclusion, potential regulatory patterns of f_H to meet oxygen demand were not masked by detrimental effects of bearing the bio-logger.

Swim tunnel U_{crit} tests are mostly attributed to indicate fatigue (Peake and Farrell, 2005). In contrast, manually chasing fish in a static tank until they become unresponsive is attributed to indicate exhaustion. While exhaustion can be defined as the state when a fish has depleted its energy storage and cannot be stimulated to resume activity at any level, fatigue describes the state where the fish has not fully depleted its energy storage, however, cannot immediately be stimulated to resume previous activity levels. Fatigue has been confirmed to be largely a behavioral response, hypothesized to preserve muscle glycogen stores, and not brought about by total depletion of energy reserves (Peake and Farrell, 2005). U_{crit} of the present study was not significantly impacted by temperatures ranging from 0 to 8°C and averaged 2.3 ± 0.3 BL/s. Therewith, it was distinctly lower than in untagged controls of Kempf (2020), which averaged 2.90 ± 0.45 BL/s U_{crit} at 100% oxygen concentrations. This earlier onset of fatigue, potentially is a consequence of rising oxygen demand due to the bio-logger bearing (further discussed below). Interestingly, while U_{crit} in BL/s (relative to fish total length) was clearly impacted by fish size, no significant differences were found in analysis of total U_{crit} in cm/s across individuals.

Similar to the U_{crit} results, a discrepancy in MMR with 6.76 ± 1.62 µmol/g/h at 2.0 ± 1°C in comparison to controls of Kempf (2020) with 4.39 ± 0.56 µmol/g/h at 2.5 ± 1°C, was observed. This potentially indicates an interactive stress effect of bio-logger bearing and highest exercise,

resulting in rising oxygen demands (e.g. due to additionally carried weight) of bio-logged fish. Considering lowered U_{crit} compared to controls, such increased oxygen demand could have consequently resulted in earlier onset of fatigue in polar cod. The high variability of individual size and weight within the present study, however, must be addressed in assessment of polar cod welfare (i.e. representative nature of measurements). Despite weight-normalization, MMR of the present study was highly variable across individuals. Although fish weight had no significant effect on MMR, due to the small sample size of n = 10, it should be considered that MMR of the two largest individuals amounted to 5.28 µmol/g/h and 4.43 µmol/g/h at $2.0 \pm 1°C$. That strongly highlights the importance to work with largest possible individuals when bio-logging polar cod. Lastly, given the high variability of M_{O2} measurements, accuracy of the present respirometry method has to be discussed. Due to the relatively high difference of fish and respirometer volume, erratic oxygen traces were obtained. Pulses of water with different oxygen tensions are typical in large respirometers, since there is no perfectly stable diffusion gradient due to fish movement disturbances. Further, oxygen leakage into the respirometer is to be considered, and these problems are amplified when M_{O2} is very low, as it was presently the case in simulation of Arctic water temperatures.

Concluding the assessment of fish welfare, what are the consequences of above-mentioned discrepancies in U_{crit} and MMR for future f_H bio-logging studies in polar cod? Firstly, the overall similarity of SMR measurements to untethered controls, as well as high sensitivity of f_H in response to temperature and swimming velocity, are strong indicators of sufficient recovery one-month post-surgery. Interactive stress effects of bio-logging at highest performance levels should be addressed by further feasibility analyses, as they would have severe implications on the representability of bio-logged polar cod in comparison to untagged individuals. Nevertheless, the extent of additional oxygen demand at maximum activity levels, not being at display in polar cod baseline metabolism has to be questioned. Furthermore, when comparing swim tunnel derived maximum performance parameters of the present study to previous controls, the additive implications, of both individual variability in M_{O2} and the behavioral component in fish fatigue, on small sample sizes of the present study should be considered. Therefore, although swim tunnel exercise might not necessarily reflect natural performance levels (Plaut, 2001), I want to emphatically stress the necessity to perform further feasibility studies of homogenously sized polar cod at maximum swimming performance, by implementing a control of simultaneously performing untagged fishes at similar treatments.

4.4. Temperature Susceptibility of Polar Cod Heart Rate

Polar cod f_H ranged from minimum 8 bpm in the respiration chamber to maximum 55 bpm during U_{crit} tests (see Figure 12 A). Although relatively low f_H are typical for polar fish due to biochemical reactions following Q_{10} rules, a f_H of 8 bpm is at the extreme low limit for fish in general. For reference, to the best of my knowledge, the routine free-swimming f_H measurement of 6 bpm in the Antarctic fish *Dissostichus mawsoni* is the lowest recorded f_H for any fish (Campbell et al., 2009). This indicates that polar cod f_H at low temperatures is primarily dictated by kinetic restraints.

