THE OTOLITH-ISOTOPE METHOD: AN OPPORTUNITY TO EXAMINE FIELD METABOLIC RATE AS AN *IN SITU* INDICATOR OF CLIMATE CHANGE WITHIN AND ACROSS JUVENILE ATLANTIC COD POPULATIONS (*GADUS MORHUA*)

by

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Abstract

Individual metabolism is a unifying variable in animal ecology, influencing all aspects of performance including growth rate, energetic efficiency, and mortality. As abiotic factors continue to fluctuate due to climate change and anthropogenic disturbance, it is becoming increasingly important to measure an individual's metabolic rate in its natural environment to assess critical energetic tradeoffs. Field metabolic rate (FMR) is the metabolic rate measured from a free-ranging organism, taking into account an individual's specific dynamic action (SDA), standard metabolic rate (SMR), and activity, yielding a holistic estimate of energy intake and expenditure. Unlike with terrestrial animals, our knowledge of the physiological processes of fishes to date is largely based on extrapolation from observations of historic populations, or from laboratory-based measures of respiratory potential. Here, I describe a newly developed proxy for FMR which pairs the stable isotope composition of the otolith with estimates of oxygen consumption and experienced temperature to yield the full temporal history of the energetic costs associated with environmental change in free-ranging fishes. I outline the opportunities this method presents to make macroecological connections between individual metabolic rate and greater ecosystem interactions, and pair gaps in knowledge in the fields of conservation, ecology, and physiology with important research questions. I also apply the otolith-isotope FMR methodology to juvenile Atlantic cod (*Gadus morhua*) populations from the Northwest Atlantic, an iconic and economically important species, to examine the relationship between vulnerable early-life stages, decreasing ocean temperature, and energy costs. I compare the FMR-thermal sensitivity and the mean FMR between life history stages within the Newman Sound population (age-0 pulse 1 and pulse 3) and between populations (Newman Sound, Newfoundland; Skagerrak Coast, Norway) to identify pulse- and population-specific behavioural and

physiological tradeoffs. I discover pulse-specific differences in mean FMR, and provide insights on the pathways of energy allocation. At the population-level, I recover *in situ* physiological trends consistent with the metabolic cold adaptation hypothesis, suggesting population-specific thermal adaptation in juvenile Atlantic cod from two populations on opposite sides of the North Atlantic. This research contributes to expanding the current knowledge base of *in situ* physiological performance for an important fisheries species in the face of changing oceanic conditions. We hope future studies can build upon this research to bridge the gap between data and policy, incorporating field metabolic rate into stock assessments to better inform recruitment forecasting, ensuring a long-term, sustainable Atlantic cod fishery.

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Co-authorship Statement

The research described herein, including design and conceptualization, data collection and analysis, and all written work, was performed by Valesca de Groot, under the supervision and guidance of Dr. Amanda Bates, with intellectual and technical input from committee members Dr. Bob Gregory and Dr. Clive Trueman. The processing of age-0 Atlantic cod otoliths was conducted by Dr. Clive Trueman at the National Oceanography Centre, Southampton, UK due to limitations on travel imposed by COVID-19. All data analyses were conducted by Valesca de Groot. The authorship of journal contributions arising from the thesis chapters will include myself, Valesca de Groot, Dr. Amanda Bates, and Dr. Clive Trueman for Chapter 2 (*Incorporating otolith-isotope inferred field metabolic rate into conservation strategies*) and myself, Valesca de Groot, Dr. Robert Gregory, Dr. Clive Trueman, and Dr. Amanda Bates for Chapter 3 (*Otolith-isotope methods reveal distinct pulse- and population- specific field metabolic trends in juvenile Atlantic cod (Gadus morhua) of the Northwest Atlantic*).

CHAPTER 1: General Introduction

Climate change's call for *in situ* physiology

The earth's current geological epoch –the "Anthropocene" - is characterized by direct (habitat transformation, species exploitation) and indirect human interference (climate change, pollution, invasive species; IPBES, 2019). Of these anthropogenic pressures, the consistent rise of atmospheric carbon dioxide is one of the most pressing drivers, with wide-ranging consequences that are irreversible on ecological timescales, and observed from the upper atmosphere to the deep seas (e.g., Jacob & Winner, 2009; Doney *et al.,* 2012; Sweetman *et al.,* 2017). The fluctuating abiotic factors associated with climate change are reshaping whole ecosystems, altering habitat quality and phenology, which drives biodiversity loss, produces community shifts, and rewires trophic relationships (e.g. Hughes *et al.,* 2003; Perry *et al.,* 2005; Wilson *et al.,* 2008; Dulvy *et al.,* 2008; Mueter & Litzow, 2008; Mitchell *et al.,* 2008; Pereira *et al.,* 2010; Bates *et al.,* 2014; Stuart-Smith *et al.,* 2018; Bartley *et al.,* 2019; Antão *et al.,* 2020). The cascading effects of climate change threatens the goods and services provided by healthy ecosystems, with direct implications for food security and human welfare (e.g., Antle *et al.,* 2001; Ding *et al.,* 2017; FAO 2016). The spatiotemporal aspects of climate change have been well examined, with impacts estimated to accelerate over time according to both best-case and worst-case climate scenarios associated with greenhouse gas emissions (e.g., SRES A1B, stable-2000 level scenario; Cheung *et al.,* 2010). Furthermore, climate-driven impacts on biodiversity (species richness and composition) are unevenly distributed across the planet. Higher temporal turnover in marine assemblages have been observed due to a lack of barriers to dispersal (e.g.,

Blowes *et al.,* 2019; Antão et al. 2020), with particularly intensified impacts in polar and temperate ecosystems (e.g., Stouffer *et al.,* 1989; Flato *et al.,* 2001).

Fluctuations in ocean conditions (stratification, nutrient input, ocean currents, coastal upwelling, ocean acidification, sea-level rise, shifts in salinity, and hypoxia events) influence whole networks of marine communities – from individual-level physiological thresholds and species interactions, to ecosystem function. Macroecological studies have used "top-down" approaches to examine climate-driven variability, studying ecological patterns at high levels of biological organization by employing multidisciplinary methods that range from field methods to model simulations (e.g., Sarmiento *et al.,* 2004; FAO 2008; Mueter & Litzow, 2008; Przeslawski *et al.,* 2008; Cheung *et al.,* 2008; Pereira et al. 2010; Mellin *et al.,* 2016). For example, significant research effort has been applied to determine how climate trends redistribute populations (e.g., Sunday *et al.,* 2012; Bates *et al.,* 2013), impact habitat quality (e.g., Craft *et al.,* 2009), and how such shifts can lead to biodiversity loss and destabilize trophic structure of communities (e.g., Johnson *et al.,* 2011; Schultz *et al.,* 2016).

Analyses at the level of ecosystems are important in understanding the cascading effects of climate variability and change, but provide little information on the direct *in situ* consequences for individual-level physiological tolerances and thresholds, behaviour, and demographic traits that can drive cascading shifts in population structure. Climate-driven variability in abiotic environmental factors is a regulating processes within individuals, challenging the physiological tolerances to which organisms are adapted. The study of physiology examines an organism's basal life-sustaining processes through gene expression, cellular function, and key biochemical properties – processes that are sensitive to environmental factors (Hill *et al.,* 2012). Physiological plasticity can provide short and long term insight on

climate change response, indirectly altering the life history traits that maintain equilibrium within an ecological community, with the potential to influence multigenerational evolutionary response (IPCC, 2007; Monaco & Helmuth 2011; Evans and Hofmann, 2012; Doney *et al.,* 2012; Chung *et al.,* 2020). Linking physiological response at the level of the individual, to broader ecological patterns (e.g., in a "bottom-up" approach) has proven difficult. Physiological measurements have historically been limited to lab-reared individuals in confined experiments that aim to replicate climate-induced fluctuations in abiotic factors. The research gap between the individual and its role within complex assemblages (population, community, ecosystem) in response to climate-driven abiotic variability requires physiological research in an ecological framework. Therefore, a multidisciplinary approach that integrates *in situ* physiology into ecological knowledge on climate response is necessary to inform conservation and fisheries management strategies.

Metabolism under climate change

A key focus in the field of physiology is the measure of metabolic rate, which sums cellular-level chemical reactions that produce adenosine triphosphate (ATP) during the synthesis of organic food molecules to produce the individual's energy supply (Hill *et al.,* 2012). Energy metabolism follows how this energy is channeled into useful functions for survival, connecting internal biochemical processes to individual-level behaviour. Physiologists have used different metabolic rate metrics to understand individual-level energy expenditure, measuring standard/basal metabolic rate (SMR- ectotherms, BMR- endotherms), maximum metabolic rate (MMR), and routine metabolic rate (RMR) under laboratory conditions (Table 2.1). While these metabolic rate metrics represent fundamental components of physiological functioning,

determining *in situ* metabolic rate is a baseline step in the effort of merging physiological research with climate change ecology.

It is field metabolic rate (FMR) - the metabolic rate of an individual in its natural habitat - that becomes relevant when highlighting the important energetic trade-offs that arise under suboptimal environmental conditions (Brown *et al.,* 2004). While the energy budget of an organism is first established by food intake and the internal processes which break down organic molecules, the assimilation of energy is further influenced by external processes (Hill *et al.,* 2012). Abiotic factors in the individual's environment (e.g., temperature, salinity, dissolved oxygen, pH) can redistribute energy allocation to various life processes influencing all aspects of performance, including growth rate, foraging, predator avoidance, reproduction, and survival (Figure 1.1). Individual field metabolic rate links abiotic factors to metabolic and behavioural trade-offs that promote survival and reproduction, which can in turn provide insight on population- (predator-prey interactions, competition, facilitation, mutualism, population dynamics), and ecosystem-level (nutrient cycling; primary and secondary productivity; resource depletion, trophic dynamics) processes (Brown *et al.,* 2004; Chabot *et al.,* 2016).

Ambient temperature is one of the most significant abiotic factors to address when examining the energy metabolism of marine fishes (Brown *et al.,* 2004). The high temperature sensitivity of fish directly influences metabolism by altering biological processing rates, subsequently shifting population productivity, species interactions, food web structure, and ecosystem functioning (Petchey *et al.,* 1999; Brown *et al.,* 2004). By measuring *in situ* metabolic rate of individuals exposed to temperature dynamics in real-time, the realized responses of animal populations to climate change can be assessed (Innis 1940; Peiss & Field, 1950).

A positive feedback loop exists between the accumulation of greenhouse gasses associated with climate change and heat uptake by the world's oceans, resulting in a rise in Sea Surface Temperature (SST) over the 20th century (Roemmich *et al.,* 2012). However, marine ambient temperature trends are not unidirectional, as the rise in SST is accompanied by alterations to hydrological cycles, disrupting net evaporation to induce saltier subtropical waters and fresher subpolar regions (Boyer *et al.,* 2007). This disruption has complicated the warming trend associated with climate change through significant periods of cooling in the subpolar North Atlantic gyre (Hanna & Cappelen, 2003; Lozier *et al.,* 2008; Thompson et al. 2010). While many studies have examined the direct link between climate change and ocean warming on both local and global fish assemblages (e.g., Pörtner *et al.,* 2001; Perry *et al.,* 2005; Nguyen *et al.,* 2011; Stuart-Smith *et al.,* 2015; Sunday *et al.,* 2015), the fluctuating abiotic factors associated with winter climate change has received less attention.

The winter season is often associated with biological dormancy, an assumption that has historically discouraged research into the ecological processes which both differentiate and drive ecosystem functioning relative to other seasons. It is the energy deficits, extended resource scarcity, and extreme environmental conditions associated with winter that merit research attention; however, these winter conditions can have significant impacts on year-round individual processes (reproduction, growth, fitness and survival) and can destabilize the network of interactions that structure communities and ecosystems (trophic shifts, migrations). Recent rapid variations in winter conditions driven by ocean warming (Campbell et al. 2005; Barnett et al. 2005; Studd *et al.,* 2021) and an altered hydrological cycle (Groisman et al. 2004; Robson *et al.,* 2014; Sgubin *et al.,* 2016) have highlighted the need to fill this important blind spot to understand how varied responses may shift the demography, ecology, and evolution of northern

species. A recent surge of studies has therefore focused on winter ecology (Kreyling 2010; Williams et al. 2015; Penczykovski et al. 2017; Contosta et al. 2019; Geissinger *et al.,* 2022), with a recent article providing a comprehensive and robust definition of winter to propel the field forward (Studd *et al.,* 2021). Despite this research momentum, studies have largely been restricted to terrestrial assemblages (Lemke et al. 2007; Kreyling 2010; Pauli et al. 2013; Williams et al. 2015; Penczykovski et al. 2017; Contosta et al. 2019). A significant gap therefore remains in our understanding of how winter climate change can impact the seasonality of marine processes (juvenile overwintering mortality, temporal migration cues) and how effects can cascade to create year-round individual and ecosystem-level shifts (Morley *et al.,* 2017).

Temperature in Newfoundland offshore and coastal waters: climate change impacts on winter

Rapid and extreme cooling weather dynamics are associated with coastal Newfoundland waters despite the global trend in increased ocean warming (Josey *et al.,* 2018; Oltmanns *et al.,* 2020). In this paper, the word extreme exemplifies quantitative measurements that are considered anomalies. This dynamic offers an important opportunity to examine the physiological stress of abnormally cold winters on marine assemblages. Interdecadal temperature oscillations have been observed in the North Atlantic subpolar gyre since the 1960s, with extreme cold spells in recent decades (Read & Gould, 1992; Chapman & Walsh, 1993; Hanna & Cappelen, 2003; Colbourne 2004; Robson *et al.,* 2014; Sgubin *et al.,* 2017). Above average basin-wide surface heat fluxes (±4 Watt per square meter) are responsible for the dramatic regional differences in SST trends between the Arctic, the nordic seas, and the western boundary current region (SST increase) and the North Atlantic SPG (SST drop; Dziewonsky *et al.,* 2008; Wu *et al.,* 2012). This regional

inconsistency has been linked to climate change, which is altering the hydrological cycle of the Earth, through the acceleration of continental glacier and sea ice melt, instability between precipitation and evaporation, and by notably increasing the freshwater budget of the subpolar North Atlantic and gin seas (Boyer *et al.,* 2007). Previous studies have identified significant links between strong individual surface freshening events, the associated increase in atmosphere-ocean interactions, and the decrease of surface SST temperature across the North Atlantic subpolar gyre, with extreme cold anomalies in the fall and winter (Oltmanns *et al.,* 2020). Significant surface freshening observed in the Arctic (Read & Gould*,* 1992; Bamber *et al.,* 2012; Haine *et al.,* 2015) over the last four decades has been attributed to the deep convection of cooler and fresher source waters in the Labrador Sea Water (LSW), which is one of the main water masses of the subpolar North Atlantic gyre (Read & Gould*,* 1992; Lozier *et al.,* 2008). Lying at the northern limit of the thermohaline "conveyor belt", oceanic conditions in the North Atlantic subpolar gyre have an expansive global reach through the Atlantic Overturning Circulation, and therefore this thermohaline variability influences large scale weather patterns. What is of interest for the current study is the North Atlantic subpolar gyre triggered cold spells over Canada (Shabbar & Bonsal, 2004), with pronounced surface freshening and cooling observed off the coast of Newfoundland (Oltmanns *et al.,* 2020). This fall-winter cold anomaly poses a threat for ectothermic organisms, such as fish, directly affecting their metabolic rate (Rose *et al.,* 1994).

Study species: Atlantic cod (*Gadus morhua*)

The fall-winter anomaly observed in Newfoundland has important implications for Newfoundland's most culturally and economically valuable fish species – the Atlantic cod (*Gadus morhua*). The Atlantic cod (*Gadus morhua*) has formed the basis of one of the largest,

most commercially valuable wild fisheries in the world. The species has been well-studied across its distribution - from temperate to arctic regions. Despite high fecundity rates and large population biomass in multiple stock components, Atlantic cod are vulnerable to both overfishing and environmental variability. At the time of the Newfoundland cod moratorium in 1992, the Atlantic cod population in the Northwest Atlantic had declined to 1% of its original standing stock biomass (Chantraine 1993; Couture *et al.,*1998; Hamilton & Butler, 2001). Recruitment overfishing played an integral role in this decline (Hutchings & Myers, 1994; Myers & Cadigan, 1995), leaving behind populations of smaller fish with slower growth rates and lower condition (Chouinard & Fréchet, 1994; Lambert & Dutil, 1997). Although considered acceptable to reopen the cod fishery at at its current biomass (~ 13-15% of population biomass), Atlantic cod are still slowly recovering, and stocks must be managed carefully and adaptively in accordance with year-to-year variation in recruitment success to ensure a long-term, sustainable fishery (Taggart et al. 1994; Cushing 1995).

Temperature and juvenile vulnerability

Since Atlantic cod populations cover a wide distribution, they also experience a wide range of temperatures leading to spatio-temporal differences in behaviour, life-history traits, and year-class survival associated with recruitment to adulthood (e.g., Neat & Righton, 2007; Rogers *et al.,* 2011; Freitas *et al.,* 2015; Holt & Jørgensen, 2015; Laurel *et al.,* 2017). Temperature-dependent metabolic adaptation specific to populations dictate climate-dependent energy budgets and juvenile fitness for recruitment (Pörtner *et al.,* 2008). Variability in temperature brought on by climate change can disrupt the crucial growth processes and energy efficiency strategies which accompany the narrow ranges of thermal tolerance to which populations are adapted.

Temperature is widely accepted as one of the most important environmental factor influencing growth in juvenile marine fish alongside food availability and availability of suitable habitat (e.g., Jobling 1988; Gotceitas & Brown, 1993; Grant & Brown, 1999). Temperature is thus a fundamental limiting factor for successful recruitment. In Newfoundland, age-0 juvenile cod pulses arrive in the small bays and nursery areas of the northeast coast of Newfoundland between May-October, hatching from fertilized eggs carried down the coast with the Labrador Current from spawning events off the Labrador coast and the Northeast Newfoundland Shelf in March/April (Templeman 1981; Lear & Green, 1984). The observed trend towards colder fall and winter temperatures through time in Newfoundland can constrain fundamental growth periods during early-life processes in late pulse individuals, and during the subsequent overwinter period. As body temperature in fish decreases with ambient temperature, the physical chemistry of the cell can depress metabolic activity, leading to dormancy or potential changes in homeostatic responses (Johnston & Dunn, 1987). Therefore, chronic exposure to cold temperatures can jeopardize vital accumulation of lipid reserves for overwinter survival, as well as increase predation risk due to slowed response, thereby reducing the likelihood of survival to adulthood (Copeman *et al.,* 2008; Heintz *et al.,* 2013). A stable metabolic budget with uncompromised allocation to growth is essential in juvenile fish as both post-settlement and overwintering mortality are largely determined by size-dependent processes, whereby larger individuals are more likely to survive (Sogard 1997; Miller *et al.,* 1988). While previous studies have examined the thermal-growth relationship in Atlantic cod (Laurel *et al.,* 2017; Gotceitas *et al.,* 1999), an ideal understanding of recruitment success would not only examine the allocation of energy towards growth, but the whole energy budget and metabolic expenditure of the individual to identify the full extent of metabolic trade-offs associated with unusually cold years.

Field metabolic rate: terrestrial benefits and aquatic gaps

The most ecologically relevant measure of individual metabolism when examining metabolic trade-offs in natural environments is the field metabolic rate (FMR; Chung *et al.,* 2019). FMR is the sum of an individual's specific dynamic action (SDA), standard metabolic rate (SMR), and activity, representing the time-averaged energy intake and expenditure of a free-ranging individual in its natural habitat (Chung *et al.,* 2019). FMR differs from conventional measurements of metabolism (SMR, MMR, RMR) conducted in the laboratory, as it captures the metabolic costs associated with fluctuating abiotic conditions in the organism's usual environment (Figure 1.2). Since 1949, the most common method to determine FMR in birds, mammals, and reptiles has been the "doubly labeled water" technique (DLW; Berteaux *et al.,* 1996; Speakman 2000; Nagy 2005). DLW follows washout rates of enriched oxygen and hydrogen isotopes in an organism's body water, which are used to measure respiratory CO_2 production, a proxy for energy metabolism over time (Nagy 2005). However, this method has not proven transferrable to aquatic species, as labelled oxygen in body water is too quickly removed by natural processes (Nagy 2005).

Until recently, determination of FMR has been restricted to terrestrial organisms (Berteaux *et al.,* 1996; Speakman 2000; Nagy 2005). Therefore, predictions of fish responses to fluctuations in oceanic conditions have largely drawn on extrapolation from observations of historic populations or from laboratory-based measures of respiratory potential (Treberg *et al.,* 2018; Chung *et al.,* 2019). Despite these alternatives, the physiology of an individual measured under laboratory conditions is unlikely to accurately reflect individual responses to the complex stressors present in natural habitats. Therefore, application of field metabolic rate measures have been limited to ectothermic marine and freshwater organisms, creating a disparity between

terrestrial and aquatic ecophysiological discoveries. In order to create energy budget models and ecosystem projections that account for current and future climatic change, scientists require a method that captures the real-time *in situ* metabolic costs of individuals. The **otolith-isotope method** has historically shown potential by linking otolith $\delta^{13}C$ values to metabolic rate (Kalish 1991; McConnaughey *et al.,* 1997; Jamieson *et al.,* 2004; Sherwood and Rose, 2005; Solomon *et al.,* 2006; Trueman *et al.,* 2013; Sinnatamby *et al*. 2015; 2014; Trueman *et al.,* 2016), however, Chung et al. (2019) only recently pushed this method forward by validating the calibration between otolith $\delta^{13}C$ and oxygen consumption, proposing it as a robust proxy to infer field metabolic rate.

Filling the gap: the otolith-isotope method

The recently developed otolith-isotope method recovers measurements of FMR by linking the stable isotope composition of carbon in carbonate biomineral ($\delta^{13}C$) of fish otoliths to estimates of oxygen consumption (Chung *et al.,* 2019). Dissolved carbon in fish blood is obtained from two isotopically distinct sources ($\delta^{13}C_{DIC}$ value, $\delta^{13}C_{diet}$ value). As the isotopic composition of dietary and seawater carbon is distinct, the proportion of metabolic carbon deposited in an otolith as it grows can be estimated through isotopic mass balance using known or inferred $\delta^{13}C_{diet}$ and $\delta^{13}C_{DIC}$ values (Chung *et al.,* 2019). The concentration of bicarbonate in body fluids is tightly regulated, therefore, changes in the rate of production of respiratory carbon are directly linked to changes in the proportion of respiratory and external carbon in blood (Chung *et al.,* 2019). The proportion of metabolic carbon in fish blood is therefore directly proportional to the rate of respiration of dietary carbon, and can be isolated to quantifiably link oxygen consumption to field metabolic rate estimates (Chung *et al.,* 2019). Furthermore, the isotopic composition of oxygen ($\delta^{18}O$) in the individual's otolith aragonite provides an estimate

of time-integrated experienced temperature (Høie 2004). Alongside age and growth determinations, an individual otolith therefore yields a time-integrated record of temperature and the associated metabolic costs experienced by the individual – the first truly holistic record of the energetic costs associated with environmental change directly measured from free-ranging fish.

Large, paired otoliths are present in almost all teleost fishes, making this method applicable to most fishes. Teleosts account for half of all extant vertebrates and 98% of all ray-finned fishes, accounting for nearly 30 000 species with tremendous morphological, physiological, and behavioural diversity (Nelson 1994; Ravi & Venkatesh, 2018). The otolith-isotope method's wide application paired with the otolith's characteristic of being a robust natural indicator of lifetime metabolic and behavioural response to environmental fluctuation, establishes this method as a valuable ecophysiological tool. Therefore, this method presents an opportunity to explore diverse energetic strategies of fish with different lifestyles in a variety of habitats (e.g., bathypelagic fishes, amphibious fish in anoxic environments such as mangrove, as well as pelagic or littoral fish from freshwater and marine ecosystems).

Research focus

The eco (*in situ*) -physiological (FMR) nature of the otolith-isotope method, along with the flexibility in its application, creates a unique opportunity to address research questions at various levels of biological organization. Measuring performance in wild fishes gives scientists a novel opportunity to examine the physiological processes of individuals within a broader ecological framework.

My overall objective in this thesis is to highlight the novel opportunity to integrate the individual field metabolic rate of free-ranging (wild) fishes, and thus, realized responses to climate and anthropogenic change: presenting general pathways for the implementation of the

stable-isotope method in an ecophysiological framework (Chapter 2), and by applying the stable-isotope method to monitor the realized response of juvenile Atlantic cod to winter climate change (Chapter 3). I provide macroecological context for the isotope-otolith method in the first part of my thesis by highlighting its potential for assessing current climate and anthropogenic pressures on individual physiology, informing models at various levels of biological organization, and making informed predictions to focus conservation efforts to the most vulnerable populations and habitats for species survival. In Chapter 3, I apply the ecophysiological advancements of the otolith-isotope method and its associated conservation and management implications to Atlantic cod in the Northwest Atlantic.

The arrival of newly settling age-0 Atlantic cod in Newfoundland is pulsed, resulting in individuals that are exposed to differing temperatures during early development, with high size variation in juvenile cod entering the first winter period (Gregory *et al.,* 2019). While this is likely not a unique characteristic within the species, Newfoundland is the only location where pulsed settlement has been conclusively demonstrated. These settlement dynamics complicate recruitment forecasting for Newfoundland Atlantic cod (Laurel *et al.,* 2017), however, they also create a unique opportunity to examine the thermal-growth relationship influencing recruitment success, which is what I aim to do in this thesis. Previous studies have identified weak recruitment signals in populations exposed to cold winters, with subsequently lower growth potential (Laurel *et al.,* 2017), and smaller overwintering cod (early recruitment pulse) resulting in mortality rates three to four times higher than the larger cod (Gotceitas *et al.,* 1999).

These studies use the indirect link between temperature and growth as a proxy for temporal energy costs, influencing recruitment success and survival. The otolith-isotope method I used here provides a more direct link between the cooling climate change dynamics of the

North Atlantic and the individual metabolic costs that may affect recruitment signals by way of post-winter juvenile mortality. Determining the thermal sensitivity of FMR between pulse groups, as well as any significant differences in mean FMR, will allow for a better understanding of the pulse-specific physiological responses to climate related changes. I will further examine the relationship between FMR and temperature at a higher level of biological organization, comparing the thermal-FMR relationship across two populations of Atlantic cod: the previously mentioned Newfoundland population, and the Skagerrak population (Chung *et al.,* 2021), which compared the FMR values across two ecotypes (Fjord and North Sea).

Study site and sampling strategy

For the purpose of my study, juvenile (age-0) Atlantic cod were collected by seine netting methods from three sites in Newman Sound, Newfoundland, Canada (Bermuda Beach, Heffern's Cove, and Narrows Beach) within Bonavista Bay in Terra Nova National Park (Figure 1.3). All three sites are located in the Northwest Atlantic, and are characterized by a sandy-rocky convoluted shoreline with minor freshwater input, boreal forests, and fjords with rocky headlands. Demersal beach seining methods were chosen as opposed to pelagic trawls because nearshore surveys offer a logistically effective means of acquiring a reliable snapshot of post-settled age-0 abundance (Tveite 1984; Ings *et al.,* 1997; Laurel *et al.,* 2017). The nearshore varies between thick kelp forests to sand-pebble-cobble habitat, providing nursery habitat benefits to juveniles through accessible cover from predators and optimal environmental conditions (temperature, macrophytes, food) for rapid growth (Gotceitas *et al.,* 1997; Laurel et al. 2003; Laurel *et al.,* 2017).

Juvenile age-0 Atlantic cod collections were conducted in the fall (October-November), following peak spawning in April/May with settlement pulses moving down the Labrador

Current over the summer months (Templeman 1981; Rideout & Rose, 2006). Fall collections allow age-0 juvenile Atlantic cod pulses to settle in nearshore coastal waters, while older juvenile stages remain here until age-2 before heading into deeper waters offshore (Dalley & Anderson, 1997; Methven & Schneider, 1998). Timing of these settlement pulses is associated with favourable combinations of episodic upwelling events followed by several days of strong onshore wind (Ings *et al.,* 2008).

Data chapter overview

Chapter 2: *Incorporating otolith-isotope inferred field metabolic rate into conservation strategies.*

This chapter provides a comprehensive review of the integration of *in situ* individual animal metabolism into the fields of ecology, management, and conservation, with particular focus on the recently filled research gap for the bony fishes – i.e., name the gap (single word). This review will serve as a practical guideline for research scientists in various fields by: 1) providing a detailed explanation of the biochemical and physiological processes involved in otolith-isotope method, 2) identifying quantifiable links between individual field metabolic rate and macroecological processes, and 3) highlighting concrete research objectives (field and modelling) for bony fish in a conservation and management framework, paying particular attention to current and projected climate change as well as controllable anthropogenic pressures.

I also place this method within the greater field of physiology by identifying gaps in the application of conventional metabolic metrics (SMR, MMR, RMR) towards conservation physiology, differentiating these from the field metabolic rate (FMR). I outline methods that have historically been used to determine FMR in terrestrial systems and explore their respective

strengths and weaknesses, while also highlighting methods that have been used to deduce similar complexity for aquatic organisms. I then highlight the limited application of field metabolic rate measures in ectothermic marine and freshwater organisms, and the discrepancies this has caused in complex metabolic-inferred ecological processes between terrestrial and aquatic systems.

Chapter 3: *Can field metabolic rate suggest thermal adaptations in juvenile Atlantic cod?*

Experimental calibrations between otolith carbon isotope ratios and oxygen consumption rates have been performed for Atlantic cod (Chung *et al.,* 2019), allowing for the estimation of FMR in free-ranging individuals of this species. Here, I put the otolith-isotope method described above to an applied use, examining whether important bottlenecks exist in the performance of juvenile Atlantic cod during changing natural conditions. I will compare differences in field metabolic rate between two recruitment pulses of age-0 Atlantic cod to determine whether cold ocean dynamics impose limiting constraints on energy expenditure. Specifically, I will examine differences in the mean FMR and the thermal sensitivity of FMR between pulses 1 and 3 in Atlantic cod to determine whether recruitment pulse has a significant impact on the metabolic budget of individuals prior to the overwinter period. Initially, this study aimed to utilize the FMR trends examined in both age-0 and age-1 individuals of the same cohort to predict overwinter survival; however, the limitations imposed by COVID-19 (laboratory closures, travel restrictions, etc.) restricted the field collection and otolith processing stages for the age-1 individuals within the scope of my master's research. Therefore, while the use of the otolith-isotope method in the field will build upon the growing foundation of *in situ* physiological data on realized responses to climate variability, it will only be able to provide potential hypotheses on impacts to overwinter survivorship and recruitment success.

Instead of extrapolating FMR trends to determine overwinter survival and recruitment success, the thermal-FMR relationship in the Newman Sound population will be compared across a higher level of biological organization - Atlantic cod populations. The FMR estimates for juvenile Atlantic cod collected from Newman Sound will be compared to those estimated for the Skagerrak population, for which Chung et al. (2021) compared FMR trends between two Atlantic cod ecotypes (North Sea vs Fjord). The Atlantic cod of the Skagerrak coast are subject to an inherently warmer system than Newman Sound, comparing field metabolic measurements can provide insight on the physiological plasticity associated with FMR under different thermal regimes. Comparing the thermal sensitivity and mean FMR within populations (pulse 1 vs pulse 3, Newman Sound) and across populations (Newman Sound vs Skagerrak) will be discussed in relation to two potential mechanisms:

The Physiological Mechanism: Inherent differences in field metabolic rate are related to the physiological plasticity or robustness associated with different populations or life stages in response to environmental factors (temperature, dissolved oxygen, pH, salinity).

The Behavioural Mechanism: Inherent differences in field metabolic rate are a realized behavioural response to the energy requirements associated with context-specific environmental factors (temperature, dissolved oxygen, pH, salinity), or environmental opportunity (more available resources), leading to ecological consequences (feeding rates, competition, and predator-prey interactions).

These mechanisms will be discussed in relation to the FMR trends observed in my research, as limitations in the current study cannot confidently isolate these mechanisms in relation to the observed metabolic responses. Examining differences in FMR between pulse groups within a population can, however, provide an opportunity to examine the physiological

differences between juvenile Atlantic cod life stages, and add general insight on *in situ* FMR data on recruitment strength to year-classes during stock assessments and as part of coastal time series. This *in situ* data integration relates back to the concepts explored in Chapter 2, improving recruitment forecasting survival (Hutchings and Myers 1994; MacKenzie et al. 2008).

Figures

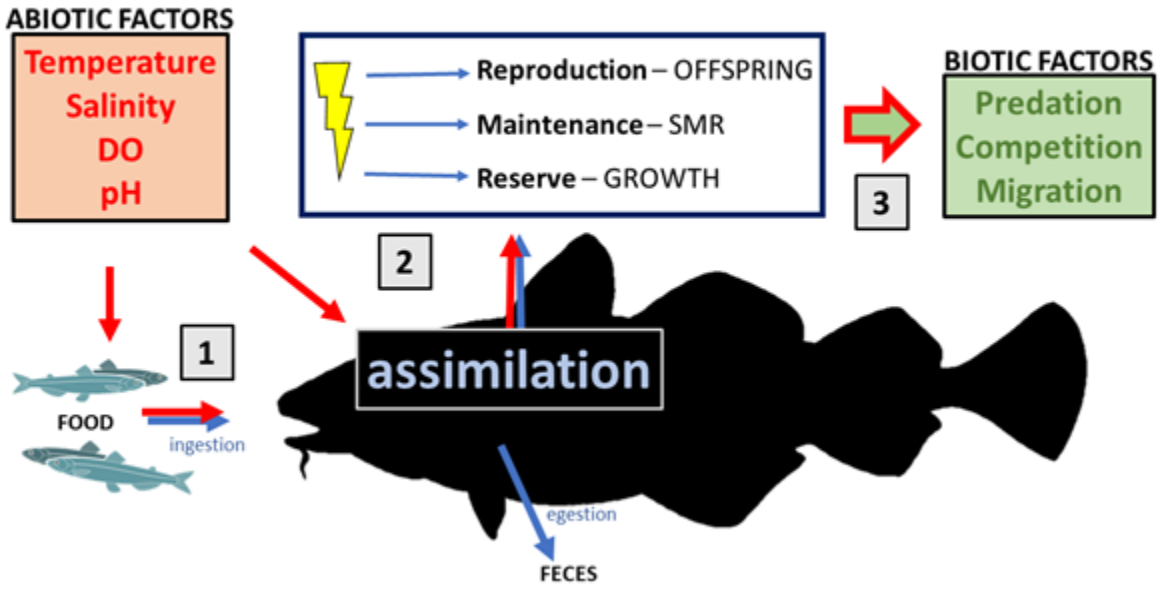


Figure 1.1. Energy flows into an organism as the food it ingests, of which some is discarded as feces, and some is further assimilated. Abiotic factors such as temperature, salinity, dissolved oxygen and pH can influence an organism's energy budget (1- red arrow) indirectly, by altering food availability (habitat destruction, species redistribution, resource depletion), or (2- blue arrow) directly, through thermal/cold stress (ectothermic disruption), hypoxia, or osmoregulatory stress. 3) Such changes can cause stress to regulatory functions, redistributing energy allocated to growth, reproduction and other activities to maintenance, ultimately influencing the way in which an organism interacts with its direct environment (predator-prey interactions, population dynamics).

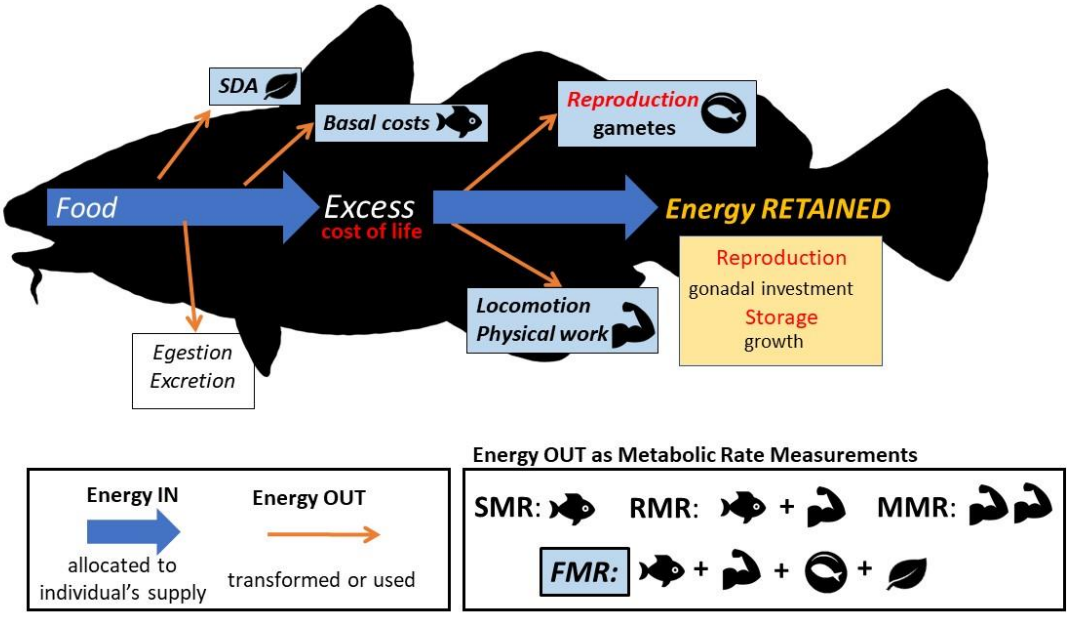


Figure 1.2. An fish's energy budget, including Energy OUT, Energy IN, and Energy RETAINED, demonstrating how these energy pathways are included in experimentally measured definitions of metabolic rate (SMR, RMR, MMR, and FMR) using associated symbols. Energy enters the individual as food, and the digestion of this food is associated with the energetic cost of the specific dynamic action (SDA), while energy is further lost through egestion and excretion (nitrogenous waste, carbon and indigestible material not assimilated). The remaining energy is first allocated to basal costs (regulatory functions, body maintenance - SMR), while the excess energy is utilized for reproduction (as Energy OUT and Energy RETAINED), growth (Energy RETAINED), and locomotion/physical work (Energy OUT) for activities such as foraging, predation, and migration (Treberg *et al.,* 2016). Unlike with SMR, RMR, and MMR, FMR (measured using the otolith isotope method) takes into account one of the first uses of energy as it enters the body - the specific dynamic action (SDA). It also includes basal costs (SMR) and any energy expenditure associated with short-term and long-term activity, such as seasonal movements and reproduction (Activity Metabolism). The "double muscle" symbol for MMR refers to purposefully enhanced activity. (Atlantic cod illustration: Cerren Richards).

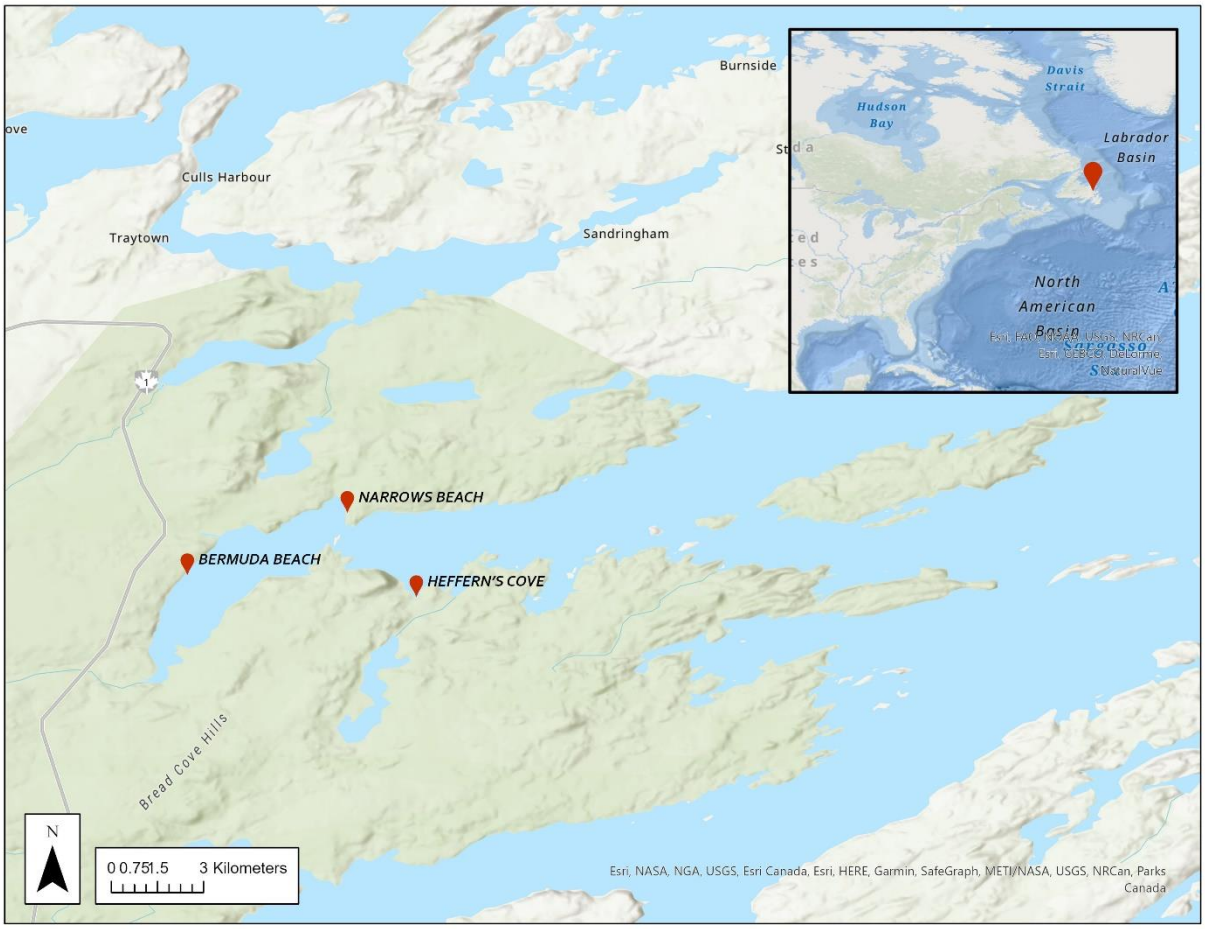


Figure 1.3 A map of Newman Sound, Bonavista Bay, Terra Nova National Park, Newfoundland, Canada indicating the three sites sampled for juvenile (age-0 and age-1) Atlantic cod (*Gadus morhua*). Narrows Beach is located closer to the outer sound, while Bermuda Beach is further inside the estuary. Heffern's Cove is the only site located in the outer sound.

References

Antão LH, Bates AE, Blowes SA, Waldock C, Supp SR, Magurran AE, Dornelas M, Schipper AM (2020) Temperature-related biodiversity change across temperate marine and terrestrial systems. *Nat Ecol Evol* 4: 927–933.

Antle J, Apps M, Beamish R et al. (2001) Ecosystems and their goods and services. in: climate change 2001: impacts, adaptation, and vulnerability (eds McCarthy JJ, Canziani OF, Leary NA et al.), pp. 237–340. Cambridge University Press, Cambridge.

Bamber J, van den Broeke M, Ettema J, Lenaerts J, Rignot E (2012) Recent large increases in freshwater fluxes from Greenland into the North Atlantic. *Geophys Res Lett* 39: 8–11.

Barnett TP, Adam JC, Lettenmaier DP (2005) Potential impacts of a warming climate on water availability in snow-dominated regions. *Nature* 438: 303–309.

Bartley TJ, Mccann KS, Bieg C, Cazelles K, Granados M, Guzzo MM, Macdougall AS, Tunney TD, Mcmeans BC (2019) Food web rewiring in a changing world. *Nat Ecol Evol* 3: 345–354.

Bates AE, Mckelvie CM, Sorte CJB, Morley A, Jones NAR, Mondon JA, Bird TJ, Quinn G, Cjb S, Sa M, *et al.* (2013) Geographical range, heat tolerance and invasion success in aquatic species. *Proc Royal Soc B* 280.

Bates AE, Pecl GT, Frusher S, Hobday AJ, Wernberg T, Smale DA, Sunday JM, Hill NA, Dulvy NK, Colwell RK, *et al.* (2014) Defining and observing stages of climate-mediated range shifts in marine systems. *Global Environmental Change* 26: 27–38.

Berteaux, D., Thomas, D. W., Bergeron, J. M., and Lapierre H (1996) Repeatability of daily field metabolic rate in female meadow voles (Microtus pennsylvanicus). *Funct Ecol* 10: 751–759.

Berteaux, D., Thomas, D. W., Bergeron, J. M., and Lapierre H (1996) Repeatability of daily field metabolic rate in female meadow voles (Microtus pennsylvanicus). *Funct Ecol* 10: 751–759.

Boyer T, Levitus S, Antonov J, Locarnini R, Mishonov A, Garcia H, Josey SA (2007) Changes in freshwater content in the North Atlantic Ocean 1955-2006. *Geophys Res Lett* 34: 1–5.

Brown JH, Gillooly JF, Allen AP, Savage VM, West GB (2004) Towards a metabolic theory of ecology. *Ecology* 85: 1771–1789.

Caesar L, Rahmstorf S, Robinson A, Feulner G, Saba V (2018) Observed fingerprint of a weakening Atlantic Ocean overturning circulation. *Nature* 556: 191–196.

Campbell JL, Mitchell MJ, Groffman PM, Christenson LM, Hardy JP (2005) Winter in northeastern North America: A critical period for ecological processes. *Front Ecol Environ* 3: 314–322.

Chabot D, Steffensen JF, Farrell AP (2016) The determination of standard metabolic rate in fishes. *J Fish Biol* 88: 81–121.

Chapman WL, Walsh JE (1993) Recent variations of sea ice and air temperature in high latitudes. *Bulletin - American Meteorological Society* 74: 33–47.

Cheung WWL, Close C, Lam V, Watson R, Pauly D (2008) Application of macroecological theory to predict effects of climate change on global fisheries potential. *Mar Ecol Prog Ser* 365: 187–197.

Cheung WWL, Lam V, Sarmiento, JL, Kaerney K, Watson R, Zeller D, Pauly D (2010) Large-scale redistribution of maximum fisheries catch potential in the global ocean under climate change. *Glob Chang Biol* 16: 24–35.

Chouinard GA, Frechet A (1994) Fluctuations in the cod stocks of the Gulf of St Lawrence. *Mar Sci Symposia* 198: 121–139.

Chung MT, Jørgensen KEM, Trueman CN, Knutsen H, Jorde PE, Grønkjær P (2021) First measurements of field metabolic rate in wild juvenile fishes show strong thermal sensitivity but variations between sympatric ecotypes. *Oikos* 130: 287–299.

Chung MT, Trueman CN, Godiksen JA, Holmstrup ME, Grønkjær P (2019) Field metabolic rates of teleost fishes are recorded in otolith carbonate. *Commun Biol* 2: 1–10.

Clarke A (2006) Temperature and the metabolic theory of ecology. *Funct Ecol* 20: 405–412.

Clarke A, Rothery P, Isaac NJB, Building M, Lane B, Gifford C (2010) Scaling of basal metabolic rate with body mass and temperature in mammals. *J Anim Ecol* 79: 610–619.

Colbourne EB (2004) Decadal changes in the ocean climate in Newfoundland and Labrador waters from the 1950s to the 1990s. *J Northwest Atl Fish Sci* 34: 43–61.

Colbourne EB, Narayanan S, Prinsenberg S (1994) Climatic changes and environmental conditions in the Northwest Atlantic, 1970-1993. *ICES Mar Sci Symposia* 198: 311–322.

Contosta AR, Casson NJ, Garlick S, Nelson SJ, Ayres MP, Burakowski EA, Campbell J, Creed I, Eimers C, Evans C, *et al.* (2019) Northern forest winters have lost cold, snowy conditions that are important for ecosystems and human communities. *Ecol Appl* 29: 1–24.

Copeman LA, Parrish CC, Gregory RS, Wells JS (2008) Decreased lipid storage in juvenile Atlantic cod (Gadus morhua) during settlement in cold-water eelgrass habitat. *Mar Biol* 154: 823–832.

