The overwintering behaviour and physiology of Northern Map Turtles (*Graptemys geographica*) in Ontario

by

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A thesis submitted to the Faculty of Graduate and Postdoctoral Affairs in partial fulfillment of the requirements for the degree of

Master of Science

in

Biology

Carleton University
Ottawa, Ontario

Abstract

Northern map turtles (*Graptemys geographica*) are a freshwater turtle species that spends months of the year overwintering submerged under ice. They are anoxia intolerant, making their ability to survive submerged without access to atmospheric oxygen physiologically impressive. Overwintering behaviour and physiology were examined to understand how this species survives the winter. Biologgers recorded locomotor activity, temperature, and depth throughout overwintering. Locomotor activity was continuous during the winter. The amount of movement differed between adult females, juvenile females, and adult males. Temperature preference for all groups was near 1°C and each moved progressively shallower as winter progressed. Respirometry was used to measure adult female standard metabolic rates at under-ice temperatures. Metabolism was lower at lower temperatures (i.e., 1°C versus 4°C), indicating considerable metabolic savings at 1°C. The behaviours observed likely reflect this species working to meet winter oxygen and energetic needs which differ based on size specific physiological needs.

Acknowledgements

I’d like to thank my supervisors Dr. Steven J. Cooke, Dr. Heath A. MacMillan, and Dr. Grégory Bulté for their support and guidance over the last two years. I am thankful for the time they have put into reviewing my work and listening to my ideas and the constructive feedback they provided which has helped to make me a better writer and researcher. I’d like to thank Dr. Bulté in particular for teaching me innumerable practical field skills and for his patience during our long excursions on the water. My appreciation extends to the members of the Cooke and MacMillan labs as well who created a friendly working environment and were always keen to offer their support virtually and in the field. I’d also like to thank the Queen’s University Biology station for allowing me to use their research facilities to complete both projects associated with my data chapters. Finally, I would like to thank my family and my friends for the endless support and encouragement as I continue to pursue my academic endeavors. I am grateful for the opportunities and experiences I was fortunate enough to have during my time at Carleton University and look forward to carrying these with me into the future.

Co-authorship Statement

Chapter 2 is comprised of a manuscript that is currently being prepared for submission to a journal. All authors contributed with the revision of the manuscript. The bibliographical details of the original article are:

Robichaud JA, Bulté G, MacMillan HA, Cooke SJ (Manuscript in preparation) Five months under ice: biologging reveals behavior patterns of overwintering freshwater turtles.

Chapter 3 is comprised of a manuscript that is currently being prepared for submission to a journal. All authors contributed with the revision of the manuscript. The bibliographical details of the original article are:

Robichaud JA, Bulté G, LaRochelle L, Cooke SJ, MacMillan HA (Manuscript in preparation) The effect of temperature on the standard metabolic rate of Northern map turtles (*Graptemys geographica*) in simulated under-ice conditions

Coauthors G Bulté, HA MacMillan, and SJ Cooke contributed to experimental design and provided revisions/edits for both manuscripts as they appear in this thesis. G Bulté and L LaRochelle provided invaluable assistance with collecting data in the field.

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Chapter 1: General Introduction

1.1 Winter in Temperate Regions

The arrival of winter in temperate, alpine, and polar environments greatly impacts life in these regions. While the definition of winter varies globally, here winter will be defined based on the maxims described in Studd et al. (2021), with emphasis on winter being the coldest, darkest, and most frozen part of the year. Winter temperatures remain near or below freezing (0°C) for up to six consecutive months (Williams et al. 2015; Studd et al. 2021). With sub-freezing temperatures comes the onset of snow and the formation of ice on bodies of water (McMeans et al. 2020). For aquatic species, these significant seasonal changes, namely ice and the snow that accumulates above it, create environmental conditions drastically different to those during spring, summer, and fall (Hampton et al. 2017). Ice formation creates a physical barrier between liquid water and the air, resulting in forced submergence for many air breathing species that spend the winter below the ice (Studd et al. 2021). Further, ice restricts gas exchange between the water and air that can lead to seasonal lows of oxygen availability, creating hypoxic environments in some instances (Kauko et al. 2017). The accumulation of snow on top of the ice blocks the transmission of sunlight, resulting in a dark environment with reduced primary production where it is increasingly difficult for animals to forage for limited food sources (Shuter et al. 2012; Hampton et al. 2017). As difficult as these environmental conditions may be, however, aquatic species can survive winter by using one of several overwintering strategies.