A holistic understanding of how temperature affects fish f_{Hmax} is still to be reached (Farrell et al., 2009). For example, in resting and exercising sockeye salmon, f_{Hmax} displayed strict thermal independence (Steinhausen et al., 2008). In contrast, polar cod f_{Hmax} was highly temperature-sensitive. Despite methodological differences, temperature-dependent f_H at maximum exercise in the present study was close to or equal to f_{Hmax} values obtained by Drost et al. (2014). Firstly, this supports that temperature is the primary modulator of intrinsic f_H (Gollock et al., 2006; Clark et al., 2008; Farrell et al., 2009) up to maximum performance in polar cod. Further, measurements displayed highest similarity, although polar cod were acclimated to 0°C in the previous study, while fish of the present study recovered at least one month at 4°C. That finding is in line with previous observations of restricted acclimation potential and consequently decreasing mitochondrial efficiency in polar cod (Leo et al., 2017). Nevertheless, highly remarkable, present f_{Hmax} was obtained during swim tunnel exercise while previous f_{Hmax} was obtained induced via intraperitoneal injection of atropine and isoproterenol. In both cases, the hearts pacemaker is regulated by hormones (Farrell, 2009). Therefore, the degree of similarity across the experiments, not only validates the relevance of both methods, but also supports the achievement of temperature-dependent physiological capacity of f_H at U_{crit}.

As the present study recorded the first f_H measurements of free-swimming polar cod over increasing performance demands, it represents a valuable opportunity in assessment of temperature susceptibility within a changing climate. Drost and colleagues (2014) observed f_{Hmax} in order to deduct temperature limitations on aerobic scope, which is difficult to accurately measure in remote locations such as the Arctic. Aerobic scope describes a fish's energy budget by subtraction of maximum energy supply (i.e. MMR) and minimum energy demands (i.e. SMR). Following the OCLTT (Pörtner et al., 2010; 2017), aerobic scope is limited by the cardiovascular capacity to supply oxygen (i.e. by f_H and cardiac output). Aerobic scope has an

optimal temperature, limited by pejus temperatures (T_p), which determine the active tolerance range. Within the active tolerance range, f_H is expected to scale sufficiently to meet temperature-dependent rise in oxygen demand, given that cardiac output (i.e. stroke volume) is not the only determinant regulating oxygen supply (see section 4.5). Passive thermal tolerance starts once f_H and/ or cardiac output fail to increase sufficiently to match oxygen demand, resulting in lowered aerobic scope and consequent sublethal limitations such as performance and abundance declines.

Incremental f_H Q_{10} values of the present study were compared across low, mid, and fast swimming velocities. They displayed similar trends across velocities and stayed elevated up to 4°C (i.e. 2.58 ± 0.69 at 2°C and 2.50 ± 0.82 at 4°C). Past that temperature, they declined to 2.00 ± 0.50 at 6°C and 1.73 ± 0.74 at 8°C. Similarly, Drost and colleagues determined f_{Hmax} Q_{10} values to be high and relatively steady between 0.5 and 5.5°C (i.e. 2.4 ± 0.5). Past that point, they once measured the distinct decline of Q_{10} values setting in at 5.5°C, and once at 8.0°C. Hence, beyond these temperatures, f_H failed to scale steadily with further temperature increase. This data is in accordance with previously obtained data on the optimal temperature range of polar cod, which was determined to be located between 2.8 and 4.4°C (Schurmann and Christiansen, 1994). The observed trends in f_H Q_{10}, with initially high values followed by a fast decrease, are typical for cold-adapted stenothermal and "sluggish" fish. Both holds true for polar cod. In comparison to eurythermal, "active" fishes, a stronger stimulus to meet the same increase in oxygen demand is experienced, causing a greater percentage cost increment and thus contributing to higher Q_{10} values and earlier thermal limitations (Pörtner et al., 2017).

Concluding, f_H Q_{10} values of polar cod start failing to scale with temperatures past 4–6°C, which fits previously described temperature susceptibilities (Leo et al., 2017) and ranges (Schurmann and Christiansen, 1994). Since polar cod was found to experience summer temperatures up to 6–8°C (Bradstreet et al., 1986; DeVries and Steffensen, 2005), it is possible that extended periods of passive thermal tolerance and consequent performance declines are already ecologically relevant. Given that Arctic temperature is projected to rise by more than 4°C relative to the present until the year 2100 (SSP1–RCP2.6 scenario; IPCC, 2021), and given the importance of polar cod within the Arctic food web, it is imperative to disentangle the temperature-dependent cardiovascular regulation of oxygen supply. The following section will take a first step in that direction as it explores the correlation between f_H and M_{O2}.

4.5. Heart Rate as a Proxy for Energy Expenditure in Polar Cod

Following the OCLTT hypothesis, temperature-specific energy budgets of fishes are determined by the temperature-dependent cardiovascular capacity to supply oxygen to the tissues (Pörtner et al., 2010; 2017). In the past, f_H has been mostly neglected as a proxy for energy expenditure, as contemporary research has identified the heart's stroke volume to be the primary determinant for said cardiovascular capacity (Farrell, 1991). Continued research, however, has led to the present state of the art, where the value of f_H in determination of energy expenditure has been highlighted in a multitude of studies (Altimiras and Larsen, 2000; Schreer et al., 2001; Cooke et al., 2003; Clark et al., 2005, 2008; Clark and Seymour, 2006) and therefore, has to be evaluated on species-specific basis.