Craft C, Clough J, Ehman J, Jove S, Park R, Pennings S, Guo H, Machmuller M (2009) Forecasting the effects of accelerated sea-level rise on tidal marsh ecosystem services. *Front Ecol Environ* 7: 73–78.

Dalley EL, Anderson JT (1997) Age-dependent distribution of demersal juvenile Atlantic cod (Gadus morhua) in inshore/offshore northeast Newfoundland. *Can J Fish Aquat Sci* 54: 168–176.

Dalley EL, Anderson JT (1997) Age-dependent distribution of demersal juvenile Atlantic cod (Gadus morhua) in inshore/offshore northeast Newfoundland. *Can J Fish Aquat Sci* 54: 168–176.

Ding Q, Chen X, Hilborn R, Chen Y (2017) Vulnerability to impacts of climate change on marine fi sheries and food security. *Mar Policy* 83: 55–61.

Doney SC, Ruckelshaus M, Duffy JE, Barry JP, Chan F, English CA, Galindo HM, Grebmeier JM, Hollowed AB, Knowlton N, *et al.* (2012) Climate change impacts on marine ecosystems. *Annu Rev Mar Sci* 4: 11–37.

Drinkwater KF (1996) Atmospheric and oceanic variability in the northwest Atlantic during the 1980s and early 1990s. *J Northwest Atl Fish Sci* 18: 77–97.

Dulvy NK, Rogers SI, Jennings S, Stelzenmüller V, Dye SR, Skjoldal HR (2008) Climate change and deepening of the North Sea fish assemblage : a biotic indicator of warming seas. *J Appl Ecol* 45: 1029–1039.

Dziewonski AM, Woodhouse JH, Romanowicz B, Romanowicz B, Helmberger D v, Dziewonski A, Trampert J, Durek J, Sumita I, Kumazawa M, *et al.* (2008) The spatial pattern and mechanismsof heat-content change in the North Atlantic. *Science (1979)* 319: 800–803.

Evans TG, Hofmann GE (2012) Defining the limits of physiological plasticity: how gene expression can assess and predict the consequences of ocean change. *Phil Trans Royal Soc B* 367: 1733–1745.

FAO (2008). Fisheries Management. The ecosystem approach to fisheries. 2.1 Best Practices in Ecosystem Modelling for Informing an Ecosystem Approach to Fisheries. Fisheries Technical Guidelines for Responsible Fisheries. Rome.

FAO (2016). The state of fisheries and aquaculture 2016. Contributing to food security and nutrition for all. Food and Agriculture Organisation of the United Nations, Rome. 200 pp.

Flato GM, Boer GJ (2001) Warming asymmetry in climate change simulations. *Geophys Res Lett* 28: 195–198.

Freitas C, Olsen EM, Moland E, Ciannelli L, Knutsen H (2015) Behavioral responses of Atlantic cod to sea temperature changes. *Ecol Evol* 5: 2070–2083.

Geissinger EA, Gregory RS, Laurel BJ, Snelgrove PVR (2022) High site-fidelity and low mortality of juvenile Atlantic cod (Gadus morhua) in subarctic coastal habitat during their first winter. *ICES Journal of Marine Science* 79: 1408–1418.

Gotceitas V, Brown JA (1993) Substrate selection by juvenile Atlantic cod (Gadus morhua): effects of predation risk. *Oecologia* 93: 31–37.

Gotceitas V, Fraser S, Brown JA (1997) Use of eelgrass beds (Zostera marina) by juvenile Atlantic cod (Gadus morhua). *Can J Fish Aquat Sci* 54: 1306–1319.

Gotceitas V, Methven DA, Fraser S, Brown JA (1999) Effects of body size and food ration on over-winter survival and growth of age-0 Atlantic cod, Gadus morhua. *Environ Biol Fishes* 54: 413–420.

Grant SM, Brown JA (1999) Variation in condition of coastal Newfoundland 0-group Atlantic cod (Gadus morhua): Field and laboratory studies using simple condition indices. *Mar Biol* 133: 611–620.

Gregory RS, Morris C, Linehan J (2019) Relative strength of the 2003 and 2004 year-classes, from nearshore surveys of demersal age 0 and 1 Atlantic cod in Newman Sound, Bonavista Bay. *Canadian Science Advisory Secretariat*.

Gregory, RS, Morris, C, & Linehan, J (2006) Relative strength of the 2003 and 2004 year-classes, from nearshore surveys of demersal age 0 and 1 Atlantic cod in Newman Sound, Bonavista Bay. *Canadian Science Advisory Secretariat*, Res Doc 2006/3848.

Groisman PY, Knight RW, Karl TR, Easterling DR, Sun B, Lawrimore JH (2004) Contemporary changes of the hydrological cycle over the contiguous United States: Trends derived from in situ observations. *J Hydrometeorol* 5: 64–85.

Haine TWN, Curry B, Gerdes R, Hansen E, Karcher M, Lee C, Rudels B, Spreen G, de Steur L, Stewart KD, *et al.* (2015) Arctic freshwater export: Status, mechanisms, and prospects. *Glob Planet Change* 125: 13–35.

Hanna E, Cappelen J (2003) Recent cooling in coastal southern Greenland and relation with the North Atlantic Oscillation. *Geophys Res Lett* 30: 30–32.

Heintz RA, Siddon EC, Farley E v., Napp JM (2013) Correlation between recruitment and fall condition of age-0 pollock (Theragra chalcogramma) from the eastern Bering Sea under varying climate conditions. *Deep Sea Res 2 Top Stud Oceanogr* 94: 150–156.

Hill R, A. Wyse G, Anderson M (2012) Themes in the Study of Animal Physiology, Third Edition. Science of the Total Environment. Sinauer Associates, Sunderland.

Høie H, Otterlei E, Folkvord A (2004) Temperature-dependent fractionation of stable oxygen isotopes in otoliths of juvenile cod (Gadus morhua L.). *ICES J Mar Sci* 61: 243–251.

Holt RE, Jørgensen C (2015) Climate change in fish: Effects of respiratory constraints on optimal life history and behaviour. *Biol Lett* 11. doi:10.1098/rsbl.2014.1032

Hughes TP, Baird AH, Bellwood DR, Card M, Connolly SR, Folke C, Grosberg R, Jackson JBC, Kleypas J, Lough JM, *et al.* (2003) Climate change, human impacts, and the resilience of coral reefs. *Science (1979)* 301: 929–934.

Hutchings JA, Myers RA (1994) Timing of cod reproduction - Interannual variability and the influence of temperature. *Mar Ecol Prog Ser* 108: 21–32.

Ings DW, Gregory RS, Schneider DC (2008) Episodic downwelling predicts recruitment of Atlantic cod, Greenland cod and white hake to Newfoundland coastal waters. *J Mar Res* 66: 529–561.

Ings DW, Schneider DC, Methven DA (1997) Detection of a recruitment signal in juvenile Atlantic cod (Gadus morhua) in coastal nursery areas 25–29.

Innis, H. A. 1940. The cod fisheries: the history of an intenational economy. University of Toronto Press. 522 pp.

IPBES. (2019). Summary for policymakers of the global assessment report on biodiversity and ecosystem services of the intergovernmental science-policy platform on biodiversity and ecosystem services. Bonn, Germany: IPBES Secretariat.

IPCC (2007) Climate Change 2007: the Physical Science Basis. Working Group I Contribution to the Fourth Assessment Report of the IPCC. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Jacob DJ, Winner DA (2009) Effect of climate change on air quality. *Atmos Environ* 43: 51–63.

Jamieson RE, Schwarcz HP, Brattey J (2004) Carbon isotopic records from the otoliths of Atlantic cod (Gadus morhua) from eastern Newfoundland, Canada. *Fish Res* 68: 83–97.

Jobling, M. (1988). A review of the physiological and nutritional energetics of cod, Gadus morhua L., with particular reference to growth under farmed conditions. *Aquaculture*, 70: 1-19.4

Johnston IA, Dunn J (1987) Temperature acclimation and metabolism in ectotherms with particular reference to teleost fish. *Symp Soc Exp Biol* 41: 67–93.

Josey SA, Hirschi JJM, Sinha B, Duchez A, Grist JP, Marsh R (2018) The recent atlantic cold anomaly: Causes, consequences, and related phenomena. *Ann Rev Mar Sci* 10: 475–501.

Kalish JM (1991) 13C and 18O isotopic disequilibria in fish otoliths: metabolic and kinetic effects. *Mar Ecol Prog Ser* 75: 191–203.

Kreyling, J (2010) Winter climate change: a critical factor for temperate vegetation performance. *Ecology*, 91: 1939-1948.

Lambert Y, Dutil JD (1997) Condition and energy reserves of Atlantic cod (Gadus morhua) during the collapse of the northern Gulf of St. Lawrence stock. *Can J Fish Aquat Sci* 54: 2388–2400.

Laurel BJ, Cote D, Gregory RS, Rogers L, Knutsen H, Olsen EM (2017) Recruitment signals in juvenile cod surveys depend on thermal growth conditions. *Can J Fish Aquat Sci* 74: 511–523.

Laurel BJ, Gregory RS, Brown JA (2003) Predator distribution and habitat patch area determine predation rates on Age-0 juvenile cod Gadus spp . *Mar Ecol Prog Ser* 251: 245–254.

Lear, W. H., & Green, J. M. (1984). Migration of the "northern" Atlantic cod and the mechanisms involved. In *Mechanisms of migration in fishes* (pp. 309-315). Springer, Boston, MA.

Lemke P, Ren JF, Allison I, Carrasco JF (2007) Observations: changes in snow, ice and frozen ground. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL, eds. Climate Change 2007: Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change . Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Linehan JE, Gregory RS, Schneider DC (2001) Predation risk of age-0 cod (Gadus) relative to depth and substrate in coastal waters. *J Exp Mar Biol Ecol* 263: 25–44.

Lozier SM, Leadbetter S, Williams RG, Roussenov V, Reed MSC, Moore NJ (2008) The spatial pattern and mechanisms of heat-content change in the North Atlantic. *Science (1979)* 319: 800–803.

MacKenzie BR, Horbowy J, Köster FW (2008) Incorporating environmental variability in stock assessment: Predicting recruitment, spawner biomass, and landings of sprat (Sprattus sprattus) in the Baltic Sea. *Can J Fish Aquat Sci* 65: 1334–1341.

McConnaughey TA, Burdett J, Whelan JF, Paull CK (1997) Carbon isotopes in biological carbonates: Respiration and photosynthesis. *Geochim Cosmochim Acta* 61: 611–622.

McGill BJ, Chase JM, Hortal J, Overcast I, Rominger AJ, Rosindell J, Borges PAV, Emerson BC, Etienne R, Hickerson MJ, *et al.* (2019) Unifying macroecology and macroevolution to answer fundamental questions about biodiversity. *Glob Ecol Biogeogr* 28: 1925–1936.

Mellin C, Mouillot D, Kulbicki M, Mcclanahan TR, Vigliola L, Bradshaw CJA, Brainard RE, Chabanet P, Edgar GJ, Fordham DA, *et al.* (2016) Humans and seasonal climate variability threaten large-bodied coral reef fish with small ranges. *Nat Commun* 7: 1–9.

Methven DA, Schneider DC (1998) Gear-independent patterns of variation in catch of juvenile Atlantic cod (Gadus morhua) in coastal habitats. *Can J Fish Aquat Sci* 55: 1430–1442.

Miller TJ, Crowder LB, Rice JA, Marschall EA (1988) Larval size and recruitment mechanisms in fishes: toward a conceptual framework. *Can J Fish Aquat Sci* 45: 1657–1670.

Mitchell NJ, Kearney MR, Nelson NJ, Porter WP (2008) Predicting the fate of a living fossil: how will global warming affect sex determination and hatching phenology in tuatara? *Proc Royal Soc B* 275: 2185–2193.

Monaco CJ, Helmuth B (2011) Tipping points, thresholds and the keystone role of physiology in marine climate change research. Advances in Marine Biology.

Morley JW, Batt RD, Pinsky ML (2017) Marine assemblages respond rapidly to winter climate variability. *Glob Chang Biol* 23: 2590–2601.

Myers RA, Cadigan NG (1995) Was an increase in natural mortality responsible for the collapse of northern cod? *Can J Fish Aquat Sci* 52: 1274–1285.

Nagy KA (2005) Field metabolic rate and body size. *J Exp Biol* 208: 1621–1625.

Neat F, Righton D (2007) Warm water occupancy by North Sea cod. *Proc Royal Soc B: Biological Sciences* 274: 789–798.

Nelson, J. S. (1994). Fishes of the world, 3rd Edition. John Wiley & Sons Inc. *New York, USA*. Johnson CR, Banks SC, Barrett NS, Cazassus F, Dunstan PK, Edgar GJ, Frusher SD, Gardner C, Haddon M, Helidoniotis F, *et al.* (2011) Climate change cascades: Shifts in oceanography, species' ranges and subtidal marine community dynamics in eastern Tasmania. *J Exp Mar Biol Ecol*.

Nguyen KDT, Morley SA, Lai C, Clark MS, Tan KS, Bates AE, Peck LS (2011) Upper temperature limits of tropical marine ectotherms : Global warming implications 6: 6–13.

Oltmanns M, Karstensen J, Moore GWK, Josey SA (2020) Rapid Cooling and Increased Storminess Triggered by Freshwater in the North Atlantic. *Geophys Res Lett* 47. doi:10.1029/2020GL087207

Pauli JN, Zuckerberg B, Whiteman JP, Porter W (2013) The subnivium: A deteriorating seasonal refugium. *Front Ecol Environ* 11: 260–267.

Peiss C, Field J (n.d.) The respiratory metabolism of excised tissues of warm- and cold-adapted fishes. Point Barrow.

Penczykowski RM, Connolly BM, Barton BT (2017) Winter is changing: Trophic interactions under altered snow regimes. *Food Webs* 13: 80–91.

Pereira HM, Leadley PW, Proença V, Alkemade R, Scharlemann JPW, Fernandez-manjarrés JF, Araújo MB, Balvanera P, Biggs R, Cheung WWL, *et al.* (2010) Scenarios for global biodiversity in the 21st century. *Science (1979)* 330: 1496–1502.

Perry AL, Low PJ, Ellis JR, Reynolds JD (2005) Climate change and distribution shifts in marine fishes. *Science (1979)* 308: 1912–1915.

Pörtner HO, Berdal B, Blust R, Brix O, Colosimo A, de Wachter B, Giuliani A, Johansen T, Fischer T, Knust R, *et al.* (2001) Climate induced temperature effects on growth performance, fecundity and recruitment in marine fish: Developing a hypothesis for cause and effect relationships in Atlantic cod (Gadus morhua) and common eelpout (Zoarces viviparus). *Cont Shelf Res* 21: 1975–1997.

Pörtner HO, Bock C, Knust R, Lannig G, Lucassen M, Mark FC, Sartoris FJ (2008) Cod and climate in a latitudinal cline: Physiological analyses of climate effects in marine fishes. *Clim Res* 37: 253–270.

Rahmstorf S, Box J, Feulner G, Mann ME, Robinson A, Rutherford S, Erik Schaffernicht E (1970) Evidence for an exceptional 20th century slowdown in atlantic ocean overturning.

Ravi V, Venkatesh B (2018) The divergent genomes of teleosts. *Annu Rev Anim Biosci* 6: 47–68.

Read JF, Gould WJ (1992) Cooling and freshening of the subpolar North Atlantic Ocean since the 1960s. *Nature* 360: 55–57.

Rideout RM, Rose GA (2006) Suppression of reproduction in Atlantic cod Gadus morhua. *Mar Ecol Prog Ser* 320: 267–277.

Robson J, Sutton R, Smith D (2014) Decadal predictions of the cooling and freshening of the North Atlantic in the 1960s and the role of ocean circulation. *Clim Dyn* 42: 2353–2365.

Roemmich D, John Gould W, Gilson J (2012) 135 years of global ocean warming between the Challenger expedition and the Argo Programme. *Nat Clim Chang* 2: 425–428.

Rogers LA, Stige LC, Olsen EM, Knutsen H, Chan KS, Stenseth NC (2011) Climate and population density drive changes in cod body size throughout a century on the Norwegian coast. *Proc Natl Acad Sci U S A* 108: 1961–1966.

Rose GA, Atkinson BA, Baird J, Bishop CA, Kulka DW (1994) Changes in distribution of Atlantic cod and thermal variations in Newfoundland waters, 1980-1992. *ICES J Mar Sci* 198: 542–552.

Sarmiento JL, Slater R, Barber R, Bopp L, Doney SC, Hirst AC, Kleypas J, Matear R, Mikolajewicz U, Monfray P, *et al.* (2004) Response of ocean ecosystems to climate warming. *Global Biogeochem Cycles* 18: 1–23.

Schultz JA, Cloutier RN, Côté IM (2016) Evidence for a trophic cascade on rocky reefs following sea star mass mortality in British Columbia. *PeerJ* 4. doi:10.7717/peerj.1980

Sgubin G, Swingedouw D, Drijfhout S, Mary Y, Bennabi A (2017) Abrupt cooling over the North Atlantic in modern climate models. *Nat Commun* 8. doi:10.1038/ncomms14375

Sinnatamby RN, Dempson JB, Reist JD, Power M (2015) Latitudinal variation in growth and otolith-inferred field metabolic rates of Canadian young-of-the-year Arctic charr. *Ecol Freshw Fish* 24: 478–488.

Shabbar A, Bonsal B (2004) Associations between low frequency variability modes and winter temperature extremes in Canada. *Atmosphere-Ocean* 42: 127–140.

Sherwood GD, Rose GA (2005) Stable isotope analysis of some representative fish and invertebrates of the Newfoundland and Labrador continental shelf food web. *Estuar Coast Shelf Sci* 63: 537–549.

Sogard SM (1997) Size-selective mortality in the juvenile stage of teleost fishes: A review. *Bull Mar Sci* 60: 1129–1157.

Solomon CT, Weber PK, Cech JJ, Ingram BL, Conrad ME, Machavaram M V., Pogodina AR, Franklin RL (2006) Experimental determination of the sources of otolith carbon and associated isotopic fractionation. *Can J Fish Aquat Sci* 63: 79–89.

Speakman J (2000) The cost of living: field metabolic rates of small mammals. *Adv Ecol Res* 30: 177–297.

Stouffer RJ, Manabe S, Bryan K (1989) Interhemispheric asymmetry in climate response to a gradual increase of atmospheric CO2. *Nature* 342: 660–662.

Stuart-Smith RD, Brown CJ, Ceccarelli DM, Edgar GJ (2018) Ecosystem restructuring along the Great Barrier Reef following mass coral bleaching. *Nature* 560: 92–96.

Stuart-smith RD, Edgar GJ, Barrett NS, Kininmonth SJ, Bates AE (2015) Thermal biases and vulnerability to warming in the world's marine fauna. *Nature* 1–17.

Studd EK, Bates AE, Bramburger AJ, Fernandes T, Hayden B, Henry HAL, Humphries MM, Martin R, McMeans BC, Moise ERD, *et al.* (2021) Nine maxims for the ecology of cold-climate winters. *Bioscience*. doi:10.1093/biosci/biab032

Sunday JM, Bates AE, Dulvy NK (2012) Thermal tolerance and the global redistribution of animals. *Nat Clim Chang* 2: 686–690.

Sweetman AK, Thurber AR, Smith CR, Levin LA, Mora C, Wei C, Gooday AJ, Jones DOB, Rex M, Yasuhara M, *et al.* (2017) Major impacts of climate change on deep-sea benthic ecosystems. *Elementa Science of the Anthropocene* 5.

Taggart CT, Anderson J, Bishop C, Colbourne E, Hutchings J, Lilly G, Morgan J, Murphy E, Myers R, Rose G, *et al.* (1994) Overview of cod stocks, biology, and environment in the Northwest

Atlantic region of Newfoundland, with emphasis on northern cod. *ICES J Mar Sci* 198: 140–157.

Templeman W (1981) Vertebral numbers in Atlantic cod, Gadus morhua, of the Newfoundland and adjacent areas, 1947-71, and their use for delineating cod stocks. *J Northwest Atl Fish Sci* 2: 21–45.

Treberg JR, Killen SS, MacCormack TJ, Lamarre SG, Enders EC (2016) Estimates of metabolic rate and major constituents of metabolic demand in fishes under field conditions: Methods, proxies, and new perspectives. *Comp Biochem Physiol A Mol Integr Physiol* 202: 10–22.

Trueman CN, Rickaby REM, Shephard S (2013) Thermal, trophic and metabolic life histories of inaccessible fishes revealed from stable-isotope analyses: A case study using orange roughy Hoplostethus atlanticus. *J Fish Biol* 83: 1613–1636.

Trueman CN, Chung MT, Shores D (2016) Ecogeochemistry potential in deep time biodiversity illustrated using a modern deep-water case study. *Philos Trans R Soc B Biol Sci* 371. doi:10.1098/rstb.2015.0223

Tveite S (1984) Zero-group cod investigations on the Norwegian Skagerrak coast. In: Dahl E, Danielssen DS, Moksness E, Solemdal P, eds. The Propagation of Cod Gadus Morhua: An International Symposium. pp 581–590.

Williams CM, Henry HAL, Sinclair BJ (2015) Cold truths: How winter drives responses of terrestrial organisms to climate change. *Biological Reviews* 90: 214–235.

Wilson SK, Fisher R, Pratchett MS, Graham NAJ, Dulvy NK, Turner RA, Cakacaka A, Polunin NVC, Rushton SP (2008) Exploitation and habitat degradation as agents of change within coral reef fish communities. *Glob Chang Biol* 14: 2796–2809.

CHAPTER 2: Incorporating otolith-isotope inferred field metabolic rate into conservation strategies

Abstract

Fluctuating ocean conditions are rearranging whole networks of marine communities – from individual-level physiological thresholds to ecosystem function. Physiological studies in the marine realm have largely focused on individual-level responses (biochemical, cellular, tissue, respiratory potential) in laboratory settings. The otolith-isotope method of recovering field metabolic rate has recently filled a gap for the bony fishes, linking otolith stable isotope composition to *in situ* oxygen consumption and experienced temperature estimates. Here, I provide a comprehensive review of the otolith-isotope method by breaking down the biochemical and physiological processes that yield estimates of field metabolic rate. I identify a multidisciplinary pathway in the application of this method, providing concrete research goals (field, modeling) aimed at linking individual-level physiological data to higher levels of biological organization. We hope that this review will provide researchers with a transdisciplinary 'roadmap', guiding the use of the otolith-isotope method to bridge the gap between individual-level physiology and field and modeling efforts, while ensuring that *in situ* data is central in marine policy-making aimed at mitigating climatic and anthropogenic threats.

General Energy Expenditure and Statement of Purpose

Earth's current geological epoch, the "Anthropocene", is characterized by profound anthropogenic disruption and climate variability (IPCC, 2013) – a threat to biodiversity that is reshaping the natural world at all levels of biological organization. Rapid alterations to natural systems have been examined at the biosphere level, from cascading changes to the hydrological cycle, for example (e.g. Boyer *et al.,* 2007; Lozier *et al.,* 2008), to individual level changes to an organism's energetic budget. Energy is the currency required for all biological functions, a unifying variable across the animal kingdom. Both the magnitude (food availability) and allocation (physiological costs) of an organism's energy supply to key life sustaining processes therefore influences survival, fitness, species interactions, and phenology (Doney *et al.,* 2012; Poloczanska *et al.,* 2013), with cascading impacts on population viability and food web dynamics, ultimately influencing overall ecosystem structure and function. It is important to measure an organism's metabolic rate directly within its own environment to not only capture the direct impacts of varying environmental factors on metabolism, but also to target interindividual and interspecific metabolic responses that might interact to reorganize communities in unpredictable ways .

Response to anthropogenic and climate change inherently begins at the level of the organism, manifesting itself through physiological (e.g. metabolic rate, osmoregulatory ability, thermal tolerance) and behavioural (predation, competition, symbiosis, parasitism) strategies resulting from altered biological rates at the cellular level (Rilov *et al.,* 2019). When faced with the energetic costs associated with environmental change, the physiology of organisms can be altered for better performance in their specific habitat – this is known as physiological plasticity

(Gaitán-Espitia *et al.,* 2017). Physiological plasticity varies in accordance with fluctuations in the abiotic environment (temperature, pH, salinity, dissolved oxygen, nutrients) with species-specific responses in accordance with metabolic genotypes (Harley *et al.,* 2017). Organisms with resilient metabolic genotypes are selected for in a population, providing a basis upon which natural selection can act (Gunderson and Stillman, 2015; Martínez *et al.,* 2015). This adaptive strategy can lead to interindividual variation in the energy budget of organisms through the adjustment of metabolic rates, and can highlight important energy allocation tradeoffs (Gaitán-Espitia *et al.,* 2017). While this can benefit certain populations (e.g. those living at cold edges), these factors can also potentially jeopardize performance, reproduction, and survival.

In order to gain a mechanistic understanding of climate and anthropogenic change in the marine realm, the energetic budget of an organism needs to be complete, quantifiable, and comparable. A heterotrophic organism's energy supply is synthesized from organic food molecules (Hill *et al.,* 2012), and is then allocated to key life processes as: supply (Energy IN), transformation or use (Energy OUT) and growth or reproductive effort (Energy RETAINED) (Figure 1.3. Metabolic rate is a proxy for the energy expended by an animal over a specific period of time, and has been studied since the thirteenth century (Hill *et al.,* 2012). Energy is transformed from organic food molecules through the process of glycolysis, producing the organic compound adenosine triphosphate (ATP) which provides energy to drive crucial body processes, such as chemical synthesis and muscle contraction (Hill *et al.,* 2012; Nelson, 2016). The ultimate indicator of energy expenditure is the heat generated by the body measured using direct calorimetry, however, this method has several disadvantages including the complex engineering, costs, and appropriate facilities required to build whole-room calorimeters (Johnson and McKenzie, 2001). In lieu, indirect calorimetry has been widely adopted by physiologists,

kinesiologists, and ecologists alike, using oxygen consumption (and carbon dioxide production) as a measurable indicator of ATP energy. Evolutionary, ecological and physiological aspects of metabolic rate can be examined at various levels of biological organization including: 1. individuals (assess tissue and cellular respiration rates, reproduction, growth, performance, mortality), 2. populations (productivity, predator-prey interactions, population dynamics, differential energy expenditure patterns), and 3. ecosystems (resource depletion, trophic dynamics) (Brown *et al.,* 2004); Chabot *et al.,* 2016).

The metabolic responses of individuals to complex environmental stressors have the ability to highlight important interindividual variability in physiological traits within and across populations, where higher levels of physiological diversity is synonymous with higher resilience, whether that be through resistance or recovery (Spicer and Gaston, 2009). These measurements have the ability to provide a reliable real-time assessment of current and future climate change threats on individuals and populations. There remains an important disconnect between individual-level physiological response and the multiple environmental stressors associated with global climate change in marine eco-physiological studies, however. While large-scale multistressor studies have been conducted to examine climate impacts at higher levels of organization, such as species range shifts (e.g. Lauchlan and Nagelkerken, 2020), determining the direct influence of climate driven physiological costs on individual performance has been more complicated.

In contrast with terrestrial research, our knowledge of the physiological processes of fishes to date has relied almost exclusively on extrapolation from observations of historic populations, or from laboratory-based measures of respiratory potential (Table 2.1). Individual-

level metabolic stress response to real-world climate impacts have been assessed using laboratory multistressor studies, in which collected organisms are exposed to a manipulated environment meant to replicate current and projected environmental factors (Schwieterman *et al.,* 2019). In these studies, factors such as aerobic scope alteration and hypoxia tolerance are measured as indicators of responses to environmental stress. While multistressor studies can highlight the interactive effects of varying environmental stressors, several crucial spatiotemporal factors (e.g. seasonality, life history, phenology, local adaptation) and co-occurring drivers (nutritional status, thermal tolerance, oxygen availability, food availability, competition, predation) associated with complex natural habitats might enhance or buffer responses between individuals in an ecosystem (e.g. Crain *et al.,* 2008; Kuo and Sanford, 2009; Doney *et al.,* 2012; Araújo *et al.,* 2014; Graiff *et al.,* 2015; Crisci *et al.,* 2017; Rilov *et al.,* 2019). Even after controlling for sources of variation related to lifestyle (e.g. benthic vs pelagic), metabolic rate can differ by a factor of up to three among individual fish of the same species, sex, and age held in similar conditions (Millidine *et al.,* 2009; Norin and Malte, 2011; Killen *et al.,* 2012). The relationship between a free-ranging organism's physiological response and its surrounding environment is further complicated by the differential severity of climate and anthropogenic impacts between species, which can shift or even reverse the nature of species interactions within a community (Kordas *et al.,* 2011), ultimately influencing broader scale ecosystem impacts (Connell *et al.,* 2017).

It is crucial to measure an individual's metabolic rate under the influence of the real abiotic and biotic present in its environment to both capture the direct impacts of varying environmental factors on metabolism, while also targeting interindividual and interspecific metabolic responses that might interact to reorganize communities in unpredictable ways (e.g.

Dong *et al.,* 2017). Experimental systems also typically expose individuals to conditions outside of those in the population's natural experience, therefore, it is currently unclear whether acclimation over lab timescales is sufficient to replicate adaptive buffering potential of multi-generational exposure to a given condition. Metabolic enzyme activity and RNA/DNA have been used as biochemical indicators of metabolic condition in free-ranging marine invertebrates and fish, representing direct change in environmental condition (Dahlhoff, 2004; Lesser, 2016). While this method represents an important *in situ* measurement of metabolic rate, it yields a snapshot of the condition of the individual at the time of sampling, with no accompanying spatiotemporal history of metabolic experience or environmental exposure (Dahlhoff, 2004). This is a crucial gap, as realistic predictions on the fate of species and populations require accurate indicators of experienced environmental conditions. This has become especially apparent in recent studies, highlighting that the frequency, intensity, and sequence of abiotic change might be more important to the alteration of population dynamics (Pearson *et al.,* 2009) and species distribution (Wethey *et al.,* 2011) than changes in mean conditions (Wahl *et al.,* 2013; Helmuth *et al.,* 2011; Benedetti-Cecchi *et al.,* 2015). To strengthen climate predictions, scientists require a tool that provides time-integrated *in situ* data on both the individual's physiological response (metabolism) and the associated spatiotemporal indicators of experienced abiotic factors (Bates *et al.,* 2018; Kelly, 2019).

The main goal here is to describe a novel way of acquiring real-time metabolic rate data for free-ranging fishes (field metabolic rate, FMR) through the isotope analysis of sagittal otoliths (Chung *et al.,* 2019b). I highlight the unique opportunity provided by the otolith record, which combines quantitative data on individual variation in metabolic traits with a time-integrated spatiotemporal record of experienced water chemistry, creating a metabolic indicator

of climate and anthropogenic change. The purpose of this paper is three-fold: 1) to compare standard measures of metabolism in fish to field metabolic rate opportunities historically confined to terrestrial organisms, 2) to highlight the otolith-isotope method as a novel and robust tool to acquire individual, time-integrated measures of field metabolic rate for fish, and 3) to establish a new definition of metabolism in the multidisciplinary fields of fish biology, encouraging a new data standard for the creation of powerful predictive models. Understanding the physiological mechanisms that are modifying and reshaping communities will improve biological forecasting on climate change threats to ecosystems and the services they provide. The ability to integrate *in situ* data on the physiological plasticity of free-ranging fish into models at various levels of biological organization has the potential to better inform conservation objectives and management strategies under current and projected climate scenarios.

An organism's realised field metabolic rate (FMR) describes the energy expenditure of a free-ranging organism in its natural environment, averaged over the duration of the observation period. Assessing FMR in free-living organisms avoids confounding factors associated with laboratory studies, such as the complications associated with acclimation and the stresses of manipulation. Furthermore, it provides an opportunity to assemble the context-dependent measures of metabolism associated with life in specific habitats, including the energetic trade-offs associated with complex ecological and environmental interactions. FMR may be a more ecologically relevant measure of energy expenditure, but as a composite measure of basal and active energetic processes, variations in FMR may be complicated to interpret. FMR encompasses different energetic pathways based on the method of measurement used (Figure 2.1. Here I focus on the **otolith-isotope method**, while alternatives will be explored in the following section of this review.

The measurement of field metabolic rate differs from metabolic rate methods that have thus far dominated the field of fish experimental physiology, and have subsequently informed the fields of ecology and behaviour (Table 2.1). Studying SMR, MMR, and aerobic scope allows us to gain mechanistic understanding of ecophysiological responses to specific drivers. While these measures of metabolism represent fundamental components of physiology functioning, they are unlikely to reflect realized physiological responses to complex environmental stressors. These metabolic metrics also contain inherent limitations in their application to free-ranging fishes. Measurements of SMR, for example, are only taken at a single point in time so that variation between individuals can represent measurement errors and random temporal fluctuations, therefore, true differences in regulatory energy expenditure aren't always clear (Metcalfe *et al.,* 2016). Methods of MMR measurements also differ between species of fish, for instance, which vary in their willingness to swim against water currents, making it difficult to exercise them to exhaustion (Norin and Clark, 2016).

The measurement of FMR encompasses the regulatory maintenance functions associated with standard metabolic rate (SMR) and daily activities ranging from foraging to locomotion (Smith 1980), while also including one of the first uses of energy, the specific dynamic action (SDA) (Figure 1.3; Table 2.1). Field metabolic rate therefore sums an individual's standard metabolic rate, its specific dynamic action, and activity metabolism, and can be represented as a time-averaged or instantaneous measure of energy intake and expenditure. While this measure represents an integrated energetic measure of the organism's response to its environment, depending on the research question, the holistic nature of the FMR value could present a drawback due to the difficulty in separating basal from active energetic contributions (SMR, SDA, activity).

The inclusion of both maintenance metabolism (SMR) and activity metabolism in the measure of field metabolic rate highlights the critical physiological tradeoffs that prioritize survival in the face of fluctuating ocean conditions (e.g. water temperature, acidification, hypoxia events), and how critical thresholds may in turn alter activity through physiological and behavioural plasticity (Treberg *et al.,* 2016; Chung *et al.,* 2021). While SMR represents the baseline metabolic requirements for maintaining organismal homeostasis, activity metabolism measures the energy allocated to foraging, feeding, predation, locomotion, and behaviours associated with reproduction, such as courtship and parental care (guarding, scattering). Activity metabolism varies dramatically between species based on the organism's lifestyle characteristics, influencing both the size of the metabolic scope as well as the baseline SMR associated with the metabolic cost of maintaining high-activity machinery (Priede, 1985; Norin and Malte, 2012). While the threshold of activity for a species can be determined in a laboratory setting (MMR), it is the daily activity of an individual interacting with its direct environment, and how survival mechanisms controlled by SMR can prioritize these interactions, which makes activity metabolism interesting within the scope of FMR.

Specific dynamic action (SDA) is a key parameter to factor into the interpretation of FMR, as it is a cost that is not included in SMR or activity metabolism, yet, is dependent on the environment in which the individual lives. The SDA of an organism is defined as the increase in oxygen consumption following a meal, and is driven by the biochemical processes involved with the digestion and mobilization of amino acids (Kleiber 1961). This SDA can vary between 5-20% depending on the organism's diet, with high-protein diets producing the highest SDA values (Priede 1985). The lack or abundance of these food resources in an environment can further alter the energy costs associated with SDA. The seasonality of food resources can therefore lead to

variations in the allocation of energy towards the various metabolic pathways contained within the estimated FMR value, from SDA, to somatic growth, activity, and reproduction. Fish that live in environments with excess food resources are known to have an increased metabolic cost of digestion, creating a metabolic conflict in energy budgeting by limiting performance (e.g. inhibited swimming activity) and prioritizing digestion (Priede 1985).The incorporation of SDA within the measurement of FMR in free-ranging fishes therefore holds a two-fold benefit as it provides integrated information on (1) resource distribution and composition in an organism's environment (high SDA - abundant resources/high protein diet, low SDA - limited resources/low protein diet) and, (2) the reallocation of energy associated with SMR and activity metabolism (high SDA - activity limiting, low SDA - activity priority).

How has field metabolic rate historically been measured?

Despite the prior definition of FMR in relation to fishes, the term 'field metabolic rate' can have different meanings across studies based on the method each uses. Thus, different studies might yield slightly different information for the same species, as they account for different energetic pathways of metabolizable energy in free-ranging animals (Figure 1.3; Table 2.2).

Activity-based measurements: Time-energy budgets and Accelerometry

Depending on the lifestyle of a species, activity-specific energy expenditure can give insight on a significant portion of the overall energy budget. Earlier methods, such as **time-energy budgets**, have tried to gain insight into free-ranging animal energy expenditure using this assumption (Gabrielsen and Ellis, 2001; Hill *et al.,* 2012). Time-energy budgets estimates the

FMR associated with specific activities, combining laboratory studies with field observations to track the amount of time the organism spends displaying different behaviours, and how this influences energetic cost (Gabrielsen and Ellis, 2001). This method relies on multiples of lab-measured basal metabolism in order to determine the total energetic cost of free-ranging animals during each activity, which oftentimes are unknown as animals roam out of sight (Figure 2.1; Burger 1981; Bernstein and Maxson, 2021).

Advances in the construction of miniature data loggers have allowed for a more direct measurement - that of **accelerometry**, which yields the same kind of information - specific daily energy expenditure associated with different locomotory modes over fine temporal scales (Wilson *et al.,* 2006; Green *et al.,* 2009; Enstipp *et al.,* 2011). Unlike simple time-energy budget models, accelerometry makes it possible to measure variation in short-term activity and behaviour, such as preening, diving, swimming, and running without constant observation (Elliott *et al.,* 2013). Accelerometry can be conducted on an animal in the field using different types corresponding acoustic transmitters and tags (Plasqui and Westerterp, 2007), such as **electromyogram telemetry** from the locomotory or opercular muscles (Weatherley *et al.,* 1982; Rogers and Weatherley, 1983; Scharold *et al.,* 1989; Briggs and Post, 1997; Cooke *et al.,* 2004) or the use of data loggers equipped with **tri-axial accelerometers** to determine overall dynamic body acceleration (Fahlman *et al.,* 2008; Murchie *et al.,* 2011; Wright *et al.,* 2014; Dalton *et al.,* 2014; Pagano and Williams, 2019), each with their own advantages and limitations. It is possible to use this method in aquatic systems, but when doing so, it is important to combine the accelerometry data associated with the swimming kinematics of a fish, for example, with environmental data logger technologies to gain insight on any environmental variables which

might influence the relationship between accelerometry data and its energetic metabolic costs (Thorarensen *et al.,* 1996; Treberg *et al.,* 2016; Lea *et al.,* 2016).

Accelerometry is still questioned as an effective way to predict energy expenditure in free-ranging animals, as there is an inherent variability in muscle efficiency that makes it difficult to confidently determine oxygen consumption rates based on mechanical power alone (Ward *et al.,* 2001; Halsey *et al.,* 2011). While locomotory, activity-based costs can encompass a significant portion of some organisms' energy budgets, for most endotherms, it usually accounts for only 15-25% of energy costs (Elliott *et al.,* 2013). Moreover, field metabolic rate estimates from accelerometry overlook important energetic changes that are independent of activity, such as growth, specific dynamic action (SDA), reproduction, thermoregulation, as well as basal and resting metabolic rate (Figure 2.1b).

Telemetered Heart Rate (HR)

Telemetered heart rate is a field-based method that considers more metabolic parameters than time-energy budgets and accelerometry (Table 2.2; Figure 2.1c). This method relies on the physiological interaction between heart rate and oxygen consumption, for which established regression relationships (Armstrong 1986) provide conversions into SMR, SDA, and activity metabolism (Lucas *et al.,* 1991). This approach provides a more holistic look at metabolic scope for fishes in the field, as it doesn't only examine activity metabolism, but also feeding state, water quality, and physiological/social stress (Treberg *et al.,* 2016). Similar to activity-based methods, the heart rate method utilizes logger and telemetry technology, eliminating the inherent bias of stress and confinement associated with laboratory-based experiments (Priede and Young, 1977; Gräns *et al.,* 2010; Clarke *et al.,* 2010). The tags used for

this method do however require precise surgical implantation of electrodes, which increases potential stress to the animal before release back into the wild (Whitney *et al.,* 2007). Some of the associated devices (satellite transmitters, salt-water indicators, speedometers, time-depth recorder) can also potentially alter organism activity in the field as they are attached externally and can increase drag during activities such as swimming (Bannasch *et al.,* 1994). Both surgical constraints and tag size limits the application of this method to larger animals, however, lab trials have been successful for Atlantic cod as small as 100g (Snelderwaard *et al.,* 2006).

Telemetered heart rate measures changes in metabolic rate on a minute-to-minute timescale; however, these measurements can be affected by cardiovascular adjustments, which are independent of energy expenditure (Hudson *et al.,* 2013; Speakman 2000; Butler *et al.,* 2004; Green *et al.,* 2009; Green, 2011; Elliott *et al.,* 2013; Stothart *et al.,* 2016). Since heart rate takes into account multiple energetic expenditure pathways, inconsistency in the influence of heart rate on cardiac output is possible, which is difficult to predict and can change with metabolic stimuli (Thorarensen *et al.,* 1996; Treberg *et al.,* 2016). Therefore, heart rate energy expenditure estimates are often applied over longer times scales and combined with temperature loggers to yield a more accurate and complete predictor of field metabolic rate in comparison to activity-based methods (Clark *et al.,* 2010).

Doubly labeled water (DLW)

Studies of field metabolic rate in animals originate from the study of **doubly labeled water (DLW)**, which is the most widely used FMR method to date, examining both terrestrial and marine vertebrates, as well as invertebrates (Butler *et al.,* 2004). The development of DLW has been central to the determination of FMR, referred to as the 'gold standard' for measuring

total energy expenditure in humans, synonymous with the term daily energy expenditure (DEE; Nagy 1989; Butler *et al.,* 2004; Plasqui and Westerterp, 2007). The DLW method takes into account the sum of all of the energetic pathways involved in a free-ranging animal's metabolizable energy, and can be applied directly to free-ranging animals in their natural habitat (Figure 2.1d; Nagy, 1989).

The doubly labeled water method was developed by Lifson *et al.* (1955) after the discovery that the oxygen present in an organism's body water undergoes a rapid isotopic exchange equilibrium with the oxygen in exhaled CO_2 (Lifson and Gordon, 1949). DLW follows washout rates of oxygen and hydrogen isotopes (^{18}O, and Deuterium ^{2}H/ Tritium ^{3}H, respectively) in an organism's body water and expired CO_2, which are present in the same proportion as the oxygen and hydrogen found in ordinary water, ^{16}O and H (Figure 2.2; Lifson *et al.,* 1955; Nagy 1989). While ^{18}O is found in the continuous flow of water (saliva, evaporative cooling, and urine) and in respiratory CO_2, $^{2}H/^{3}H$ is only eliminated in water molecules (Figure 2.2; Speakman 2000). The differential elimination of ^{18}O enrichment, which declines at a quicker rate than $^{2}H/^{3}H$, therefore allows for a quantitative estimate of the average rate of CO_2 production (Figure 2.2). The rapidity of this differential elimination is measurable in the animal's blood sample, which is compared to a background blood sample taken before the enrichment injection (Speakman 2000). Once the average rate of CO_2 production has been determined for the study period, it can be converted to energy expenditure (kJ/day) by using the respiratory quotient (RQ) specific to the species in question (Speakman 2000; Butler *et al.,* 2004).

By combining the doubly labeled water method with other behavioral, ecological, and physiological measurements, DLW has allowed for insight at several levels of biological

organization, including the function of organ systems (Shoemaker and Nagy, 1984; Nagy and Shoemaker, 1984), how individuals alter behaviours within their environment to promote survival (Karasov, 1983; Scantlebury *et al.,* 2014; Rojano-Doñate *et al.,* 2018), and how daily food requirements, for example, can suggest impacts on ecosystem trophic dynamics (Brekke and Gabrielsen, 1994; Berteaux *et al.,* 1996; Nagy *et al.,* 1999; Nagy, 2001). One of the reasons for this method's wide application is its ability to be conducted on any air-breathing organism with a large range in body size, e.g., from walruses to bumblebees, a difference in size of a magnitude of 10^6 (1,310 kg to 0.0003 kg) (Wolf *et al.,* 1996; Butler *et al.,* 2004; Acquarone *et al.,* 2007). This has resulted in an influx of species-specific validation studies comparing the results of DLW to those resulting from telemetered heart rate (Nolet *et al.,* 1992; Boyd 1995), dynamic body acceleration (Elliott *et al.,* 2013; Stothart *et al.,* 2016; Pagano and Williams, 2019), open-flow respirometry (Sparling *et al.,* 2008), simultaneous materials balance trials (Gales *et al.,* 1988), or combinations of these methods (Dalton *et al.,* 2014). FMR values resulting from such studies have been used in meta-analyses aimed at investigating the allometric relationship of FMR, determining scaling differences based on traits such as body size, thermal physiology, taxonomic differences, as well as the effects of age, diet, habitat, and season on FMR in animals (Nagy *et al.,* 1999; Nagy, 2005; Acquarone *et al.,* 2007; Hudson *et al.,* 2013), including their influence on human health (Plasqui and Westerterp, 2007).

Doubly labeled water: Inapplicable to the largest class of organisms – the bony fishes

The primary concern with the doubly labeled water method is that it has not been transferrable to aquatic species due to their high whole body water turnover rates (Motais *et al.,* 1969; Maloiy 1979). In water-breathing and amphibious organisms, labelled oxygen in body

water is quickly removed, while very little isotopic oxygen leaves the animal as CO_2 (Nagy 1989). In order for the DLW method to work, a substantial fraction (~15%) of isotopic oxygen needs to leave the animal as CO_2. In teleost fish, however, unidirectional water influx rates cause body water turnover rates as fast as 10 to 100+% per hour, and osmoconforming species such as salmonids and Anguilliformes can even exceed this (Nagy 1989; Schmidt-Nielsen, 1990). Because of the smaller ratio of CO_2 production to water, the error involved in detecting and quantifying CO_2 production in water-breathing and amphibious animals increases dramatically, meaning that it is not practical for use in fishes.

Acquiring metabolic information on fish, both bony (teleost, lungfish) and cartilaginous (sharks, skates, and rays), has therefore been restricted to methods such as electromyogram telemetry (Rogers and Weatherley, 1983; Hinch *et al.,* 1996; Briggs and Post, 1997; Cooke *et al.,* 2004; Quintella *et al.,* 2009), heart rate monitoring (Priede and Young, 1977; Priede, 1983; Priede 1985; Lucas *et al.,* 1991; Sureau and Lagardére, 1991; Thorarensen *et al.,* 1996; Clark *et al.,* 2005), ventilation frequency (Frisk *et al.,* 2012), tail beat frequency (Ross *et al.,* 1981), acoustic tri-axial accelerometer transmitters (Kaseloo *et al.,* 1992; Metcalfe *et al.,* 2016), intermittent-flow respirometry (Murchie *et al.,* 2011), field-based respirometry (Bailey *et al.,* 2002; Farrell *et al.,* 2003) or a combination of these techniques (Barnett *et al.,* 2016). However, these approaches only yield short-term “snapshots" into the energy budget of free-ranging fish (Martino 2020). The review by Treberg *et al.* (2016) summarizes an array of available methods and their potential to capture the routine energy expenditure of bony fishes in the field. A more recent technique has been recognized since Treberg *et al.* (2016), regarding the relationship between biomineral carbon in fish otoliths and oxygen consumption – the otolith-isotope method (Chung *et al.,* 2019b).