Species adapt to winter conditions using a variety of strategies. For example, some species avoid the season and escape winter conditions by leaving their spring through fall range to go elsewhere for the winter season. This is commonly facilitated by migration, wherein species move to habitats with less harsh conditions (i.e., warmer temperatures and/or more productive environments) (Alerstam et al. 2003). Alternatively, species who cannot migrate due to physical (i.e., limited mobility) or environmental (i.e., connectivity) barriers, adapting to seasonal environmental changes is a more feasible overwintering strategy. Adaptation here refers to any overwintering strategy that allows an organism to stay in the same habitat they inhabit for the rest of the year and is enabled by behavioural and physiological adaptions (Studd et al. 2021). For many species, adapting to winter is heavily dependent on managing their energy budget during this season given the scarcity of resources. Energy budgets are managed namely by (1) storing energy prior to the winter months and (2) minimizing energy use (Shuter et al. 2012; Speers-Roesch et al. 2018).

For freshwater ectotherms such as fish or turtles, energy use can be minimized through dormancy, where metabolic rate decreases due to inactivity (Costa 2013; Speers-Roesch et al. 2018). Alternatively, energy use can be minimized through changes to metabolism in response to cold temperatures (Brown et al. 2004). Some ectotherms actively engage in metabolic depression to conserve energy during times of stress (i.e., limited resources, lack of oxygen), while others appear to benefit from the passive effect of temperature on metabolism, wherein metabolic suppression occurs as a direct result of low temperature (Jackson and Ultsch 2010). Under the ice, temperature stability facilitates tolerance by creating a stable environment with more predictable metabolic costs (McMeans et al. 2020). Temperatures under the ice usually remain between 0°C and 4°C for the duration of the ice-on period, and these temperatures are cold enough to either cause a depression or have a passive effect on ectotherm metabolic rates (Lemons and Crawshaw 1985). This reduction in metabolism can therefore help conserve energy, which can result in significant energy savings over extended dormancy (Gregory 1982; Lemons and Crawshaw 1985). However, energy supplies are not the only concern throughout the winter. Oxygen also appears to be a limiting factor for some aquatic ectotherms at this time of year, and this is particularly true for several species of freshwater turtles who cannot survive prolonged periods without oxygen.

1.2 Freshwater Turtle Winter Biology

All eight species of semi-aquatic and aquatic freshwater turtles found in Canada overwinter under the ice (Ultsch 2006). While this environment offers refuge to turtles from sub-freezing temperatures, it also restricts them from atmospheric oxygen. For some species, a lack of oxygen is not a problem as they can withstand anoxic conditions for prolonged periods of time. Such examples include Painted turtles (*Chrysemys picta*) and Snapping turtles (*Chelydra serpentina*), both of which have been noted to conserve winter energy by engaging in anoxia-induced metabolic depression, and by exhibiting little to no locomotor activity during this season (Taylor and Nol 1989; Meeks and Ultsch 1990; Jackson 2002; Ultsch 2006). These species are dependent on anerobic metabolic pathways to survive and, consequently, experience physiological challenges such as increases in lactate and decreases in blood pH (Edge et al. 2009; Jackson and Ultsch 2010). Anaerobic pathways have proven useful for survival; however, they do run the risk of the potentially fatal conditions respiratory acidosis or metabolic acidosis (Herbert and Jackson 1985). To overcome this, turtles can buffer the build-up of lactic acid through calcium and magnesium carbonate stores in their skeletal system (Reese et al. 2003). Because of their ability to survive without oxygen, anoxia tolerant species are often observed hibernating in environments more prone to anoxia such as ponds, marshes, wetlands, or swamps (Brown and Brooks 1994; Ultsch 2006; Rollinson et al. 2008).