For polar cod, M_{O2} of the present study was significantly correlated to f_H at 0, 2, and 8°C. Overall, the spearman rank correlation coefficient rho amounted to 0.42, indicating a positive relationship of the parameters. Hence, an increase in f_H was overall significantly accompanied by an increase in M_{O2}. The absence of significant correlation at 4 and 6°C could potentially draw back to the acclimation of polar cod at 4°C. The corresponding hypotheses would be that, in consequence to specific temperature acclimation, either oxygen demand is homogenous across the swimming performance and U_{crit} is not limited by MMR, or the regulatory mechanisms of oxygen supply are variable and the contribution of f_H diminishes. Temperature-dependent variation in the relationship of f_H and M_{O2}, however, is opposed by the general similarities in slope at 0, 2 and 8°C. Furthermore, homogeneity in f_H increase across temperature regimes, as well as similarity in f_{Hmax} values to measurements of Drost and colleagues (2014), leave doubt of polar cod's overall acclimation potential (see section 4.4). Consequently, implications of high inter-individual variability in M_{O2} are brought to focus. Regression analysis of the $M_{O2}-f_H$ relationship did not display significant results ($R^2 < 0.4$). Hence, sample size of n = 9 was clearly insufficient to perform high-power modeling of the $M_{O2}-f_H$ relationship. Consequently, the necessity to conduct further trials of f_H bio-logging within the swim tunnel respirometer in the future has to be highlighted.

Nevertheless, the present study provided multiple further results that support the contribution of f_H in oxygen supply regulation and hence, the value of f_H as a proxy for energy expenditure in polar cod. Firstly, the significant (p < 0.0001) impact of swimming velocity on f_H, potentially indicates the presence in regulatory function of f_H, addressing performance-related oxygen demands. Further, in the present study, f_H at U_{crit} was close to equal to previously researched

f_{Hmax} after intraperitoneal atropine and isoproterenol injection (Drost et al., 2014). This supports the hypothesis of a temperature-dependent linkage of f_{Hmax} to maximum performance limitations (Pörtner et al., 2010; 2017; Farrell, 2009).

Lastly, the interaction of swimming velocity and temperature did not significantly ($p = 0.71$) impact M_{O2}. Furthermore, the significant linear regression ($R^2 = 0.75$) of f_H with increasing swimming velocity was homogenous across all temperature regimes, and amounted to 4.65 ± 0.43 bpm for an increase in 1 BL/s. In addition, the M_{O2} regression with increasing f_H displayed slopes of high similarity at 0, 2, and 8°C. Consequently, the M_{O2}–f_H relationship potentially is temperature-independent, which would highly simplify the determination of energy expenditure based on *in situ* f_H bio-logging.

Due to small sample size and high variability in respiratory measurements, further swim tunnel trials with bio-logged polar cod will be necessary in order to describe the M_{O2}–f_H relationship in statistical significance. Nevertheless, in final conclusion of the present paper, polar cod f_H of the present study was clearly scaling with both temperature and swimming performance. f_{Hmax} at U_{crit} mirrored values previously obtained by humoral injections, indicating causal relationship of f_H and performance limitations. I assess the contribution of f_H in regulation, and ultimately limitation, of oxygen supply in response to temperature- and performance-related energy demand, as highly probable. Therefore, the potential of f_H as a proxy for energy expenditure in polar cod was both highlighted and not contradicted over the course of my research. Given both the cost- and time-intensive nature of present f_H bio-logging methodology, as well as the general necessity to implement stroke volume and cardiac output into holistic energy budget models, additional laboratory studies, observing stroke volume as well as arterial and venous oxygen content, are highly advisable.

Previous sections have validated the present Star-Oddi micro HRT (G2) bio-loggers as highly robust in response to Arctic temperature regimes and highest swimming activities. Although additional feasibility analyses at maximum performance levels have to be performed, SMR measurements and behavioral observations have supported the f_H bio-logging methodology species-specifically for polar cod. Furthermore, the novel description of free-swimming polar cod f_H has supported previous results, stating its alarming temperature susceptibility at projected Arctic climate change until the year 2100 (Kunz et al., 2016; Leo et al. 2017). Therefore, continuing polar cod f_H bio-logging is not only plausible, but also highly advisable. I strongly believe that driving bio-logging of Arctic fish further towards *in situ* implementation,

is accompanied by unraveling most essential knowledge of fish ecophysiology, and represents one of our best chances in understanding conservation demands within a changing climate.

5. References

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Acknowledgements – Danksagung