The otolith-isotope method and field metabolic rate

The otolith: the fish's spatiotemporal record

The otolith is an acellular, unvascularised, incremetally grown structure. Its acellular nature means that once deposited, otolith mineral cannot be resorbed. The otolith therefore provides a lifetime record of the chemical environment in the endolymph fluid at the time of deposition. Three bilaterally symmetric sets of otoliths (the sagittae, lapilli, and asterisci) are located in the endolymph fluid of the vestibular apparatus of the fish's inner ear, and are involved in sensory perception (Solomon *et al.,* 2006). In many fishes, the asteriscus may be made of vaterite, but it is the aragonite that is of interest with this method (Tomas and Geffen, 2003). The precipitation rate of aragonite (crystal $CaCO_3$) from the endolymph fluid is linked to fish growth through the deposition of distinct mineral bands onto a protein-sugar matrix, expanding the otolith structure over the individual's lifetime (Campana and Neilssra, 1985). In section, the otolith appears to have opaque and translucent bands due to variations in the proportion of mineral compared to organic constituents, where organic-rich layers absorb more light, giving an opaque appearance when viewed in transmitted light. These alternating bands of organic-rich and organic-poor layers are deposited in accordance with daily and annual growth increments providing morphological information on age and growth, while the isotopic composition of the associated mineral yields information on experienced water chemistry ($\delta^{18}O$ value) as well as diet and metabolic expenditure ($\delta^{13}C$ value).

The isotopic composition of both oxygen and carbon in otolith aragonite have historically been used to quantitatively link a fish's individual biochemistry to its direct environment, providing insight into dietary composition (Nonogaki *et al.* 2006; Elsdon *et al.,* 2010; von Biela

et al., 2015), population structure (Ashford and Jones, 2007), geography, migration, and spatial ecology (Secor *et al.,* 2001; Ashford and Jones, 2007; Trueman *et al.,* 2012; Kimirei *et al.,* 2013; Javor and Dorval, 2014; Currey *et al.,* 2014; Gerard *et al.,* 2015), as well as stock identification and life history ecology (Gao and Beamish, 1999; Gao *et al.,* 2001; Bastow *et al.,* 2002; Gao *et al.,* 2010; Correia *et al.,* 2011; Shen and Gao, 2012). Stable isotopes of oxygen undergo equilibrium fractionation during incorporation into precipitating aragonite, with the fractionation effect predictably related to the temperature of precipitation, and apparently relatively consistent across taxa (Høie *et al.,* 2004). The temperature experienced by a fish during otolith growth can therefore be estimated from otolith $\delta^{18}O$ values providing an estimate of the isotopic composition of oxygen in the ambient water is available (Trueman *et al.,* 2012). Isotopic analysis of otolith aragonite typically proceeds via evolution of CO_2 gas following acid dissolution. Each analysis simultaneously determines $\delta^{18}O$ and $\delta^{13}C$ values for the same otolith sample. The ability to examine the otolith $\delta^{18}O$ value alongside the $\delta^{13}C$ value therefore allows us to infer both the temperature experienced by the individual fish, and the FMR manifested at this temperature, averaged over the same time period. The incremental structure of the otolith provides additional information relating to growth achieved under each observed combination of temperature and FMR. Because the otolith is an inert acellular structure, it does not undergo metabolic reworking or turnover (Thorrold *et al.,* 1997; Campana, 1999; Solomon *et al.,* 2006), thus, collecting the otolith at any point during a fish's lifetime provides an uninterrupted, tangible record of somatic growth linked to experienced water temperature and dietary information useful for age estimations (Gauldie, 1996), life-history trait reconstruction (Gao *et al.,* 2010; Javor and Dorval, 2014), and the reconstruction of anthropogenically altered environmental histories (Schloesser *et al.,* 2009; Fraile *et al.,* 2016). The integrity of the otolith and its composition is also useful in

examining species that can no longer be monitored due to extinction (archived in museums and research institutes; Schutkowski *et al.,* 1999; Wurster and Patterson, 2003; Disspain *et al.,* 2016) or that live in unattainable environments, such as the deep sea (Shephard *et al.,* 2007; Trueman *et al.,* 2013).

Two sources of carbon are incorporated into the fish's otolith, and these are dependent on, and representative of, the organism's direct environment: 1) dissolved inorganic carbon (DIC) from the surrounding water incorporated through the gill and/or the gut ($\delta^{13}C_{DIC}$ value) and 2) metabolically derived carbon from the cellular respiration of food ($\delta^{13}C_{diet}$ value) (Figure 2.3; Kalish 1991; McConnaughey *et al.,* 1997; Chung *et al.,* 2019a). It is the mixing of these two isotopically distinct sources of dissolved carbon ($\delta^{13}C_{DIC}$ value, $\delta^{13}C_{diet}$ value) that changes the overall isotope ratio into an interpretable measure of metabolically sourced carbon (Figure 2.3).

The otolith/field metabolic rate link

The biochemical relationship between the fish's respiration rate (O_2 consumption, CO_2 production), the rate of oxidation of dietary carbon, and the otolith's isotopic carbon ratio ($\delta^{13}C_{oto}$ value) highlights a unique proxy to infer field metabolic rate (Kalish, 1991; Solomon et al,. 2006, Trueman *et al.,* 2013, 2016; Figure 2.3). When a fish experiences an increase in metabolic demand, there is a subsequent increase in respiration and the rate of production of metabolic CO_2, increasing the proportion of metabolically sourced carbon (C_{resp}) in the blood. Fish must regulate blood HCO_3^- concentrations to maintain body pH and avoid acidosis. Therefore, increases in rate of production of metabolic CO_2 must increase the proportional contribution of metabolic C to blood, the endolymph, and subsequently, the aragonite of the otolith (Figure 2.3; Solomon *et al.,* 2006; Martino 2020). Metabolically sourced carbon (C_{resp})

has lower $\delta^{13}C$ values than DIC ($\delta^{13}C_{DIC}$, $\delta^{13}C_{diet}$; ~1 and -16%, respectively; (Sherwood and Rose, 2005; Tagliabue and Bopp, 2008), resulting in a net decrease in the $\delta^{13}C_{oto}$ value (Kalish 1991; Høie *et al.,* 2003 Dufour *et al.,* 2007). If the $\delta^{13}C_{DIC}$, and $\delta^{13}C_{diet}$ values are known or can be inferred, the proportion of carbon in the otolith derived from metabolic sources (C_{resp}) can be distinguished using a two-component mixing model (Chung *et al.,* 2019a, b; Martino 2020). The inversely proportional relationship between oxygen consumption rate and the $\delta^{13}C_{oto}$ value is therefore a result of the metabolic oxidation of dietary carbon, and the metabolically derived source of carbon (C_{resp}) can be further isolated to provide a proxy for precise patterns in metabolism (FMR; Figure 2.3).

In the past, field studies have linked $\delta^{13}C_{oto}$ values to metabolism by examining swimming capacity in marine fish (Sherwood and Rose, 2003), or by examining the intra-otolith variations in $\delta^{13}C_{oto}$ value in the temperature-driven metabolic cycles of archived freshwater drums (*Aplodinotus grunniens*; Wurster and Patterson, 2003). While these studies represent the important first steps towards understanding the pattern between metabolism and the $\delta^{13}C_{oto}$ value, they remain constrained to qualitative estimates of FMR differences between life stages and individuals due to the inability to link otolith stable isotope composition directly to oxygen consumption measurements. Recent validation studies have strengthened and solidified the relationship between oxygen consumption, otolith carbon stable isotope signatures, and metabolic metrics by using intermittent-flow respirometry to directly relate individual-level measurement of oxygen consumption to a $\delta^{13}C_{oto}$ value in laboratory reared Atlantic cod (*Gadus morhu*a; Chung *et al.,* 2019b) and Australasian snapper (*Chrysophrys auratus*; Martino 2020). These studies are crucial in promoting the translation of this method from the laboratory to the field, as they highlight species-specific statistical calibrations between the isotopic composition

of the otolith ($\delta^{13}C_{oto}$, C_{resp}) and alternative metabolic metrics (e.g. O_2 ml/min/kg) providing strong evidence of this method's accuracy as a proxy for FMR (Chung *et al.,* 2019b; Martino 2020). Species-specific calibration equations (Chung *et al.,* 2019a, b; Martino 2020) serve as the baseline for field-based experiments, in which C_{resp} can be associated with time-integrated, retrospective estimates of individual-level FMR expressed by fish in their natural environment, expressed either as C_{resp} values or equivalent oxygen consumption rates (Chung *et al.,* 2021).

The otolith-isotope method presents an opportunity to explore the metabolic components of organisms in the aquatic realm; specifically, the individual field metabolic rate of the vast diversity and abundance of bony fish species (>30 000 spp; Ravi and Venkatesh, 2018). Progress over the last ten years have helped advance the application of otolith $\delta^{13}C$ values as a metabolic proxy, demonstrating robust and consistent relationships between the proportion of metabolic carbon in the otolith (C_{resp} values) and fish body size, temperature and activity (e.g. Kalish 1991; Wurster and Patterson, 2003; Sherwood and Rose, 2005; Solomon 2006; Trueman *et al.* 2013; Sturrock *et al.,* 2015; Chung *et al.,* 2019a). More recently, laboratory experiments have been used to manipulate metabolic rates through ambient temperature, thereby calibrating C_{resp} values against oxygen consumption rates (Chung *et al.,* 2019b; Martino 2020). Otolith C_{resp} values have subsequently been used to demonstrate differences in FMR among sympatric ecotypes of cod (Chung *et al.,* 2021), to infer relative FMR among myctophid species (Alewijnse *et al.,* 2021), and infer historic variations in FMR in northern cod populations (Smoliński *et al.,* 2021).

Limitations, considerations and potential solutions

The use of the otolith as a biogeochemical indicator of lifetime metabolic histories in wild fish provides a comprehensive tool for ecologists and physiologists alike, however, inherent limitations and logistical constraints call for further research effort to confidently use this method between and within species. Variation in diets, lifestyles, and locations/migratory patterns may complicate the precision of estimates of $\delta^{13}C$ values for diet and DIC, which could yield inaccurate estimates of individual FMR. Moreover, applying this method to wild fishes in a macroecological framework might benefit from species-specific laboratory validations. Here we provide a brief summary of key limitations and developing solutions in relation to the major steps associated with the $\delta^{13}C_{oto}$ method: $\delta^{13}C_{DIC}$ and $\delta^{13}C_{diet}$ value variation, fractionation, C_{resp} determination, and oxygen consumption estimation, while a comprehensive review is available in Chung *et al.* (2019a).

C_{resp} determination

The proportion of metabolically sourced carbon in the otolith (C_{resp}) of an individual is dependent on the representative accuracy of estimates of both $\delta^{13}C_{DIC}$ and $\delta^{13}C_{diet}$ values in the two-component mixing model (Figure 2.3). Past studies on a variety of fish species (summarized in Chung *et al.,* 2019a) have determined that C_{resp} values estimated from two-component mixing models generally fall in the range of 0-0.5. It is currently uncertain whether C_{resp} values that fall outside of this range yield inaccurate C_{resp} estimations (Wurster and Patterson, 2003; Hanson *et al.,* 2013), or whether these are consistent with species-specific metabolic lifestyles, such as the high metabolism experienced by tuna. The proportion of metabolically derived carbon in otoliths is known to vary based on time of day (Tohse and Mugiya, 2008), and within fish functional

groups consistent with lifestyle ($\delta^{13}C_{diet}$ variation), and migratory status/location ($\delta^{13}C_{DIC}$ variation) (Chung *et al.,* 2019a), but this should not affect calibrations to oxygen consumption, yielding an FMR

$\delta^{13}C_{DIC}$ value variation: The proportion of respiratory carbon in otoliths is typically less than 40%, therefore, estimates of C_{resp} values are more sensitive to uncertainty in $\delta^{13}C_{DIC}$ values than to $\delta^{13}C_{diet}$ values, which is helpful in marine settings where $\delta^{13}C_{DIC}$ values are better constrained. Variation in water $\delta^{13}C_{DIC}$ values occurs based on the environmental source(s) and isotopic compositions of dissolved organic carbon, as well as the extent and of type of photosynthesis involved and the size of the water body (in essence the degree of isotopic buffering). In open marine systems, DIC levels remain relatively uniform between c. -0.5 and 1.5‰ due to the carbonate buffer system. However, temperature-driven differences in the solubility of CO_2 in seawater and variations in the rate of removal of CO_2 through photosynthetic fixation impose systematic variation on oceanic $\delta^{13}C_{DIC}$ values. Databases and models (Tagliabue and Bopp, 2008; Schmittner *et al.,* 2013) describing oceanic $\delta^{13}C_{DIC}$ values provide reasonable estimates (and uncertainties) suitable for otolith C_{resp} mixing models. Non-oceanic, coastal, estuarine and freshwater systems are typically more seasonally and spatially variable, however, resulting in highly variable water DIC concentration and $\delta^{13}C_{DIC}$ values. Larger lakes may exhibit relatively seasonally stable $\delta^{13}C_{DIC}$ values, assisting the application of otolith carbon isotopes to infer metabolism (Solomon *et al.,* 2006; Weidel *et al.,* 2007; Gerdeaux and Dufour, 2015).

While $\delta^{13}C_{DIC}$ values are relatively uniform in oceanic environments (especially the deep-sea; Tagliabue and Bopp, 2008; Schmittner *et al.,* 2013; Becker *et al.,* 2016), temporal effects also cause variation in the $\delta^{13}C_{DIC}$ values. The Suess effect (Figure 2.4) is important to

keep in mind when making use of otoliths from historic archives, and comparing these over long time periods. The Suess effect is reflected by the reduction of the $\delta^{13}C$ value of atmospheric carbon resulting from the burning of fossil fuels with relatively low $\delta^{13}C$ values (Keeling, 1979; Eide *et al.,* 2017; Chung *et al.,* 2019a). There is a mismatch between the atmospheric and biological effects of the Suess effect over time, in which amplified phytoplankton growth rates increase the biological fractionation of carbon, reducing the $\delta^{13}C$ values of organic carbon more rapidly than those of DIC. While there are good records of the extent of the atmospheric and biological effects of the Suess effect across ocean basins (e.g. Körtzinger *et al.,* 2003; Tagliabue and Bopp, 2008; Young *et al.,* 2013; de la Vega *et al.,* 2019), this discrepancy should be addressed when conducting analyses involving FMR comparisons across large timeframes using historical otolith records.

$\delta^{13}C_{diet}$ value and fractionation variation: The isotopic composition of diet carbon sources can be estimated from stable isotope analyses of muscle tissue of the fish under study, or from compilations of isotopic data for prey sources. As diet contributes proportionally less carbon to the otolith than ambient water, uncertainty in $\delta^{13}C$ diet values will contribute less to overall uncertainty, particularly in less active fishes where the contribution of dietary carbon to the otolith is minimised (Figure 2.4; Chung *et al.,* 2019a). To incorporate uncertainty in $\delta^{13}C_{DIC}$ and $\delta^{13}C_{diet}$ values as well as uncertainty in the fractionation coefficient between species (Dufour *et al.,* 2007; Chung *et al.,* 2019a), Monte Carlo resampling, potentially within a Bayesian framework should be used for isotopic mixing models (Stock *et al.,* 2018; Chung *et al.,* 2019a).

C_{resp} and oxygen consumption: C_{resp} values are a proxy for field metabolic rate, however it is helpful to express FMR estimates in units common to respirometry (e.g. oxygen consumption

rates). Laboratory-based calibrations between C_{resp} values and oxygen consumption rates (Chung *et al.* 2019b; Martino 2020) inferred a nonlinear exponential decay-type relationship, setting an upper boundary term to describe departures from non-linearity at high measured oxygen consumption rates. It is not clear whether the determined upper boundary term is appropriate, as at high temperatures, energetic costs above SMR may contribute to increases in SMR (and hence oxygen consumption rates), but these costs are incorporated into otolith FMR estimates at all temperatures. Therefore the relationship between C_{resp} values and oxygen consumption rates may be better captured by linear calibrations particularly in natural settings where sustained field metabolic rates are less likely to approach maximum metabolism. However, more experimental work is needed to address this issue.

Intra-specific variation

While uncertainty can arise during the conversion of C_{resp} to alternative proxies for FMR, variation in lifestyles, diet, or locations, there are also considerations to keep in mind when conducting FMR comparisons between individuals or populations of the same species (Figure 2.4). Body size and temperature are important covariates influencing all metrics of metabolic rate, and should be similarly considered for the FMR proxy. Body size has a major influence on metabolic rate, as energy demands increase allometrically with biomass (Glazier, 2005; Killen *et al.,* 2010). While the otolith provides an opportunity to measure the long-term time integrated metabolic rate in wild fish, limitations are present when making intra-specific comparisons across life stages. For example, the metabolic rate of fish is influenced by a mass-specific decrease with age, which is reflected in the otolith as an increase in $\delta^{13}C_{oto}$ values across its lifetime (Fidhiany and Winckler, 1998; Rosenfeld *et al.,* 2015; Peck and Moyano, 2016;

Trueman *et al.,* 2016; Chung *et al.,* 2019a). Sampling should therefore be focused on individuals of the same size and temperature exposure, or alternatively, a scaling relationship should be determined to correct for differences in temperature and size effects (Chung *et al.,* 2019a).

Water temperature is another factor that might influence FMR estimates, changing rapidly in coastal systems and varying markedly across seasons. The ability to analyse otolith the $\delta^{18}O$ value alongside the $\delta^{13}C$ value, however, allows for the reconstruction of time-specific experienced temperature in wild fishes (Kalish 1991; Kitagawa *et al.,* 2013). Otolith isotope-derived FMR values can therefore be standardized in accordance with individual-specific experienced temperature and season, allowing for confident estimations of temperature-influenced processes, such as food conversion efficiency, and growth potential at coarser time scales.

Analytical flexibility: determining the resolution and precision of field metabolic rate data

Pairing the recording capacity of the otolith's structure with differing otolith processing techniques would allow researchers to use the otolith to target trends at varying time scales and resolutions. The precision applied during aragonite powder extraction within or across time-integrated mineral bands determines the temporal scale covered by the isotopic environmental tracers (days-lifetime). While other techniques of micro-sampling are potentially available (SIMS, ion microprobe analysis, laser ablation techniques), these are generally not suitable for biomineral $\delta^{13}C$ analysis as measurements are confounded by the presence of the organic matrix which is also ablated during probe-based sampling.

The appropriate temporal resolution (i.e. choice of otolith growth over which to integrate a sample) depends on the research question under investigation. Hand drilling otolith outer surfaces provides the highest temporal resolution as the full surface area of the otolith is available for sampling. Micromilling sectioned otoliths allows multiple sampling over ontogeny for a single individual (Weidel *et al.,* 2007; Chung *et al.,* 2019b). Serial sampling or temporally targeted sampling provides an opportunity to capture retrospective estimates of metabolic costs associated with long-term natural behaviours (seasonal movements, reproduction, established trophic interactions) over life history periods of interest (ontogeny), or site-specific environmentally-significant time periods (El Niño, overwintering periods, chronic eutrophication, long-scale habitat alteration projects, local anthropogenic/agricultural runoff). However sampling from otolith sections generally comes at a cost of lower temporal resolution and with less precise estimates of body size compared to sampling otolith edges. The ability to examine the FMR proxy over a long time scale can be beneficial in minimizing the impact of extreme or abnormal behaviours on net C_{resp}, allowing for the analysis of averaged, yet robust, trends at several levels of biological organization (Figure 2.5). While obtaining a weighted average C_{resp} value over a larger time period can be ecologically relevant, such measures warrant additional caution, as temporal covariance between temperature and FMR could be mismatched. Otolith processing techniques that increase the temporal resolution of the FMR-proxy present an opportunity to examine daily periodicity of metabolic rate and experienced temperature to target the acute physiological effects of short-term environmental (weather, marine heat waves, plumes, upwelling, environmental/anthropogenic runoff, acute eutrophication) or behavioural (transition between life history stages, short-term migration, prey selection, spawning, habitat choice) dynamics (Weidel *et al.,* 2007). Targeting C_{resp} over short-time periods at a high resolution

provides an alternative to long-term FMR-proxies, which might not have the appropriate high frequency environmental sampling data to support studies aimed at analysing physiological responses to climate (Weidel *et al.,* 2007). The ability to examine acute physiological trends at high resolutions without the need for large-scale environmental data integration is also valuable for examining archived otoliths, allowing for the reconstruction of thermal histories and energetic time-series as useful indicators of past climates.

It is important keep in mind that the temporal resolution of otolith isotope based-FMR estimates relies on the amount of aragonite powder that can be removed and collected for analysis (IRMS typically requiring c.5 to 50 µg powder), and will translate to different timescales of otolith growth based on otolith size and fish growth rate. While otolith size has historically limited high-resolution isotope profiles over narrow timescales, recent advances in otolith processing techniques have allowed for increased precision during both the aragonite extraction and isotope analysis phases. The smaller amounts of aragonite material obtained by milling along the otolith growth axis has previously limited the maximal resolution of metabolic information by both the growth rate and the size of the otolith, and therefore, high-frequency time-series were constrained to fish with notably large otoliths (Wurster and Patterson 2003; Wurster *et al.,* 2005; Chung *et al.,* 2019b). Sampling the outer surface of otoliths (as opposed to sectioned otoliths) maximises the surface area available for sampling, and reduces uncertainty in estimates of water $\delta^{13}C$ DIC and $\delta^{18}O$ values as the smaller timeframe sampled relates more directly to the location at which the fish was caught.

Incorporating otolith-isotope inferred energetics into conservation strategies

While the otolith-isotope method has previously been applied to wild fishes (Kalish 1991; Jamieson *et al.,* 2004; Trueman *et al.,* 2013; Sinnatamby *et al*. 2015; 2014; Trueman *et al.,* 2016,), the first study to contrast inferred oxygen consumption rates among populations of wild fishes is recent (Chung *et al.,* 2021). This study outlined the potential for exciting conservation gains at various levels of biological organization. After laboratory validation studies aimed at reliably expanding the method towards ecologically and economically important fish species (Chung *et al.,* 2019b; Martino 2020), the otolith-isotope method was applied to two sympatric ecotypes of Atlantic cod off the Skagerrak coast of southern Norway to examine the thermal sensitivity of FMR (Chung *et al.,* 2021). This study aimed to examine the context-dependent relationship between FMR ($\delta^{13}C$ values) and experienced temperature ($\delta^{18}O$ values) to gain insight on population-level responses to climate change, while accounting for any behavioural responses that might influence individual energy balance in free-ranging fish (Chung *et al.,* 2021). The reconstruction of ecotype-specific individual-level FMR and its thermal sensitivity highlights unique metabolic strategies between thermally adapted populations under a continually warming climate (Chung *et al.,* 2021). Here, the cold-adapted Fjord population demonstrated both a lower thermal sensitivity of FMR and a lower maximum FMR at high temperatures, suggesting that while Fjord populations have a metabolic advantage at lower temperatures, this is not consistent at higher temperatures (Chung *et al.,* 2021). Such measures create a link between the individual's physiological response and its greater environment by suggesting impacts at higher levels of biological organization, in this case, being changes in ecotype-specific competitive interactions within populations (Figure 2.5). The thermal sensitivity of FMR and the mean FMR of individuals provides an important foundation for energetic

modelling at the population level (fish growth and viability - for population/production modeling, fisheries projection models), as well as the community and ecosystem level (distribution/habitat use – trophic network models). This first *in situ* use of the otolith-isotope method provides a great example of how field measurements provide an analytical framework to inform conservation and management strategies. The application of this method has also recently been expanded to historical otolith collections (Smoliński *et al.,* 2021), examining differences in FMR between Atlantic cod (*Gadus* morhua) populations from Iceland and the Barents Sea over a nearly a century (1914-2013). Studies are also expanding the otolith FMR method to other species, for example estimating relative field metabolic rates in myctophid fishes to their role in ocean carbon export (Alewijnse *et al.,* 2021).

Assessing individual response to complex environmental stressors is important in determining the underlying mechanisms that drive the trend toward either vulnerability or resilience in the face of climate and anthropogenic change. Resilience can be further divided into two pathways - that of resistance to, or recovery from, destabilizing anthropogenic and climatic stressors. Organisms with resilient metabolic genotypes are selected for in a population, providing a basis upon which natural selection can act (Gunderson and Stillman, 2015; Martínez *et al.,* 2015). The metabolic responses of individuals to environmental stressors have the ability to highlight important interindividual variability in physiological traits within and across populations, in which higher levels of physiological diversity is synonymous with higher resilience (Spicer and Gaston, 2009). Physiological diversity represents the variability in physiological traits (metabolic rate, hemoglobin-oxygen affinity, thermal tolerance, osmoregulatory ability, resting membrane potential) among organisms in a population or community (Spicer and Gaston, 2009). This physiological diversity does not only stem from

responses to direct environmental stressors, but also from unique genetic and developmental factors (Spicer and Gaston, 2009). Understanding how the underlying physiological responses of individuals are situated within higher levels of biological organization is crucial to create realistic predictions of ecosystem vulnerability or resilience. Furthermore, since climate threats are unpredictable and do not necessarily follow latitudinal gradients, the determination of physiological vulnerability at regional and local scales might reveal climate risks that could otherwise go undetected (e.g. Burrows *et al.,* 2011).

Otolith-isotope inferred FMR-proxies can provide insight into the energy requirements, physiological responses, behavioural adaptations, and trophic interactions of free-ranging fishes exposed to environmental change and anthropogenic disturbance (Figure 2.6; Nagy 1999; Chung *et al.,* 2021; Martino 2020). It is difficult to use individual field metabolic rate alone as an indicator for anthropogenic and climatic physiological stress, however, combining stress indicators at multiple levels of biological organization can start to paint a holistic picture of *in situ* impacts. Combining individual-level examinations of metabolic rate fluctuations with population-level analyses of reproductive output and physiological diversity can create context for the allocation of energy associated with the field metabolic rate value.

Figure 2.6 connects the many steps associated with the otolith-isotope method and its ability to address research questions from various fields (physiology, toxicology, animal behaviour, evolution, food web ecology) to infer conservation strategies. Because of the inherent limitations associated with interspecific studies, Step 1 will restrict the initial sampling effort to make comparisons between individuals and populations of the same species in areas influenced by climate variability or anthropogenic change. The FMR ($\delta^{13}C$) and water chemistry ($\delta^{18}O$)

values obtained from the otolith record serve as indicators for biological processes, such as daily energy requirements and seasonal movement, respectively, which in turn can be linked to larger-scale ecological dynamics (Figure 2.6; Step 2 and 3). The intra-specific physiological stressors associated with fluctuating environments can influence species interactions, trophic dynamics, habitat use, and life history stages, providing crucial *in situ* information (Step 4) for the development of effective conservation strategies (Figure 2.6; Step 5).

The unique precision, yet flexibility, of the otolith as a spatiotemporal tool linking FMR to experienced temperature and location provides four key interlinked opportunities to inform and advance conservation and management strategies:

1- **Physiological response to regional climate pressures.** The otolith-isotope method can be applied to capture the impact of local (e.g. agricultural/industrial runoff, eutrophication) and wide-scale (acidification, El Niño events, temperature, heat waves) climate stressors on the FMR of free-ranging fishes. Moreover, the otolith's ability to gather this FMR data through a spatiotemporal lens allows for a three-dimensional assessment of climate impacts - examining the influence of environmental fluctuations on individual metabolism across heterogeneous environments and seasonal, inter-annual, and decadal timescales. Understanding how acute and chronic climate stressors alter the physiological diversity of natural populations over space and time can provide insight on the metabolic and behavioural strategies adopted by individuals and populations in the face of climate variability with the potential for behavioural thermoregulation, niche variation, range expansion, and alterations in migratory routes, which all have cascading impacts on wider ecosystem processes. The capacity to track how the critical physiological phenotypes of individuals differ within and between populations can inform conservation

objectives on the vulnerability or the inherent resilient responsivity of populations under different climate stressors. *In situ* data on physiological costs therefore allow for informed protected area spatial planning consistent with the vulnerability of populations in the face of climate change.

2- **Physiological response to local anthropogenic stressors.** Additional drivers of physiological stress can be identified by using the otolith-isotope method to target populations impacted by anthropogenic pressures, such as habitat modification (shoreline development, dam and wave energy conductor construction), species exploitation (commercial or recreational fishing, changes in predator abundance), or pollution exposure (pesticides, metals, pharmaceuticals, light, sound). While humans cannot strategically reduce the current impacts of climate change, an opportunity arises to use the otolith-isotope method as a tool to produce warning signals of harmful and irreversible anthropogenic pressure on individuals under climate stress. This method allows us to identify whether local anthropogenic conditions amplify or dampen the effects of climatic stressors. For example, questions regarding the influence of urbanised vs natural settings, or varying fishing intensity, on the thermal optimum for FMR or the metabolic phenotypic diversity of a population can be explored. The otolith's inherent ability to record the energetic costs of free ranging fish over large temporal scales provides a unique opportunity to examine how sudden alterations in global human activity, such as occurred during the COVID 19 lockdown in 2020, affects the energetic budgets of fish populations, for instance. How a system responds to direct and indirect anthropogenic stressors can provide further insight into the behavioural and physiological adaptations involved in driving resilience (resistance or recovery), informing stakeholders and policy makers on any necessary alterations to

anthropogenic pressures to ensure the health of overall ecosystems and the sustainable productivity of fish stocks.

3- **Improve energetic models.** Examining individual variation in FMR provides insight on the full energetic budget of a free-ranging fish exposed to real-time climate variability and anthropogenic pressure, with the ability of examining the potential physiological costs associated with extreme temperatures (critical thermal limits) and resource depletion (daily energy requirements). This valuable *in situ* data increases the predictive power of modeling efforts at various levels of biological organization, including energetic models, population and productivity models, fisheries-catch projections, spatial distribution models, and trophic network models (Figure 2.5). By integrating real-time *in situ* data into these models, they will provide better estimates of the long-term growth and productivity of populations under current and projected climate scenarios, with the ability of including the additional physiological costs associated with anthropogenic activity to assess real-time ecosystem stability.

4- **Assessing restoration efforts.** While the otolith-isotope method can be used to inform and implement marine protected areas and maximum sustainable yield (MSY) estimates, it can also be used to assess any differences in physiological phenotypes between protected and unprotected populations. Incorporating the otolith-isotope method into long-term monitoring programs aimed at assessing the physiological performance of protected versus unprotected populations can provide valuable information on the effectiveness of conservation initiatives, and can help weigh the direct costs and benefits of implementation.

A prime **example** of the four key opportunities associated with the otolith-isotope method is its application to fishery science, with the aim of providing well-informed projections that ensure

consistency between stock management and conservation objectives. Real-time *in situ* knowledge on the vulnerability of fish populations to climate events (1) provides a unique opportunity to alleviate any additional pressures caused by anthropogenic activities (2) to ensure ecosystem health, while also allowing for the maximum productivity of fish stocks for socioeconomic stability. This means integrating *in situ* physiological costs into stock assessments to create maximum sustainable yield (MSY) estimates and fisheries catch projections (3) that account for real-time environmental and anthropogenic pressures on fish populations. When combined with long-term fisheries catch projections informed by greenhouse gas emissions, the otolith-isotope method can provide valuable information on the physiological costs associated with acute climate events for particular regions of concern (Cheung *et al.,* 2011; Figure 2.6). Incorporating *in situ* data into maximum sustainable yield estimates will inform conservation initiatives aimed at alleviating adjustable anthropogenic pressures on populations under climatic stress, while subsequently encouraging management practices that ensure a sustainable equilibrium state between fisheries capture rates and fish population productivity. By integrating the otolith-isotope method into long-term monitoring programs (4), the energetic performance of populations under climate-informed fisheries practices can be continually assessed to determine whether conservation and management practices actively contribute to reliable long-term global food security.

Conclusion and future directions

The potential to examine the dynamic balance between maintenance and activity metabolism within the scope of FMR can highlight physiological and behavioural plasticity in the face of climate variability and anthropogenic stressors, with direct implications for species

interactions and population dynamics (Chung *et al.,* 2021). Predicting the cascading effects of individual metabolism on behaviour is especially important in marine systems, with its relative lack of barriers to dispersal and greater colonization rates, as it provides insight into the metabolic drivers associated with species distribution, community compositional turnover, and trophic interactions (Poloczanska *et al.,* 2013; Burrows *et al.,* 2019; Pinsky *et al.,* 2019).

The otolith-isotope method outlines an exciting path forward in the field of ecophysiology, allowing flexibility in answering *in situ* questions, such as the physiological costs of locomotion over specific migration routes, for example. This presents a novel opportunity for various branches of biology and physiology, as measuring the activity involved in trophic interactions, such as foraging and predation, is difficult within a laboratory setting, usually calling for the use of microcosm and mesocosm experiments, which are difficult to replicate, costly, and can lead to oversimplification of ecosystem processes due to their narrow spatial and temporal scales (Coelho *et al.,* 2013). While mesocosms can demonstrate the metabolic balance of planktonic communities within imitated wetland and mangrove habitats (Oviatt *et al.,* 1986; Lv *et al.,* 2017), mesocosms have not been used to measure individual metabolism of vertebrates.

The measurement of FMR with the otolith-isotope method presents the first truly holistic view of the energetic costs associated with environmental change directly measured from free-ranging fish, providing insight on somatic growth, dietary breadth, experienced temperature, and metabolism. Future opportunities include applying the aragonite-based method to a wider range of taxa which incorporate metabolically derived carbon into their carbonate structures, such as the shells of mollusks ($\delta^{13}C$ value: oxygen consumption) and foraminifera ($\delta^{13}C$ value: symbiont photosynthetic activity), with interpretable deviations of ~5‰ between $\delta^{13}C$ values and inorganic

aragonite precipitation (Grossman and Ku, 1986; Prado *et al.,* 2013). There is also potential to extend this method to extant and archived urchin species (Grossman and Ku, 1986; Spero and Williams, 1987; Prado *et al.,* 2013) which incorporate metabolically derived carbon into their aragonite tests, previously been used for diet analysis (Prado *et al.,* 2013). Similar assessments of other calcium carbonate based benthic consumers and photosynthesizers, such as crustaceans, gastropods, and corals, could provide insight into field based metabolism. When examining photosynthetic organisms, such as foraminifera and coral, it is important to examine the relationship between $\delta^{13}C$ signatures and respired CO_2 to allow for species-specific calibrations that accurately depict metabolically derived carbon signatures (Swart, 1983; Høie *et al.* 2003). Despite the inherent limitations associated with the otolith-isotope method, the increasing use of this method will mirror the development of new technologies, increasing its relevance to various fields of ecophysiology and taxa, allowing scientists to refine their lens on the climatic and anthropogenic changes to aquatic environments, and determine the best ways to move forward in mitigating them.

Figures

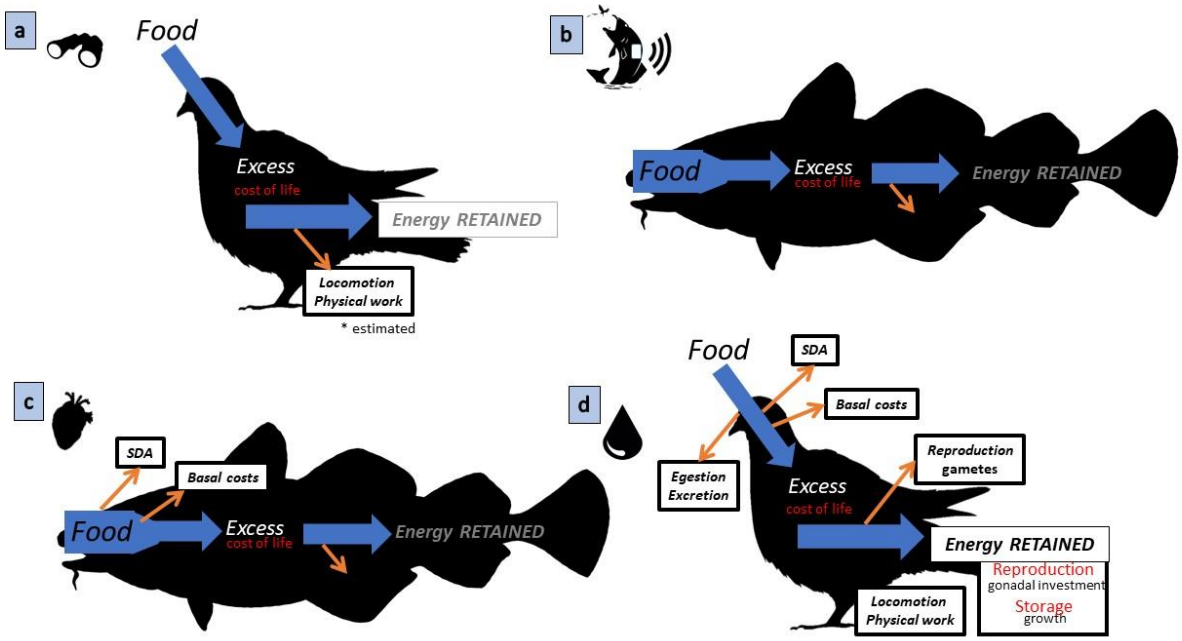


Figure 2.1. A summary of each FMR method discussed and the energetic pathways they incorporate into the measurement of FMR. The methods that can be applied to fishes have been represented by a fish, while the methods that only function for air-breathing animals have been represented by a pigeon. (a) **Time energy budget** provides an estimate of locomotion and physical work (Energy OUT) by using multiples of lab deduced basal metabolism. (b) **Accelerometry** measures the variation in specific daily energy costs associated with different locomotory modes, which means that remaining metabolic costs must be incorporated in energetic models to determine the total field metabolic rate. (c) **Heart rate telemetry** utilizes measurements of heart rate fluctuations to track short-term changes in metabolism associated with feeding state (SDA), basal costs (SMR), and activity metabolism. (d) **Doubly-labeled water** measures potential changes in energy expenditure associated with growth and reproduction (Energy RETAINED and Energy OUT), activity, thermoregulation (in endothermic animals), as well as basal costs (BMR) and feeding state (SDA). (Atlantic cod illustration: Cerren Richards).

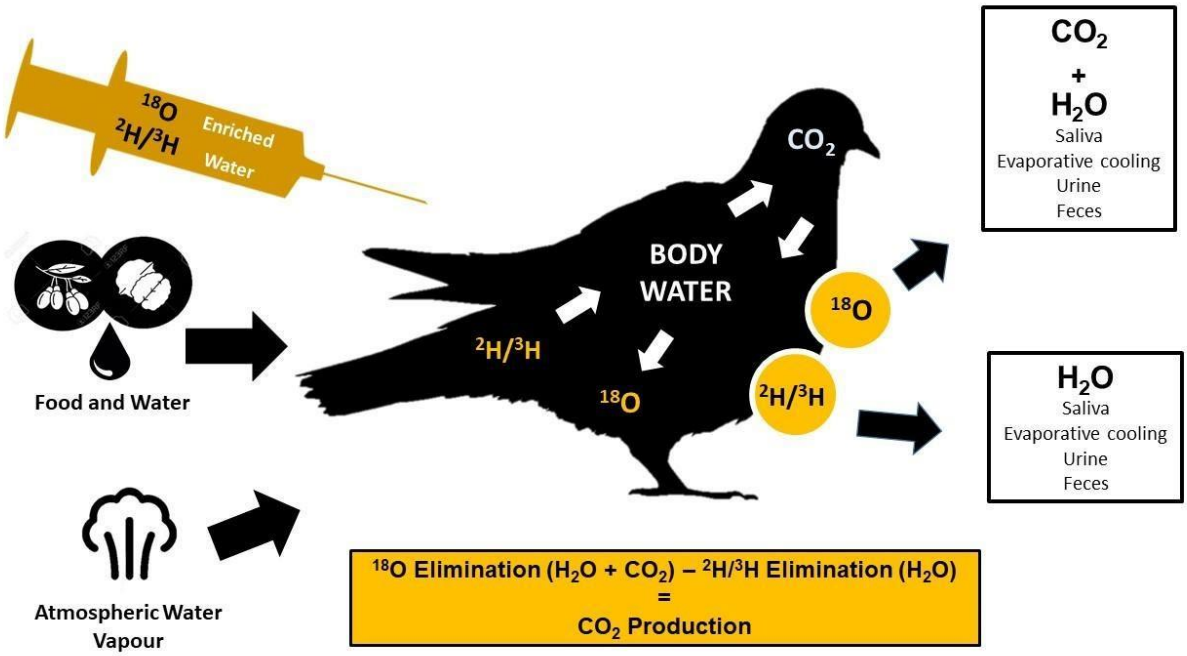


Figure 2.2. The doubly labeled water method follows the washout rate of a measured quantity of oxygen and hydrogen isotopes (^{18}O and $^{2}H/^{3}H$) from their injection into the body to their elimination in the organism's body water. Following isotope injection, the organism is released to behave freely in its natural habitat, where food and water, as well as atmospheric water vapour, may be added to the organism's body content. The oxygen and hydrogen isotopes mix into the organism's body water, undergoing a steady isotope enrichment in the blood, and declining over time as isotope elimination processes become dominant through expired CO_2 and H_2O release (^{18}O in H_2O and CO_2, $^{2}H/^{3}H$ in H_2O exclusively). The rapidity of this differential elimination is measurable in the animal's blood sample by comparing enrichment levels to an initial background level sample, and allows for the quantitative estimate of the average rate of CO_2 production over the study period.

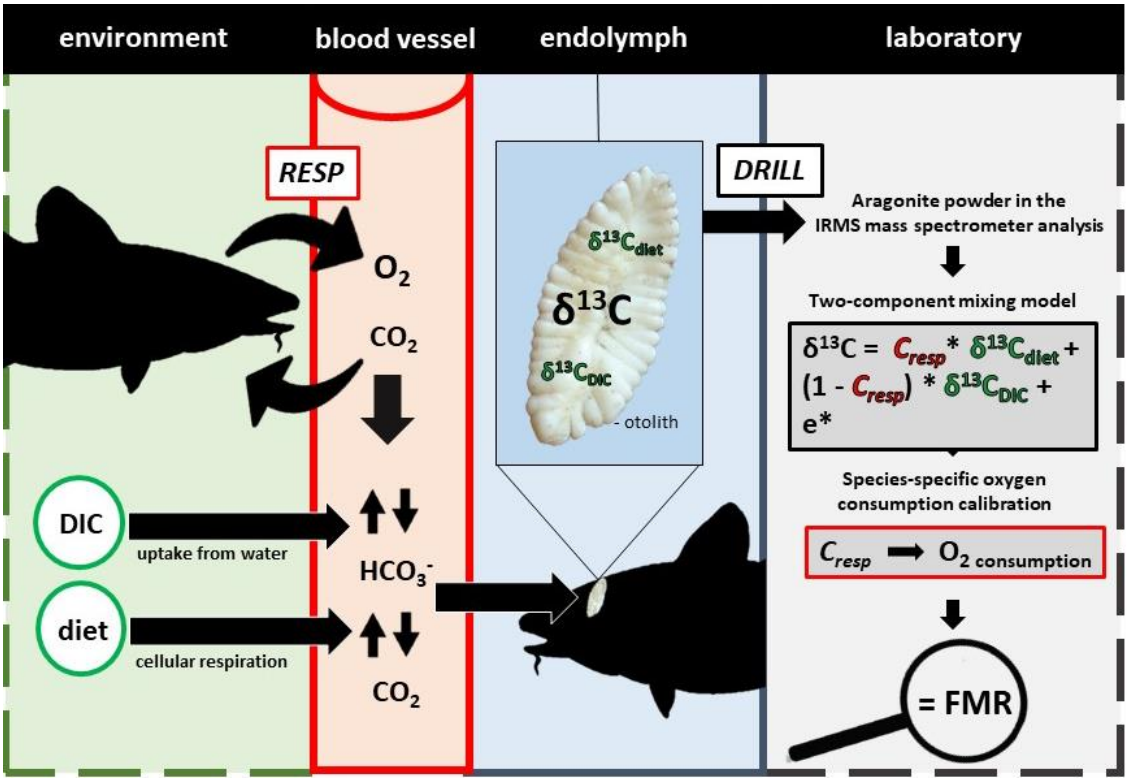


Figure 2.3. The stable isotope otolith method is dependent on biochemical processes, in which respiration alters the carbon concentration of two isotopically distinct sources of carbon in the blood ($\delta^{13}C_{DIC}$, $\delta^{13}C_{diet}$), which then undergo fractionation and are deposited into the otolith as an interpretable stable isotope signature (Solomon *et al.,* 2006; Chung *et al.,* 2019a). The stable isotope signatures from DIC and diet are isotopically distinct, and the proportion of carbon derived from metabolic sources (C_{resp}) can be isolated using a two-component mixing model. C_{resp} can then be converted to O_2 consumption using a species-specific statistical calibration equation (Chung *et al.,* 2019b, Martino *et al.,* 2020), and then converted to field metabolic rate (FMR). The abbreviation RESP stands for respiration (O_2 consumption, CO_2 production) and the e* in the two-component mixing model stands for the fractionation coefficient. The panels surrounded by solid lines represent processes that occur within the organism, while the dashed lines represent those outside the organism. (Atlantic cod illustration: Cerren Richards).