Other species who cannot withstand prolonged anoxia are dependent on overwintering habitats that retain some amount of dissolved oxygen during the winter, which we refer to here as normoxic conditions. These habitats are commonly rivers, streams, or lakes (Ultsch 2006; Ultsch and Reese 2008). Examples of anoxic intolerant species include Blanding's turtles, Spiny softshell turtles (*Apolone spinifera*), Musk turtles (*Sternotherus odoratus*), Northern map turtles (*Graptemys geographica*), and Wood turtles (*Glyptemys insculpta*) (Reese et al. 2001; Reese et al. 2003; Greaves and Litzgus 2008; Edge et al. 2009). Anoxia intolerant species are believed to have more efficient extra-pulmonary routes of oxygen uptake relative to anoxia tolerant species, allowing them to extract dissolved oxygen (DO) from the water and maintain aerobic pathways (Ultsch and Cochran 1994; Reese et al. 2001; Greaves and Litzgus 2008). As a result, they do not experience physiologically notable changes in lactate and blood pH like anoxia tolerant species do. Nonetheless, these species are still susceptible to metabolic acidosis if they experience anoxia (Jackson and Ultsch 2010). Spiny softshell turtles and Northern map turtles, for example, will die from metabolic acidosis after approximately 11 and 45 days respectively in unoxygenated water at 3°C (Reese et al. 2001; Reese et al. 2003). Therefore, normoxic conditions are critical to support the aerobic respiration of these species, which suggests oxygen is likely a key consideration when selecting overwintering habitat (Crocker et al. 2000; Reese et al. 2001). Notably, regardless of their anoxia tolerance, most freshwater turtle species appear to select overwintering sites where temperatures remain near 1°C (Brown and Brooks 1994; Litzgus et al. 1999; Crocker et al. 2000; Greaves and Litzgus 2007; Rollinson et al. 2008). The reasoning behind this choice remains unclear, but it is likely that low temperature environments reduce both energy use and oxygen demand (Greaves and Litzgus 2008; Rollinson et al. 2008).

1.3 Northern map turtles

With respect to overwintering ecology, the Northern map turtle is a particularly interesting case. In some instances, Northern map turtles have been observed to overwinter communally in groups by the hundreds and demonstrate long-term fidelity to these overwintering sites, suggesting that overwintering sites with ideal temperatures and/or adequate winter oxygen supplies are limited (Ultsch and Jackson 1995; Ultsch et al. 2000; Graham et al. 2000). At this time, few observations have been made of other freshwater turtles moving during the winter, but there are numerous accounts of Northern map turtle locomotor activity during this season (Newman 1906; Graham and Graham 1992). Field observations have reported Northern map turtles walking along the bottom of the overwintering site after ice has formed and that they are responsive when deliberately disturbed (Evermann and Clark 1916; Graham and Graham 1992). This behaviour is peculiar given that movement requires them to expend their limited winter energy stores. The extent and purpose of this movement remains unknown, although it is a behaviour likely linked to their oxygen requirements. Interestingly, these accounts also note that adult males appear to be more alert than adult females (Graham and Graham 1992). Northern map turtles show pronounced sexual dimorphism, where females weight between 800 and 3880 g and males weigh no more than 400 g in some populations in Ontario (Bulté, unpublished data). This notable size difference is likely the reasoning behind the observed differences in movement, however the specific difference behind this behaviour is unknown. It is also unknown how this behaviour is related to overwintering metabolic physiology.

Multiple physiological mechanisms have been proposed that may help Northern map turtles survive the winter. These include (1) metabolic depression, (2) improved efficiency of extrapulmonary gas exchange, and (3) adaptations of the blood-oxygen transport system (Jackson et al. 2001; Reese et al. 2001; Maginniss et al. 2004). Currently, metabolic depression has only been suggested based on behavioural observations made in a laboratory setting under anoxic conditions (Crocker et al. 2000). This information does not provide meaningful evidence to suggest the same would be true in the wild, especially given their apparent need for normoxic conditions. There is evidence to suggest that the blood-oxygen transport system of Northern map turtles promotes extrapulmonary gas exchange, which again is useful given their dependence on winter oxygen availability (Maginniss et al. 2004). Overall, there remains a knowledge gap with respect to how Northern map turtles overwinter, particularly with respect to how physiological mechanisms affect behaviour and how these both work to allow this species to tolerate harsh winter conditions for almost half of their life.