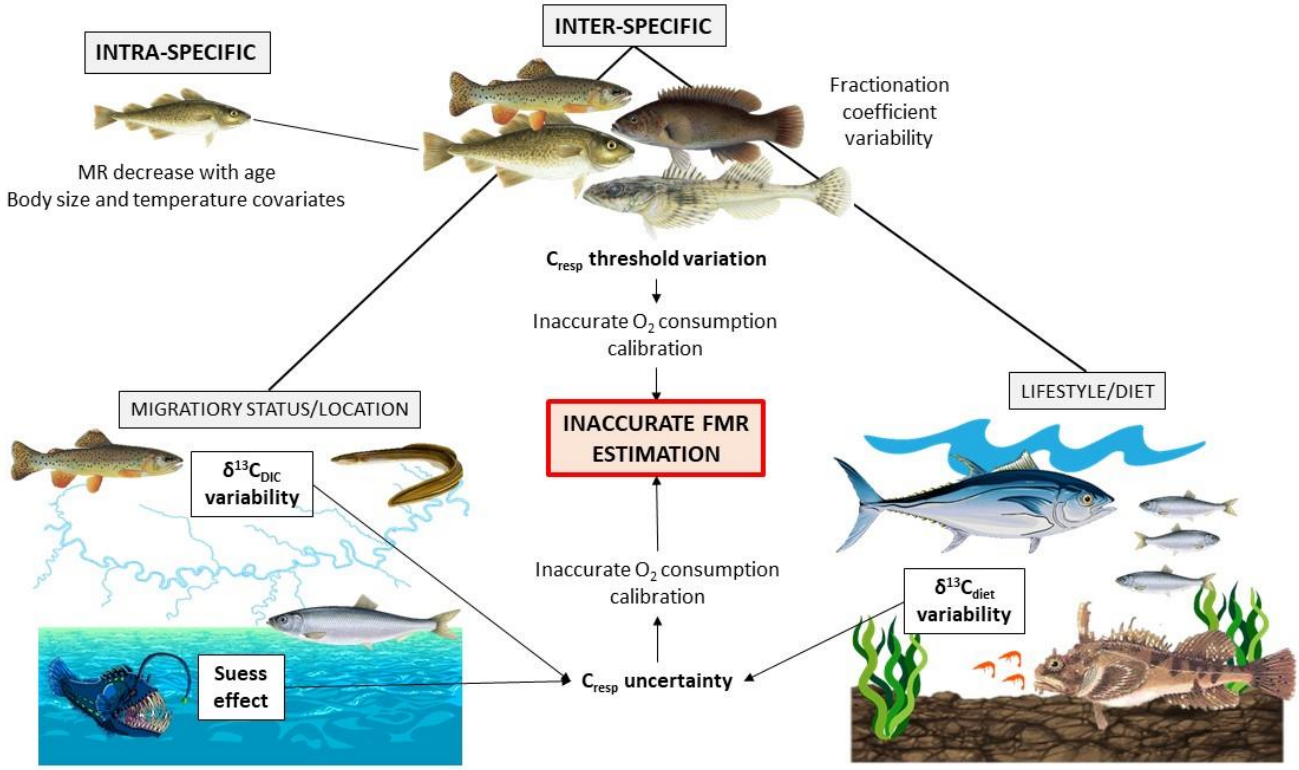
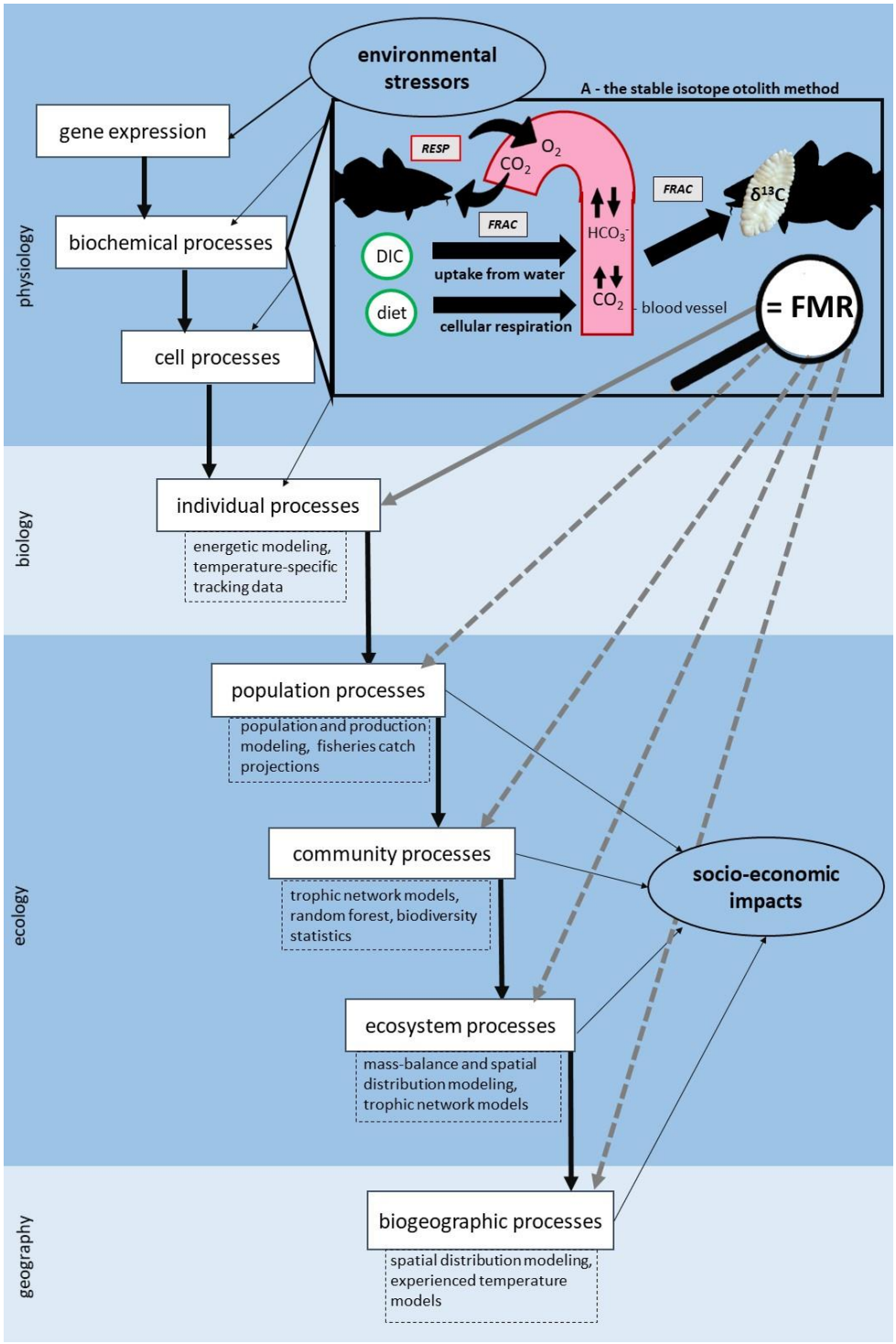


Figure 2.4. An illustration of the limitations to keep in mind when conducting studies aimed at examining differences in FMR through an intra-specific and inter-specific lens. The inter-specific category is further divided into migratory status and location (migrating, freshwater, marine, deep-sea), as well as lifestyle and diet in order to highlight specific processes that might affect the accuracy of FMR estimation.

Figure 2.5. An illustration of where the otolith-isotope method fits into the hierarchical levels of biological complexity (A-biochemical processes). Processes are identified as interlinked boxes overlaid on a background (light and darker blue) of increasing biogeographic scale. The impacts of environmental stressors on, and socio-economic outputs from, these processes are indicated by the thin black arrows. The grey arrows depict the ability to utilize otolith-derived FMR data to directly (solid) and indirectly (dashed) predict processes at multiple levels of organisation. This figure has been adapted from Le Quesne & Pinnegar (2011) and Metcalfe *et al.,* (2012). (Atlantic cod illustration: Cerren Richards).



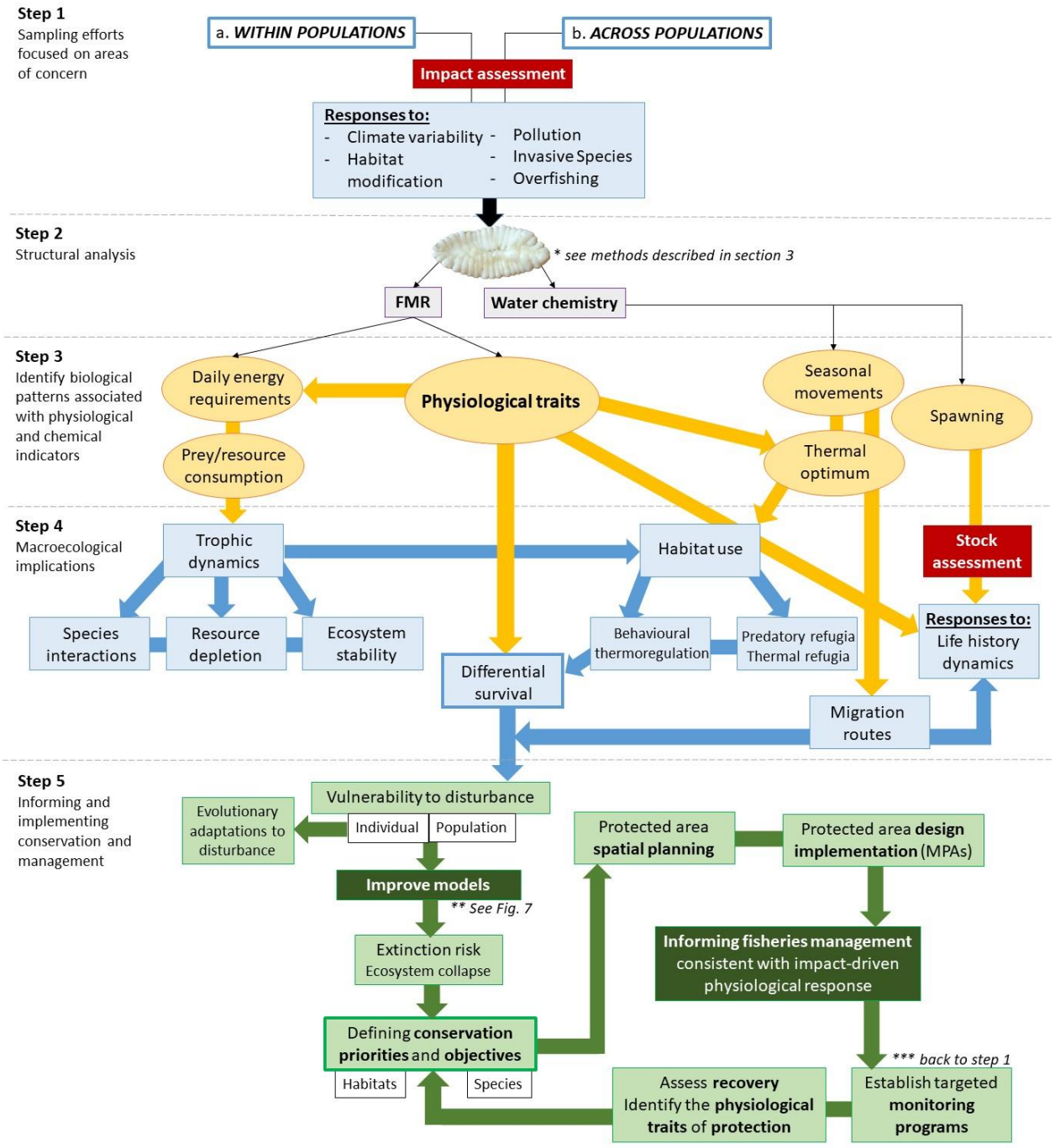


Figure 2.6. Phases for integrating the otolith-isotope method into conservation strategies. These phases include (Step 1) determining whether comparisons will be made within populations or across populations, subsequently dictating the sampling effort and size associated with fieldwork, as well as the targeting of individuals in areas under anthropogenic or climatic pressure, (Step 2) undergoing the isotopic analysis of the otolith, (Step 3) identifying biological patterns associated with FMR and water chemistry and employing any necessary calibrations, (Step 4) creating links between biological and macroecological processes, and, (Step 5) implementing vulnerability, structure, and system response into energetic models to inform conservation objectives and lead protected area design and sustainable fisheries initiatives.

Tables

Table 2.1. Laboratory measurements of different metabolic rate metrics (SMR, RMR, MMR, FMR, AS) for fishes, their biological meaning, the required condition of the organism at measurement, and the methods of measurement commonly used (Blažka *et al.,* 1960; Brett, 1964; Steffensen et al. 1984; Beamish, 1979; Killen *et al.,* 2010; Killen *et al.,* 2012; Metcalfe *et al.,* 2016; Treberg *et al.,* 2016; Killen *et al.,* 2016; Norin & Clark, 2016; Nelson 2016). The energy pathway each measurement represents can be followed in Figure 1.2.

Metabolic Measurement	Biological Meaning	Conditions for Measurement	Methods Examples	Figure 1.2 Pathway
Standard Metabolic Rate (SMR)	Baseline metabolic rate needed to sustain life in an ectotherm by maintaining organismal homeostasis (protein and DNA repair, maintenance of ion gradients, ATP production, anabolism and catabolism of tissues, etc.)	**Rest** (coaxed to truly minimal levels of activity) **Post-absorptive state** (SDA effects absent) Knowledge of the ambient **temperature** at which measured*	Continuous Flow Respirometry: oxygen consumption measurement	Basal costs
Routine Metabolic Rate (RMR)**	Baseline costs (SMR) + normal spontaneous activity, maintenance of posture/equilibrium or voluntary activity. Behaviour/activity must be carefully specified and defined, and the amount of activity should be quantifiable	Influenced by **random activity** under experimental conditions **Post absorptive state** (SDA effects absent) **Protected from outside stimuli**	Continuous Flow Respirometry: oxygen consumption measurement	Basal costs + Any specified random activity

Maximum Metabolic Rate (MMR)	The upper threshold of metabolic capacity attainable by the individual at a given temperature under any ecologically relevant circumstance	Fish exposed to **critical swimming speed tests**, **burst-swimming protocols**, and **exhaustive chases** until a defined critical swimming speed has been reached (point of fatigue, max O_2 consumption reached) **Post absorptive state** (SDA effects absent)	Blažka-, Brett- and Steffensen-type swim flume respirometers	Enhanced activity (locomotion/work)
Field Metabolic Rate (FMR)* **	Baseline costs (SMR) + specific dynamic action (SDA) + and activity metabolism. Represents time-averaged energy intake and expenditure.	Measured on a free-ranging organism in its natural habitat, undisturbed by confinement and manipulation.	Otolith-Isotope Method	Basal costs (SMR) + SDA + Activity (locomotion/work)
Aerobic Scope (AS)	Difference between MMR and SMR under the same environmental conditions. Defines the overall capacity of the animal to increase its metabolism in relation to non-regulatory	N/A	Measured MMR – SMR under the same laboratory conditions	Enhanced activity (locomotion/ work)

	requirements, such as locomotion for migration, energy for predation, reproduction, etc.		**OR** FMR – SMR under the same field conditions	- Basal costs **OR** Field Metabolic Rate - Basal costs

* As opposed to the thermoneutral zone assumption in Basal Metabolic Rate (BMR) measurements for endotherms.
** Extra caution should be applied when using the term RMR for fishes, as its definitions can be somewhat vague and can sometimes be used interchangeably with SMR.
*** Energy pathways demonstrated for field metabolic rate measured using the isotope otolith method.

Table 2.2. Summary of historical FMR methods.

Method	**Time energy budget**	**Accelerometry**	**Heart Rate Telemetry**	**Doubly Labeled Water**
Energy pathways included in FMR value	Figure 2.1a	Figure 2.1b	Figure 2.1c	Figure 2.1d
Period of measured FMR	Timescale of observation	Total time of transmitter tracking (days to months)	Total time of transmitter tracking (days to months)	Time of species specific isotope washout rate (24h – 28 days)
FMR temporal resolution	Observation error associated with changes in activity limit fine scale FMR information	Fine temporal scales	Fine temporal scales (minute to minute changes in FMR)	Only available over a large temporal scale, (FMR average per 24h period)
Applicable to	Terrestrial animals, continually observed	Terrestrial and aquatic animals, limited by tag size	Terrestrial and aquatic animals, limited by tag size and surgical vulnerability	Terrestrial vertebrates and invertebrates, aquatic mammals, no size limit
Pros	Minimal field equipment required Low cost	Combine with environmental loggers to track abiotic factor influence on energy output	Combine with environmental loggers to track abiotic factor influence on energy output	FMR value encompasses all metabolic pathways
Cons	**No direct metabolic rate measurements:** Uncertainty	**Uncertainty in O_2 consumption conversion**: Variation in	**Uncertainty in O_2 consumption conversion**: Inconsistent relationship	**Additional uncertainty associated with CO_2 production conversion*:** Unknown species-specific respiratory quotient

	in basal metabolism to activity metabolism conversions Limited to tracking **short-term** energy trends **Organisms must remain in sight**: bias towards organisms/life cycles that are tied to specific sites (nesting/breeding) **High sampling effort/high probability of observer error**: continual observation and good knowledge of activity/behavior required.	muscular efficiency between species Limited to tracking **short-term** energy trends **Changes in behavior related to surgical stress and externally attached devices**	between heart rate and cardiac output across metabolic stimuli Limited to tracking **short-term** energy trends **Changes in behavior related to surgical stress**	increases variability in conversion values compared to O_2 consumption conversions. Limited to tracking **short-term** energy trends **Potential changes in behaviour associated with method handling*** **Washout rate imposes a narrow recapture window*:** potential need for back calculations, increased cost of study, limited sample sizes.

*See supporting information for additional information

Supporting Information

DLW: Behavioural uncertainty and recapture limitations

The doubly labeled water technique can capture the natural behaviour of individuals. While this is certainly so when compared to laboratory experiments, the behaviour of the individual can however still be significantly altered by the two periods of captivity required for this method. During a DLW study, the organism of interest is captured and handled twice, once to get weighed, marked, injected with the DLW, and have a background blood sample taken; then again a variable amount of time later, to weigh and sample the blood again (Nagy 1989). Certain studies have identified changes in behaviour in DLW-injected organisms, leading to the abandonment of offspring (Stothart *et al.,* 2016; Schultner *et al.,* 2010; Hinsley *et al.,* 2011) or changes in foraging parameters such as dive depths, dive angles and foraging ranges in seabirds (Bannasch *et al.,* 1994). This in turn leads to false estimates of everyday energy expenditure (Schultner *et al.,* 2010; Hinsley *et al.,* 2011). Altered methods have been developed in order to reduce disturbance, by injecting the doubly labeled water intravenously, or by following a single-sampling approach (Stothart *et al.,* 2016; Ricklefs et al. 1986; Webster & Weathers 1989; Williams 1993; Williams & Dwinnel 1990). However, using the single-sampling approach for animals weighing more than 4-5 kg has led to other problems, such as overestimation of metabolic energy expenditure (Gabrielsen & Ellis, 2001). Therefore, it is important to find a balance between using the proper approach for the species in question, while also making sure the animal's behaviour is not significantly altered by manipulation.

Between equilibration at initial capture and the final enrichment measurement at recapture, the animal is released back into its natural habitat for a length of time dependent on the rate of flow of isotopes throughout the body (Butler *et al.,* 2004). This is the period of FMR

measurement in the organism, and varies from 28 days in large organisms to as little as 24 h in smaller vertebrates with high washout rates, which have sometimes called for multiple injections (Berteaux *et al.,* 1996) or more expensive forms of analysis able to detect very low levels of isotopes (Pettit *et al.,* 1988). This washout-rate-limited measurement period makes it difficult to track concrete, long-term relationships between metabolic parameters, such as the FMR of organisms during critical growth periods between life stages, or over phenological or reproductive periods.

As expected, when studying free ranging animals, problems arise in the recapturing of animals within specific time windows, especially for the above-mentioned organisms with high washout rates (sampling period >24h; Gabrielsen & Ellis, 2001; Butler *et al.,* 2004). If all the isotopes have already been eliminated by the time of recapture, a proper CO_2 production calculation is impossible. Animals that have not been recaptured within the necessary time interval, even by just an hour, require a back calculation, introducing additional uncertainty into the measurement (Gabrielsen & Ellis, 2001). Failure in recapturing study animals within the expected time frame adds to the cost of a study, while also rendering it difficult to achieve adequate sample sizes (Butler *et al.,* 2004). In order to increase the chances of recapture, investigators, especially those studying seabirds, are focusing their research on animals tied to specific nesting sites during the breeding season. This has created an important bias in DLW seabird studies, as well as the bioenergetic models they create, as they focus solely on the energetic costs associated with reproduction (incubation and chick rearing). More recently, researchers have revisited some of these species and applied accelerometry, and therefore, trackable tags, alongside DLW (Elliott *et al.,* 2013; Stothart 2016). This allows for more robust

models, integrating good energetics data not only from breeding colonies, but also from chicks, juveniles, and non-breeding birds.

DLW: The uncertain step between CO_2 production and energy expenditure

It is important to note that while DLW yields direct average estimations of CO_2 production over an interval of time, it is less accurate than the conversion to energy expenditure from O_2 consumption utilized by the heart rate method (Speakman 1997). This results from the higher variability in energy released per milliliter of CO_2 produced compared to the equivalent of O_2 consumed. For example, when an animal mobilizes carbohydrates, the energy produced by 1 ml of both O_2 and CO_2 is 20.9 kJ, but for fat mobilization, it varies from 19.66 kJ to 28.1 kJ for 1 ml of O2 consumption and CO_2 production, respectively (Butler *et al.,* 2004). The difference between the lowest and highest conversion values is therefore much larger for CO_2 than for O_2 (approximately 34% and 6% respectively). This variability in conversion values can become problematic when the respiratory quotient (RQ) of an animal is unknown (Speakman 1997). The respiratory quotient (RQ) is a dimensionless number representing the ratio of moles of CO_2 produced by a cell to the moles of O_2 simultaneously consumed, and is used in the conversion of CO_2 production to energy expenditure (Hill *et al.,* 2012).

The RQ value is dependent on the oxidation of different substrates, and therefore, the knowledge of the macronutrient compositions of the diet of the organism, as well as the rates of water and carbon dioxide (Lifson *et al.,* 1955; Speakman 1997). When this information is known for a group of individuals, a theoretical RQ can be calculated (Lifson *et al.,* 1955). However, when these components are unknown, the potential error in the use of the RQ in the conversion from CO_2 to energy is even greater than the conversion from O_2 consumption, and can therefore reflect inaccurate FMR values. Precise dietary composition is widely unknown for free-ranging

animals, therefore, researchers have grown accustomed to formulating 'hypothetical' diets (Ricklefs *et al.,* 1986, Roby & Williams, 1986; Costa & Gentry, 1986), and using assumptions on their assimilation and oxidation for convenience (Reilly & Fedak,, 1991). The assumption that diets between populations of the same species, or during different times of the year, are the same are also often made (Adams, Brown & Nagy, 1986). When analyzing the FMR of harbor porpoises (*Phocoena phocoena*), for example, Rojano-Doñate et al. (2018) used reported RQ values from captive harbor porpoises (Reed *et al.,* 2000; Boutilier *et al.,* 2001), as well as reported RQ values from other marine mammals with similar high protein and fat diets (Kooyman *et al.,* 1981; Feldkamp, 1987; Boily and Lavigne, 1995) to estimate an intermediate RQ value. While researchers use "best guess" estimates with the RQ values that are available for captive animals, the lack of information specific to the diet of wild organisms, and its subsequent composition, assimilation and oxidation processes, limits the accuracy of the DLW method in free-living animals (Speakman & Racey, 1987).

Seasonal change also impacts RQ, and therefore, the resulting FMR values. A study by Dalton et al. (2014) on northern fur seal (*Callorhinus ursinus*) energetics found that seasonal change contributed to significant differences between DLW-acquired FMR and respirometry-acquired FMR. Seasonally appropriate RQ values are therefore necessary, whether it be estimated or determined experimentally through captive studies (Dalton *et al.,* 2014). Previous studies have also noted that changes in ambient air and water temperature can explain discrepancies when examining energetic expenditure over prolonged periods of time, and that these results can affect the accuracy of CO_2 production estimates by 10-15% (LeFebvre, 1964; Lifson *et al.,* 1955; Speakman, 1997; Tiebout & Nagy, 1991). This is because differences in the air temperature can affect the physical fractionation and concentration of heavy isotopes such as

^{18}O and $^{2}H/^{3}H$ when they leave the body in the gaseous state compared to the concentration remaining in the body water (Speakman 1997).

References

Acquarone M, Born EW, Speakman JR (2007) Field metabolic rates of Walrus (Odobenus rosmarus) measured by the doubly labeled water method. *Aquat Mamm* 32: 363–369.

Adams, N. J., Brown, C. R., & Nagy, K. A. (1986). Energy expenditure of free-ranging wandering albatrosses Diomedea exulans. *Phys Zool 59*(6): 583-591.

Alewijnse S, Stowasser G, Saunders R, Belcher A, Crimmen O, Cooper N, Trueman C (2021) Otolith-derived field metabolic rates of myctophids (Family Myctophidae) from the Scotia Sea (Southern Ocean). *Mar Ecol Prog Ser* 675: 113–131.

Antão, LH, Bates, AE, Blowes, SA, Waldock, C, Supp, SR, Magurran, AE, Dornelas, M, Schipper, AM (2020) Temperature-related biodiversity change across temperate marine and terrestrial systems. *Nat Ecol Evol 4*(7): 927–933. https://doi.org/10.1038/s41559-020-1185-7

Ashford J, Jones C (2007) Oxygen and carbon stable isotopes in otoliths record spatial isolation of Patagonian toothfish (Dissostichus eleginoides). *GCA* 71: 87–94.

Bailey DM, Jamieson AJ, Bagley PM, Collins MA, Priede IG (2002) Measurement of in situ oxygen consumption of deep-sea fish using an autonomous lander vehicle. *Deep Res Part I Oceanogr Res Pap* 49: 1519–1529.

Bannasch R, Wilson R, Culik B (1994) Hydrodynamic aspects of design and attachment of a back-mounted device in penguins. *J Exp Biol* 194: 83–96.

Barnett A, Payne NL, Semmens JM, Fitzpatrick R (2016) Ecotourism increases the field metabolic rate of whitetip reef sharks. *Biol Conserv* 199: 132–136.

Bastow TP (2002) Elevated salinity and isotopic composition of fish otolith carbonate : stock delineation of pink snapper, Pagrus auratus , in Shark Bay. *Mar Biol* 801–806.

Bates AE, Helmuth B, Burrows MT, Duncan MI, Garrabou J, Guy-Haim T, Lima F, Queiros AM, Seabra R, Marsh R, *et al.* (2018) Biologists ignore ocean weather at their peril. *Nature* 560: 299–301

Beamish FWH (1978) Swimming capacity. In: Hoar WS, Randall DJ, eds. Fish Physiology, pp 349-401.

Becker M, Andersen N, Erlenkeuser H, Humphreys MP, Tanhua T (2016) An internally consistent dataset of δ 13 C-DIC in the North Atlantic Ocean – NAC13v1 559–570.

Benedetti-Cecchi L, Tamburello L, Maggi E, Bulleri F (2015) Experimental perturbations modify the performance of early warning indicators of regime shift. *Curr Biol* 25: 1867–1872.

Bernstein NP, Maxson SJ (2021) Reproductive energetics of blue-eyed shags in Antarctica. *Wilson Bull* 97: 450–462.

Berteaux, D., Thomas, D. W., Bergeron, J. M., and Lapierre H (1996) Repeatability of daily field metabolic rate in female meadow voles (Microtus pennsylvanicus). *Funct Ecol* 10: 751–759.

Blazka, P., Volf, M., & Cepela, M. (1960). A new type of respirometer for the determination of the metabolism of fish in an active state. *Physiol. Bohemoslov* 9(6): 553-558.

Blowes, SA, Supp, SR, Antão, LH, Bates, A, Bruelheide, H, Winter, M, Bjorkman, AD, Bowler, DE, Byrnes, JEK, Gonzalez, A. (2019). The geography of biodiversity change in marine and terrestrial assemblages. *Science 345*: 339–345.

Boily, P, Lavigne, DM (1995) Resting metabolic rates and respiratory quotients of gray seals (Halichoerus grypus) in relation to time of day and duration of food deprivation. *Phys Zool* 68(6): 1181-1193.

Boutilier, RG, Reed, JZ, Fedak, MA (2001) Unsteady-state gas exchange and storage in diving marine mammals: the harbor porpoise and gray seal. *Am J Physiol Regul Integr Comp Physiol*

281(2): 490-494.

Boyd IL (1995) Validation of heart rate and doubly labelled water as measures of metabolic rate during swimming in California sea lions 9: 151–160.

Boyer T, Levitus S, Antonov J, Locarnini R, Mishonov A, Garcia H, Josey SA (2007) Changes in freshwater content in the North Atlantic Ocean 1955-2006. *Geophys Res Lett* 34: 1–5.

Brekke B, Gabrielsen GW (1994) Assimilation efficiency of adult Kittiwakes and Brünnich's Guillemots fed Capelin and Arctic Cod. *Polar Biol* 14: 279–284.

Brett JR (1964) The respiratory metabolism and swimming performance of young sockeye salmon. *J Fish Res Board Can* 21: 1183–1226.

Briggs CT, Post JR (1997) Field metabolic rates of rainbow trout estimated using electromyogram telemetry. *J Fish Biol* 51: 807–823.

Brown, JH, Gillooly, JF, Allen, AP, Savage, VM, West, GB (2004) Toward a metabolic theory of ecology. *Ecology* 85(7): 1771-1789.

Burger AE (1981) Time budgets, energy needs and kleptoparasitism in breeding Lesser sheathbills. Oxford University Press pp 106–112.

Burrows MT, Bates AE, Costello MJ, Edwards M, Edgar GJ, Fox CJ, Halpern BS, Hiddink JG, Pinsky ML, Batt RD, *et al.* (2019) Ocean community warming responses explained by thermal affinities and temperature gradients. *Nat Clim Chang* 1–5.

Burrows MT, Schoeman DS, Buckley LB, Moore P, Poloczanska ES, Brander KM, Brown C, Bruno JF, Duarte CM, Halpern BS, *et al.* (2011) The pace of shifting climate in marine and terrestrial ecosystems. *Science* 334: 652–656.

Butler PJ, Green JA, Boyd IL, Speakman JR (2004) Measuring metabolic rate in the field: The pros and cons of the doubly labelled water and heart rate methods. *Funct Ecol* 18: 168–183.

Campana SE (1999) Chemistry and composition of fish otoliths: Pathways, mechanisms and applications. *Mar Ecol Prog Ser* 188: 263–297.

Campana SE, Neilssra D (1985) Microstructure of Fish Otoliths. *Can J Fish Aquat Sci* 42.

Chabot D, Steffensen JF, Farrell AP (2016) The determination of standard metabolic rate in fishes. *J Fish Biol* 88: 81–121.

Chung MT, Jørgensen KEM, Trueman CN, Knutsen H, Jorde PE, Grønkjær P (2021) First measurements of field metabolic rate in wild juvenile fishes show strong thermal sensitivity but variations between sympatric ecotypes. *Oikos* 130: 287–299.

Chung MT, Trueman CN, Godiksen JA, Grønkjær P (2019a) Otolith δ13C values as a metabolic proxy: Approaches and mechanical underpinnings. *Mar Freshw Res* 70: 1747–1756.

Chung MT, Trueman CN, Godiksen JA, Holmstrup ME, Grønkjær P (2019b) Field metabolic rates of teleost fishes are recorded in otolith carbonate. *Commun Biol* 2: 1–10.

Clark TD, Ryan T, Ingram BA, Woakes AJ, Butler PJ, Frappell PB (2005) Factorial aerobic scope is independent of temperature and primarily modulated by heart rate in exercising Murray cod (Maccullochella peelii peelii). *Physiol Biochem Zool* 78: 347–355.

Clarke A, Rothery P, Isaac NJB, Building M, Lane B, Gifford C (2010) Scaling of basal metabolic rate with body mass and temperature in mammals. *Journal of Animal Ecology* 79: 610–619.

Coelho FJRC, Santos AL, Coimbra J, Almeida A, Cunha Â, Cleary DFR, Calado R, Gomes NCM (2013) Interactive effects of global climate change and pollution on marine microbes: The way ahead. *Ecol Evol* 3: 1808–1818.

Connell SD, Doubleday ZA, Hamlyn SB, Foster NR, Harley CDG, Helmuth B, Kelaher BP, Nagelkerken I, Sarà G, Russell BD (2017) How ocean acidification can benefit calcifiers. *Curr Biol* 27: 95–96.

Cooke SJ, Thorstad EB, Hinch SG (2004) Activity and energetics of free-swimming fish: Insights from electromyogram telemetry. *Fish Fish* 5: 21–52.

Correia AT, Barros F, Sial AN (2011) Stock discrimination of European conger eel (Conger conger L .) using otolith stable isotope ratios. *Fish Res* 108: 88–94.

Costa D P, Gentry RL (2014). Free-ranging energetics of northern fur seals. Fur seals, Princeton University Press, pp 79-101.

Crain CM, Kroeker K, Halpern BS (2008) Interactive and cumulative effects of multiple human stressors in marine systems. *Ecol Lett* 11: 1304–1315.

Crisci C, Ledoux JB, Mokhtar-Jamaï K, Bally M, Bensoussan N, Aurelle D, Cebrian E, Coma R, Féral JP, la Rivière M, *et al.* (2017) Regional and local environmental conditions do not shape the response to warming of a marine habitat-forming species. *Sci Rep* 7. doi:10.1038/s41598-017-05220-4

Currey LM, Heupel MR, Simpfendorfer CA, Williams AJ (2014) Inferring movement patterns of a coral reef fish using oxygen and carbon isotopes in otolith carbonate. *J Exp Mar Biol Ecol* 456: 18–25.

Dahlhoff EP (2004) Biochemical indicators of stress and metabolism: Applications for marine ecological studies. *Annu Rev Physiol* 66: 183–207.

Dalton AJM, Rosen DAS, Trites AW (2014) Season and time of day affect the ability of accelerometry and the doubly labeled water methods to measure energy expenditure in northern fur seals (Callorhinus ursinus). *J Exp Mar Biol Ecol* 452: 125–136.

de la Vega C, Jeffreys RM, Tuerena R, Ganeshram R, Mahaffey C (2019) Temporal and spatial trends in marine carbon isotopes in the Arctic Ocean and implications for food web studies. *Glob Chang Biol* 25: 4116–4130.

Disspain MCF, Ulm S, Gillanders BM (2016) Otoliths in archaeology: Methods, applications and future prospects. *J Archaeol Sci Rep* 6: 623–632.

Doney SC, Ruckelshaus M, Duffy JE, Barry JP, Chan F, English CA, Galindo HM, Grebmeier JM, Hollowed AB, Knowlton N, *et al.* (2012) Climate change impacts on marine ecosystems. *Annu Rev Mar Sci* 4:11-37

Dong Y wei, Li XX, Choi FMP, Williams GA, Somero GN, Helmuth B (2017) Untangling the roles of microclimate, behaviour and physiological polymorphism in governing vulnerability of intertidal snails to heat stress. *Proc R Soc B Biol Sci* 284. doi:10.1098/rspb.2016.2367

Dufour E, Gerdeaux D, Wurster CM (2007) Whitefish (Coregonus lavaretus) respiration rate governs intra-otolith variation of δ 13 C values in Lake Annecy. *Can J Fish Aquat Sci* 64: 1736–1746.

Edmonds, J. S. (1995). Population discrimination by variation in concentrations of minor and trace elements in sagittae of two Western Australian teleosts. In: Recent developments in fish otolith research. University of South Carolina Press.

Eide M, Olsen A, Ninnemann US, Johannessen T (2017) A global ocean climatology of preindustrial and modern ocean δ13C. *Global Biogeochem Cycles* 31: 515–534.

Elliott KH, Vaillant M le, Kato A, Speakman JR, Ropert-coudert Y (2013) Accelerometry predicts daily energy expenditure in a bird with high activity levels. *Biol Lett* 9. doi:10.1098/rsbl.2012.0919

Elsdon TS, Ayvazian S, Mcmahon KW, Thorrold SR (2010) Experimental evaluation of stable isotope fractionation in fish muscle and otoliths. *Mar Ecol Prog Ser* 408: 195–205.

Enstipp MR, Ciccione S, Gineste B, Milbergue M, Ballorain K, Ropert-Coudert Y, Kato A, Plot V, Georges JY (2011) Energy expenditure of freely swimming adult green turtles (Chelonia mydas) and its link with body acceleration. *J Exp Biol* 214: 4010–4020.

Fahlman Å, Arnemo JM, Persson J, Segerström P, Nyman G (2008) Capture and medetomidine-ketamine anesthesia of free-ranging wolverines (Gulo gulo). *J Wildl Dis* 44: 133–142.

Farrell, A. P., Lee, C. G., Tierney, K., Hodaly, A., Clutterham, S., Healey, M., Hinch, S., & Lotto, A. (2003). Field-based measurements of oxygen uptake and swimming performance with adult Pacific salmon using a mobile respirometer swim tunnel. *Journal of Fish Biology*, *62*(1), 64–84. https://doi.org/10.1046/j.1095-8649.2003.00010.x

Feldkamp SD (1987) Swimming in the California sea lion: morphometrics, drag and energetics. *J Exp Biol* 131: 117–135.

Fidhiany L, Winckler K (1998) Influence of body mass, age, and maturation on specific oxygen consumption in a freshwater cichlid fish, Cichlasoma nigrofasciatum. *Comp Biochem Physiol* 119: 613–619.

Fraile I, Arrizabalaga H, Groeneveld J, Kölling M, Neves M, Macías D, Addis P, Dettman DL, Karakulak S, Deguara S, *et al.* (2016) The imprint of anthropogenic CO2 emissions on Atlantic blue fi n tuna otoliths. *J Mar Syst* 158: 26–33.

Frisk M, Skov PV, Steffensen JF (2012) Thermal optimum for pikeperch (Sander lucioperca) and the use of ventilation frequency as a predictor of metabolic rate. *Aquaculture* 151–157.

Gabrielsen G, Ellis H (2001) Energetics of Free-Ranging Seabirds. In: Biology of Marine Birds. CRC Press LLC, pp 359–408.

Gaitán-Espitia JD, Villanueva PA, Lopez J, Torres R, Navarro JM, Bacigalupe LD (2017) Spatio-temporal environmental variation mediates geographical differences in phenotypic responses to ocean acidification. *Biol Lett* 13. doi:10.1098/rsbl.2016.0865

Gales R, Green B, Stahel C (1988). The energetics of free-living little penguins Eudyptula-Minor (Spheniscidae), during molt. *Aust J Zool* 36: 159-167.

Gao Y, Dettman DL, Piner KR, Wallace FR (2010) Isotopic Correlation (δ 18 O versus δ 13 C) of Otoliths in Identification of Groundfish Stocks . *Trans Am Fish Soc* 139: 491–501.

Gao YW, Beamish RJ (1999) Isotopic composition of otoliths as a chemical tracer in population identification of sockeye salmon (Oncorhynchus nerka). *Can J Fish Aquat Sci* 56: 2062–2068.

Gao YW, Joner SH, Bargmann GG (2001) Stable isotopic composition of otoliths in identification of spawning stocks of Pacific herring (Clupea pallasi) in Puget Sound. *Can J Fish Aquat Sci* 58: 2113–2120.

Gauldie RW (1996) Biological factors controlling the carbon isotope record in fish otoliths: Principles and evidence. *Comp Biochem Physiol - B Biochem Mol Biol* 115: 201–208.

Gerard T, Malca E, Muhling BA, Mateo I, Lamkin JT (2015) Isotopic signatures in the otoliths of reef-associated fishes of southern Florida : Linkages between nursery grounds and coral reefs. *Reg Stud Mar Sci* 2: 95–104.

Gerdeaux D, Dufour E (2015) Life history traits of the fish community in Lake Annecy: evidence from the stable isotope composition of otoliths. *Knowl Manag Aquat Ecosyst* 416: 1–22.

Glazier DS, Glazier DS (2005) Beyond the ‘ 3 / 4-power law ’: variation in the intra- and interspecific scaling of metabolic rate in animals *Biol Rev* 80: 611–662.

Graiff A, Bartsch I, Ruth W, Wahl M, Karsten U (2015) Season exerts differential effects of ocean acidification and warming on growth and carbon metabolism of the seaweed Fucus vesiculosus in the Western Baltic Sea. *Front Mar Sci* 2. doi:10.3389/fmars.2015.00112

Gräns A, Olsson C, Pitsillides K, Nelson HE, Cech JJ, Axelsson M (2010) Effects of feeding on thermoregulatory behaviours and gut blood flow in white sturgeon (Acipenser transmontanus) using biotelemetry in combination with standard techniques. *J Exp Biol* 213: 3198–3206.

Green JA (2011) The heart rate method for estimating metabolic rate: Review and recommendations. *Comp Biochem Physiol - A Mol Integr Physiol* 158: 287–304.

Green JA, Halsey LG, Wilson RP, Frappell PB (2009) Estimating energy expenditure of animals using the accelerometry technique: Activity, inactivity and comparison with the heart-rate technique. *J Exp Biol* 212: 745–746.

Grossman EL, Ku TL (1986) Carbon and oxygen isotopic fractionation in biogenic aragonite - temp effects. *Chem Geol* 59: 59–74.

Gunderson AR, Stillman JH (2015) Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. *Proc R Soc B Biol Sci* 282. doi:10.1098/rspb.2015.0401

Halsey LG, White CR, Enstipp MR, Wilson RP, Butler PJ, Martin GR, Grémillet D, Jones DR (2011) Assessing the validity of the accelerometry technique for estimating the energy expenditure of diving double-crested cormorants phalacrocorax auritus. *Physiol Biochem Zool* 84: 230–237.

Hanson NN, Wurster CM, Eimf, Todd CD (2013) Reconstructing marine life-history strategies of wild Atlantic salmon from the stable isotope composition of otoliths. *Mar Ecol Prog Ser* 475: 249–266.

Hanson NN, Wurster CM, Todd CD (2010) Comparison of secondary ion mass spectrometry and micromilling / continuous flow isotope ratio mass spectrometry techniques used to acquire intra-otolith d 18 O values of wild Atlantic salmon (Salmo salar). *Rap Commun Mass Spectrom* 24: 2491–2498.

Harley CDG, Connell SD, Doubleday ZA, Kelaher B, Russell BD, Sarà G, Helmuth B (2017) Conceptualizing ecosystem tipping points within a physiological framework. *Ecol Evol* 7: 6035–6045.

Hill R, A. Wyse G, Anderson M (2012) Themes in the Study of Animal Physiology, Third Edition. Science of the Total Environment. Sinauer Associates, Sunderland.

Hinch SG, Diewert RE, Lissimore TJ, Prince AMJ, Healey MC, Henderson MA (1996) Use of electromyogram telemetry to assess difficult passage areas for river-migrating adult sockeye salmon. *Trans Am Fish Soc* 125: 253–260.

Hinsley SA, Bellamy PE, Rothery P, Redman P, Furness L, Speakman JR (2011) Effects of the doubly labelled water procedure on Great Tits Parus major feeding young. *Bird Study* 58: 151–159.

Høie H, Folkvord A, Otterlei E (2003) Effect of somatic and otolith growth rate on stable isotopic composition of early juvenile cod (Gadus morhua L) otoliths. *J Exp Mar Biol Ecol* 289: 41–58.

Hudson LN, Isaac NJB, Reuman DC (2013) The relationship between body mass and field metabolic rate among individual birds and mammals. *J Anim Ecol* 82: 1009–1020.

IPCC (2013) Climate change 2013: the physical science basis. Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Jamieson RE, Schwarcz HP, Brattey J (2004) Carbon isotopic records from the otoliths of Atlantic cod (Gadus morhua) from eastern Newfoundland, Canada. *Fish Res* 68: 83–97.

Javor B, Dorval E (2014) Geography and ontogeny influence the stable oxygen and carbon isotopes of otoliths of Pacific sardine in the California Current. *Fish Res* 154: 1–10.

Johnson RK, McKenzie D (2001). Energy requirement methodology, In: Boushey C, Coulston AM, Rock L, MonsenCash E, eds. Nutrition in the Prevention of Treatment of Disease.

Kalish JM (1991) 13C and 18O isotopic disequilibria in fish otoliths: metabolic and kinetic effects. *Mar Ecol Prog Ser* 75: 191–203.

Karasov WH (1983) Wintertime energy conservation by huddling in antelope ground squirrels Ammospermophilus leucurus). *J Mammal* 64: 341–345.

Kaseloo PA, Weatherley AH, Lotimer J, Farina MD (1992) A biotelemetry system recording fish activity. *J Fish Biol* 40: 165–179.

Keeling CD (1979) The suess effect : 13carbon-14carbon interrelations. *Environ Int* 2: 229–300.

Kelly M (2019) Adaptation to climate change through genetic accommodation and assimilation of plastic phenotypes. *Phil Trans R Soc B* 374.

Killen SS, Atkinson D, Glazier DS (2010) The intraspecific scaling of metabolic rate with body mass in fishes depends on lifestyle and temperature. *Ecol Lett* 13: 184–193.

Killen SS, Fu C, Wu Q, Wang YX, Fu SJ (2016a) The relationship between metabolic rate and sociability is altered by food deprivation. *Funct Ecol* 30: 1358–1365.

Killen SS, Glazier DS, Rezende EL, Clark TD, Atkinson D, Willener AST, Halsey LG (2016b) Ecological influences and morphological correlates of resting and maximal metabolic rates across teleost fish species. *Am Nat* 187: 592–606.

Killen SS, Marras S, Steffensen JF, Mckenzie DJ (2012) Aerobic capacity influences the spatial position of individuals within fish schools. *Proc R Soc B Biol Sci* 279: 357–364.

Kimirei IA, Nagelkerken I, Mgaya YD, Huijbers CM (2013) The mangrove nursery paradigm revisited : Otolith stable isotopes support nursery-to-reef movements by indo-pacific fishes. *PloS one* 8. doi:10.1371/journal.pone.0066320

Kitagawa T, Ishimura T, Uozato R, Shirai K, Amano Y, Shinoda A, Otake T, Tsunogai U, Kimura S (2013) Otolith δ18O of Pacific bluefin tuna Thunnus orientalis as an indicator of ambient water temperature. *Mar Ecol Prog Ser* 481: 199–209.

Kleiber M (1961) The fire of life: an introduction to animal energetics. John Wiley & Sons, Inc., New York.

Kooyman GL, Castellini MA, Davis RW (1981) Physiology of diving in marine mammals. *Annu Rev Physiol* 43: 343–356.

Kordas RL, Harley CDG, O'Connor MI (2011) Community ecology in a warming world: The influence of temperature on interspecific interactions in marine systems. *J Exp Mar Biol Ecol* 400: 218–226.

Körtzinger A, Quay PD, Sonnerup RE (2003) Relationship between anthropogenic CO2 and the 13C suess effect in the North Atlantic Ocean. *Global Biogeochem Cycles* 17. doi:10.1029/2001gb001427

Kuo ESL, Sanford E (2009) Geographic variation in the upper thermal limits of an intertidal snail: Implications for climate envelope models. *Mar Ecol Prog Ser* 388: 137–146.

Lauchlan SS, Nagelkerken I (2020) Species range shifts along multistressor mosaics in estuarine environments under future climate. *Fish Fish* 21: 32–46.

le Quesne WJF, Pinnegar JK (2012) The potential impacts of ocean acidification: Scaling from physiology to fisheries. *Fish and Fisheries* 13: 333–344.

Lea JMD, Keen AN, Nudds RL, Shiels HA (2016) Kinematics and energetics of swimming performance during acute warming in brown trout Salmo trutta. *J Fish Biol* 88: 403–417.

LeFebvre (1964) The use of D2O18 for measuring energy metabolism in columba livia at rest and in flight. *Oxford Journals* 81: 403–416.

Lesser MP (2016) Climate change stressors cause metabolic depression in the blue mussel, Mytilus edulis, from the Gulf of Maine. *Limnol Oceanogr* 61: 1705–1717.

Lewis SL, Maslin MA. 2015. Defining the anthropocene. *Nature* 519:171–180.

Lifson N, Gordon GB (1949) The fate of utilized molecular oxygen and the source of the oxygen of. *J Biol Chem* 180: 803–811.

Lifson N, Gordon GB, McClintock R (1955) Measurement of total carbon dioxide production by means of D2O18. *J Appl Physiol 7*:704-710.

Lozier SM, Leadbetter S, Williams RG, Roussenov V, Reed MSC, Moore NJ (2008) The spatial pattern and mechanisms of heat-content change in the North Atlantic. *Science (1979)* 319: 800–803.

Lucas MC, Priede IG, Armstrong JD, Gindy ANZ, de Vera L (1991) Direct measurements of metabolism, activity and feeding behaviour of pike, Esox Zucius L ., in the wild, by the use of heart rate telemetry. *J Fish Biol* 39: 325–345.

Lv T, Zhang Y, Carvalho PN, Zhang L, Button M, Arias CA, Weber KP, Brix H (2017) Microbial community metabolic function in constructed wetland mesocosms treating the pesticides imazalil and tebuconazole. *Ecol Eng* 98: 378–387.

Maloiy G (1979) Comparative physiology of osmoregulation in animals. London, Academic Press.

Martínez B, Arenas F, Trilla A, Viejo RM, Carreño F (2015) Combining physiological threshold knowledge to species distribution models is key to improving forecasts of the future niche for macroalgae. *Glob Chang Biol* 21: 1422–1433.

Martino JC (2020) Experimental support towards a metabolic proxy in fish using otolith carbon isotopes. *J Exp Biol* 223 doi:10.1242/jeb.217091

McConnaughey TA, Burdett J, Whelan JF, Paull CK (1997) Carbon isotopes in biological carbonates: Respiration and photosynthesis. *Geochim Cosmochim Acta* 61: 611–622.

Metcalfe NB, Van Leeuwen TE, Killen SS (2016) Does individual variation in metabolic phenotype predict fish behaviour and performance? *J Fish Biol* 88: 298–321.

Millidine KJ, Armstrong JD, Metcalfe NB (2009) Juvenile salmon with high standard metabolic rates have higher energy costs but can process meals faster. *Proc R Soc B Biol Sci* 276: 2103–2108.

Mittelbach GG, Ballew NG, Kjelvik MK (2014) Fish behavioral types and their ecological consequences. *Can J Fish Aquat Sci* 71: 927–944.

Monaco, C. J., & Helmuth, B. (2011). Tipping points, thresholds and the keystone role of physiology in marine climate change research. *Adv Mar Biol*, *60*, 123-160.

Motais R, Isaia J, Rankin JC, Maetz J (1969) Adaptive changes of the water permeability of the teleostean gill epithelium in relation to external salinity. *J Exp Biol* 51: 529–546.

Murchie KJ, Cooke SJ, Danylchuk AJ, Suski CD (2011) Estimates of field activity and metabolic rates of bonefish (Albula vulpes) in coastal marine habitats using acoustic tri-axial accelerometer transmitters and intermittent-flow respirometry. *J Exp Mar Biol Ecol* 396: 147–155.

Nagy K (2001) Food requirements of wild animals: predictive equations for free-living mammals, reptiles, and birds. *Nutr Abstr Rev* 71: 21–31.

Nagy KA (1989) Doubly-labeled water studies of vertebrate physiological ecology. In: Stable Isotopes in Ecological Research. Springer-Verlag, New York, pp 270–287.

Nagy KA (2005) Field metabolic rate and body size. *J Exp Biol* 208: 1621–1625.

Nagy KA, Girard IA, Brown TK (1999) Energetics of free-ranging mammals, reptiles, and birds. *Annu Rev Nutr* 19: 247–277.

Nagy KA, Shoemaker VH (1984) Field energetics and food consumption. *Physilogical Zool* 57: 281–290.

Nelson JA (2016) Oxygen consumption rate v. rate of energy utilization of fishes: A comparison and brief history of the two measurements. *J Fish Biol* 88: 10–25.

Nolet BA, Butler PJ, Masman D, Woakes AJ (1992) Estimation of daily energy expenditure from heart rate and doubly labeled water in exercising geese. *Physiol Zool* 65: 1188–1216.

Nonogaki H, Nelson JA, Patterson WP (2007) Dietary histories of herbivorous loricariid catfishes : Evidence from d13C values of otoliths. *Environ Biol Fishes*. doi:10.1007/s10641-006-9074-8

Norin T, Clark TD (2016) Measurement and relevance of maximum metabolic rate in fishes. *J Fish Biol* 88:122–151.

Norin T, Malte H (2011) Repeatability of standard metabolic rate, active metabolic rate and aerobic scope in young brown trout during a period of moderate food availability. *J Exp Biol* 214: 1668–1675.

Norin T, Malte H (2012) Intraspecific variation in aerobic metabolic rate of fish: Relations with organ size and enzyme activity in brown trout. *Physiol Biochem Zool* 85: 645–656.

Oviatt C, Keller A, Sampou P, Beatty L (1986) Patterns of productivity during eutrophication: a mesocosm experiment. *Mar Ecol Prog Ser* 28: 69–80.

Pagano AM, Williams TM (2019) Estimating the energy expenditure of free - ranging polar bears using tri-axial accelerometers : A validation with doubly labeled water. *Ecol Evol* 9: 4210–4219.

Pearson GA, Lago-Leston A, Mota C (2009) Frayed at the edges: Selective pressure and adaptive response to abiotic stressors are mismatched in low diversity edge populations. *J Ecol* 97: 450–462.

Peck MA, Moyano M (2016) Measuring respiration rates in marine fish larvae: challenges and advances. *J Fish Biol* 88: 173–205.

Pettit TN, Nagy KA, Ellis HI, Whittow GC (1988) Incubation energetics of the Laysan Albatross. *Oecologia* 74: 546–550.

Pinsky ML, Eikeset AM, McCauley DJ, Payne JL, Sunday JM (2019) Greater vulnerability to warming of marine versus terrestrial ectotherms. *Nature* 569: 108–111.

Plasqui G, Westerterp KR (2007) Physical activity assessment with accelerometers: an evaluation against doubly labeled water. *Obesity* 15: 2371–2379.

Poloczanska ES, Brown CJ, Sydeman WJ, Kiessling W, Schoeman DS, Moore PJ, Brander K, Bruno JF, Buckley LB, Burrows MT, *et al.* (2013) Global imprint of climate change on marine life. *Nat Clim Chang* 3: 919–925.

Prado P, Heck KLJ, Watts SA, Cebrian J (2013) δ13C and δ18O signatures from sea urchin skeleton : importance of diet type in metabolic contributions. *Mar Ecol Prog Ser* 476: 153–166.

Priede IG (1983) Heart-rate telemetry from fish in the natural-environment. *Comp Biochem Physiol - A Mol Integr Physiol* 76: 515–524.

Priede IG (1985) Metabolic scope in fishes. In: Tytler P, ed. Fish Energetics. pp 33–64.

Priede IG, Young AH (1977) The ultrasonic telemetry of cardiac rhythms of wild brown trout (Salmo trutta L.) as an indicator of bio-energetics and behaviour. *J Fish Biol* 10: 299–318.

Quintella BR, Póvoa I, Almeida PR (2009) Swimming behaviour of upriver migrating sea lamprey assessed by electromyogram telemetry. *J Appl Ichthyol* 25: 46–54.

Ravi V, Venkatesh B (2018) The divergent genomes of teleosts. *Annu Rev Anim Biosci* 6: 47–68.

Reed JZ, Chambers C, Hunter CJ, Lockyer C, Kastelein R, Fedak MA, Boutilier RG (2000) Gas exchange and heart rate in the harbour porpoise, Phocoena phocoena. *J Comp Physiol B* 170: 1–10.

Reilly JJ, Fedak MA (1991) Rates of water turnover and energy expenditure of free-living male common seals (Phoca vitulina). *J Zool* 223: 461–468.

Ricklefs R, Roby D, William J (2019) Daily energy expenditure by adult leach's storm-petrels during the nesting cycle. *Physiol Zool* 59: 649–660.

Ricklefs RE, Konarzewski M, Daan S (1996) The relationship between basal metabolic rate and daily energy expenditure in birds and mammals. *Am Nat* 147: 1047–1071.

Rojano-Donãte, L., McDonald, B. I., Wisniewska, D. M., Johnson, M., Teilmann, J., Wahlberg, M., Højer-Kristensen, J., & Madsen, P. T. (2018). High field metabolic rates of wild harbour porpoises. *Journal of Experimental Biology*, *221*(23). https://doi.org/10.1242/jeb.185827

Rilov G, Mazaris AD, Stelzenmüller V, Helmuth B, Wahl M, Guy-Haim T, Mieszkowska N, Ledoux JB, Katsanevakis S (2019) Adaptive marine conservation planning in the face of climate change: What can we learn from physiological, ecological and genetic studies? *Glob Ecol Conserv* 17. doi:10.1016/j.gecco.2019.e00566

Rogers SC, Weatherley AH (1983) The use of opercular muscle electromyograms as an indicator of the metabolic costs of fish activity in rainbow trout, Salmo gairdneri Richardson, as determined by radiotelemetry. *J Fish Biol* 23: 535–547.

Rosenfeld J, Leeuwen T van, Richards J, Allen D (2015) Relationship between growth and standard metabolic rate: measurement artefacts and implications for habitat use and life-history adaptation in salmonids. *J Anim Ecol* 84: 4–20.

Ross LG, Watts W, Young AH (1981) An ultrasonic biotelemetry system for the continuous monitoring of tail-beat rate from free-swimming fish. *J Fish Biol* 18: 479–490.

Scantlebury DM, Mills MGL, Wilson RP, Wilson JW, Mills MEJ, Durant SM, Bennett NC, Bradford P, Marks NJ, Speakman JR (2014) Flexible energetics of cheetah hunting strategies provide resistance against kelptoparasitism. *Science* 346: 79–82.

Schloesser RW, Rooker JR, Louchuoarn P, Neilson JD, Secor DH (2009) Interdecadal variation in seawater d13C and d18O recorded in fish otoliths. *Limnol Oceanogr* 54: 1665–1668.

Schmidt-Nielsen K (1990) Animal physiology: adaptation and environment. Cambridge University Press.

Schmittner A, Gruber N, Mix C, Key RM, Tagliabue A (2013) Biology and earth air – sea system gas exchange δC in the ocean. *Biogeosciences Discuss* 10: 8415–8466.

Schultner J, Welcker J, Speakman JR, Nordoy ES, Gabrielsen GW (2010) Application of the two-sample doubly labelled water method alters behaviour and affects estimates of energy expenditure in black-legged kittiwakes. *J Exp Biol* 213: 2958–2966.

Schutkowski H, Herrmann B, Grupe G (1999) Diet, status and decomposition at Weingarten: trace element and isotope analyses on early mediaeval skeletal material. *J Archaeol Sci* 26: 675–685.

Schwieterman GD, Crear DP, Anderson BN, Lavoie DR, Sulikowski JA, Bushnell PG, Brill RW (2019) Combined effects of acute temperature change and elevated pCO2 on the metabolic rates and hypoxia tolerances of clearnose skate (Rostaraja eglanteria), summer flounder (Paralichthys dentatus), and thorny skate (Amblyraja radiata). *Biology* 8(56). doi:10.3390/biology8030056

Secor DH, Hendersn-Arzapalo A, Piccoli PM (1995) Can otolith microchemistry chart patterns of migration and habitat utilization in anadromous fishes. *J Exp Mar Biol Ecol* 192: 15-33.

Shen J, Gao Y (2012) Otolith isotopic signatures in identification of natal sources and stock differences of Chinese silver carp stable isotope analyses in otoliths of silver carp : a pilot study in identification of natal sources and stock differences. *Environ Biol Fishes* 95: 445–453.

Shephard, S., Trueman, C., Rickaby, R., & Rogan, E. (2007). Juvenile life history of NE Atlantic orange roughy from otolith stable isotopes. *Deep-Sea Research Part I: Oceanographic Research Papers*, *54*(8), 1221–1230. https://doi.org/10.1016/j.dsr.2007.05.007

Sherwood GD, Rose GA (2003) Influence of swimming form on otolith δ13C in marine fish. *Mar Ecol Prog Ser* 258: 283–289.

Sherwood GD, Rose GA (2005) Stable isotope analysis of some representative fish and invertebrates of the Newfoundland and Labrador continental shelf food web. *Estuar Coast Shelf Sci* 63: 537–549.

Shoemaker VH, Nagy KA (1984) Osmoregulation in the Galapagos marine iguana, Amblyrhynchus cristatus. *Physiol Zool* 57: 291–300.

Sinnatamby RN, Dempson JB, Reist JD, Power M (2015) Latitudinal variation in growth and otolith-inferred field metabolic rates of Canadian young-of-the-year Arctic charr. *Ecol Freshw Fish* 24: 478–488.

Smith RR (1980) Nutritional bioenergetics in fish. In: FAO/UNDP Training Course in Fish Feed Technology. United Nations Development Programme: Food and Agriculture Organization of the United Nations, Seattle.

Smoliński S, Denechaud C, von Leesen G, Geffen AJ, Grønkjær P, Godiksen JA, Campana SE (2021) Differences in metabolic rate between two Atlantic cod (Gadus morhua) populations estimated with carbon isotopic composition in otoliths. *PLoS One* 16: 1–19.

Snelderwaard PC, Van Ginneken V, Witte F, Voss HP, Kramer K (2006) Surgical procedure for implanting a radiotelemetry transmitter to monitor ECG, heart rate and body temperature in small Carassius auratus and Carassius auratus gibelio under laboratory conditions. *Lab Anim* 40: 465–468.

Solomon CT, Weber PK, Cech JJ, Ingram BL, Conrad ME, Machavaram M V., Pogodina AR, Franklin RL (2006) Experimental determination of the sources of otolith carbon and associated isotopic fractionation. *Can J Fish Aquat Sci* 63: 79–89.

Sparling CE, Thompson D, Fedak MA, Gallon SL, Speakman JR (2008) Estimating field metabolic rates of pinnipeds: Doubly labelled water gets the seal of approval. *Funct Ecol* 22: 245–254.

Speakman JR (2000) The Cost of Living: Field Metabolic Rates of Small Mammals. *Adv Ecol Res* 30: 177–297.

Spero HJ, Williams DF (1987) Extracting environmental information from planktonic foraminiferal d13C data. *Nature* 335: 7–9.

Spicer J, Gaston K (2009). Physiological diversity: ecological implications. John Wiley and Sons.

Steffensen, J F, Johansen K, Bushnell PG (1984) An automated swimming respirometer. *Comp Biochem and Physiol* 79:437-440.

Stock BC, Jackson AL, Ward EJ, Parnell AC, Phillips DL, Semmens BX (2018) Analyzing mixing systems using a new generation of Bayesian tracer mixing models. *Peer J* 6: 1–27.

Stothart MR, Elliott KH, Wood T, Hatch SA, Speakman JR (2016) Counting calories in cormorants : dynamic body acceleration predicts daily energy expenditure measured in pelagic cormorants. *J Exp Biol* 219: 2192–2200.

Sturrock AM, Hunter E, Milton JA, Johnson RC, Waring CP, Trueman CN, EIMF (2015) Quantifying physiological influences on otolith microchemistry. *Methods Ecol Evol* 6: 806–816.

Sureau D, Lagardére J -P (1991) Coupling of heart rate and locomotor activity in sole, Solea solea (L.), and bass, Dicentrarchus labrax (L.), in their natural environment by using ultrasonic telemetry. *J Fish Biol* 38: 399–405.

Swart PK (1983) Carbon and oxygen isotope fractionation in scleractinian corals : a review. *Earth Sci Rev* 19: 51-80. doi:10.1016/0012-8252(83)90076-4

Tagliabue A, Bopp L (2008) Towards understanding global variability in ocean carbon-13. *Global Biogeochem Cycles* 22: 1–13.

Thorarensen H, Gallaugher PE, Farrell AP (1996) The limitations of heart rate as a predictor of metabolic rate in fish. *J Fish Biol* 49: 226–236.

Thorrold SR, Campana SE, Jones CM, Swart PK (1997) Factors determining d13C and d18O fractionation in aragonitic otoliths of marine fish. *Geochim Cosmochim Acta* 61: 2909–2919.

Tiebout H, Nagy K (1991) validation of the doubly labeled water method (3hh180) for measuring water flux and CO2 production in the tropical hummingbird Amazilia saucerottei. *Physiol Zool* 64: 362–374.

Tohse H, Mugiya Y (2008) Sources of otolith carbonate: experimental determination of carbon incorporation rates from water and metabolic CO2, and their diel variations. *Aquat Biol* 1: 259–268.

Tomas J, Geffen AJ (2003) Morphometry and composition of aragonite and vaterite otoliths of deformed laboratory reared juvenile herring from two populations. *J Fish Biol* 63: 1383–1401.

Treberg JR, Killen SS, MacCormack TJ, Lamarre SG, Enders EC (2016) Estimates of metabolic rate and major constituents of metabolic demand in fishes under field conditions: Methods, proxies, and new perspectives. *Comp Biochem Physiol -Part A Mol Integr Physiol* 202: 10–22.

Trueman CN, Chung MT, Shores D (2016) Ecogeochemistry potential in deep time biodiversity illustrated using a modern deep-water case study. *Philos Trans R Soc B Biol Sci* 371. doi:10.1098/rstb.2015.0223

Trueman CN, Mackenzie KM, Palmer MR (2012) Identifying migrations in marine fishes through stable-isotope analysis. *J Fish Biol* 81: 826–847.

Trueman CN, Rickaby REM, Shephard S (2013) Thermal, trophic and metabolic life histories of inaccessible fishes revealed from stable-isotope analyses: A case study using orange roughy Hoplostethus atlanticus. *J Fish Biol* 83: 1613–1636.

von Biela VR, Newsome SD, Zimmerman CE (2015) Examining the utility of bulk otolith δ13C to describe diet in wild-caught black rockfish Sebastes melanops. *Aquat Biol* 23: 201–208.

Wahl M, Hinrichsen H-H, Lehmann A, Lenz M (2013) Natural variability in hard-bottom communities and possible drivers assessed by a time-series study in the SW Baltic Sea: know the noise to detect the change. *Biogeosciences* 10: 5227–5242.

Ward S, Möller U, Rayner JMV, Jackson DM, Bilo D, Nachtigall W, Speakman JR (2001) Metabolic power, mechanical power and efficiency during wind tunnel flight by the European starling Sturnus vulgaris. *J Exp Biol* 204: 3311–3322.

Weatherley AH, Rogers SC, Pincock DG, Patch JR (1982) Oxygen consumption of active rainbow trout, Salmo gairdneri Richardson, derived from electromyograms obtained by radiotelemetry. *J Fish Biol* 20: 479–489.

Weidel BC, Ushikubo T, Carpenter SR, Kita NT, Cole JJ, Kitchell JF, Pace ML, Valley JW (2007) Diary of a bluegill (Lepomis macrochirus): daily δ13C and δ18O records in otoliths by ion microprobe. *Can J Fish Aquat Sci* 64: 1641–1645.

Whitney NM, Papastamatiou YP, Holland KN, Lowe CG (2007) Use of an acceleration data logger to measure diel activity patterns in captive whitetip reef sharks, Triaenodon obesus. *Aquat Living Resour* 20: 299–305.

Williams JB (1993) Energetics of Incubation in free-living orange-breasted sunbirds in South Africa. *Condor* 95: 115–126.

Williams JB, Dwinnel B (1990) Field metabolism of free-living female savannah sparrows during incubation: a study using doubly labeled water. *Physiol Zool* 63: 353–372.

Wilson RP, White CR, Quintana F, Halsey LG, Liebsch N, Martin GR, Butler PJ (2006) Moving towards acceleration for estimates of activity-specific metabolic rate in free-living animals: The case of the cormorant. *J Anim Ecol* 75: 1081–1090.

Wolf TJ, Ellington CP, Davis S, Feltham MJ (1996) Validation of the doubly labelled water technique for bumblebees Bombus terrestris (L.). *J Exp Biol* 199: 959–972.

Wright S, Metcalfe JD, Hetherington S, Wilson R (2014) Estimating activity-specific energy expenditure in a teleost fish, using accelerometer loggers. *Mar Ecol Prog Ser* 496: 19–32.

Wurster CM, Patterson WP (2003) Metabolic rate of late holocene freshwater fish: Evidence from δ13C values of otoliths. *Paleobiology* 4: 492–505.

Wurster CM, Patterson WP, Stewart DJ, Bowlby JN, Stewart TJ (2005) Thermal histories, stress, and metabolic rates of Chinook salmon (Oncorhynchus tshawytscha) in Lake Ontario: Evidence from intra-otolith stable isotope analyses. *Can J Fish Aquat Sci* 62: 700–713.

Young JN, Bruggeman J, Rickaby REM, Erez J, Conte M (2013) Evidence for changes in carbon isotopic fractionation by phytoplankton between 1960 and 2010. *Global Biogeochem Cycles* 27: 505–515

CHAPTER 3: Otolith-isotope methods reveal distinct pulse- and population- specific field metabolic trends in juvenile Atlantic cod (*Gadus morhua*) from the Northwest Atlantic

Abstract

I examine context-dependent relationships between field metabolic rate and experienced temperature to provide a base understanding of the effects of climate change on age-0 Atlantic cod (*Gadus morhua*) pulses in the Northwest Atlantic, a system that experiences substantial winter cooling (dropping from 16 to -1.5 ºC annually). Examining otolith $\delta^{13}C$ and $\delta^{18}O$ stable isotope signatures from two age-0 pulse groups (pulse 1 and 3) from the 2019 Atlantic cod cohort from Newman Sound, Newfoundland, I recover unique pulse-specific mean FMR at identical temperatures, despite nearly identical FMR-thermal sensitivity trends. I expand these data to include FMR trends recovered for age-0 juvenile Atlantic cod from the Skagerrak coast of Norway, a system which is nearly two times warmer. Between the Newman Sound Atlantic cod pulses and the Skagerrak Atlantic cod ecotypes, significant differences in FMR-thermal sensitivity occur between Atlantic cod with different habitat types and life history characteristics across populations (Fjord and Newman Sound juveniles vs North Sea juveniles), while mean FMR differs significantly between all groups except for the Fjord and pulse 3 Atlantic cod. This study reveals both pulse- and population-specific differences in the otolith-isotope inferred field metabolic rate of individuals exposed to different environmental temperatures, suggesting 1) physiological diversity in the response of age-0 juvenile Atlantic cod to cooling trends in the Northwest Atlantic, 2) and potential evidence for population-specific thermal adaptation consistent with the metabolic cold adaptation hypothesis in Atlantic cod using *in situ* measurements that incorporate the environmental complexities of two different populations.

Individual-level *in situ* physiological data would benefit recruitment forecasting and possibly guide management actions regarding future fisheries management decisions.

Introduction

Climate change is driving fluctuations in abiotic factors (stratification, nutrient input, ocean currents, coastal upwelling, ocean acidification, sea-level rise, shifts in salinity, and hypoxia events). These drivers, in turn, alter biotic processes across all levels of biological organization. The response of marine fishes to changing temperature has received much research attention due to their physiological vulnerability as ectotherms (e.g., Rose *et al.,* 1994). Fluctuating temperatures destabilize the biological processing rates of fishes, which can cause cascading shifts to organisms (energy budgets), populations (productivity), communities (species-interactions), and ecosystems (food web structure). While many studies have examined fish response to increasing ocean temperatures and marine heat waves across both local and global assemblages (e.g., Pörtner *et al.,* 2001; Sunday *et al.,* 2015; von Biela *et al.,* 2019), the temperature fluctuations at the other end of the spectrum – i.e., winter climate change – have received little attention until now.

The winter season is often defined by predictable changes in abiotic process, including shorter photoperiods and colder air and water temperatures (Farmer et al. 2015; Sutton *et al.,* 2021), influencing annual individual-level processes (growth, reproduction fitness, and survival). However, climate change has reduced the predictability of winter and its impact on ecological processes, introducing additional abiotic variability through climate events such as mid-winter warm and cold extremes (Bokhorst et al. 2009; Humphries and Umbanhowar 2007). These deviations from the median norms of winter are referred to as “winter weirding”, and may have

far greater impacts on biodiversity than previously thought (Sutton *et al.,* 2021). While recent studies have shifted their focus to winter ecology and associated responses to climate change, these have largely focused on terrestrial processes and species assemblages (Lemke et al. 2007; Kreyling 2010; Pauli et al. 2013; Williams et al. 2015; Penczykovski et al. 2017; Contosta et al. 2019). There remains a gap in our understanding of how climate variability influences the pre-existing winter stresses experienced by aquatic organisms, and how this might impact seasonality of marine processes (e.g., juvenile overwintering mortality, temporal migration cues); affecting annual individual and ecosystem-level shifts (Morley *et al.,* 2017).

While a continual rise in Sea Surface Temperature (SST) throughout the 20th century (Roemmich *et al.,* 2012) is clear, marine ambient temperature trends are not unidirectional and are further influenced by accompanying changes to the hydrological cycle (continental glacier and sea ice melt, destabilized precipitation and evaporation). With its connection to the North Atlantic Subpolar Gyre and the expansive Atlantic Meriodional Overturning Circulation, the coastal areas of eastern Newfoundland have experienced pronounced surface freshening and cooling (Oltmanns *et al.,* 2020); with cold periods reaching far back as the 1960s (Read & Gould*,* 1992; Colbourne 2004). The recently updated NLCI (Newfoundland & Labrador Climate Index) incorporating environmental conditions for 70 years (1951 – 2020) has highlighted consistent cooling trends since 2011, in which 2014-2017 was the coldest period since the early 1990s (Cyr & Galbraith, 2021). These cooling events are tightly linked with increased intensification of convection currents in the Labrador Sea, spreading denser and cooler waters, resulting in the largest volume of Labrador Sea Water (LSW) since the 1990s (Cyr & Galbraith, 2021). The fall-winter cold anomaly associated with the east coast of Newfoundland presents an important opportunity to examine climate change variability at the opposite end of the thermal

spectrum, and the response of its most culturally valuable fish species – the Atlantic cod (*Gadus morhua*).

The Atlantic cod has been well-studied across its temperate and arctic range, and makes up one of the largest, most valuable wild fisheries in the world. It is its value in fisheries that reduced the Atlantic cod population in the Northwest Atlantic to 1% of its original biomass (Chantraine 1993), declaring it as Endangered in 2003 by the Committee On the Status of Endangered Wildlife in Canada (COSEWIC, 2003) partially due to recruitment overfishing (Myers & Cadigan, 1995). An extensive dataset of annual length frequency and abundance data has shown that age-0 juvenile Atlantic cod in Newman Sound, Bonavista Bay, off the northeast coast of Newfoundland (NAFO Divisions 3KL) settle in the nearshore in several distinct pulses, generally ranging from July-November (Gregory *et al.,* 2019). Temperature is known to be a fundamental environmental factor driving settlement processes, influencing inputs to energy budgets through processes such as somatic growth (Copeman *et al.,* 2008; Heintz *et al.,* 2013), which eventually lead to recruitment to the offshore adult population (Laurel *et al.*, 2017; Lunzmann-Cooke *et al.,* 2021).

The cooling dynamics evident in the northwest Atlantic can jeopardize the energy budget of age-0 Atlantic cod by altering metabolic rates during the first overwintering period (Gaitán-Espitia *et al.,* 2017). While pulse classification (body length) and the severity of winter can pose physiological challenges, the outcome on overwintering mortality and recruitment success isn't always evident. This has demonstrated by a recent analysis of age-0 juvenile Atlantic cod showing unusually low size-structured mortality over the first winter period (Geissinger *et al.,* 2022). The fall-winter temperature anomaly associated with this system has the potential to

differentially impact the metabolic rate of age-0 Atlantic cod from different pulses, with unknown outcomes on overwintering success, recruitment to adulthood, and productivity (Regular *et al.,* 2022). The northern population of Atlantic cod in Canada is still recovering today; therefore, cod stocks must be managed adaptively, taking into account the *in situ* environmental fluctuations associated with climate change and its potential impacts on annual variation in recruitment success.

A sustainable wild fishery relies on the long-term growth and productivity of fish populations, and this in turn depends on individual-level physiological response to the complex environmental stressors associated with current and projected climate scenarios. Determining metabolic response to temperature from laboratory-based measurements is difficult given the unpredictability of "winter weirding" in already complex natural habitats (Treberg *et al.,* 2016; Sutton *et al.,* 2020). It is important to measure the metabolic rate of an organism directly within its own environment, not only to capture the direct impacts of varying environmental factors on metabolism, but also any inter-individual behavioural responses that may dampen or exaggerate thermal effects on energy budget (Pettersen *et al.,* 2018). It is the field metabolic rate (FMR) – a proxy for the energy expenditure of a free-ranging organism in its natural environment - that must be examined in order to highlight important energetic trade-offs among individuals, distinct life history stages (age-0 pulses), and populations (Chung *et al.,* 2019b). FMR avoids the confounding factors associated with laboratory studies, such as the stresses of manipulation and confinement, and takes into account all pathways of metabolizable energy (SMR, SDA, and activity metabolism) to yield *in situ* time-averaged information on energy expenditure (Chung *et al.,* 2019b).

The ability to measure *in situ* metabolism in wild aquatic organisms is relatively new, with recent studies identifying links between the biomineral carbon stable isotope composition of fish otoliths and estimates of oxygen consumption to give retrospective measurements of FMR (Chung et al. 2019a, b, Martino et al. 2020). Dissolved carbon in fish blood is derived from two isotopically distinct sources ($\delta^{13}C_{diet}$ value; $\delta^{13}C_{DIC}$ value), from which the proportion of metabolic carbon in an otolith can be estimated through isotopic mass balance (Chung *et al.,* 2019). This proportion of metabolic carbon in blood is directly proportional to the rate of respiration of dietary carbon, creating a quantifiable link to energy use through the oxygen consumption proxy. By converting oxygen consumption to estimates of field metabolic rate, data can be easily compared across studies (Chung *et al.,* 2019). Chung et al. (2021) recently identified differences in thermal FMR sensitivity between juvenile Atlantic cod ecotypes on the Skagerrak coast of Norway (Fjord vs North Sea). Despite a recent surge in the use of the otolith-isotope method, more research is needed to identify whether the thermal sensitivity of FMR differs across different life-history stages (e.g., individual settlement pulses), or whether FMR thermal-relationships are comparable across populations experiencing different environmental conditions.

I will apply the FMR-otolith methodology to an Atlantic cod population from the Northwest Atlantic to identify important bottlenecks in the performance of juveniles during changing natural conditions. Experimental calibrations between otolith carbon isotope ratios and oxygen consumption rates have been performed for Atlantic cod (Chung *et al.,* 2019b), making this approach possible. My overarching objective is to test evidence of limiting constraints of cold ocean dynamics on energy expenditure across two settlement pulses of age-0 Atlantic cod. Specifically, I will examine differences in the mean FMR and the thermal sensitivity of FMR

between pulses 1 and 3 from the 2019 cohort (the year of hatch) to determine whether separate life history stages and their associated experienced temperatures have a significant impact on the metabolic budget of individuals going into the overwintering period.

The thermal sensitivity of FMR in the Newfoundland population will be further compared to that of the Skagerrak population, in which Chung et al. (2021) compared trends between two Atlantic cod ecotypes (North Sea vs Fjord). The Skagerrak population is based in an inherently warmer system, with a mean Sea Surface Temperature 2.14x higher (10.46°C vs 4.88°C) than Newman Sound over the four sampling years included in this study (2000, 2003, 2008, and 2019). Comparing field metabolic measurements will provide insight on the ability of juvenile Atlantic cod to perform under different thermal regimes. Field metabolic rate is expected to demonstrate significant differences between pulses within a population (pulse 1 vs pulse 3, Newfoundland) and across populations (Newfoundland vs Skagerrak) due to differences in experienced temperature. Two potential mechanisms will be discussed:

The Physiological Mechanism: Inherent differences in field metabolic rate are related to the physiological plasticity or robustness associated with different populations or life stages in response to environmental factors (temperature, dissolved oxygen, pH, salinity).

The Behavioural Mechanism: Inherent differences in field metabolic rate are a realized behavioural response to the energy requirements associated with context-specific environmental factors (temperature, dissolved oxygen, pH, salinity), or environmental opportunity (more available resources), leading to ecological consequences (feeding rates, competition, and predator-prey interactions).

Due to limitations in the current study, I cannot isolate either of these mechanisms in association with FMR differences across or within populations. However, differences in FMR trends between pulse classifications and populations exposed to differing temperature dynamics can provide an opportunity to examine the concept of physiological plasticity and the possibility for population-specific thermal adaptation.

Methods

Applying the otolith-isotope measure to obtain a reliable FMR-proxy requires four main steps: (1) field collection of juvenile (age-0) Atlantic cod with pulse determinations, (2) otolith and muscle tissue dissection and preparation for stable isotope analysis, (3) $\delta^{18}O$-inferred reconstructions of experienced temperature, and (4) $\delta^{13}C$-inferred FMR reconstructions. We then compare the relationship between experienced temperature, and the proportion of respiratory carbon (C_{resp})/FMR within pulse groups and between pulse groups for the age-0 individuals. We also compare the thermal-FMR relationship between two populations of Atlantic cod: the Newman Sound population collected in this study, and the Skagerrak population collected by Chung et al. (2021), which compared the FMR values across two ecotypes (Fjord and North Sea).

Field collection, dissection, and pulse classification

Juvenile (age-0) Atlantic cod were collected by seine netting methods at three sites in Newman Sound, Newfoundland, Canada (Bermuda Beach, Heffern's Cove, and Narrows Beach) within Bonavista Bay in Terra Nova National Park (Figure 3.1). Age-0 Atlantic cod were collected during two sampling periods, October 15/16th and November 12/13th, 2019. Sampling was conducted alongside the Department of Fisheries and Oceans' monitoring programme which

has been conducted since 1996 (Laurel *et al.,* 2017). Fish were collected using a 25 m long × 2 m high Danish bag seine net with a 9 mm stretch mesh in the wings, belly and codend, a 24.4 m headrope, and a 26.2 m footrope (Gregory *et al.,* 2019). Aluminum poles (75 cm-long, 25 mm-diameter) are located on each wing to maintain the spread between the headrope and footrope (Gregory *et al.,* 2004). The net was deployed approximately 55m from shore from a 6m boat, and retrieved by two individuals standing 16m apart on shore. The net samples from the seabed to 2 m above bottom, in a total area of approximately 880 m^2, with low escapement rates for juvenile cod (<5%; Gotceitas et al. 1997). All fish collected were identified, and previously established age-length relationships for Newfoundland Atlantic cod in late autumn were used to determine which Atlantic cod age groups to sample. Assignments to tentative pulse groups in the field were later cross-referenced with length frequency trajectories through time for the full Newman Sound age-0 2019 cohort (Gregory *et al.,* 2020).

Age-0 (n=170) Atlantic cod were euthanized and kept in a cooler in the field until their transfer to a refrigerator. Dissections for otolith and muscle tissue samples were conducted within 48 hours of collection. Water temperature and salinity were recorded at each site at a depth of approximately 30 cm using a YSI 30 (YSI Inc, Yellow Springs, Ohio). Each individual was measured to standard length (mm) and was weighed (±0.01 g), with age-0 fish ranging from 29 to 98 mm Standard Length. The otoliths were extracted by cutting a groove behind the gills on the ventral side of the organism, clearing the tissue out of the way, piercing the brain case, and carefully removing the otoliths. The muscle plugs were removed dorsolaterally behind the dorsal fin and stored in a -20°C freezer.

Individual age-0 Atlantic cod were assigned to one of 3 settlement pulses based on the length frequency trajectories through time established for the age-0 2019 cohort, with pulse assignment data input from Gregory et al. 2021 (Table 3.1). The age-0 Atlantic cod collected for this study were grouped into three pulse groups; pulse 1 (n=78), pulse 2 (n=33), and pulse 3 (n=59) (Table 3.1). The Atlantic cod population data for the age-0 2019 cohort was overlaid with the individuals collected for the current study in order to place them in the context of the larger and broader population pulse structure (Figure 3.2). Comparisons between the specimens sampled in this study and the age-0 2019 cohort (Gregory et al. 2021) ensures that the individuals I collected demonstrate pulse-specific growth rates that are representative of the Newman Sound population (Figure 3.2).

Otolith $\delta^{13}C$ and $\delta^{18}O$ determination

The otoliths were mounted on a resin block in order to secure them for the milling stage. To ensure there was no resin overlap onto the otolith drilling surface, each individual otolith was examined below the microscope and wiped with ethanol. The otoliths were sampled using a dremel. The distal surface was sampled to achieve a sample weight of approximately 30 micrograms at a depth at the order of tens of microns. The conversion of the sampling depth to the estimated timeframe of carbonate deposition in the aragonite samples obtained is estimated to be on the order of weeks, as much less than 10% of the otolith radius was collected. Stable isotope ($\delta^{13}C$ and $\delta^{18}O$) analyses were performed using a Thermo Scientific Kiel IV carbonate device coupled to a MAT 253 Isotope ratio mass spectrometer at the University of Southampton, UK, a delta notation was expressed relative to a VPDB (Vienna Pee Dee Belemnite) Standard. Analytical errors were <0.18‰ for δ13C and <0.33‰ for δ18O values in otoliths. A standard of

precision and accuracy better than 0.1 per mil (‰) was obtained through repeat analyses of international carbonate standards (NBS18, NBS19 and LSVEC). Instrument drift and the mass effect were corrected using a certified in-house cod otolith standard (Chung *et al.,* 2019). Due to the varying constraints associated with conducting a graduate degree in 2020/2021 (e.g., COVID-19 imposed closures, travel restrictions), and sampling complications which are part of field and lab study (e.g., otoliths too small and sample stuck in trap), the sample size was reduced from n= 170 caught individuals to n= 90 otoliths analysed for stable isotope composition. Remaining otoliths have been labeled and are stored for use in future studies.

Muscle carbon stable isotope analysis

Age-0 (n=170) muscle tissue samples were dried in a drying oven (VWR, Canada) at 60°C for 48 h and then powdered by hand using a mortar and pestle. Dried muscle tissue samples were crushed and 1g ± 10 mg tissues were packed in tin cups for stable isotope analysis. The δ13C values were standardised using a Gelatine A standard with a known isotopic value of -21.8‰. Samples were analysed using Thermo Scientific Delta V plus IRMS equipped with Elemental Analyser at the University of Southampton, UK. Delta notation of carbon isotope was relative to VPDB. Only the muscle tissue samples belonging to specimens which were also sampled for otolith stable isotope composition (n=90) were analysed for $\delta^{13}C$ stable isotope diet composition. The remaining muscle tissue samples have been labeled and are stored for use in future studies.

Water sample collection and DIC analysis

The water $\delta 13C_{DIC.W}$ values are determined simultaneously at the point of sampling to ensure a precise and accurate estimation. The methods for the water sample collection and DIC

analysis are largely based on Olack et al. (2018). Four water samples (1 ml) were collected at each site in pre-acidified (100 ul of 85% H_3PO_4) 12 mL ExetainerVR borosilicate tubes flushed with Helium (5.0 quality, TERRA) to inhibit microbial respiration. The water samples were given 18 h to equilibrate at 24 °C before CO_2 headspace measurements. Water samples from each site were analysed for $\delta^{13}C_{DIC}$ by headspace analysis using a Gasbench II connected to a Delta V Plus IRMS (Thermo) following standard protocols (TERRA, Memorial University of Newfoundland; Torres et al. 2005). Any droplets present in the samples were centrifuged down (2600 3 g, 1 min, swinging bucket, Sorvall Legend RT1, Thermo), and additional droplets under the cap were also removed after overnight incubation at 26°C. The headspace was sampled using a two-hole needle with a 0.5 mL/min He carrier gas. DIC standard solutions were analyzed alongside the crystalline salt form of the reagents used for the DIC standard solutions, as well as phosphoric-acid digested powdered crystalline carbonate $\delta^{13}C$ standards. Preliminary $\delta^{13}C$ readings were calculated against the working gas and final values were assigned by correction to calibrated laboratory standards. Accuracy was monitored through repeat analyses of laboratory standards within an expected 0.1 per mil (‰) accuracy.

$\delta^{18}O$ based reconstructions of experienced temperature

Individual experienced time-averaged temperature for the time period of otolith growth sampled by drilling was reconstructed using otolith $\delta^{18}O$ values following the Atlantic cod-specific equation (Høie *et al*., 2004):

1) $$\delta^{18}O_{oto} - \delta^{18}O_{water} = 3.90(\pm 0{:}24) - 0.20(\pm 0{:}019)T$$

which can be rewritten as:

$$T = \frac{(\delta^{18}O_{oto} - \delta^{18}O_{water}) - 3.9}{-0.2}$$

where T is temperature in Celsius (°C), and $\delta^{18}O_{oto}$ and $\delta^{18}O_{water}$represent the oxygen-18 isotope value recorded otolith (VPDB) and ambient water (VSMOW), respectively. Since cod collections were conducted within a bay with significant freshwater input, the salinity measurements were quite low (20.9-24 ppt), and therefore the salinity-isotope relationship was extrapolated from Benetti *et al*., (2016) to an approximate value of -2.25 per mille, with further confirmation from the global NASA GISS oxygen-18 seawater isotope compilation (Schmidt 1999).

Population-specific growth rate determination

A comparable population-specific growth rate value was determined from a subsample of the population dataset used to calculate FMR to ensure that growth rate is not driving population-specific FMR patterns. Individual sampling dates (Julian date) were regressed with length (mmSL), and the resulting slope for each population (Skagerrak, Newman Sound) acts as a standardized growth rate (mmSL/Julian date) for comparisons across populations.

While the Newfoundland analyses for this paper were done on a subset of the Newman Sound population (Heffern's Cove), the growth rate was determined using all data available for pulse 1 and 3 Atlantic cod from Julian date 301-317, including the age-0 2019 cohort dataset (Gregory et al. 2021). The decision of using the cohort dataset was made due to the low sample

size of collected pulse 1 individuals immediately prior to the 316 sampling date, and the non-existent appearance of pulse 3 individuals until this same date, creating a dataset with temporal gaps in length measurements. The subsample for the Skagerrak population solely included individuals collected in 2000, as this was the only dataset with a sufficiently long sampling period. The growth rate value for both the Newman Sound and Skagerrak populations were therefore calculated over a similar sampling period (16 and 17 days apart, respectively). Using data that reflects growth rate (16-17 days) over a similar time period as the carbonate deposition timeframe for otolith-inferred FMR values (~14 days) allows us to determine a pulse-specific growth rate measurement that is consistent with otolith-inferred FMR values.

$\delta^{13}C$ based field metabolic rate reconstructions

The total time-averaged FMR of individuals can be estimated from the isotopic composition of otolith carbonate in two key steps. First, the relative proportion of respiratory carbon in the otolith carbonate (C_{resp}) is estimated from a two-component mixing model:

$$\delta^{13}C_{oto} = C_{resp} * \delta^{13}C_{diet} + (1 - C_{resp}) * \delta^{13}C_{DIC.W} + \varepsilon \quad (2)$$

which can be rewritten as:

$$C_{resp} = (\delta^{13}C_{oto} - \delta^{13}C_{DIC.W}) \,/\, (\delta^{13}C_{diet} - \delta^{13}C_{DIC.W})$$

where $\delta^{13}C_{oto}$ is the carbon isotope value recorded in the otolith, $\delta^{13}C_{diet}$ is the $\delta^{13}C$ value derived from muscle tissue analysis minus 1.5‰ reflecting the relationship between diet and tissue enrichment (Sweeting *et al.*, 2007), and $\delta^{13}C_{DIC.W}$ represents the $\delta^{13}C$ values of dissolved inorganic carbon (DIC) measured from seawater. The ε term is the total net isotopic fractionation

during carbon exchange from the environment to the otolith aragonite. Similar to Chung et al. (2021, 2019b), ε was set as 0 in accordance with the findings in Solomon et al. (2006). Determining the absolute value of ε is not necessary in this study because C_{resp} values are calibrated against measured oxygen consumption rates for the study species (Chung *et al.,* 2019b). Like Chung et al. (2021), the term C_{resp} has been adopted to represent the relative proportion of respiratory carbon in otolith carbonate instead of M (Schwarcz et al. 1998, Solomon et al. 2006, Chung *et al.,* 2019a, b), which has the potential to be confused with abbreviations associated with body mass and oxygen consumption in moles.

Once C_{resp} has been calculated, individual FMR, in units of oxygen consumption rate, can be estimated using a statistical calibration for cod (Chung *et al.,* 2019b), with the assumption that the relationship between oxygen consumption rate and the proportion of respired carbon in the blood is constant across life stages:

3) $$C_{resp} = C\ (1 - e^{-k(oxygen\ consumption)})$$

which can be rewritten as:

$$Oxygen\ consumption = \frac{ln(1 - \frac{C_{resp}}{C})}{-k}$$

where C is an upper bound fitted as 0.4, a common high end value applied across multiple taxa in cool waters, and k is a decay constant with a fitted value of 8.80*10E-3. Each calculated oxygen consumption value represents the individual-specific FMR value with the units $mgO_2kg^{-1}h^{-1}$.

Standard metabolic rate determination

We calculated the likely theoretical SMR using the Metabolic Theory of Ecology (MTE) to understand the relationship between $\delta^{13}C$ and average metabolic rates as predicted by size and temperature (Brown *et al.,* 2004; Gillooly *et al.,* 2001). The SMR was estimated for each individual, using its respective body mass and experienced temperature:

4) $$Theoretical\ SMR = B_0 \times M^{a} \times e^{-\left(\frac{0.65}{(8.62\times10^{-5})\times K}\right)}$$

where the B_0 is the normalised constant (1.64E+13; Martino *et al.,* 2020), M is the body mass (g), K is temperature in Kelvin, and α is the allometric scaling exponent of body mass. The allometric scaling exponent of body mass is still highly debated. Here, we use the 0.21 value for teleost fish compiled from Clarke (2006), reflecting a whole organism mass scaling exponent of 0.79. It is important to note that the 0.65 term used here to represent the thermal sensitivity of metabolic rate is also still under debate. The theoretical SMR was compared to the estimated FMR for each individual.

Statistical analyses

A subset of the population was used to compare trends between pulse 1 and pulse 3 individuals. The Heffern's Cove individuals (n= 52) were used as they contain the largest sample size for pulse 1 (n= 24) and pulse 3 (n= 28) over the same sampling period, allowing me to isolate metabolic response under the same environmental conditions. The sample size for the pulse 2 organisms was insufficient to draw any conclusions (n=7) and were; therefore, not

included in my analyses. These latter samples not used in the analyses for the current research will be available for use in future undergraduate and graduate research.

First, C_{resp} values and $\delta^{18}O$-inferred experienced temperatures were compared to both standard length measurements and pulse classification using a simple linear regression and a t-test, respectively. The relationship of metabolic rate and temperature theoretically follows the Arrhenius model (Brown et al. 2004):

5) $$metabolic\ rate \propto e^{-\frac{E}{kT}}$$

where T is absolute temperature in Kelvin, k is Boltzmann's constant (8.62E-5 eVK−1), and E is the activation energy. To evaluate the thermal relationship of FMR, the FMR of each individual was scaled according to theoretical mass scaling of FMR. This was then modelled with inverse temperature:

$$\ln(FMR/M^{-0.13}) \textit{ vs. } 1/kT$$

A generalised least squares fit was conducted on the linear modeling in order to detect significant differences between the slope (thermal sensitivity of FMR) and the intercept (mean FMR) across population subgroups - NL pulses (P1 and P3) and Skagerrak ecotypes (North Sea and Fjord). In order to differentiate between slopes, a Q_{10} value was also determined using the *respirometry* package in RStudio (<www.r-project.org>), which determined the best fitting Q10 for each subpopulation over its range of FMR and temperature measurements. The linear regression, t-tests and GLS were performed in R, and figures were produced using the package

ggplot2. GLS models were constructed using base R functions, incorporating the *varIdent* function to account for the difference in variance structures across the Skagerrak and Newfoundland datasets. All analyses can be found in the RMarkdown sheet appended to the end of this thesis.

Results

Temperature reconstruction

Otolith $\delta^{18}O$ values for all of the sampled individuals (n=90) varied between 0.97 and −1.14‰, suggesting that experienced temperatures ranged from 3.38 to 13.97°C (Supporting information). The reconstructed temperature corresponded to the range of in-situ temperature (5.5–10.5°C) recorded at the sampling locations using the YSI in 30 cm of water. This suggests that the otolith drill samples only integrate temperature differences over a short timeframe (days/weeks). All the experienced temperatures on the higher end of the thermal spectrum (6.48-13.97°C) are consistent with individuals sampled at Narrows Beach and Bermuda Beach in October, while individuals sampled at Heffern's Cove in November demonstrated a shift in experienced temperatures associated with the cooler water (3.38-9.21°C) in fall. A significant overlap of experienced temperature remains apparent between sampling events and sites. While neither the lowest nor the highest otolith-derived temperatures were measured in the field (YSI), this could be related to measuring *in situ* temperature in shallow water (~30cm), and therefore, missing the lower end of the temperature spectrum. Temperature records from the Department of Fisheries and Oceans, Canada (DFO) record temperatures as low as 2.87°C with a HydroCAT (Sea-Bird Scientific, USA) at a depth of 15-17m for the sampling dates associated with this dataset. The higher end of the spectrum was not measured using the HydroCAT or as a mean

daily temperature (depth of ~3m), but several individuals recorded experienced temperatures above 10°C (n=24). These high values could be associated with errors resulting from estimating water δ18O values based on salinity.

A significant relationship was observed between experienced temperature and the standard length of juvenile Atlantic cod at Heffern's Cove. This data indicates that smaller (41-58 mm SL) juvenile Atlantic cod individuals (pulse 3) frequented nearshore areas with higher temperatures, while longer (70-94 mm SL) juvenile Atlantic cod individuals (pulse 1) frequent nearshore areas with lower temperatures (Figure 3.3a). Experienced temperature also appears to be significantly different between juvenile Atlantic cod pulse classifications (Figure 3.3b), following the same trend (pulse 1 – colder temperatures, pulse 3 – warmer temperatures).

Field metabolic rate reconstruction

Otolith and muscle $\delta^{13}C$ values for all the sampled individuals (n=90) ranged from −5.12 to −2.40 ‰ and from −22.45 to −20.91 ‰, respectively. An example of the raw data necessary for calculation can be found in Table 3.2, and details about all individuals sampled are given in the Supporting Information. Using Eq. 2, otolith C_{resp} values varied between 0.18 and 0.29. These values correspond to individual FMR estimates ranging from 68.14 to 148.51 mg O_2 kg^{-1} h^{-1}. C_{resp} values and FMR varied significantly across pulse classifications, and followed an inversely proportional linear trend with standard length (Figure 3.4).

Relationship between otolith C_{resp} *values and experienced temperature*

The otolith C_{resp} values for individuals belonging to both pulses generally increased with increasing experienced temperature. While outliers slightly alter the slope of the linear

regressions (Figure 3.5a,b,c), they do not change the significant relationship observed between C_{resp} and experienced temperature among all individuals (Figure 3.5b). A lack of significance is observed between experienced temperature and the C_{resp} values in the pulse 3 individuals regardless of the presence of outliers (Figure 3.5c), and the absence of outliers also renders the relationship between experienced temperature and C_{resp} values insignificant for the pulse 1 individuals (Figure 3.5b). Transformations in accordance with the Metabolic Theory of Ecology are performed on the data in subsequent analyses.