1.4 Challenges with Studying Winter Biology

Although winter conditions can last for nearly half the year at high latitudes, winter is traditionally understudied compared to other seasons. This is largely attributed to limitations associated with temporal, physical, and technological barriers. Researchers are often limited in how much time they can spend in the field during this season given the bulk of the academic calendar occurs during this time of the year (Campbell et al. 2005). Graduate and undergraduate students who would normally be available to help conduct field studies are usually unavailable at this time due to full-time academic commitments (Marsden et al. 2020; McMeans et al. 2020). The weather conditions of winter are logistically challenging for humans for many of the same reasons they are challenging to other animals; extreme cold, deep snow, short days, and the presence of ice can all make field work more difficult and dangerous (Brown and Duguay 2010; Shuter et al. 2012). For aquatic research, many sampling sites are restricted due to boat inaccessibility or spotty ice coverage, and winter snorkelling or scuba diving requires specialized training and equipment which, even then, exposes swimmers to dangerous conditions (Marsden et al. 2020). Further, much of the sampling equipment used during the warmer months of the year does not work under winter weather conditions, requiring researchers to invest in specialized sampling gear (Studd et al. 2021). Despite these limitations, winter research is important for addressing unknowns about annual energy budgets and bioenergetics and is becoming increasingly important as we attempt to anticipate the impact of climate change (Marsden et al. 2020; Studd et al. 2021). This is particularly true for organisms at mid to high latitudes, who are expected to be the most affected by global climate warming relative to those in lower latitude regions (Dillion et al. 2010). Luckily, advancements in technology in recent years has made studying winter more accessible for researchers.

1.5 Biologging

Biologgers are a type of electronic tagging technology that can be fixed to an animal to record data using different types of sensors (Cooke et al. 2016). Recent advancements in biologging technology have allowed researchers to record and quantify more detailed activity patterns of free-ranging animals (Wilson et al. 2006; Gleiss et al. 2011; Fosette et al. 2012; Wright et al. 2014; Brownscombe et al. 2018). Biologgers equipped with tri-axial accelerometers, temperature, and pressure (depth) sensors are becoming a common approach to record the behaviour of aquatic vertebrates without direct human interference. Acceleration biologgers are useful for capturing fine-scale information on locomotion and body position, that can then be used to quantify animal behaviour and energy expenditure as it related to activity (Shepard et al. 2008; Brown et al. 2014). An accelerometer works by recording the acceleration of an animal in three spatial dimensions, including dynamic (animal movement) and static (gravity) acceleration (Halsey et al. 2009; Gleiss et al. 2011; Cooke et al. 2016). Acceleration values are used to calculate overall dynamic body acceleration, which is a proxy for locomotor activity and field metabolic rate (Wilson et al. 2006, Gleiss et al. 2011; Halsey et al. 2011). This makes it useful for studying ectotherm behaviour and bioenergetics. When attached securely without interference to an animal's normal locomotion capabilities, these loggers can be used for an extended period of time to capture the animal's behaviour in the wild. For researchers conducting studies during the winter, this hands-off approach to recording data can help eliminate some of the issues associated with sampling restrictions. With specific consideration of aquatic ectotherms like turtles, individuals can be caught and tagged during the warmer months of the year and recaptured after the winter once ice-thaw has occurred. Further, biologger data can help address questions related to winter bioenergetics given that locomotor activity (i.e., energy expenditure) and temperature are important parts of an ectotherm's energy budget (Cooke et al. 2016).

1.6 Respirometry and Metabolic Rates

Another technique for assessing bioenergetics is the determination of the metabolic rate. This is the rate at which an individual transforms and expends energy, usually measured indirectly with respirometry trials (Mochancez et al. 2017). Respirometry is a long-standing method used to measure aquatic oxygen consumption rates (MO_2) in flow-through (Niimi 1978), intermittent-flow (Steffensen 1989), or closed-flow (Ege and Krogh 1914; Scholander et al. 1943) systems. Commonly, MO_2 values are then used to estimate standard metabolic rate (SMR) that are standardized for mass allowing us to make comparisons across different sized individuals in a population (Dillon et al. 2010). An individual's SMR refers to the energy expended for strictly subsistence living (i.e., no locomotor activity, digestion, or growth) which essentially supports only homeostatic activities (Chabot et al. 2016). In ectothermic species, SMR is influenced by both temperature (Hulbert and Else 2004) and by body mass (Brown et al.

2004). Therefore, calculating SMR for a species of interest can inform us of how individuals are influenced by different environmental conditions and allow us to predict how certain temperatures may influence energy stores over time. With respect to ectotherms in winter, namely Northern map turtles, estimating SMR can allow us to project for if a specific temperature at overwintering sites can afford them greater energy savings over time (Gregory 1982). Alternatively, if not for energy savings, then choosing overwintering sites with a specific temperature range may be a mechanism that allows turtles meet their oxygen demands by either decreasing their demand of extrapulmonary respiration to reduce their overall oxygen demand or by simply providing higher concentrations of dissolved oxygen (Greaves and Litzgus 2008).