For each observed temperature, individual fish from both pulses exhibited a range of C_{resp} values (5 °C: 0.18-0.20; 6 °C: 0.18-0.24; 7 °C: 0.19-0.25; 8 °C: 0.19-0.29; Figure 3.5d,e,f). The maximum among-individual variation in C_{resp} values was observed at the experienced temperatures of 7°C. It is important to remember that the variance is influenced by the sample size for each experienced temperature, and proportionally more individuals (35% of individuals) recorded time averaged temperatures of 7°C (Figure 3.5g,h,i). No clear trends are observed in the C_{resp} distribution relative to temperature in either pulse 1 or pulse 3 individuals (Figure 3.5e,f).

Relationship between FMR values, theoretical SMR, and experienced temperature

Reconstructed FMR values were compared to theoretical SMR values estimated using the Metabolic Theory of Ecology for each individual at its isotope-otolith determined experienced temperature (Fig. 3.6). Within the range of observed temperatures (3.38-9.21°C), 84% of reconstructed FMR values were within the expected range of theoretical SMR to FMR ratio (1:3). The FMR values for pulse 1 and 3 individuals fell within 1.51-2.16x and 1.64 - 4.17 x the theoretical SMR, respectively. (Figure 3.6a,b,c), suggesting that certain pulse 3 individuals demonstrate a higher FMR than would be expected based on calculated theoretical SMR.

Population-specific growth rate comparisons

The regression for the Skagerrak population subsample (2000, n=41) resulted in a growth rate of 0.24 mmSL/day (Figure 3.7b), similar to the 0.28 mmSL/day (Figure 3.7a) observed in the Newman Sound population subsample (2019, n=430). It is important to note, that the best fitting LMER for the Newman Sound population included pulse as a random effect, while the individuals collected from the Skagerrak coast of southern Norway are not divided by pulse classification due to the current lack of research on this type of recruitment structure in this population. The difference in juvenile Atlantic cod life-history classification between these two populations may reflect slight discrepancies in the date-length relationship behind growth rate determination; however, the difference in growth rate isn't significant enough to suggest this.

We also want to stress that the growth rate calculated for the purpose of this study was standardized to show a snapshot of growth over a comparable timespan, for the individuals for which FMR was estimated. Therefore, the population-specific growth rate values above will not match the population and pulse-specific composite growth rates determined using larger population datasets. This is apparent when examining the full Newman Sound population dataset (Gregory et al. 2021), in which an increased sample size and a longer sampling period ultimately changes the steepness of the slope, and therefore, the growth rate value (Figure 3.7c).

Given that the calculated growth rates for the Skagerrak and Newman Sound populations only differ by 0.04 mmSL/day, it is safe to assume that potential differences in FMR values across populations are not solely driven by differences in energy expenditure allocated to somatic growth. Regarding within-population growth rates, there is a difference in growth rate

between pulse 1 and pulse 3 individuals (0.19 mmSL/day and 0.31 mmSL, respectively). This will be discussed in relation to their mean FMR values in the following section.

Thermal FMR relationships within and between populations

When comparing the thermal FMR relationship under the Arrhenius model, the best fitting GLS identified varying differences in FMR-thermal sensitivity (slopes) and mean FMR (intercept) between populations (Newman Sound, NL vs Skagerrak, Norway), and within populations across ecotypes and pulse classification (pulse 1 and pulse 3 individuals, North Sea and Fjord ecotypes).

In terms of FMR thermal sensitivity, the only significant difference in slopes, occurs within the Skagerrak population between the North Sea and Fjord ecotypes. The North Sea ecotype demonstrates a significantly higher slope than the Fjord ecotype (NS: $t=-2.04$ $p= 0.044$, Fj: $t= 2.04$, $p= 0.043$; Figure 3.8). The high FMR thermal sensitivity of the North Sea ecotype is also reflected in its Q10 value, with a high 2.77 (North Sea) in contrast to 1.59 for the Fjord ecotype. The slopes representing pulse 1 and 3 individuals in the Newman Sound population share similarities with both the higher FMR thermal sensitivity of the North Sea ecotype, and the more robust FMR-thermal relationship observed in the Fjord ecotype ($p>0.05$; Figure 3.8). When examining the Q10 values, however, the pulse 1 and 3 Newman Sound individuals demonstrate a similarly low FMR thermal sensitivity to the Fjord individuals, with a Q10 of 1.84 and 1.92, respectively. The activation energy (E) derived from the Arrhenius model is consistent with this result, demonstrating low values for pulse 1 and 3 individuals (0.39 and 0.43, respectively) that are consistent with the activation energy observed in the Fjord ecotype individuals (0.37; Figure 3.8). The activation energies for pulse 1 and the Fjord ecotype fall just

shy of the commonly recovered estimates of activation energies for SMR (0.41–0.74, Gillooly et al. 2001), while the pulse 3 individuals are within its lower end and the North Sea surpasses this range (0.79).

The model prediction for mean FMR at a temperature of 6.4°C revealed a significantly lower mean FMR in the North Sea ecotype compared to the Fjord ecotype and both Newman Sound pulses (Figure 3.8). On the other end of the spectrum, pulse 1 individuals demonstrated a significantly higher mean FMR than pulse 3 and the two Skagerrak ecotypes (Figure 3.8). Despite similar thermal sensitivities between the Newman Sound pulses and the Skagerrak ecotypes, both pulses of Newman Sound individuals are predicted to maintain a significantly higher mean FMR than both Skagerrak ecotypes at the lower Newfoundland temperatures (Figure 3.8). Within each population, significant differences in mean FMR are observed both between pulse classifications, as well as Skagerrak ecotypes. The growth rate for the pulse 1 individuals was lower than that of the pulse 3 individuals by a factor of 1.63, which is inconsistent with the assumption that elevated growth implies higher FMR.

Discussion

By comparing the FMR response of the Newman Sound population (cold ecosystem) to the Skagerrak population (warm ecosystem), I make a few key observational findings. I notice significant differences between mean FMR values within (pulse classifications, sympatric ecotypes) and across populations (Newman Sound, Skagerrak Coast). I calculate the theoretical SMR using the Metabolic Theory of Ecology to examine how SMR values predicted by size and temperature fits within the scope of estimated mean FMR. The mean FMR estimates contain pulse-specific deviations from the FMR-SMR relationship, demonstrating a potential mismatch

between the high proportion of FMR in the FMR-SMR relationship of pulse 3 individuals and the high mean FMR observed in pulse 1 individuals. While this mismatch provides an opportunity to discuss differential pathways of energy allocation within the FMR value (SDA, SMR, activity metabolism) it is important to stress that the SMR values determined in this study are theoretical. When examining FMR-thermal sensitivity, similarities are observed between individuals from the Fjord ecotype in the Skagerrak population and both pulses from the Newman Sound population, with a strong deviation from this trend in the North Sea individuals.

Context dependent relationships between FMR and experienced temperature can be interpreted by comparing the y-intercepts of the Arrhenius equation, which represent pulse- or ecotype-specific mean FMR values. A significantly higher mean FMR is observed at 6.4 ºC in pulse 1 individuals, and this relationship is not expected to change with shifts in temperature due to the identical thermal sensitivity of FMR between pulses in the Newman Sound population. When interpreting mean FMR, it is important to remember that FMR estimates using the otolith-isotope method represent a holistic value of the energetic expenditure experienced by free-ranging individuals, including the specific dynamic action (SDA) and activity metabolism in addition to the standard metabolic rate (SMR). The inclusion of the specific dynamic action and activity metabolism in the measurement of FMR has the potential to exaggerate the FMR in excess to the maintenance costs associated with SMR. This suggests that while the magnitude of FMR might overall be higher in pulse 1 individuals than pulse 3, the allocation of energy associated with the "field" portion of the FMR (SDA & activity), must be weighed against its maintenance costs (SMR) to better understand the mechanism behind pulse-specific energy expenditure.

The expected relationship between FMR and theoretical SMR values estimated using the Metabolic Theory of Ecology is 3:1, respectively (Brown & Sibly, 2012). While the upper limit of metabolic rate was not measured (MMR) to compare the range of FMR to aerobic scope (MMR-SMR), the theoretical SMR was calculated for each individual fish based on mass and temperature to determine whether the FMR-SMR ratio followed that described by Brown & Sibly, (2012). It is important to note here that these theoretical SMR estimates are quite vague, assuming a constant B_0 across individuals and pulses, and will therefore only be used to give a rough approximation of the magnitude of SMR within the energy budget of free-ranging individuals. The FMR from pulse 1 fell within the expected SMR:FMR relationship (1.51-2.16x) averaging at 1.73x the SMR, however, the pulse 3 individuals demonstrated a higher FMR (1.64 - 4.17x) than would be expected based on theoretical SMR, averaging at 2.65x the FMR, with 29% of individuals surpassing the 3:1 FMR-SMR relationship. Due to the theoretical nature of the calculated SMR values, the FMR-SMR deviation in the pulse 3 individuals could be a consequence of inconsistencies in size (pulse 3 - smaller body size - larger difference in estimated SMR), or to differences in temperature between pulse 1 and 3 not being accurately represented by the 0.65 scaling exponent. Alternatively, the relationship between theoretical SMR and otolith-inferred FMR could be a sign of a greater energetic allocation of the overall energy budget to the specific dynamic action and activity metabolism in pulse 3 individuals (Figure 3.9).

The deviation from the theoretical 3:1 FMR-SMR relationship in pulse 3 individuals may be explained by a behavioural response to the energy requirements associated with context-specific environmental factors (temperature, dissolved oxygen, pH, salinity), leading to ecological consequences (e.g., feeding rates, competition, and predator-prey interactions). Pulse-

specific differences in feeding strategies and food availability (SDA), or differential energy expenditure associated with predator avoidance and locomotion (activity metabolism) can represent costly energetic processes in the individual's budget unrelated to SMR. While examining metabolic differences between pulse classifications within the same environment and population can help isolate habitat-specific effects; fine-scale, spatially heterogeneous biological processes are known to influence age-0 success across year-classes in Newman Sound, Newfoundland (Ings *et al.,* 2008). Size-specific trends in Newman Sound have been associated with high plankton availability, for example, where smaller age-0 juvenile Atlantic cod show reduced feeding efficiency due to increased feeding rates (Geissinger *et al.,* 2021). The increase in plankton production in the fall is consistent with the timing of the smaller sized pulse 3 individuals' recruitment to the nearshore (Lunzmann-Cooke, 2021). The high proportion of energy allocated to SDA and activity metabolism could therefore potentially reflect the full use of enhanced zooplankton production to increase lipid stores prior to the timely overwintering period, disproportionately increasing energy allocation to SDA in relation to SMR.

While exposed to the same environment, the energy allocation within the FMR value (FMR-SMR relationship) and the overall magnitude of the mean FMR itself, is different in pulse 1 individuals. Here, there is a higher pulse-specific mean FMR and a proportionally larger energy expenditure associated with maintenance costs (SMR). The energy allocation trend derived from theoretical SMR calculations suggests that environmental as opposed to behavioural factors could pose a larger impact on underlying physiological processes by way of physiological plasticity associated with different populations or life stages in response to environmental factors (temperature, dissolved oxygen, pH, salinity). Environmental temperatures are known to directly alter the biological processing rates of fishes, and can be especially

detrimental to the vital accumulation of lipid reserves for overwintering survival in juveniles, increasing predation risk and reducing the likelihood of survival to adulthood (Brown *et al.,* 2004; Pörtner *et al.,* 2008). In this study, pulse 1 Atlantic cod from the age-0 2019 cohort settled into the nearshore of Newman Sound in late July in comparison to pulse 3 individuals which did so in early November (Figure 3.2). Thus pulse 1 individuals were exposed to warmer temperatures during their early development, with a mean daily temperature of 13.36ºC during their first month post-settlement, as opposed to 4.55ºC in pulse 3 individuals. The thermal-FMR relationship examined in the Arrhenius equation integrates the experienced temperature derived from otolith $\delta^{18}O$ stable isotope composition. This measure of temperature is acute, meaning that it is the temperature experienced by the organism at the time of the sampled measure of field metabolic rate. While pulse 1 individuals were collected during the same sampling events with only slight differences in this temperature experience, the difference in pulse-specific temperature experienced during the early developmental stage could drive differences in mean FMR. Ontogeny, evolutionary history, and thermal history can modify metabolic response across a temperature tolerance window (Pörtner *et al.,* 2008). Thermal effects on early life stages have been examined, but largely in relation to chronic and acute warming in a laboratory setting, demonstrating interclutch variation in metabolic rates, significant changes in metabolic performance curve shape and height, and altered metabolic rate temperature sensitivity (e.g., Flynn & Todgham, 2018).

In addition to differences in metabolic rate and temperature experienced during early development, pulse 1 individuals are also entering the overwintering period at approximately 1.75x larger sizes than pulse 3 individuals. In order to examine whether the tradeoff between field metabolic rate, temperature at development, and size drives significant pulse-specific trends

in recruitment success, FMR trends must be examined in the "survivors" of the overwintering period (age-1 2020 cohort) and represents an important future research direction.

The thermal sensitivity of FMR can provide insight on the thermal adaptation of populations to climate change. Here, there are different trends within (Skagerrak), and across populations adapted to different thermal regimes (cold - Newman Sound, warm - Skagerrak). The nearly identical slope of the Arrhenius model for pulses 1 and 3 in the Newman Sound population suggests that, despite running at significantly different metabolic rates, changes in temperature affects the field metabolic rate of individuals in the same way. There is also an absence of a marked drop in FMR at either end of the thermal spectrum in Newman Sound individuals, suggesting that behavioural strategies may lead individuals to remain within thermal ranges that match *in situ* energetic requirements, preventing the acute drop or increase in FMR associated with a crossover into critical thermal thresholds. The lack of a difference in thermal sensitivity between pulses in Newman Sound contrasts results from North Sea and Fjord ecotypes of the Skagerrak population, where a clear difference in FMR thermal sensitivity is evident (Chung *et al.,* 2021).

Similarities in the FMR-thermal sensitivity (slope) between the Fjord ecotype and the two pulses in the Newman Sound population could be attributed to parallels in habitat and life history strategies. The Fjord ecotype from Norway is spawned and lives its entire life in the cold Skagerrak fjords, while the North Sea ecotype is transported into the fjord as egg or larvae (Knutsen *et al.,* 2018). Similarly, the majority of age-0 juvenile Atlantic cod from NAFO Divisions 3KL are spawned in Bonavista Bay by adults travelling along the coast each year from

Trinity Bay to Notre Dame Bay, and settle in the inshore waters of the Northeast coast of Newfoundland (Gregory *et al.,* 2019).

While the Fjord ecotype has a distinct genetic signature that differentiates it from the North Sea ecotypes (Sodeland *et al.,* 2016), Newman Sound individuals experience genetic variation within pulses based on pre-recruitment origin from offshore or inshore locations (Beacham *et al.,* 2000). This suggests that, unlike the Skagerrak population, genetic differences do not functionally isolate age-0 Newman Sound Atlantic cod, suggesting that environmental conditions experienced in the earliest life history stages (egg, larvae), may not differentiate the FMR-thermal sensitivity of individuals post-settlement. The physiological plasticity of age-0 Atlantic cod at recruitment, and the nursery refuge provided by Newman Sound may therefore set a baseline FMR-thermal sensitivity in response to changing climatic conditions despite differences in the magnitude of the metabolic rate.

When we examine mean FMR and FMR-thermal sensitivity in combination, we find that colder adapted fish within their population range are less sensitive to temperature change, and maintain higher metabolic rates, than warmer adapted populations at the cold edge of their range. Across the four groups we also found that, despite having similar thermal-FMR sensitivities, cold-adapted Newman Sound individuals from both pulses maintain higher metabolic rate than the cold-adapted Fjord fish would at this same temperature. This trend is consistent with the metabolic cold adaptation hypothesis (MCA), which states that animals from colder environments have higher metabolic rates at a given environmental temperature than their temperate counterparts (Hazel & Prosser 1974). This hypothesis suggests that energy allocation to important physiological processes, such as somatic growth, is maximised for individuals

adapted to environments with limited periods of optimal conditions (Lardies *et al.,* 2004). The metabolic cold adaptation hypothesis has received support in previous Atlantic cod studies, one of which demonstrated that northernmost populations of Atlantic cod from the Barents Sea have a higher capacity of aerobic enzymes, and therefore, higher rates of oxygen consumption along a latitudinal cline (Lannig *et al.,* 2003; Pörtner *et al.,* 2008). While the current study shows no significant difference in growth across populations, differences in mean FMR between populations reflect variation in the magnitude of the overall energy budget, and may also vary in the strategic proportion of FMR-SMR energy allocation, as seen between pulses in the Newman Sound population. While previous studies have only examined MCA trends within the constraints of the laboratory, the results in my study suggest population-specific thermal adaptation in juvenile Atlantic cod based on *in situ* measurements, incorporating the environmental complexities associated with both populations.

Unique pulse-specific trends in field metabolic rate exist between early and late pulse individuals, but both the mechanism behind these differences and their ultimate fate are hard to deduce from this study alone. Attribution of physiological or behavioural mechanisms to one factor, whether it be increased food availability or temperature during early development, may be overly simplistic. While the use of the otolith-isotope method to deduce estimates of field metabolic rate allows us to examine energetic expenditure of free-ranging fish in their natural environment, the complexity of ocean environments complicates the interpretation of metabolic response. It is important to remember that FMR is influenced by a variety of *in situ* parameters; therefore, experiments are needed to identify the influences of numerous abiotic (dissolved oxygen, pH, salinity) and biotic (predator-prey interactions, food availability) factors before

confidently attributing differences in FMR between and within populations to environmental temperature.

Despite these limitations, two key takeaways at different levels of biological organisation emerge from this study: 1) the opportunity to examine *in situ* measurements of FMR in relation to theoretical SMR can provide insight on the potential physiological pathways of energy allocation in response to changing environmental conditions within populations, and 2) the combined examination of mean FMR and FMR-thermal sensitivity demonstrate patterns that are consistent with the metabolic cold adaptation hypothesis, suggesting that population-specific thermal adaptation might be at play. It is important to remember that the 3:1 FMR-SMR relationship only provides a loose metric of establishing the pathways of metabolic energy, and this metric might be further complicated given the real-world nature of the data. However, detecting the potential pathways for energy allocation can provide insights on climate impacts at higher levels of biological organization, and future studies might pair *in situ* FMR studies with laboratory meaures of oxygen consumption to provide more robust estimates of SMR. Differentiating maintenance costs from SDA and activity metabolism energy allocation can highlight climate impacts at both community (activity-induced species interactions) or ecosystem (high SDA = elevated resource use) levels.

Here, I find that the pulse- and population-specific differences in the otolith-isotope inferred field metabolic rate suggests physiological diversity in the response of juvenile Atlantic cod to their cold environment. We discover that examining pulse-specific differences in mean FMR, the FMR-SMR relationship, and thermal sensitivity prior to the overwintering period can provide insight on *in situ* behavioural and physiological tradeoffs. However, it is the success of

these strategies that are especially important when considering the implications of these data on recruitment to older age groups. Therefore, future studies should compare the FMR estimates of the "survivors" after the overwintering period, in combination with pulse-specific FMR estimates prior to winter. The *in situ* FMR data I have collected in this study is also a preliminary application of the otolith-isotope method, providing relevant data on year-class recruitment strength to stock assessments and coastal time series. By routinely quantifying pulse-specific measures of mean FMR and FMR-thermal sensitivity in juvenile Atlantic cod populations of the Northwest Atlantic, *in situ* energetic response to more variable ocean dynamics can be used as a tool to better support fisheries management.

Figures

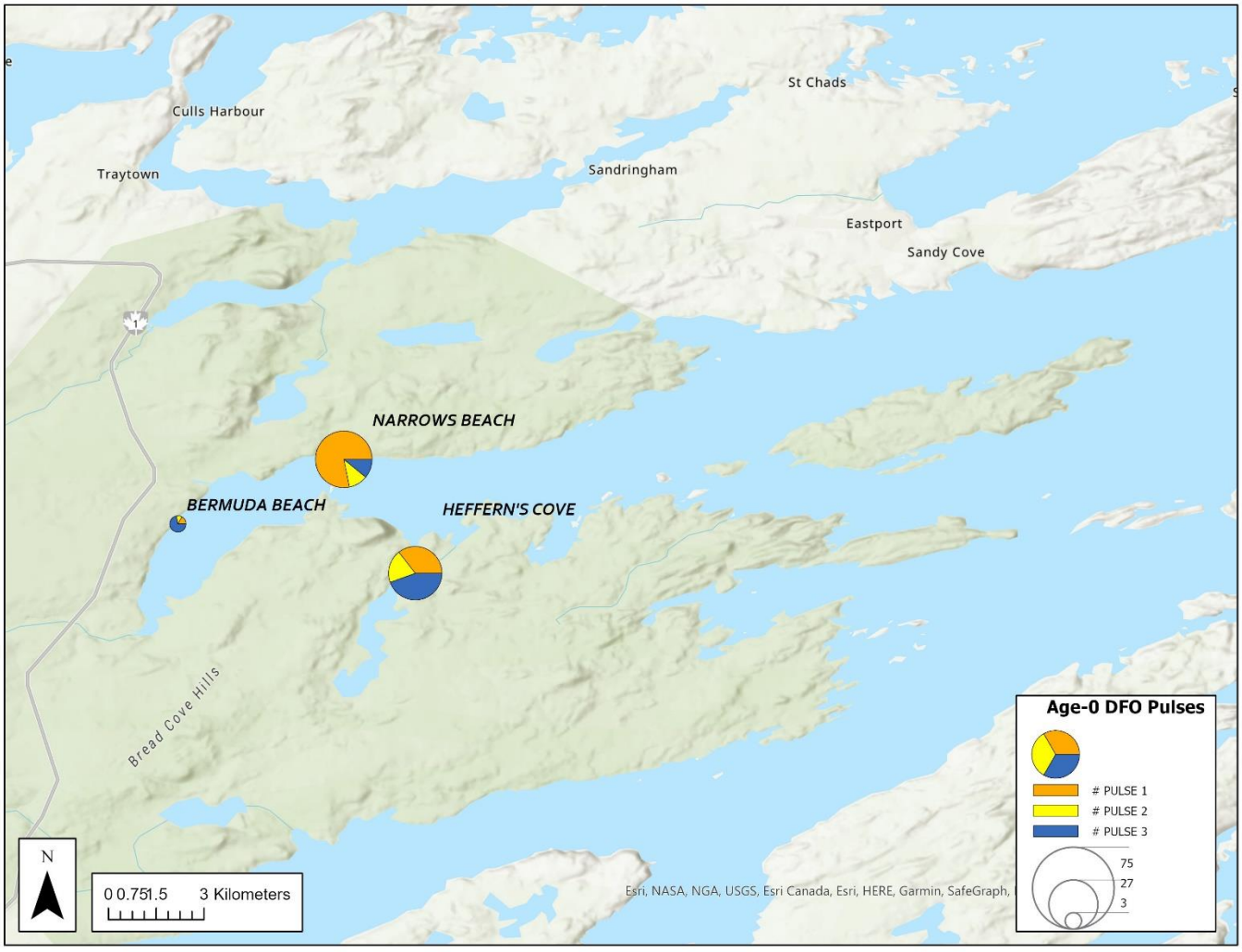


Figure 3.1. A map of Newman Sound, Bonavista Bay, Terra Nova National Park, Newfoundland, indicating the three sampling sites (Heffern's Cove, Bermuda Beach, Narrows Beach). The size of the circle at each site is proportional to the number of age-0 collected for otolith isotope analysis, while the pie charts represent the proportion of sampled age-0 individuals from verified pulse classifications (Gregory et al. 2021), representing pulses 1 (orange), 2 (yellow), and 3 (blue).

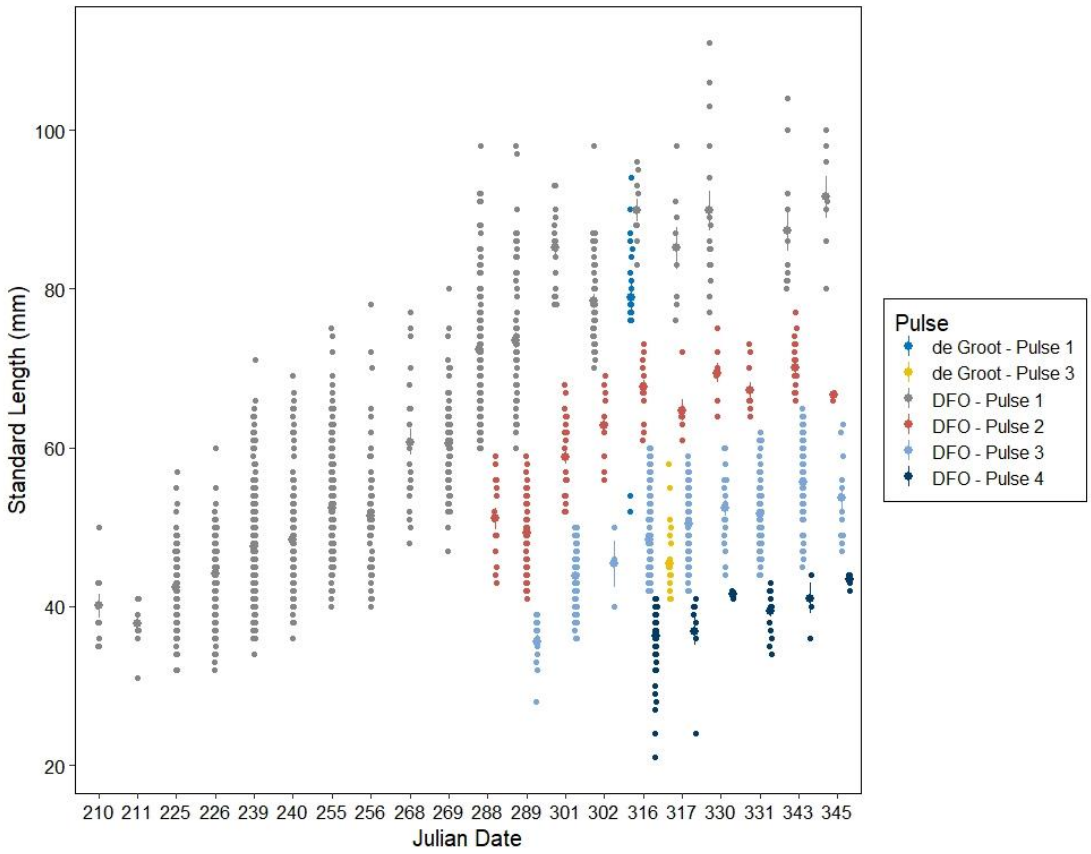


Figure 3.2. The sizes of age-0 Atlantic cod captured by beach seine in Newman Sound, Bonavista Bay from July 29th to December 11th 2019 (Julian date 210-345), and their potential settlement pulse structure. The light blue and yellow points represent the sampled pulse 1 and 3 Atlantic cod used in this study, and demonstrate a general consistency in pulse-specific size and growth when compared to the larger population dataset.

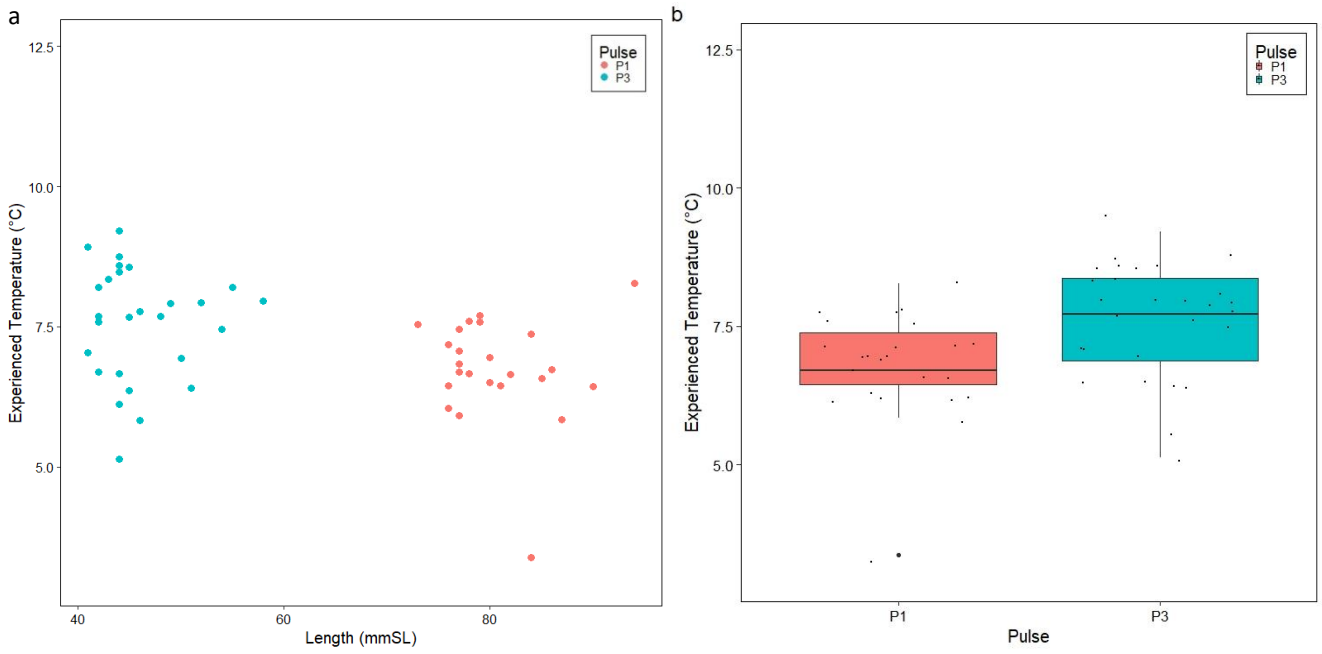


Figure 3.3. Individual reconstructed experienced temperature (°C) plotted against (a) standard length and (b) age-0 Atlantic cod 2019 cohort pulse classification. The box in the boxplot represents the 25–75 percentile, the horizontal line in the box is median, and the vertical line is the box ± 1.5 times IQR (interquartile range). The jitter points on the box plot represent individual observations shifted by a random value to distinguish overlapping data points. Here, the jitter is shifted by 30% vertically and horizontally in both positive and negative directions, occupying a total of 60% of the original resolution of the data. The relationship between individual reconstructed experienced temperature and length is consistent with the pulse structure for the age-0 Atlantic cod 2019 cohort.

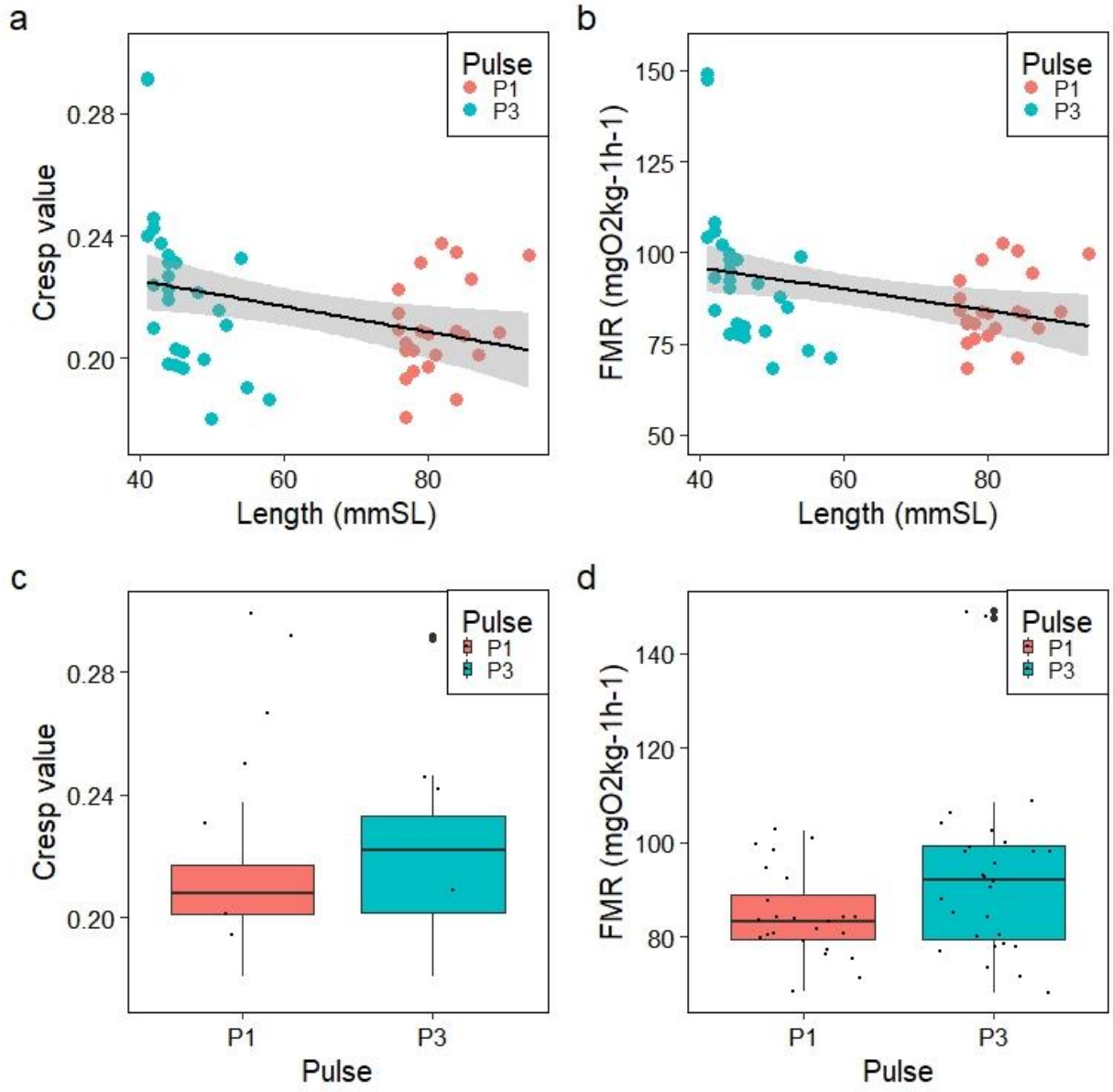


Figure 3.4. Individual reconstructed C_{resp} (a, c) and FMR (b, d) values plotted against (a, b) standard length and (c, d) age-0 Atlantic cod 2019 cohort pulse classification. The grey areas in the simple linear regressions are the 95% confidence interval, while the boxes in the boxplots represents the 25–75 percentile, the horizontal line in the box is median, and the vertical line is the box ± 1.5 times IQR (interquartile range). The jitter points on the box plot represent individual observations shifted by a random value to identify any potential hidden trends. Here, the jitter is shifted by 30% vertically and horizontally in both positive and negative directions, occupying a total of 60% of the original resolution of the data. The relationship between individual reconstructed C_{resp}/FMR and length is consistent with the pulse structure for the age-0 Atlantic cod 2019 cohort.

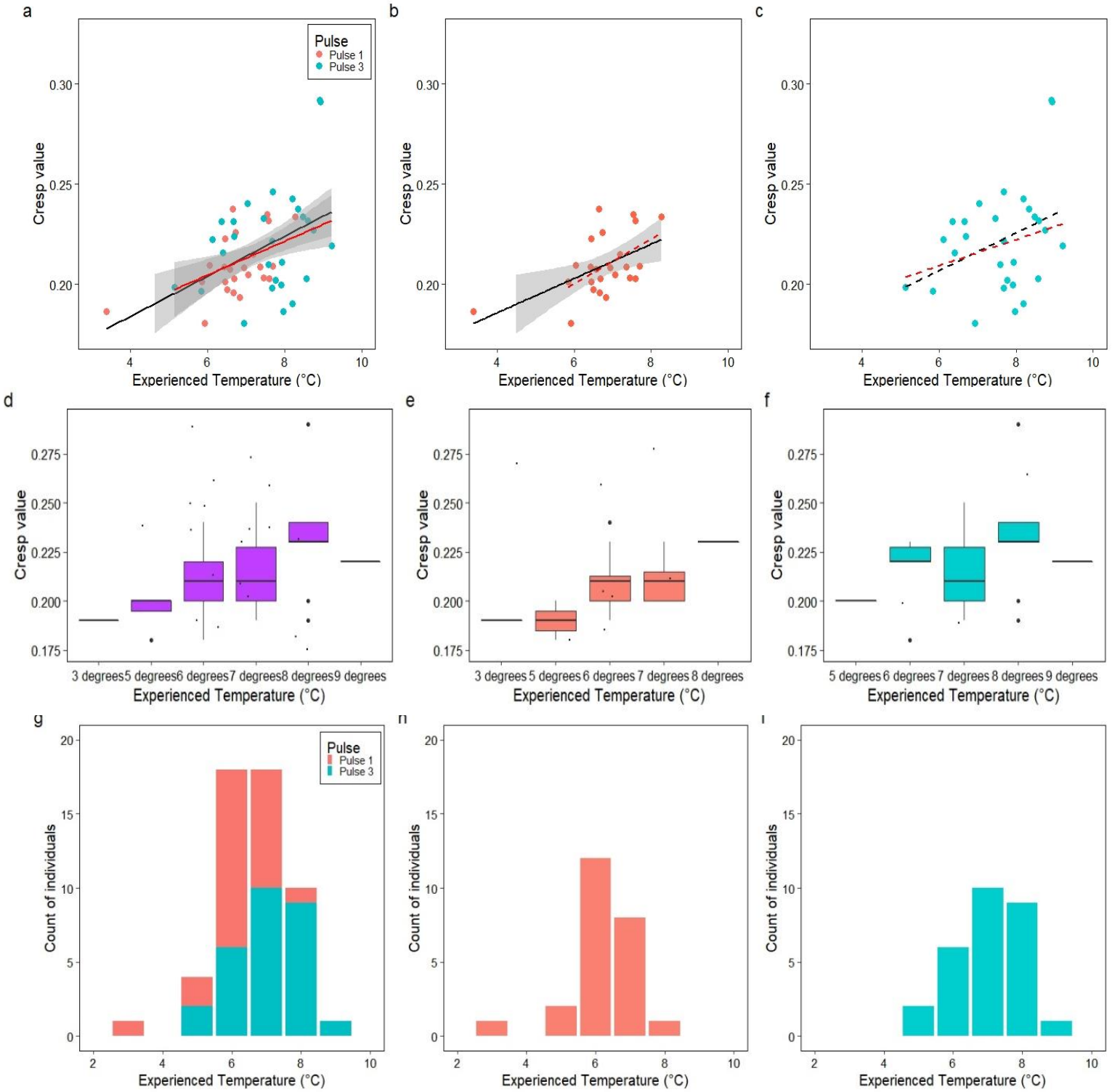


Figure 3.5. The relationship of estimated C_{resp} values and temperature among (a, d, g) all individuals, (b, e, h) pulse 1 and (c, f, i) pulse 3 individuals. Individual C_{resp} values are plotted against experienced temperature (a-c) with outliers (black line) and without (red line). The grey areas in the simple linear regressions are the 95% confidence interval, and represent significant relationships between C_{resp} values and experienced temperature. The dashed lines without the confidence intervals represent a lack of significance between experienced temperature and C_{resp} values. The boxes in the boxplots represent the 25–75 percentile, the horizontal line in the box is median, and the vertical line is the box ± 1.5 times IQR (interquartile range). The jitter points on the box plot represent individual observations shifted by a random value to identify any potential hidden trends. Here, the jitter is

shifted by 30% vertically and horizontally in both positive and negative directions, occupying a total of 60% of the original resolution of the data.

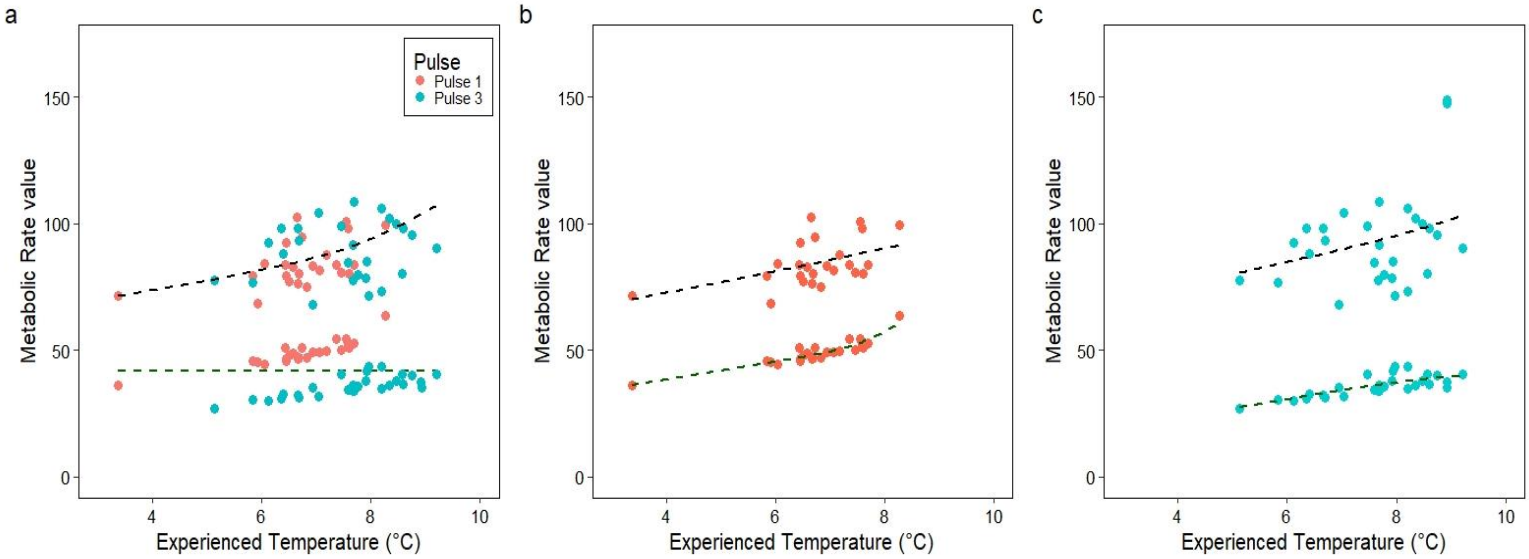


Figure 3.6. The thermal relationship of field metabolic rate (FMR - black line) in relation to theoretical standard metabolic rate (SMR - dark green line) and temperature among (a) all individuals, (b) pulse 1 individuals and (c) pulse 3 individuals. Individual FMR and SMR are plotted corresponding to experienced temperature. The theoretical SMR was calculated in accordance with the Metabolic Theory of Ecology.

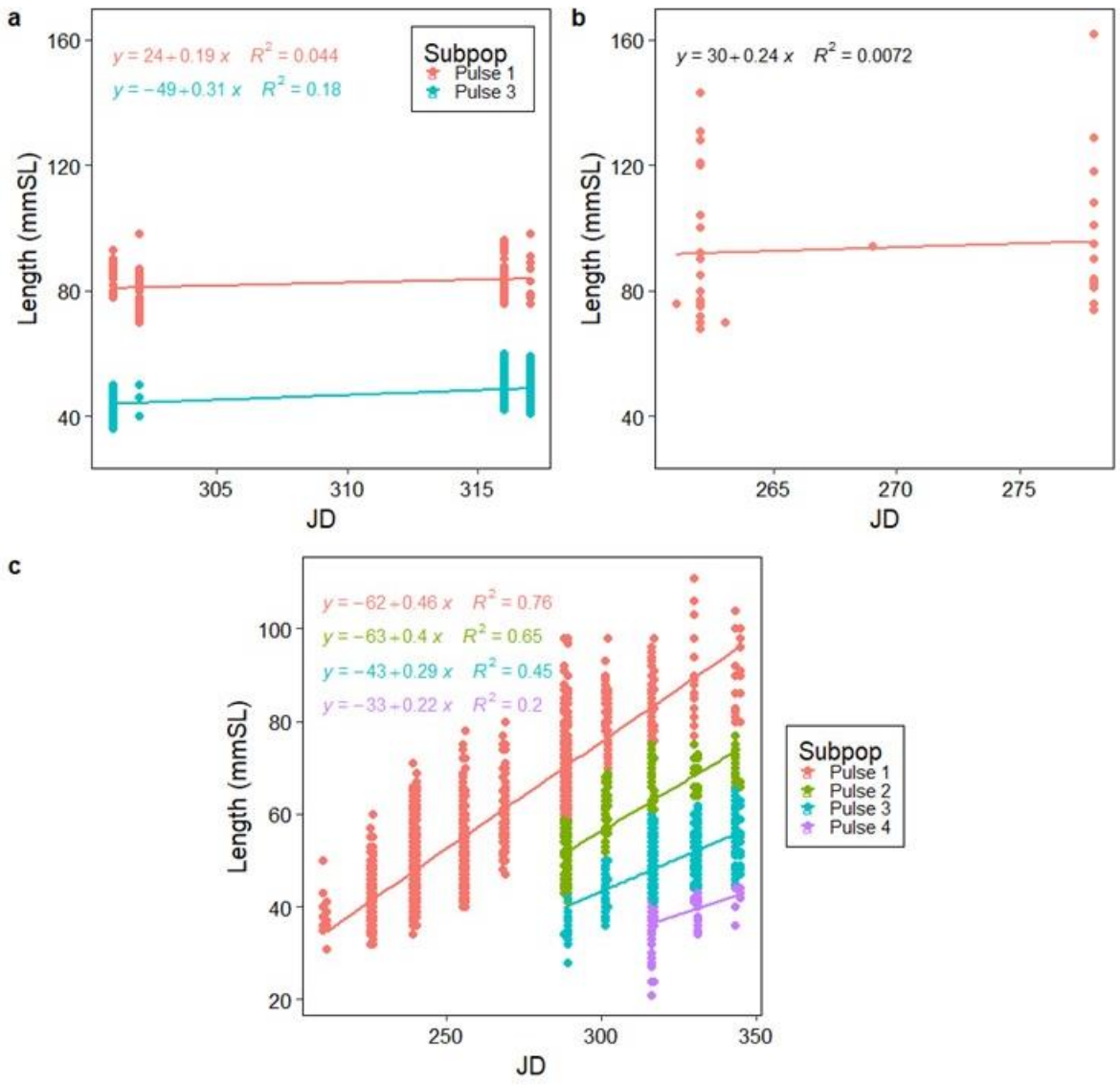


Figure 3.7. Growth rate regressions plotting Julian date (JD) versus standard length (mm) for juvenile Atlantic cod from a) age-0 pulses 1 and 3, collected in Newman Sound, Newfoundland and b) age-0 Atlantic cod, collected on the Skagerrak coast of Southern Norway. The growth rate for the Newman Sound population was determined using pulse as a random effect. Full Newman Sound population data reported in Gregory et al. (2021) c) demonstrates how the growth rate value shifts given a larger range of temporal data. The growth rate value for a) and b) are therefore only used for growth rate comparisons, and not as a stand-alone representation of growth for sampled individuals from the Newman Sound and Skagerrak populations.

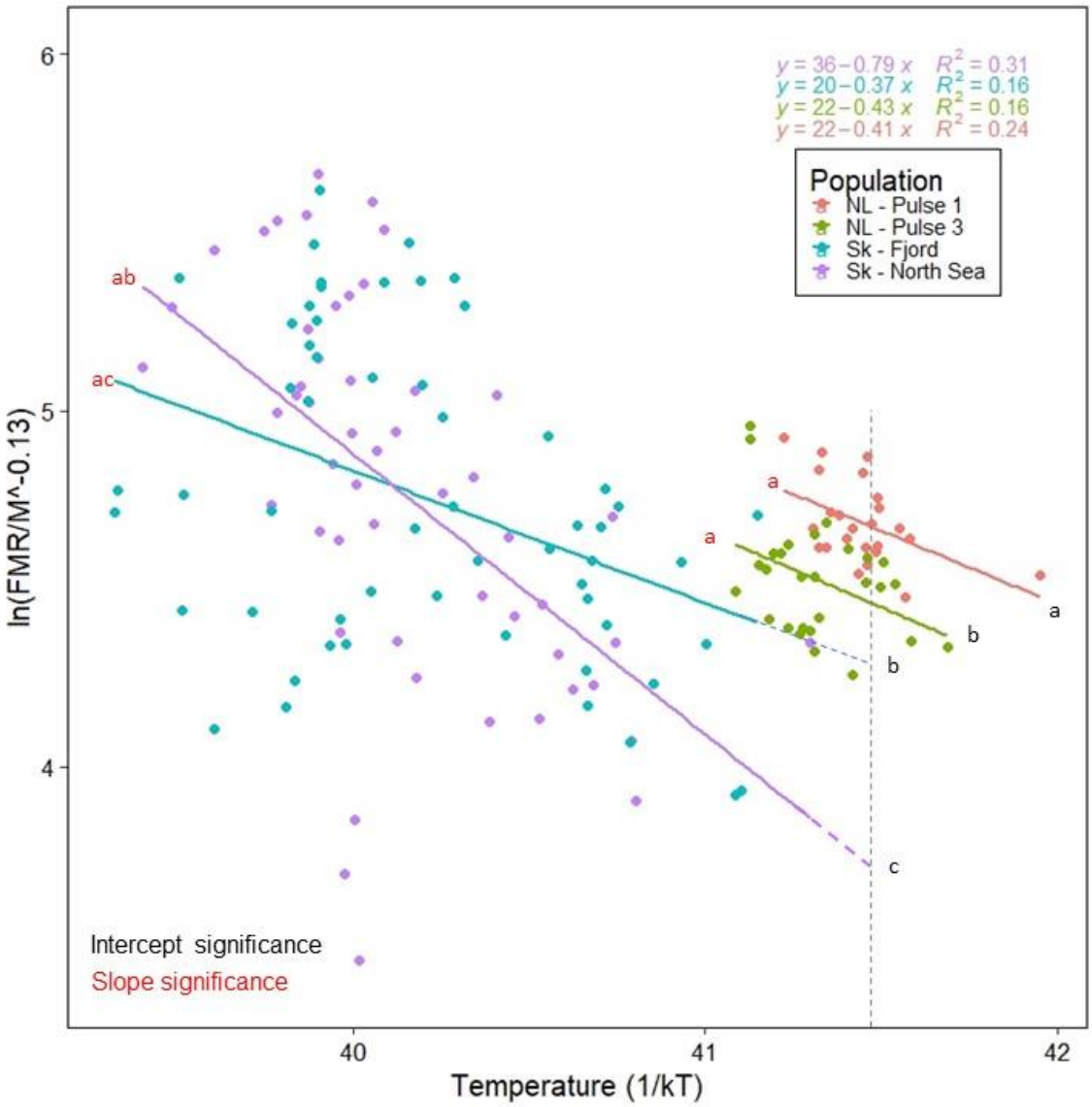


Figure 3.8. The relationship of field metabolic rate (FMR) and temperature following the Arrhenius model for pulses 1 and 3 from the Newman Sound population, and for the Fjord and North Sea ecotypes of the Skagerrak population. k is Boltzmann's constant (8.62 × 10−5eVK−1), T is absolute temperature in K and M is body mass in grams. The grey dashed line represents the temperature (6.4°C) at which mean FMR for each group was determined. Significant differences in mean FMR were determined between populations (Newman Sound vs Skagerrak) and within populations (pulse 1 versus pulse 3, Fjord versus North Sea ecotypes). The linear trends for the Fjord and North Sea ecotypes were extrapolated to predict the mean FMR at 6.4°C. The red letters at the top left corner of each linear regression demonstrates the significant difference between the slope of each population/ecotype/pulse (FMR thermal sensitivity), while the black letters to the right of the dashed line represents the significant difference between the intercept of each population/ecotype/pulse (mean FMR). The regression slopes themselves are all significantly different from 0 (Pulse 1: F-value = 7.08, $p = 0.014$, $R^2 = 0.209$, Pulse 3: F-value = 4.93, $p = 0.035$, $R^2 = 0.127$, North Sea: F-value = 22.0, $p << 0.01$, $R^2 = 0.29$; Fjord: F-value = 10.15, $p < 0.01$, $R^2 = 0.14$).

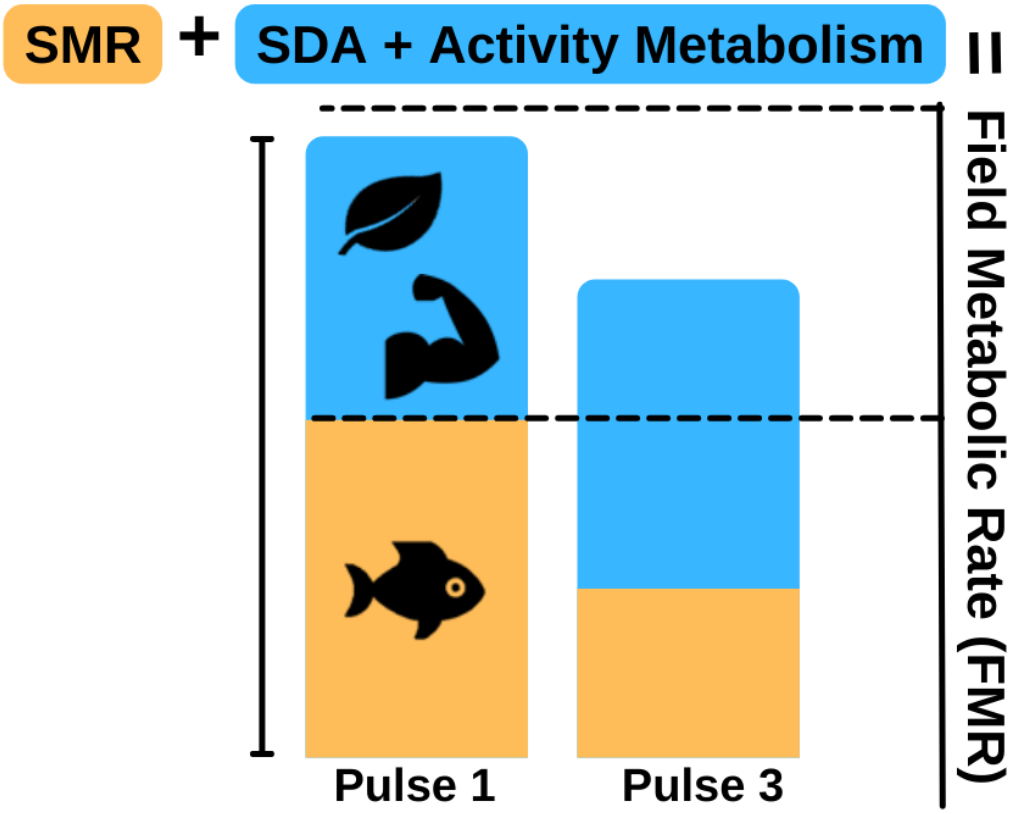


Figure 3.9. A conceptual representation showing the approximate proportion of theoretical SMR estimated from the FMR-SMR relationship compared to the magnitude of the predicted otolith-inferred mean FMR calculated through the Arrhenius equation. This figure suggests that, while the mean FMR can be higher for a subgroup of individuals, it does not necessarily mean that this increase is due to changes associated with SDA or activity metabolism. In this example, pulse 1 and pulse 3 Atlantic cod individuals demonstrate different mean FMR values (height of bars), and predicted energy allocations to SMR (yellow) and SDA+Activity metabolism (blue). Determining the proportion of the energetic budget allocated to maintenance costs (SMR) can provide insight on the potential mechanism associated with energy allocation (physiological or behavioural), hinting at how individuals interact within their ecosystem under periods of environmental stress.

Tables

Table 3.1. The recruitment pulses of age-0 Atlantic cod (Pulse 1-3) and the associated standard length measurement range specific to each sampling period.

Sampling Period	Time-Length Based Pulse Classification		
	Pulse 1	**Pulse 2**	**Pulse 3**
October 15-16, 2019	60 - 98 mmSL	41 - 59 mmSL	28 - 40 mmSL
November 12-13, 2019	76 – 110 mmSL	61 – 75 mmSL	38 – 60 mmSL

Table 3.2. A summary of the sample information used in this study, including the resulting experienced temperature, Cresp, FMR values estimated from the otolith information, and the calculated theoretical SMR (Equations 1, 2, 3, 4). The information for all individuals are shown in the Supporting information.

OTOLITH AND ENVIRONMENTAL DATA								*EQ 1:* Exp Temp	*EQ 2:* CRESP	*EQ 3:* FMR	EQ4:	
Pulse #	AC ID	Weight (g)	Length (mmSL)	otolith d13C	otolith d18O	diet d13C	Water DIC d13C				Theo. SMR	FMR:SMR
1	HC 20	7.82	87	-2.92	0.48	-21.81	1.83	5.85	0.20	79.35	45.80	1.73
1	HC 25	12.15	94	-3.48	0.00	-20.91	1.83	8.27	0.23	99.62	63.41	1.57
1	HC 35	7.02	77	-2.40	0.47	-21.60	1.83	5.92	0.18	68.28	45.10	1.51
1	HC52	6.28	77	-2.97	0.24	-21.59	1.83	7.06	0.20	81.52	49.17	1.66
3	HC 60	0.97	44	-3.77	-0.04	-22.13	1.83	8.47	0.23	99.78	38.03	2.62
3	HC61	1.00	46	-2.98	0.10	-21.98	1.83	7.77	0.20	79.87	35.77	2.23
3	HC64	0.93	44	-3.39	-0.19	-21.97	1.83	9.21	0.22	90.19	40.43	2.23
3	HC65	1.09	44	-3.57	-0.10	-21.95	1.83	8.75	0.23	95.30	40.01	2.38

References

Beacham TD, Gregory RS, Brattey J, Station PB, Centre OS (2000) Origins of two recruitment pulses of 0-group Atlantic cod (Gadus morhua) in Bonavista Bay, Newfoundland during 1999, determined from genetic variation. *Canadian Stock Assessment Secretariat*.

Benetti, M., Reverdin, G., Pierre, C., Khatiwala, S., Tournadre, B., Olafsdottir, S., & Naamar, A. (2016). Variability of sea ice melt and meteoric water input in the surface Labrador Current off Newfoundland. *Journal of Geophysical Research: Oceans*, 121(4), 2841-2855.

Bokhorst, SF, Bjerke, JW, Tømmervik, H, Callaghan, TV, Phoenix, GK (2009) Winter warming events damage sub-Arctic vegetation: consistent evidence from an experimental manipulation and a natural event. *J Ecol*, *97*: 1408-1415.

Brown JH, Gillooly JF, Allen AP, Savage VM, West GB (2004) Towards a metabolic theory of ecology. *Ecology* 85: 1771–1789.

Brown JH, Sibly RM (2012) The metabolic theory of ecology and its central equation. *Metabolic Ecology: A Scaling Approach* 21–33.

Chantraine, P (1993) The last cod-fish: life and death of the Newfoundland way of life (Vol. 1). Robert Davies Publications.

Clarke A (2006) Temperature and the metabolic theory of ecology. *Funct Ecol* 20: 405–412.

Clarke A, Rothery P, Isaac NJB, Building M, Lane B, Gifford C (2010) Scaling of basal metabolic rate with body mass and temperature in mammals. *J Anim Ecol* 79: 610–619.

Colbourne EB (2004) Decadal changes in the ocean climate in Newfoundland and Labrador waters from the 1950s to the 1990s. *Journal of Northwest Atlantic Fishery Science* 34: 43–61.

Contosta AR, Casson NJ, Garlick S, Nelson SJ, Ayres MP, Burakowski EA, Campbell J, Creed I, Eimers C, Evans C, *et al.* (2019) Northern forest winters have lost cold, snowy conditions that are important for ecosystems and human communities. *Ecol Appl* 29: 1–24.

Copeman LA, Parrish CC, Gregory RS, Wells JS (2008) Decreased lipid storage in juvenile Atlantic cod (Gadus morhua) during settlement in cold-water eelgrass habitat. *Mar Biol* 154: 823–832.

COSEWIC (2003) COSEWIC assessment and update status report on the status of Atlantic cod Gadus morhua in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. xi-76

Cyr F, Galbraith PS (2021) A climate index for the Newfoundland and Labrador shelf 1807–1828.

Farmer, TM, Marschall, EA, Dabrowski, K, & Ludsin, SA (2015) Short winters threaten temperate fish populations. *Nat Commun* 6: 1-10.

Flynn, EE, Todgham, AE (2018) Thermal windows and metabolic performance curves in a developing Antarctic fish. *J Comp Physiol B*, 188: 271-282.

Gaitán-Espitia JD, Villanueva PA, Lopez J, Torres R, Navarro JM, Bacigalupe LD (2017) Spatio-temporal environmental variation mediates geographical differences in phenotypic responses to ocean acidification. *Biol Lett* 13. doi:10.1098/rsbl.2016.0865

Geissinger EA, Gregory RS, Laurel BJ, Snelgrove PVR (2021) Food and initial size influence overwinter survival and condition of a juvenile marine fish (age-0 Atlantic cod). *Can J Fish Aquat Sci* 482: 472–482.

Geissinger EA, Gregory RS, Laurel BJ, Snelgrove PVR (2022) High site-fidelity and low mortality of juvenile Atlantic cod (Gadus morhua) in subarctic coastal habitat during their first winter. *ICES Journal of Marine Science* 79: 1408–1418.

Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M., & Charnov, E. L. (2001). Effects of size and temperature on metabolic rate. *Science*, *293*(5538), 2248–2251. https://doi.org/10.1126/science.1061967

Gregory, RS, Geissinger, EA, Newton, BH, Macrobert, E, Lancaster D. Submitted. Strength of three cohorts (2018-20) of Atlantic cod, from nearshore surveys of demersal age 0 and 1 juveniles in Newman Sound, Bonavista Bay relative to the 2J3KL stock. *DFO Can Sci Advis Sec (CSAS) Res Doc* 2021/nnn. (submitted 2021-3-26)

Gregory, RS., Dalley, KL, Newton, BH, Sargent, PS, & Cooke, EL (2019). Relative strength of three cohorts (2015-17) of Atlantic Cod, from nearshore surveys of demersal age 0 and 1 juveniles in Newman Sound, Bonavista Bay. *DFO Canadian Science Advisory Secretariat (CSAS), Res Doc* 2019/012.

Hazel, J. R., & Prosser, C. L. (1974). Molecular mechanisms of temperature compensation in poikilotherms. *Physiological reviews*, 54(3), 620-677.

Heintz RA, Siddon EC, Farley E v., Napp JM (2013) Correlation between recruitment and fall condition of age-0 pollock (Theragra chalcogramma) from the eastern Bering Sea under varying climate conditions. *Deep Sea Res 2 Top Stud Oceanogr* 94: 150–156.

Hill R, A. Wyse G, Anderson M (2012) Themes in the Study of Animal Physiology, Third Edition. Science of the Total Environment. Sinauer Associates, Sunderland.

Høie, H., Otterlei, E., & Folkvord, A. (2004). Temperature-dependent fractionation of stable oxygen isotopes in otoliths of juvenile cod (Gadus morhua L.). *ICES Journal of Marine Science*, *61*, 243–251. https://doi.org/10.1016/j.icesjms.2003.11.006

Humphries, M.M, Umbanhowar, J (2007) Filtering environmental variability: activity optimization, thermal refuges, and the energetic responses of endotherms to

temperature. In *The impact of environmental variability on ecological systems* (pp. 61-87). Springer, Dordrecht.

Ings DW, Gregory RS, Schneider DC (2008) Episodic downwelling predicts recruitment of Atlantic cod, Greenland cod and white hake to Newfoundland coastal waters. *J Mar Res* 66: 529–561.

Knutsen H, Jorde PE, Hutchings JA, Hemmer-Hansen J, Grønkjær P, Jørgensen KEM, André C, Sodeland M, Albretsen J, Olsen EM (2018) Stable coexistence of genetically divergent Atlantic cod ecotypes at multiple spatial scales. *Evol Appl* 11: 1527–1539.

Kreyling, J (2010) Winter climate change: a critical factor for temperate vegetation performance. *Ecology*, 91: 1939-1948.

Lannig, G., Eckerle, L., Serendero, I., Sartoris, F. J., Fischer, T., Knust, R., ... & Pörtner, H. O. (2003). Temperature adaptation in eurythermal cod (*Gadus morhua*): a comparison of mitochondrial enzyme capacities in boreal and Arctic populations. *Marine Biology*, 142: 589-599.

Lardies MA, Bacigalupe LD, Bozinovic F (2004) Testing the metabolic cold adaptation hypothesis: an intraspecific latitudinal comparison in the common woodlouse. *Evol Ecol Res* 6: 567–578.

Lemke P, Ren JF, Allison I, Carrasco JF (2007) Observations: changes in snow, ice and frozen ground. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL, eds. Climate Change 2007: Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change . Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Lunzmann-Cooke E, Gregory R, Snelgrove P, Cote D, Fuentes-Yaco C (2021) Spatial, temporal, and environmental influences on Atlantic cod Gadus morhua offshore recruitment signals in Newfoundland. *Mar Ecol Prog Ser* 673: 151–164.

Martino JC (2020) Experimental support towards a metabolic proxy in fish using otolith carbon isotopes. *J Exp Biol* 223 doi:10.1242/jeb.217091

Morley JW, Batt RD, Pinsky ML (2017) Marine assemblages respond rapidly to winter climate variability. *Glob Chang Biol* 23: 2590–2601.

Myers RA, Cadigan NG (1995) Was an increase in natural mortality responsible for the collapse of northern cod? *Canadian Journal of Fisheries and Aquatic Sciences* 52: 1274–1285.

Olack, G. A., Colman, A. S., Pfister, C. A., & Wootton, J. T. (2018). Seawater DIC analysis: The effects of blanks and long-term storage on measurements of concentration and stable isotope composition. *Limnology and Oceanography: Methods*, *16*(3), 160–179.

Oltmanns M, Karstensen J, Moore GWK, Josey SA (2020) Rapid cooling and increased storminess triggered by freshwater in the north atlantic. *Geophys Res Lett* 47. doi:10.1029/2020GL087207

Pauli JN, Zuckerberg B, Whiteman JP, Porter W (2013) The subnivium: A deteriorating seasonal refugium. *Front Ecol Environ* 11: 260–267.

Penczykowski, RM, Connolly, BM, Barton, BT. (2017). Winter is changing: Trophic interactions under altered snow regimes. *Food Webs*, 13: 80–91. https://doi.org/10.1016/j.fooweb.2017.02.006

Pettersen AK, Marshall DJ, White CR (2018) Understanding variation in metabolic rate. *Journal of Experimental Biology* 221. doi:10.1242/jeb.166876

Pörtner HO, Berdal B, Blust R, Brix O, Colosimo A, de Wachter B, Giuliani A, Johansen T, Fischer T, Knust R, *et al.* (2001) Climate induced temperature effects on growth performance, fecundity

and recruitment in marine fish: Developing a hypothesis for cause and effect relationships in Atlantic cod (Gadus morhua) and common eelpout (Zoarces viviparus). *Cont Shelf Res* 21: 1975–1997.

Pörtner HO, Bock C, Knust R, Lannig G, Lucassen M, Mark FC, Sartoris FJ (2008) Cod and climate in a latitudinal cline: Physiological analyses of climate effects in marine fishes. *Clim Res* 37: 253–270.

Read JF, Gould WJ (1992) Cooling and freshening of the subpolar North Atlantic Ocean since the 1960s. *Nature* 360: 55–57.

Regular PM., Buren, AD, Dwyer, KS, Cadigan, NG, Gregory, RS, Koen-Alonso, M, Rideout, RM, Robertson, GJ, Robertson, MD, Stenson, GB, Wheeland, LJ, Zhang, F (2022) Indexing starvation mortality to assess its role in the population regulation of northern cod. *Fish Res* 247. doi.org/10.1016/j.fishres.2021.106180 Accepted 2021-11-12; MS# Fish11159

Roemmich D, John Gould W, Gilson J (2012) 135 years of global ocean warming between the Challenger expedition and the Argo Programme. *Nat Clim Chang* 2: 425–428.

Rose GA, Atkinson BA, Baird J, Bishop CA, Kulka DW (1994) Changes in distribution of Atlantic cod and thermal variations in Newfoundland waters, 1980-1992. *ICES Marine Science Symposia* 198: 542–552.

Schmidt, G. A., Forward modeling of carbonate proxy data from planktonic foraminifera using oxygen isotope tracers in a global ocean model, *Paleoceanography*, 14, 482-497, 1999.

Sodeland M, Jorde PE, Lien S, Jentoft S, Berg PR, Grove H, Kent MP, Arnyasi M, Olsen EM, Knutsen H (2016) “Islands of Divergence” in the atlantic cod genome represent polymorphic chromosomal rearrangements. *Genome Biol Evol* 8: 1012–1022.

Solomon CT, Weber PK, Cech JJ, Ingram BL, Conrad ME, Machavaram M V., Pogodina AR, Franklin RL (2006) Experimental determination of the sources of otolith carbon and associated isotopic fractionation. *Can J Fish Aquat Sci* 63: 79–89.

Sunday JM, Pecl GT, Frusher S, Hobday AJ, Hill N, Holbrook NJ, Edgar GJ, Stuart-smith R, Wernberg T, Watson RA, *et al.* (2015) Species traits and climate velocity explain geographic range shifts in an ocean-warming hotspot. *Ecol Lett* 18: 944–953.

Sutton AO, Studd EK, Fernandes T, Bates AE, Bramburger AJ, Cooke SJ, Hayden B, Henry HAL, Humphries MM, Martin R, *et al.* (2021) Frozen out: Unanswered questions about winter biology. *Environmental Reviews* 29: 431–442.

Sweeting, C. J., Barry, J., Barnes, C., Polunin, N. V. C., & Jennings, S. (2007). *Effects of body size and environment on diet-tissue δ 15 N fractionation in fishes*. *340*, 1–10. https://doi.org/10.1016/j.jembe.2006.07.023

Treberg JR, Killen SS, MacCormack TJ, Lamarre SG, Enders EC (2016) Estimates of metabolic rate and major constituents of metabolic demand in fishes under field conditions: Methods, proxies, and new perspectives. *Comp Biochem Physiol A Mol Integr Physiol* 202: 10–22.

von Biela VR, Newsome SD, Zimmerman CE (2015) Examining the utility of bulk otolith δ13C to describe diet in wild-caught black rockfish Sebastes melanops. *Aquat Biol* 23: 201–208.

Williams CM, Henry HAL, Sinclair BJ (2015) Cold truths: How winter drives responses of terrestrial organisms to climate change. *Biological Reviews* 90: 214–235.

Windle MJS, Rose GA, Devillers R, Fortin MJ (2010) Exploring spatial non-stationarity of fisheries survey data using geographically weighted regression (GWR): An example from the Northwest Atlantic. *ICES Journal of Marine Science* 67: 145–154.

CHAPTER 4: General Conclusion

In this thesis, I demonstrated the utility of the otolith-isotope method to infer field metabolic rate for the bony fishes, and how this can link individual physiology to larger ecosystem functions. I outline a multidisciplinary pathway (physiology, toxicology, animal behaviour, evolution, food web ecology) to tie the ecophysiological insights of this method into management and conservation strategies, and highlight potential research questions. I identify the biochemical processes which connect otolith stable isotopes to estimates of oxygen consumption, and explore the use of this method at differing resolutions and levels of precision, highlighting remaining gaps and limitations. I further contribute to expanding the current knowledge base of the use of the otolith-isotope method to infer *in situ* field metabolic rate measures for an important fisheries species. By following the otolith-isotope method outlined in the first section of this thesis, I reveal differential mean field metabolic rates and thermal-FMR sensitivity trends between juvenile Atlantic cod within (i.e., pulse classifications, Newman Sound) and across populations (i.e., Skagerrak Coast, Newman Sound).

The initial goal of this thesis was to examine FMR trends in both age-0 and age-1 individuals of the same cohort to predict overwintering survival. Here, I included a review chapter due to the inability to process the age-0 otoliths collected in the Fall of 2019 for over a year following the start of the COVID-19 pandemic. Laboratory closures and travel restrictions delayed the completion of the micromilling and stable isotope sampling stages in my research that began on the age-0 Atlantic cod otoliths at the National Oceanography Centre in March 2019. The inaccessibility to conduct data analyses on the age-0 Atlantic cod otoliths for over a year pushed back the planned analyses for age-1 otoliths individuals, and thus the overwintering predictions fell outside the time period of my master's research.

Here, I summarise the key concepts and findings associated with each chapter and discuss their overall implications to my thesis (Figure 4.1). I also outline important limitations and areas of improvement to consider, and identify considerations for the future of this method with regards to advancing the relevance of ecophysiological data in a management and conservation framework.

Thesis summary

Chapter 2 reviewed the otolith-isotope method and outlined how this method can fill the historic field metabolic rate research gap for bony fishes. This chapter provides researchers from various fields with a 'roadmap' by 1) outlining the unique importance of both the field metabolic rate and the stable-isotope method in a changing world, highlighting limitations in historic metabolic metrics and methods, 2) breaking down the physiological and biochemical processes involved in the otolith-isotope method, and 3) identifying important research goals (field and modelling) for various scientific fields in a conservation and management context (Figure 4.1). I highlight that, while the otolith-isotope method focuses on metabolic trends at the individual level, the *in situ* nature of physiological data provides links to higher levels of biological organisation, providing an opportunity to inform modelling efforts aimed at mitigating climatic and anthropogenic threats.

In Chapter 3, I examine context-dependent relationships between FMR and experienced ocean temperature to provide a base understanding of the effects of climate change on age-0 Atlantic cod pulses in the Northwest Atlantic, an ecosystem that experiences cool winters. This chapter addresses the primary steps of the application of the FMR method for management and conservation purposes outlined in Chapter 2 (Figure 4.2). I focus my research efforts between

and within populations of juvenile Atlantic cod experiencing climate variability (Step 1) and conduct a structural analysis of otolith $\delta^{13}C$ and $\delta^{18}O$ isotopes to infer individual-specific FMR values and their temperature experience (Step 2). I report concrete findings on the physiological traits (mean FMR, FMR-thermal sensitivity) of pulse 1 and 3 age-0 juvenile Atlantic cod from a subsample of the 2019 Newman Sound cohort, and provide potential insights on trends to daily energy requirements and habitat use (energy allocation pathways, population-specific thermal adaptation; Step 3; Figure 4.2).

While FMR-thermal sensitivity remained constant, unique pulse-specific trends in mean FMR were examined between pulse classifications (pulse 1 and 3) of the Newman Sound population, where pulse 1 individuals exhibited higher metabolic rates at the same temperature compared to pulse 3 individuals in 2019 (Figure 4.1). Energy allocation pathways cannot be confidently predicted; however, SMR:FMR relationships reveal pulse-specific variations in behavioural and physiological energetic strategies within the FMR metric (SMR, SDA, activity metabolism). This variation suggests physiological and behavioural plasticity between life history stages of Atlantic cod of the same population, and may reflect tradeoffs between field metabolic rate, temperature at development, and size prior to the overwintering period. Even estimated differentiations between behavioural (SDA, activity) and physiological (SMR) energy allocation strategies may provide some context to infer interactions at higher levels of biological organisation such as predator-prey interactions, resource use, and competition.

I took the FMR method one step further to understand whether FMR-thermal relationships in juvenile Atlantic cod also differ across populations experiencing different environmental conditions (Newman Sound vs. Skagerrak Coast). By comparing FMR-thermal

sensitivity and mean FMR trends at this higher level of biological organisation, I notice that cold adapted fish within their population range (Newman Sound pulses) are less sensitive to temperature change, and maintain higher metabolic rates, than warm adapted populations (Skagerrak coast ecotypes) at the cold edge of their range (Figure 4.1). This finding is consistent with the metabolic cold adaptation hypothesis, as variation in the magnitude of the FMR seems to be consistent with the environmental temperature to which individuals from different populations are adapted. This cross-population analysis of thermal FMR trends provides an early opportunity to standardise protocols, highlighting how parallels and differences in field collections and laboratory analyses across studies can potentially improve the precision in these comparisons.

Future directions

In this thesis I discribe linkages between individual-level field metabolic rate and their interactions at higher levels of biological organisation (daily energy requirements and habitat use), but these cannot be confidently predicted (Figure 4.2). There are two specific limitations concerning these pathways: 1) the inability to confidently separate the energy allocation pathways (SMR, SDA, and activity metabolism) within the FMR value, and 2) the inability to attribute physiological or behavioural mechanisms to one environmental factor alone (e.g., increased food availability or temperature during early development) within or between populations).

The use of the otolith-isotope method to estimate field metabolic rate provides a unique opportunity to examine energetic expenditure of free-ranging fish in their natural environment; however, the complexity of ocean environments can complicate the interpretation of metabolic

response. Various *in situ* parameters can influence estimates of field metabolic rate; therefore, I suggest that laboratory experiments be conducted alongside field experiments such as these in order to identify the influence of numerous abiotic (e.g., dissolved oxygen, pH, salinity) and biotic (e.g., predator-prey interactions, food availability) factors. Such research is necessary in order to confidently attribute differences in FMR between and among populations to factors such as environmental temperature. It is also a necessary step for researchers interested in taking individual-level FMR to infer trends at higher levels of biological organisation, such as habitat use (community-level interactions) and daily energy expenditure (to infer trophic dynamics). Extrapolating results in this way adds additional uncertainty. Future research aimed at comparing otolith-isotope inferred thermal FMR trends across populations will be required to standardise both field and laboratory methods, especially for analysing growth rate. This can be applied by targetting individuals with known comparable life-history stages, or by recovering growth rate determinations directly from the otolith using daily otolith increments (Jørgensen *et al.,* 2020).

In this thesis, I recover pulse- and population-specific differences in the otolith-isotope inferred field metabolic rate, suggesting physiological diversity in the response of age-0 juvenile Atlantic cod to the cooling waters in the Northwest Atlantic. Pulse-specific differences in the FMR-SMR relationship, the mean FMR, and the thermal sensitivity of FMR in age-0 Atlantic cod prior to the overwintering period can provide insight on *in situ* behavioural and physiological tradeoffs. However, a gap remains here, which prevents us from using this research to inform conclusions regarding differential survival, specifically, overwinter survival and recruitment success (Figure 4.2). Pulse-specific differences in mean FMR can either represent robust pre-determined optima or physiological plasticity in response to fluctuating conditions. It is the success of these energetic strategies that represent a crucial step towards understanding

overwinter survival. In order to mechanistically link pulse-specific variations in field metabolic rate estimates to differences in recruitment success, future studies should focus on sampling the overwinter "survivors" - the age-1 of the 2020 cohort.

I encourage the use of the otolith-isotope method in future fisheries studies to yield routine measures of mean FMR and FMR-thermal sensitivity in juvenile Atlantic cod. Varying oceanic conditions in the Northwest Atlantic will continue to affect vulnerable age-0 Atlantic cod in unpredictable ways, but recovering field metabolic rate can give us an *in situ* snapshot of physiological performance to infer recruitment success and overwinter survival. Building on this research may provide concrete steps toward integrating individual-level *in situ* physiological data into recruitment forecasting. My research indicates that studies incorporating field metabolic rate can guide management action in a changing climate.

Figures

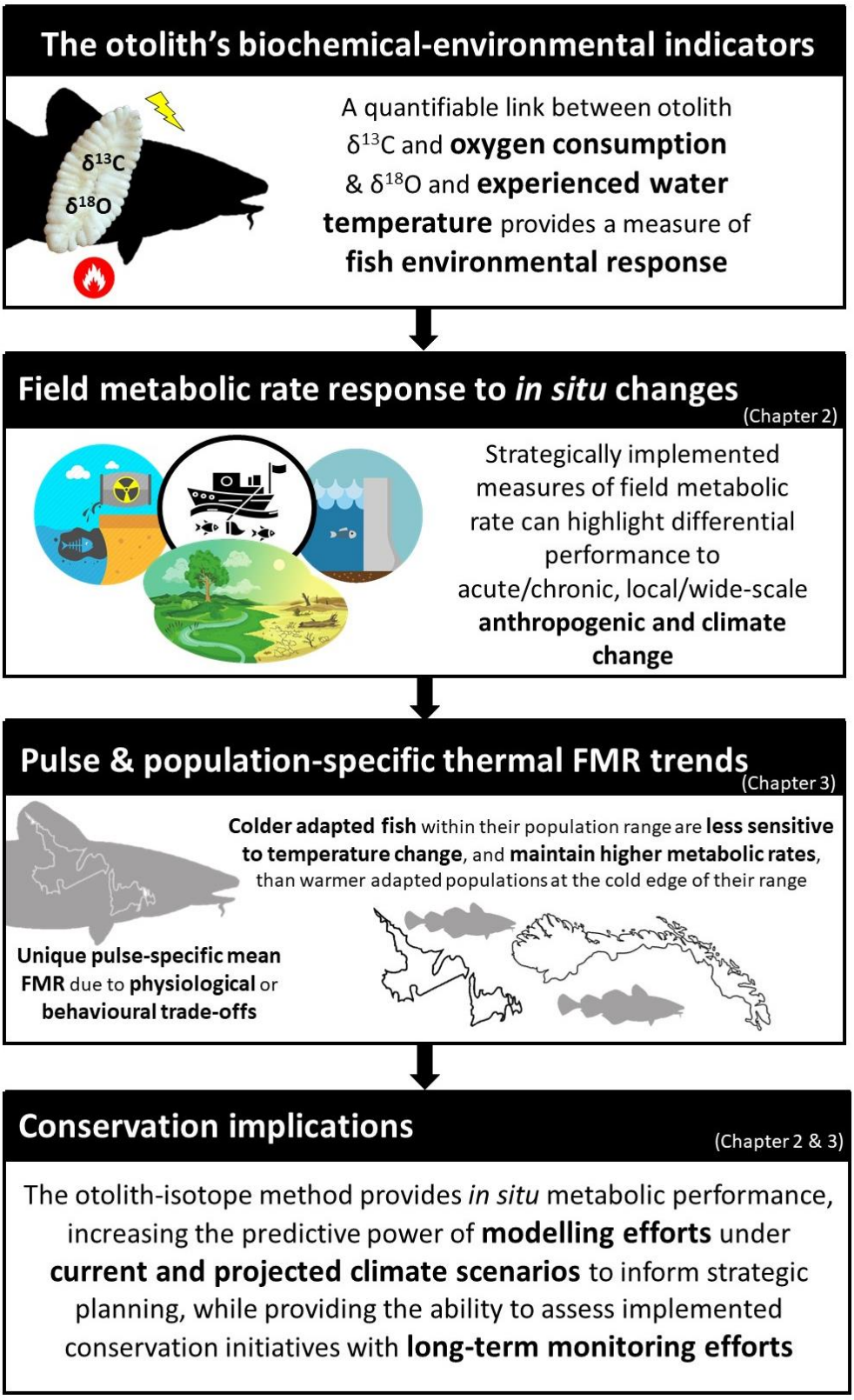


Figure 4.1. The major findings and broader implications explored in this thesis.

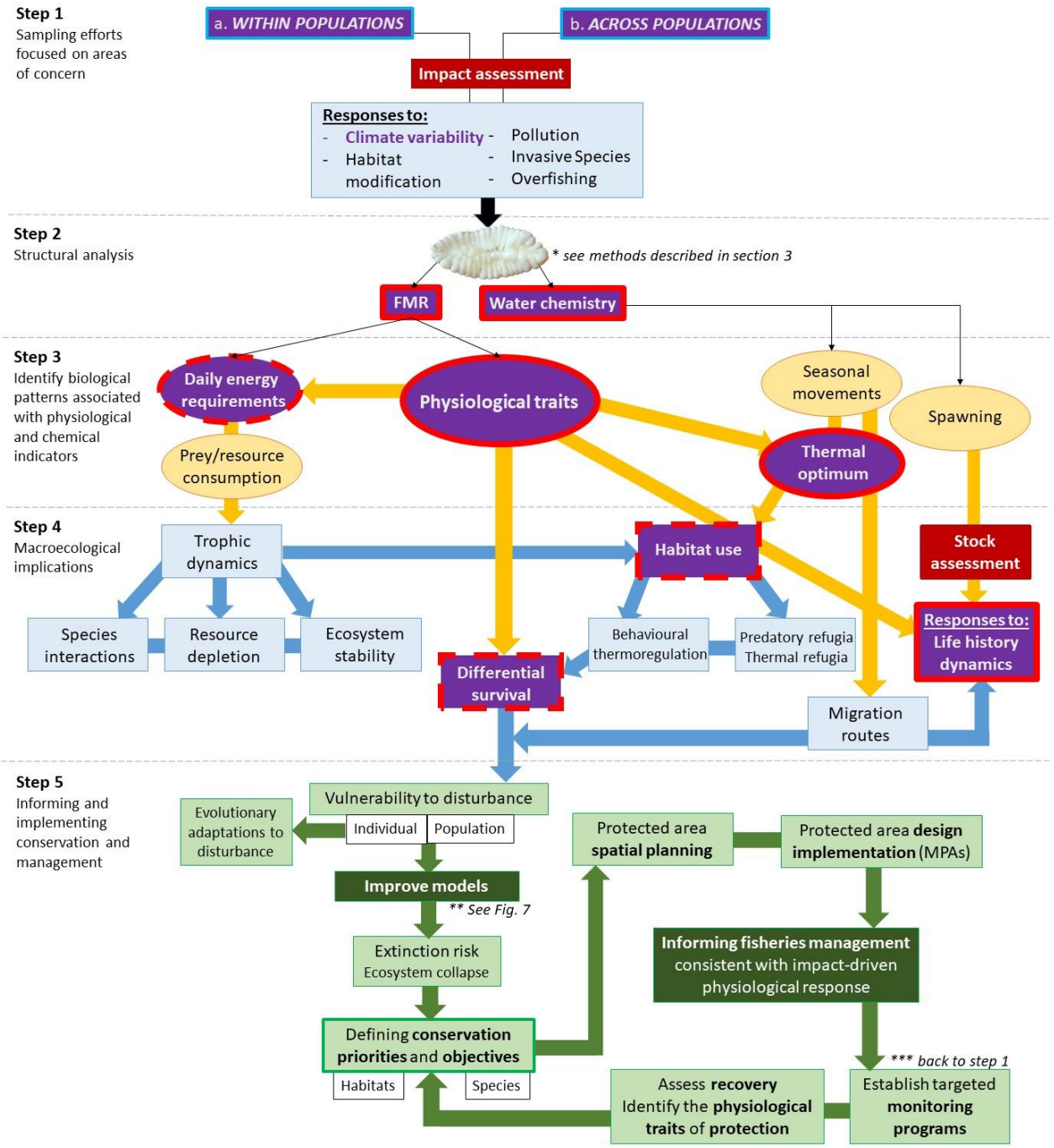


Figure 4.2. The phases for integrating the otolith-isotope method into conservation strategies outlined in Chapter 2. The purple pathway demonstrates the primary steps explored using the otolith-isotope method in Chapter 3 of this thesis. The boxes and circles displaying solid red lines represent concrete findings presented in this research, while the dashed lines represent topics for which insights were discussed and where future research efforts are necessary.

References

Jørgensen, K. E. M., Neuheimer, A. B., Jorde, P. E., Knutsen, H., & Grønkjær, P. (2020). Settlement processes induce differences in daily growth rates between two co-existing ecotypes of juvenile cod Gadus morhua. *Marine Ecology Progress Series*, *650*, 175-189.

Chapter 3 Atlantic cod FMR Analyses

Valesca de Groot

28 February, 2022

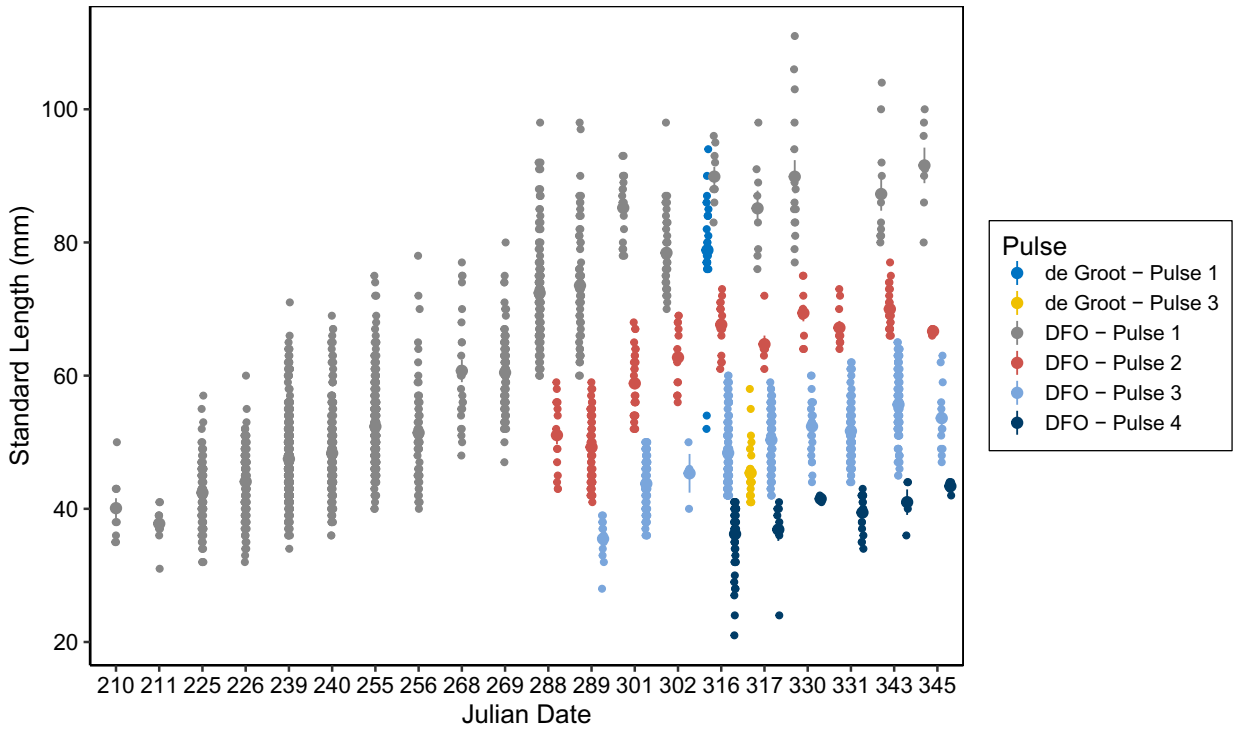
Contents

Newman Sound Dataset

Population pulse structure

The Atlantic cod population data for this study were collected from the age-0 2019 cohort found in Newman Sound, Bonavista Bay, Newfoundland in Terra Nova National Park. A regular monitoring program to assess cod recruitment has been conducted here since 1996. The individuals sampled by the Department of Fisheries and Oceans provides a representation of the larger population, and here, was overlaid with the individuals collected for the current study in order to place them in the context of the larger and broader population pulse structure.

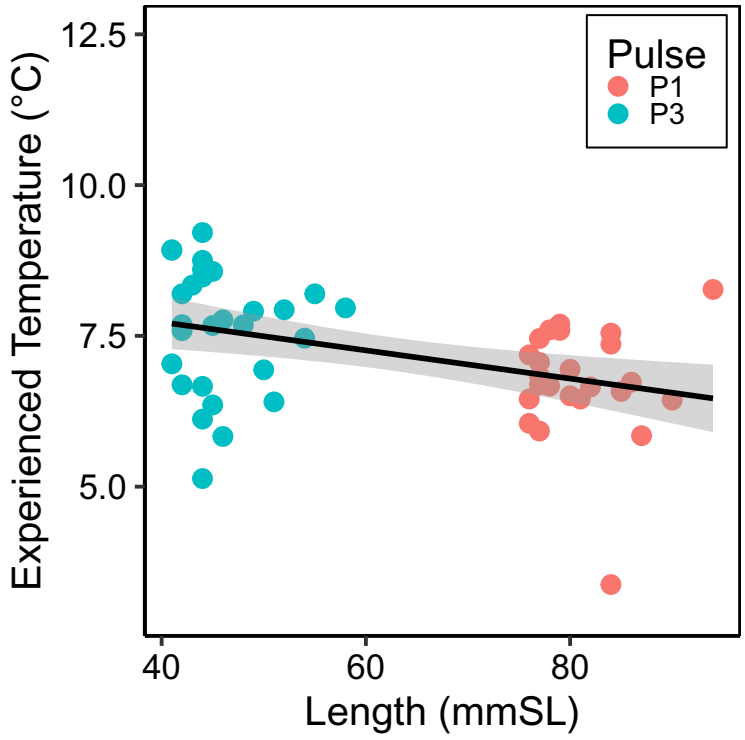
This was represented in a jitter plot representing the sizes of age-0 Atlantic cod captured by beach seine in Newman Sound, Bonavista Bay from July 29th to December 11th 2019 (Julian date 210-345), and their potential settlement pulse structure. The light blue and yellow points represent the sampled pulse 1 and 3 Atlantic cod used in this study, and demonstrate a general consistency in pulse-specific size and growth when compared to the larger population dataset.



Experienced Temperature and Atlantic cod size

Experienced Temperature vs Standard Length (mmSL)

Experienced temperature (°C) was regressed against standard length (mmSL) using a linear model.

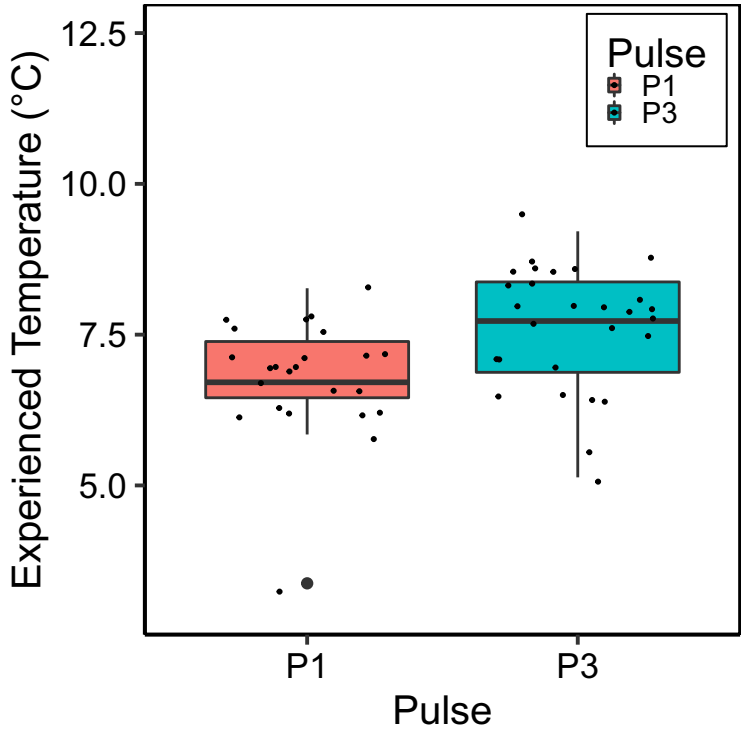



```
##
## Call:
## lm(formula = Temp ~ Len, data = TempLen)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -3.3211 -0.4621  0.0716  0.7029  1.8058
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)  8.664073   0.492122  17.606  < 2e-16 ***
## Len         -0.023408   0.007607  -3.077  0.00339 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.9916 on 50 degrees of freedom
## Multiple R-squared:  0.1592, Adjusted R-squared:  0.1424
## F-statistic: 9.469 on 1 and 50 DF,  p-value: 0.003387
```

Experienced Temperature vs Pulse Classification

Experienced temperature (°C) was plotted against age-0 juvenile Atlantic cod classifications assigned by the Department of Fisheries and Oceans based on date-length settlement pulse structure for the Newman Sound age-0 juvenile Atlantic cod 2019 cohort.

The jitter points on the box plot represent individual observations shifted by a random value to identify any potential hidden trends. Here, the jitter is shifted by 30% vertically and horizontally in both positive and negative directions, occupying a total of 60% of the original resolution of the data. All the following boxplots will follow this same rule.

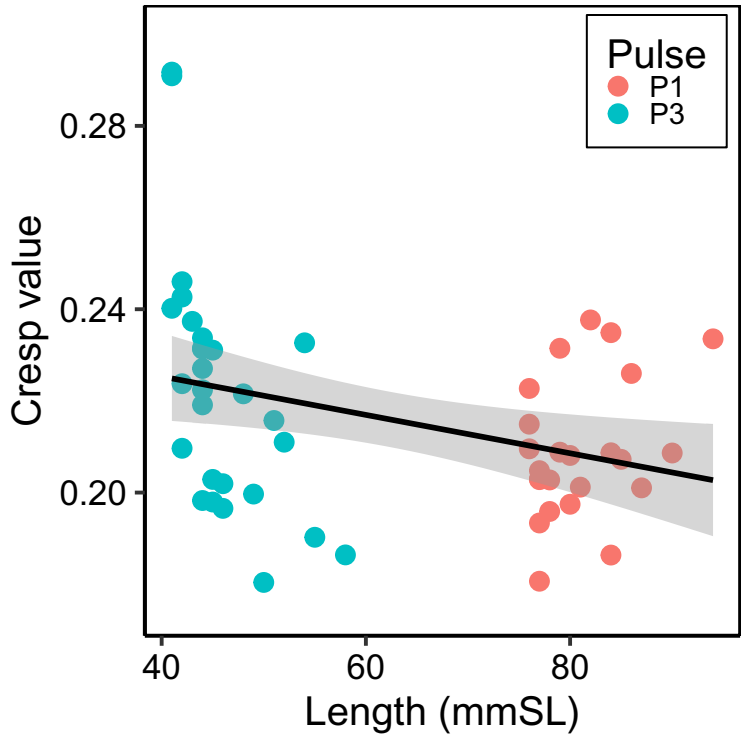



```
##
## Call:
## lm(formula = Temp ~ Pulse, data = TempPulse)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -3.3692 -0.3763  0.0758  0.7166  1.6055
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)   6.7459     0.2018  33.428  < 2e-16 ***
## PulseP3       0.8621     0.2750   3.135  0.00288 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.9886 on 50 degrees of freedom
## Multiple R-squared:  0.1643, Adjusted R-squared:  0.1475
## F-statistic: 9.827 on 1 and 50 DF,  p-value: 0.002877
```

Respiratory carbon and Atlantic cod size

C_{resp} vs Standard Length (mm SL)

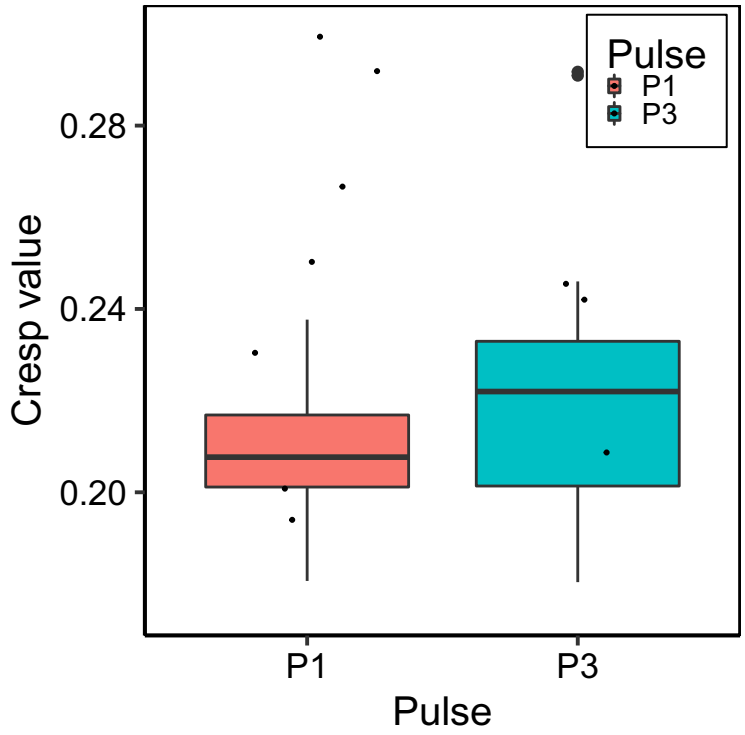
The primary proxy for field metabolic rate, the proportion of respiratory carbon, was regressed against standard length (mm) using a linear model.




```
##
## Call:
## lm(formula = Cresp ~ Len, data = CrespLength)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.040738 -0.013855 -0.000719  0.010703  0.066830
##
## Coefficients:
##               Estimate Std. Error t value Pr(>|t|)
## (Intercept)  0.2420693  0.0107474   22.52   <2e-16 ***
## Len         -0.0004187  0.0001661   -2.52    0.015 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.02166 on 50 degrees of freedom
## Multiple R-squared:  0.1127, Adjusted R-squared:  0.09497
## F-statistic: 6.351 on 1 and 50 DF,  p-value: 0.01497
```

C_{resp} vs Pulse Classification

The proportion of respiratory carbon was plotted against age-0 juvenile Atlantic cod classifications.

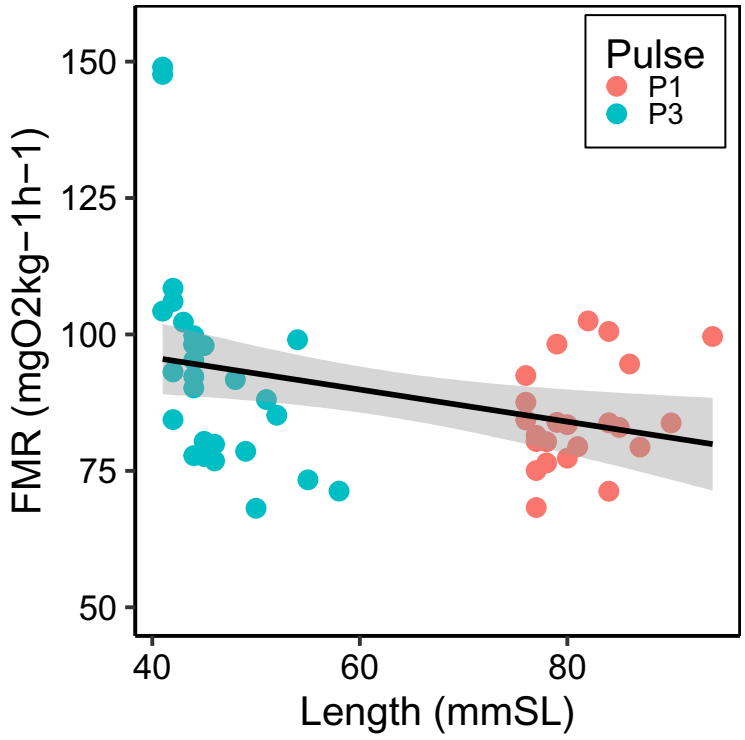



```
##
## Call:
## lm(formula = Cresp ~ Pulse, data = CrespLength)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.041524 -0.012532 -0.000863  0.011025  0.069813
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) 0.209228   0.004504  46.453   <2e-16 ***
## PulseP3     0.012693   0.006138   2.068   0.0438 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.02207 on 50 degrees of freedom
## Multiple R-squared:  0.07879,    Adjusted R-squared:  0.06037
## F-statistic: 4.277 on 1 and 50 DF,  p-value: 0.04383
```

FMR and Atlantic cod size

FMR vs Standard Length (mm SL)

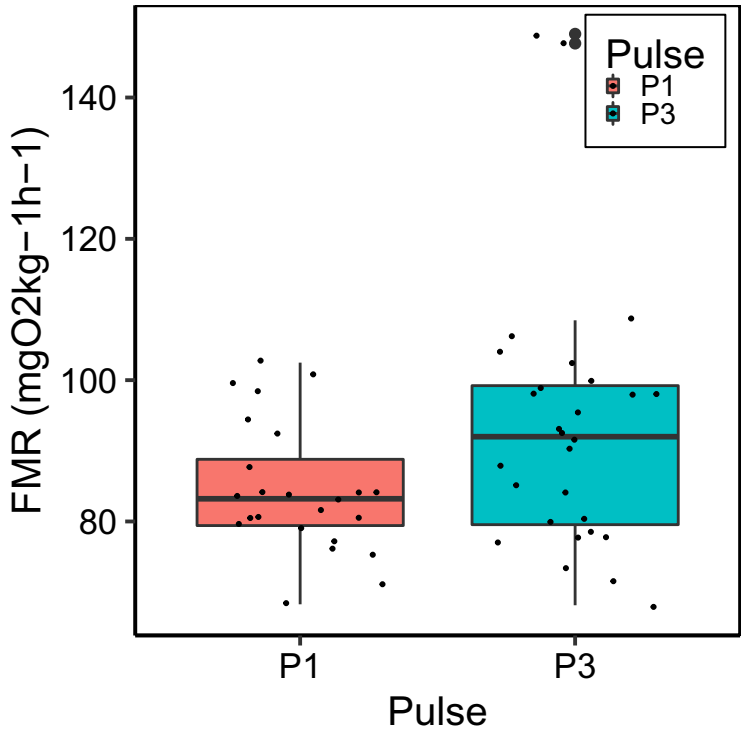
The field metabolic rate proxy was regressed against standard length (mm) using a linear model.




```
##
## Call:
## lm(formula = FMR ~ Len, data = CrespLength)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -24.703  -8.579  -1.889   5.694  53.508
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) 107.5532     7.4635   14.41   <2e-16 ***
## Len          -0.2942     0.1154   -2.55   0.0139 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 15.04 on 50 degrees of freedom
## Multiple R-squared:  0.1151, Adjusted R-squared:  0.09737
## F-statistic: 6.502 on 1 and 50 DF,  p-value: 0.01389
```

FMR vs Pulse Classification

The field metabolic rate proxy was plotted against age-0 juvenile Atlantic cod classifications.




```
##
## Call:
## lm(formula = FMR ~ Pulse, data = CrespLength)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -25.250  -8.399  -1.317   5.830  55.609
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)   84.484      3.130  26.995   <2e-16 ***
## PulseP3        8.907      4.265   2.089   0.0419 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 15.33 on 50 degrees of freedom
## Multiple R-squared:  0.08024,    Adjusted R-squared:  0.06184
## F-statistic: 4.362 on 1 and 50 DF,  p-value: 0.04186
```

Respiratory carbon and Experienced Temperature: Thermal C_{resp} trends

Regressions

Respiratory carbon was regressed against experienced temperature with outliers (black line) and without (red line) for all individuals (a), pulse 1 (b), and pulse 3 (c) individuals. The dashed lines without the confidence intervals represent a lack of significance between experienced temperature and C_{resp} values.

a. All indivdiuals

```
##
## Call:
## lm(formula = Cresp ~ Temp, data = CrespTempAll)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.037185 -0.012909 -0.001146  0.013198  0.058614
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) 0.144099   0.019345   7.449 1.19e-09 ***
## Temp        0.009981   0.002654   3.760 0.000446 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.0203 on 50 degrees of freedom
## Multiple R-squared:  0.2204, Adjusted R-squared:  0.2048
## F-statistic: 14.14 on 1 and 50 DF,  p-value: 0.0004459
```

```
##
## Call:
## lm(formula = Cresp ~ Temp, data = CrespTempAllnooutliers)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.034750 -0.012262 -0.001475  0.013438  0.062539
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) 0.154083   0.021556   7.148 4.36e-09 ***
## Temp        0.008422   0.002949   2.855  0.00633 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.01883 on 48 degrees of freedom
## Multiple R-squared:  0.1452, Adjusted R-squared:  0.1274
## F-statistic: 8.152 on 1 and 48 DF,  p-value: 0.006334
```

b. Pulse 1

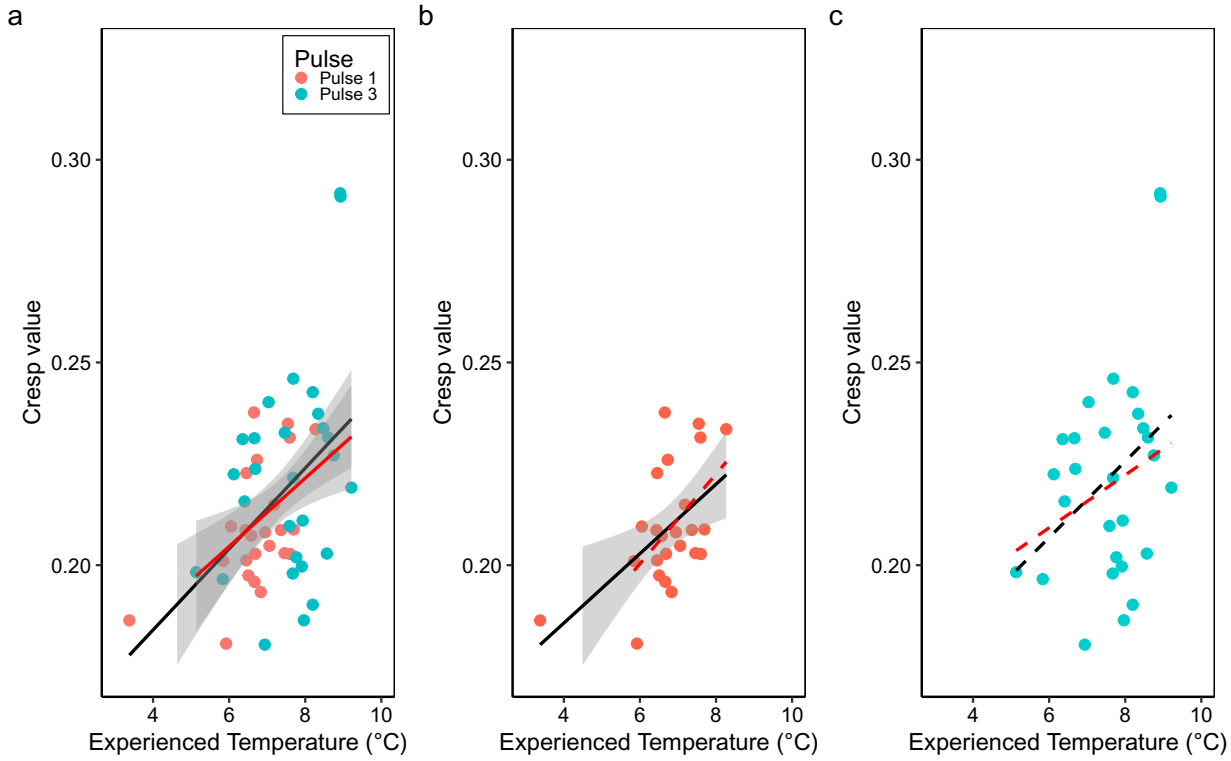
```
##
## Call:
## lm(formula = Cresp ~ Temp, data = CrespTempP1)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.021505 -0.008843 -0.001695  0.007522  0.029235
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) 0.151458   0.019769   7.662  1.2e-07 ***
## Temp        0.008564   0.002904   2.949  0.00742 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.01306 on 22 degrees of freedom
## Multiple R-squared:  0.2833, Adjusted R-squared:  0.2508
## F-statistic: 8.698 on 1 and 22 DF,  p-value: 0.007416
```

```
##
## Call:
## lm(formula = Cresp ~ Temp, data = CrespTempP1nooutliers)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.018806 -0.009400 -0.002717  0.008358  0.030097
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)  0.13389    0.03155   4.243 0.000363 ***
## Temp         0.01107    0.00456   2.428 0.024237 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.01321 on 21 degrees of freedom
## Multiple R-squared:  0.2192, Adjusted R-squared:  0.1821
## F-statistic: 5.897 on 1 and 21 DF,  p-value: 0.02424
```

c. Pulse 3

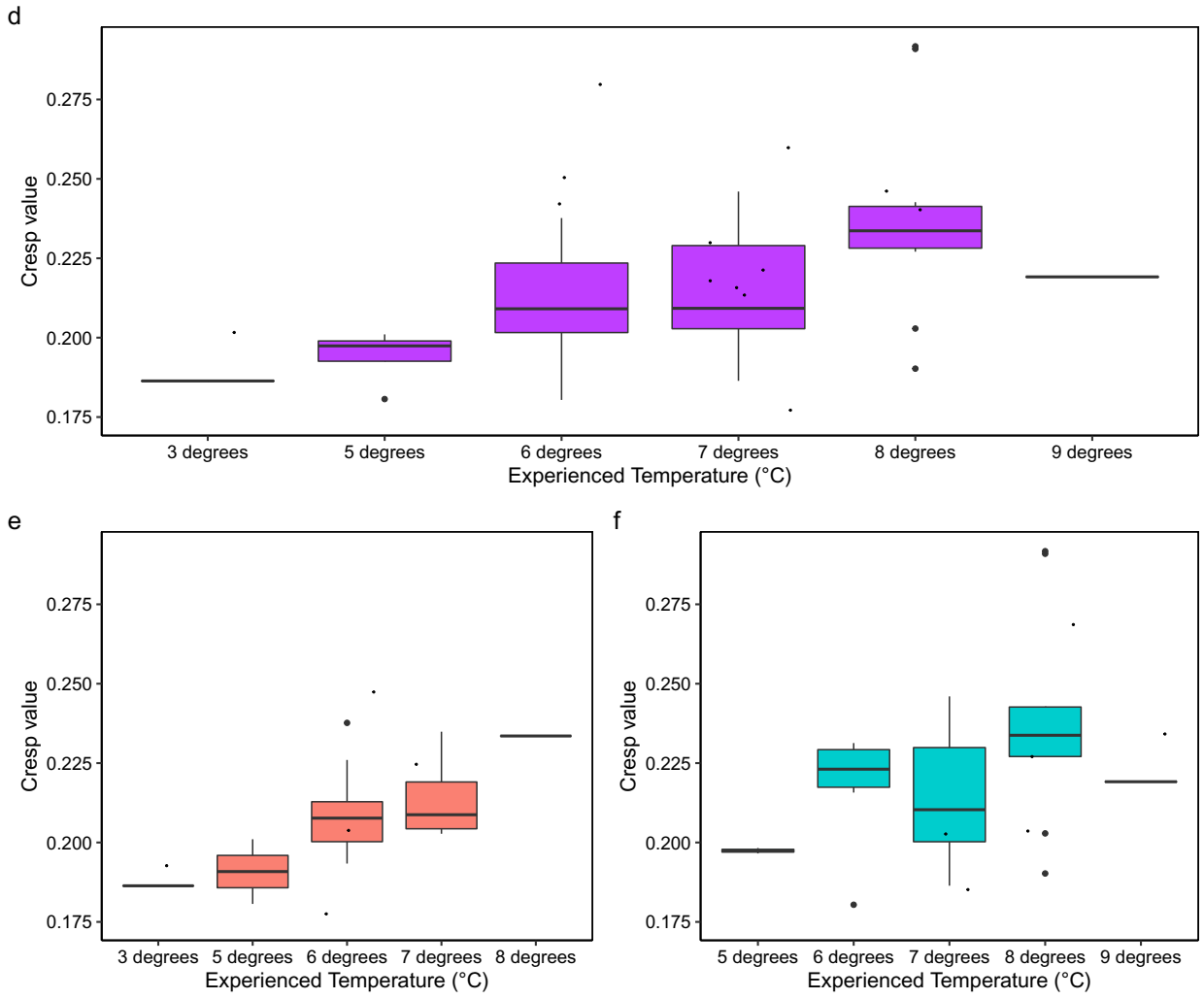
```
##
## Call:
## lm(formula = Cresp ~ Temp, data = CrespTempP3)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.038863 -0.018765 -0.000073  0.014657  0.057500
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) 0.150470   0.036219   4.155 0.000312 ***
## Temp        0.009392   0.004719   1.990 0.057188 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.02525 on 26 degrees of freedom
## Multiple R-squared:  0.1322, Adjusted R-squared:  0.09882
## F-statistic: 3.961 on 1 and 26 DF,  p-value: 0.05719
```

```
##
## Call:
## lm(formula = Cresp ~ Temp, data = CrespTempP3nooutliers)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.035597 -0.015177  0.001385  0.013422  0.063524
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) 0.170207   0.033612   5.064 3.16e-05 ***
## Temp        0.006503   0.004408   1.475    0.153
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.02283 on 25 degrees of freedom
## Multiple R-squared:  0.08008,    Adjusted R-squared:  0.04328
## F-statistic: 2.176 on 1 and 25 DF,  p-value: 0.1526
```


a
b
c
Pulse
Pulse 1
Pulse 3
Cresp value
0.30
0.25
0.20
4
6
8
10
Experienced Temperature (°C)

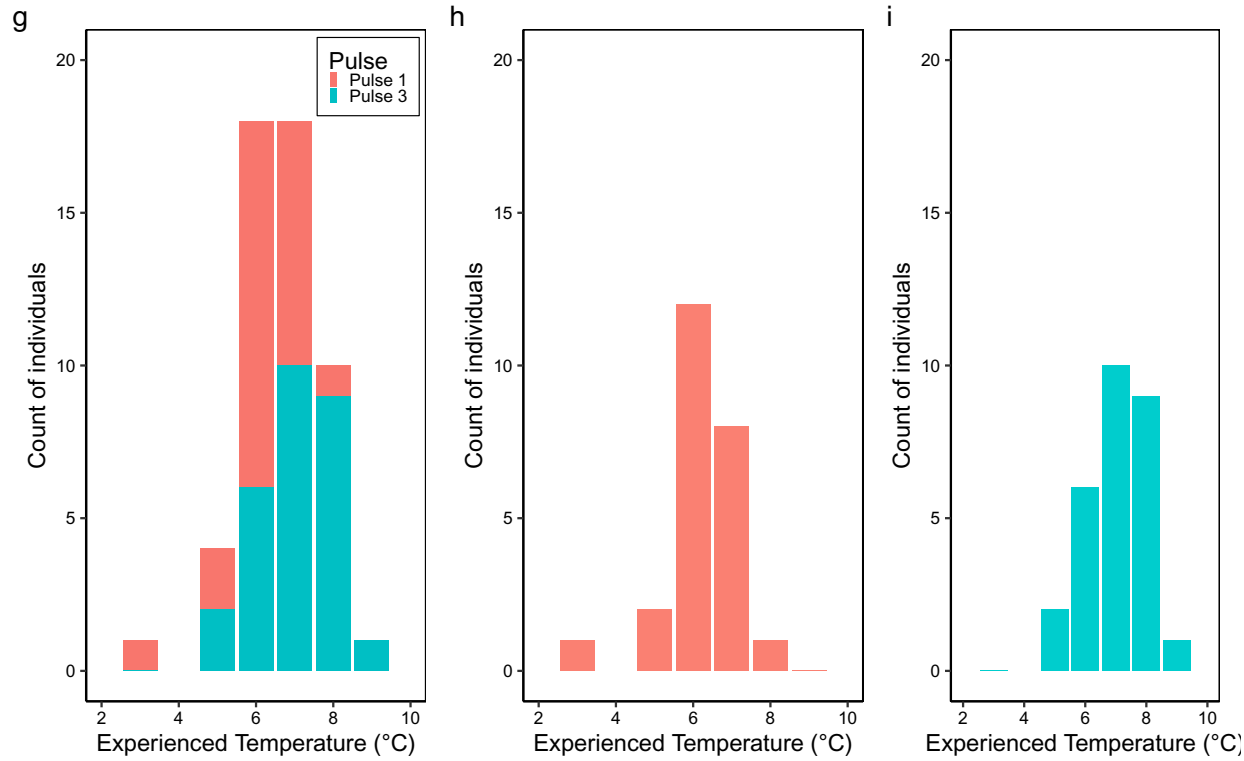
Temperature Classes

For each observed temperature, individuals from both pulses exhibited a range of C_{resp} values, with variability between pulses and between specific temperature bins. Variability in C_{resp} values was plotted using 1 degree temperature bins for (a) all individuals, (b) pulse 1, and (c) pulse 3 individuals.



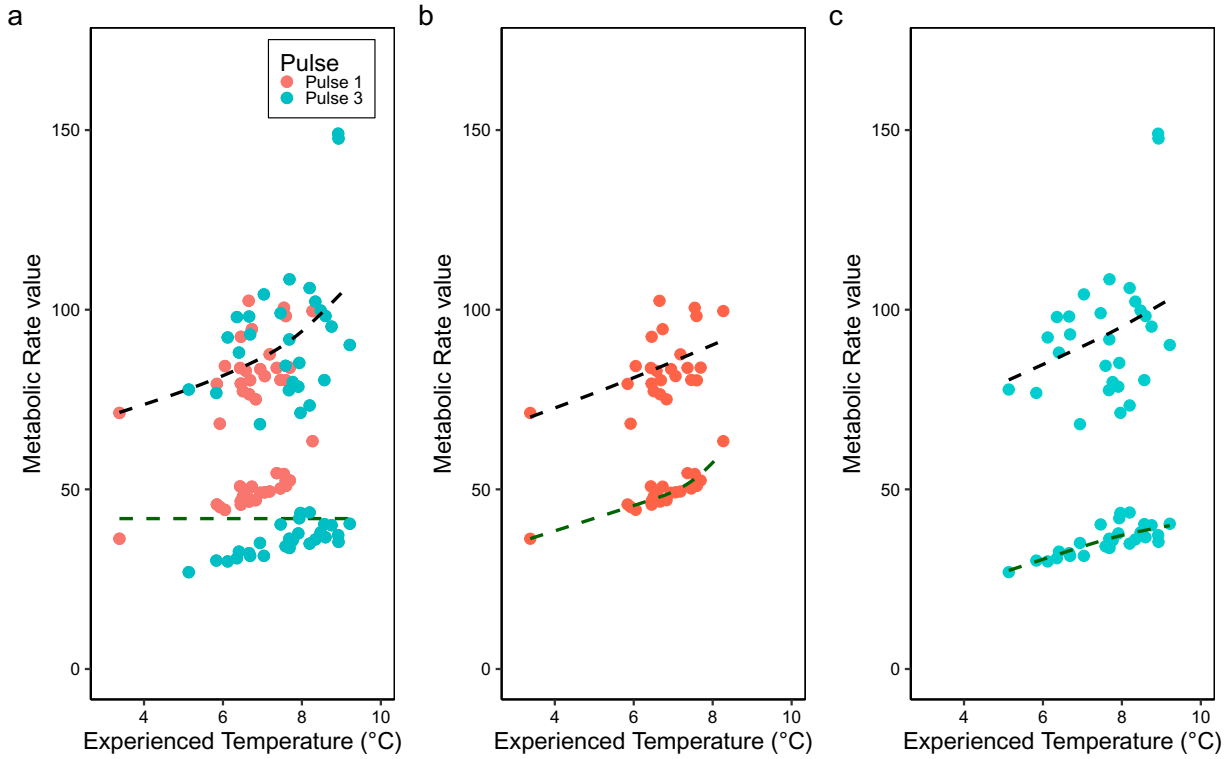
Counts

It is important to remember that the variance is influenced by the sample size for each experienced temperature, therefore the count of individual for each temperature bin is demonstrated below for (g) all individuals, (h) pulse 1, and (i) pulse 3 individuals.



FMR-SMR comparissons

Reconstructed FMR values were compared to theoretical SMR values estimated using the Metabolic Theory of Ecology (MTE) for each individual at its isotope-otolith determined experienced temperature. The thermal relationship of field metabolic rate (FMR - black line) in relation to theoretical standard metabolic rate (SMR - dark green line) is plotted against experienced temperature for (a) all individuals, (b) pulse 1, and (c) pulse 3 individuals.



Inter-population comparisons: Newman Sound vs Skagerrak Coast

Growth rate comparisons

A comparable population-specific growth rate value was determined from a subsample of the population dataset used to calculate FMR to ensure that growth rate is not driving population-specific FMR patterns. Individual sampling dates (Julian date) were regressed with length (mmSL), and the resulting slope for each population (Skagerrak, Newman Sound) acts as a standardized growth rate (mmSL/Julian date) for comparisons across populations. The growth rate relationship was examined for the Newman Sound population (a), the Skagerrak coast population (b), and for the overall Newman Sound age-0 2019 cohort (c).

a. Newfoundland

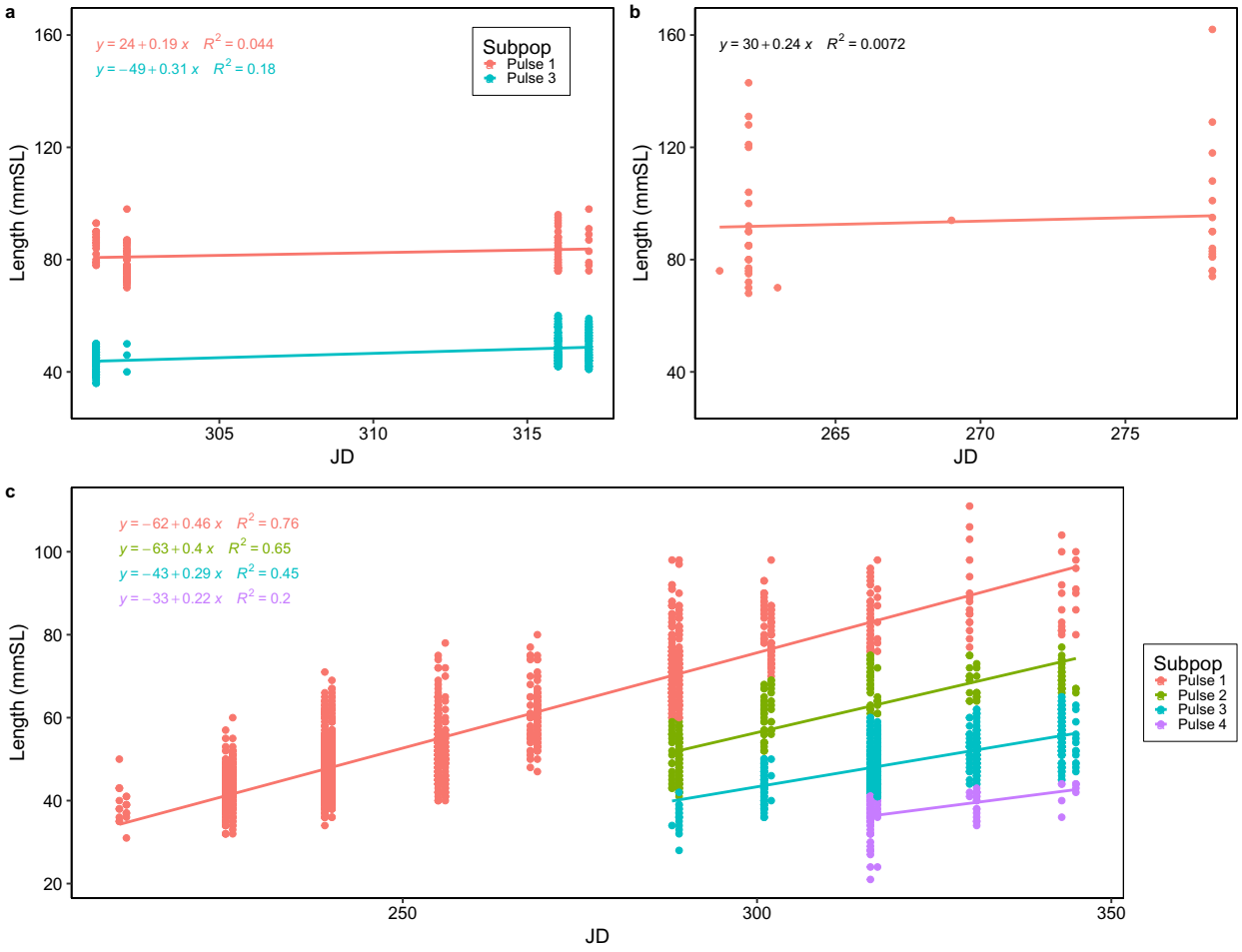
```
## Linear mixed model fit by REML ['lmerMod']
## Formula: Length ~ JD + (1 | Subpop)
##    Data: Newf300
##
## REML criterion at convergence: 2645.5
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -2.0122 -0.8404 -0.1214  0.7064  3.4016
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  Subpop   (Intercept) 648.01   25.456
##  Residual              26.75    5.172
## Number of obs: 430, groups:  Subpop, 2
##
## Fixed effects:
##              Estimate Std. Error t value
## (Intercept) -22.47052   21.06262  -1.067
## JD            0.28106    0.03529   7.964
##
## Correlation of Fixed Effects:
##    (Intr)
## JD -0.519
```

b. Norway

```
##
## Call:
## lm(formula = Length ~ JD, data = Norway2000)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -23.821 -14.821  -6.821   8.179  66.386
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)  29.7068   119.8987   0.248    0.806
## JD            0.2371     0.4465   0.531    0.598
##
## Residual standard error: 22.15 on 39 degrees of freedom
## Multiple R-squared:  0.007177,   Adjusted R-squared:  -0.01828
## F-statistic: 0.2819 on 1 and 39 DF,  p-value: 0.5985
```

c. Newfoundland whole cohort population

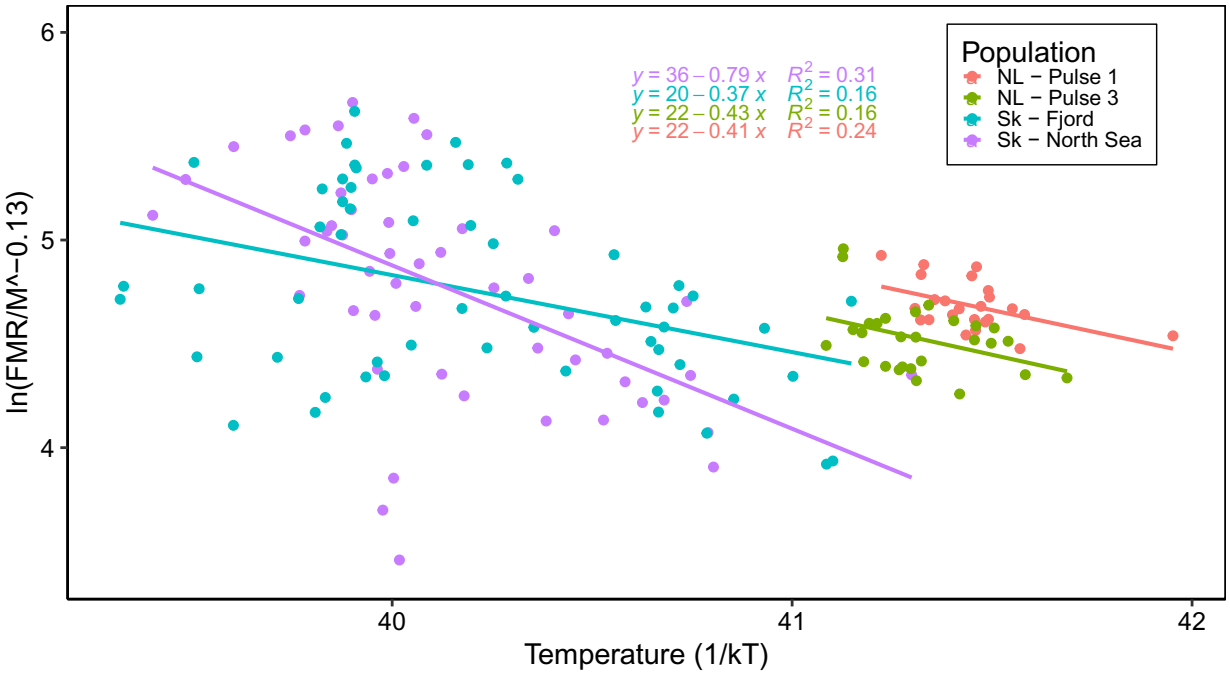
```
##
## Call:
## lm(formula = Length ~ JD, data = NewfoundlandAll)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -36.826  -6.975  -1.826   5.071  51.565
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept) 21.503499   1.535766   14.00   <2e-16 ***
## JD           0.114943   0.005574   20.62   <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 11.38 on 2915 degrees of freedom
## Multiple R-squared:  0.1273, Adjusted R-squared:  0.127
## F-statistic: 425.2 on 1 and 2915 DF,  p-value: < 2.2e-16
```


a
y = 24 + 0.19 x R² = 0.044
y = −49 + 0.31 x R² = 0.18
Subpop
Pulse 1
Pulse 3
Length (mmSL)
JD
b
y = 30 + 0.24 x R² = 0.0072
Length (mmSL)
JD
c
y = −62 + 0.46 x R² = 0.76
y = −63 + 0.4 x R² = 0.65
y = −43 + 0.29 x R² = 0.45
y = −33 + 0.22 x R² = 0.2
Length (mmSL)
JD
Subpop
Pulse 1
Pulse 2
Pulse 3
Pulse 4

Thermal FMR relationship: Mean FMR and FMR thermal sensitivity

A generalised least squares fit was conducted on the linear modeling in order to detect significant differences between the slope (thermal sensitivity of FMR) and the intercept (mean FMR) across population subgroups - NL pulses (P1 and P3) and Skagerrak ecotypes (North Sea and Fjord).

When comparing the thermal FMR relationship under the Arrhenius model, the best fitting GLS identified varying differences in FMR-thermal sensitivity (slopes) and mean FMR (intercept) between populations (Newman Sound, NL vs Skagerrak, Norway), and within populations across ecotypes and pulse classification (pulse 1 and pulse 3 individuals, North Sea and Fjord ecotypes).



Significance

The significant difference between the slopes (thermal sensitivity) and intercepts (mean FMR) was determined for each ecotype and pulse classification between and within populations. The table below idicates the statistical significance of each, with the subpopulation in the top row indicating the reference group. An alpha value of 0.05 was chosen, meaning p<0.05 indicates a difference in the slope/intercept, and p>0.05 indicates no significant difference between the slope/intercept. The p-values at the intersection of the group itself indicates the signficiance of the regression slope.The numbers in bold indicate significant values, and therefore, a significant differance between values.

Intercept: Mean FMR

Significance	Pulse 1	Pulse 3	North Sea	Fjord
Pulse 1	**0.014**	**0.000**	**0.01e-02**	*0.017*
Pulse 3	**0.000**	**0.035**	**0.002**	0.298
North Sea	**0.01e-02**	**0.002**	**2.13e-05**	*0.045*
Fjord	**0.017**	0.298	**0.045**	*0.002*

Slope: FMR Thermal Sensitivity

Significance	Pulse 1	Pulse	North Sea	Fjord
Pulse 1	**0.014**	0.952	0.101	0.837
Pulse 3	0.952	**0.035**	0.158	0.808
North Sea	0.101	0.158	**2.13e-05**	**0.043**
Fjord	0.837	0.808	**0.043**	**0.002**

```
## Generalized least squares fit by REML
##   Model: lnFMR ~ (Tscale) * Population
##   Data: FMRtempArrCOMB
##        AIC      BIC    logLik
##   99.69639 136.0616 -37.84819
##
## Variance function:
##  Structure: Different standard deviations per stratum
##  Formula: ~1 | Population
##  Parameter estimates:
##   NL - Pulse 1   NL - Pulse 3     Sk - Fjord Sk - North Sea
##       1.000000       1.461211       3.990538       4.316211
##
## Coefficients:
##                                    Value  Std.Error   t-value p-value
## (Intercept)                     4.662244 0.02259075 206.37843  0.0000
## Tscale                         -0.410611 0.15430218  -2.66108  0.0086
## PopulationNL - Pulse 3         -0.214846 0.05004983  -4.29264  0.0000
## PopulationSk - Fjord           -0.387371 0.16056629  -2.41253  0.0170
## PopulationSk - North Sea       -0.964326 0.23741926  -4.06170  0.0001
## Tscale:PopulationNL - Pulse 3  -0.014730 0.24586091  -0.05991  0.9523
## Tscale:PopulationSk - Fjord     0.039816 0.19328040   0.20600  0.8371
## Tscale:PopulationSk - North Sea -0.376032 0.22782953  -1.65050  0.1009
##
##  Correlation:
##                                (Intr) Tscale PNL-P3 PplS-F PpS-NS T:P-P3
## Tscale                          0.357
## PopulationNL - Pulse 3         -0.451 -0.161
## PopulationSk - Fjord           -0.141 -0.050  0.064
## PopulationSk - North Sea       -0.095 -0.034  0.043  0.013
## Tscale:PopulationNL - Pulse 3  -0.224 -0.628  0.635  0.032  0.021
## Tscale:PopulationSk - Fjord    -0.285 -0.798  0.129  0.600  0.027  0.501
## Tscale:PopulationSk - North Sea -0.242 -0.677  0.109  0.034  0.730  0.425
##                                T:PS-F
## Tscale
## PopulationNL - Pulse 3
## PopulationSk - Fjord
## PopulationSk - North Sea
## Tscale:PopulationNL - Pulse 3
## Tscale:PopulationSk - Fjord
## Tscale:PopulationSk - North Sea  0.541
##
## Standardized residuals:
##          Min           Q1          Med           Q3          Max
## -13.58575774  -1.62873306   0.03636131   1.86632713   7.29116144
```

```
##
## Residual standard error: 0.1033833
## Degrees of freedom: 161 total; 153 residual

## Generalized least squares fit by REML
##   Model: lnFMR ~ (Tscale) * Population
##   Data: FMRtempArrCOMB
##        AIC      BIC    logLik
##   99.69639 136.0616 -37.84819
##
## Variance function:
##  Structure: Different standard deviations per stratum
##  Formula: ~1 | Population
##  Parameter estimates:
##   NL - Pulse 3   NL - Pulse 1     Sk - Fjord Sk - North Sea
##      1.0000000      0.6843639      2.7309802      2.9538587
##
## Coefficients:
##                                    Value  Std.Error  t-value p-value
## (Intercept)                     4.447398 0.04466143 99.58029  0.0000
## Tscale                         -0.425341 0.19141166 -2.22213  0.0277
## PopulationNL - Pulse 1          0.214846 0.05004983  4.29264  0.0000
## PopulationSk - Fjord           -0.172526 0.16512370 -1.04483  0.2978
## PopulationSk - North Sea       -0.749480 0.24052485 -3.11602  0.0022
## Tscale:PopulationNL - Pulse 1   0.014730 0.24586091  0.05991  0.9523
## Tscale:PopulationSk - Fjord     0.054546 0.22402359  0.24348  0.8080
## Tscale:PopulationSk - North Sea -0.361302 0.25443182 -1.42004  0.1576
##
##  Correlation:
##                                (Intr) Tscale PNL-P1 PplS-F PpS-NS T:P-P1
## Tscale                          0.769
## PopulationNL - Pulse 1         -0.892 -0.686
## PopulationSk - Fjord           -0.270 -0.208  0.241
## PopulationSk - North Sea       -0.186 -0.143  0.166  0.050
## Tscale:PopulationNL - Pulse 1  -0.599 -0.779  0.635  0.162  0.111
## Tscale:PopulationSk - Fjord    -0.657 -0.854  0.586  0.647  0.122  0.665
## Tscale:PopulationSk - North Sea -0.579 -0.752  0.516  0.156  0.732  0.586
##                                T:PS-F
## Tscale
## PopulationNL - Pulse 1
## PopulationSk - Fjord
## PopulationSk - North Sea
## Tscale:PopulationNL - Pulse 1
## Tscale:PopulationSk - Fjord
## Tscale:PopulationSk - North Sea  0.643
##
## Standardized residuals:
##         Min          Q1         Med          Q3         Max
## -9.29760159 -1.11464604  0.02488437  1.27724683  4.98980738
##
## Residual standard error: 0.1510648
## Degrees of freedom: 161 total; 153 residual

## Generalized least squares fit by REML
```

```
##   Model: lnFMR ~ (Tscale) * Population
##   Data: FMRtempArrCOMB
##        AIC      BIC    logLik
##   99.69639 136.0616 -37.84819
##
## Variance function:
##  Structure: Different standard deviations per stratum
##  Formula: ~1 | Population
##  Parameter estimates:
##     Sk - Fjord   NL - Pulse 3   NL - Pulse 1 Sk - North Sea
##      1.0000000      0.3661681      0.2505919      1.0816096
##
## Coefficients:
##                                     Value Std.Error   t-value p-value
## (Intercept)                      4.274873 0.1589694 26.891175  0.0000
## Tscale                          -0.370795 0.1163967 -3.185616  0.0018
## PopulationNL - Pulse 3           0.172526 0.1651239  1.044825  0.2978
## PopulationNL - Pulse 1           0.387371 0.1605665  2.412528  0.0170
## PopulationSk - North Sea        -0.576955 0.2848312 -2.025602  0.0445
## Tscale:PopulationNL - Pulse 3   -0.054546 0.2240235 -0.243483  0.8080
## Tscale:PopulationNL - Pulse 1   -0.039816 0.1932802 -0.206001  0.8371
## Tscale:PopulationSk - North Sea -0.415848 0.2040718 -2.037754  0.0433
##
##  Correlation:
##                                 (Intr) Tscale PNL-P3 PNL-P1 PpS-NS T:P-P3
## Tscale                           0.939
## PopulationNL - Pulse 3          -0.963 -0.904
## PopulationNL - Pulse 1          -0.990 -0.930  0.953
## PopulationSk - North Sea        -0.558 -0.524  0.537  0.553
## Tscale:PopulationNL - Pulse 3   -0.488 -0.520  0.647  0.483  0.272
## Tscale:PopulationNL - Pulse 1   -0.566 -0.602  0.544  0.600  0.316  0.313
## Tscale:PopulationSk - North Sea -0.536 -0.570  0.516  0.530  0.957  0.296
##                                 T:P-P1
## Tscale
## PopulationNL - Pulse 3
## PopulationNL - Pulse 1
## PopulationSk - North Sea
## Tscale:PopulationNL - Pulse 3
## Tscale:PopulationNL - Pulse 1
## Tscale:PopulationSk - North Sea  0.343
##
## Standardized residuals:
##          Min           Q1          Med           Q3          Max
## -3.404488018 -0.408148172  0.009111869  0.467687446  1.827109848
##
## Residual standard error: 0.4125556
## Degrees of freedom: 161 total; 153 residual

## Generalized least squares fit by REML
##   Model: lnFMR ~ (Tscale) * Population
##   Data: FMRtempArrCOMB
##        AIC      BIC    logLik
##   99.69639 136.0616 -37.84819
##
```

```
## Variance function:
##  Structure: Different standard deviations per stratum
##  Formula: ~1 | Population
##  Parameter estimates:
## Sk - North Sea     Sk - Fjord   NL - Pulse 3   NL - Pulse 1
##      1.0000000      0.9245466      0.3385402      0.2316847
##
## Coefficients:
##                                    Value Std.Error   t-value p-value
## (Intercept)                     3.697918 0.2363420 15.646468  0.0000
## Tscale                         -0.786643 0.1676220 -4.692960  0.0000
## PopulationSk - Fjord            0.576955 0.2848311  2.025603  0.0445
## PopulationNL - Pulse 3          0.749480 0.2405249  3.116020  0.0022
## PopulationNL - Pulse 1          0.964326 0.2374193  4.061700  0.0001
## Tscale:PopulationSk - Fjord     0.415848 0.2040717  2.037755  0.0433
## Tscale:PopulationNL - Pulse 3   0.361302 0.2544318  1.420036  0.1576
## Tscale:PopulationNL - Pulse 1   0.376032 0.2278295  1.650499  0.1009
##
##  Correlation:
##                                (Intr) Tscale PplS-F PNL-P3 PNL-P1 T:PS-F T:P-P3
## Tscale                          0.965
## PopulationSk - Fjord           -0.830 -0.801
## PopulationNL - Pulse 3         -0.983 -0.948  0.815
## PopulationNL - Pulse 1         -0.995 -0.961  0.826  0.978
## Tscale:PopulationSk - Fjord    -0.793 -0.821  0.957  0.779  0.789
## Tscale:PopulationNL - Pulse 3 -0.636 -0.659  0.528  0.732  0.633  0.541
## Tscale:PopulationNL - Pulse 1 -0.710 -0.736  0.589  0.698  0.730  0.604  0.485
##
## Standardized residuals:
##          Min           Q1          Med           Q3          Max
## -3.147612084 -0.377352515  0.008424359  0.432399424  1.689250485
##
## Residual standard error: 0.4462241
## Degrees of freedom: 161 total; 153 residual
```