1.7 Research Objectives

The overall goal of my thesis was to address key knowledge gaps about Northern map turtle overwintering behaviour and physiology. Using both recent advances in technology and conventional scientific methods, the focus of my thesis is to better understand how Northern map turtles survive the coldest part of their annual cycle (i.e., winter). In Chapter 2, my first objective was to quantify the locomotory activity of Northern map turtles under the ice to confirm previous field observations of such behaviour. Using biologgers, I recorded locomotor activity, water temperature, and depth use before, during, and after the ice period wherein they experienced prolonged submergence under ice. This information was used to examine which factors may influence locomotor activity. Additionally, I used these data to test for differences in overwintering behaviour between three demographic classes (i.e., adult females, adult males, juvenile females). In Chapter 3, my second objective was to understand the temperature dependence of metabolism at winter water temperatures using values informed by the results of my biologging study. Here I attempted to quantify the differences in mass-specific standard metabolic rates of adult females. This information was used to assess factors that may have influenced behavioural differences observed with the biologgers in Chapter 2. Together, these findings will help fill in knowledge gaps and inform future studies of ectotherm winter behaviour and physiology. Moreover, these findings will be useful as we continue to project and prepare for changes to annual cycles and the subsequent adaptations of all species in conjunction with our changing global climate.

Chapter 2: Five months under ice: biologging reveals behavior patterns of overwintering freshwater turtles

2.1 Abstract

Winter in temperate regions is characteristically the coldest period of the year. Species in these regions must adapt to freezing temperatures with physiological or behavioural mechanisms to mitigate the threats of cold exposure. For aquatic species, taking refuge under the ice minimizes the risk of experiencing potentially lethal freeze injury. The Northern map turtle (*Graptemys geographica*) is one example of an organism which overwinters under the ice of lakes and rivers. Here, we observed the behaviour of free ranging overwintering Northern map turtles throughout an entire winter using biologgers equipped with tri-axial acceleration, temperature, and depth sensors. We observed that Northern map turtles maintain localized locomotor activity throughout the overwintering period. However, the extent and patterns of locomotor activity and habitat use varied among adult females, juvenile females, and adult males. All three demographic classes remained at temperatures near freezing (0.98 –1.39°C) and at average depths ranging from 1.34 – 1.7 m. Patterns of locomotor activity, depth, and temperature use are consistent with a strategy to survive the winter while remaining aerobic.

Keywords: biologging, freshwater, *Graptemys geographica*, locomotor activity, overwintering

2.2 Introduction

Winter in temperate regions is the coldest period of the year, where temperatures remain around or below freezing (0°C), for several months (Williams et al. 2015). Animals in these areas must avoid prolonged exposure to the cold, which can lead to freeze injury or death. Consequently, temperate species display a variety of adaptations to avoid the threats of cold exposure. While some species survive freezing temperatures through physiological adaptations such as freeze tolerance and freeze resistance, others avoid freezing temperatures altogether by migrating or selecting habitats buffered against freezing (Storey and Storey 2017; Studd et al. 2021). Freshwater environments can offer a refuge from freezing temperatures in the winter for certain aquatic and semi-aquatic species including many amphibians and reptiles (Ultsch 2006; Jackson and Ultsch 2010; Storey and Storey 2017). Overwintering underwater, however, may present a substantial physiological challenge for air breathing vertebrates. Freezing temperatures allow ice to form on surface waters which can force aquatic and semi-aquatic species to be submerged for several months without access to atmospheric oxygen (Greaves and Litzgus 2007; Edge et al 2009; Hampton et al. 2017; Studd et al. 2021).

Most species of freshwater turtles – including the eight species found in Canada – escape freezing winter temperatures by overwintering in liquid water under the ice (Ultsch 2006). Some species including the Painted turtle (*Chrysemys picta*) and the Snapping turtle (*Chelydra serpentina*), can survive several months submerged in anoxic water (Ultsch 2006). Other species, however, cannot survive an entire winter without oxygen and rely on extra-pulmonary routes to extract dissolved oxygen (DO) from the water (Ultsch and Cochran 1994; Reese et al. 2001; Reese et al. 2003). The Northern map turtle (*Graptemys geographica*) is such a species. Laboratory studies indicate that Northern map turtles die from metabolic acidosis after 45 days in anoxic water at 3°C, making normoxic conditions a requirement to support aerobic respiration throughout the winter (Crocker et al. 2000; Reese et al. 2001).

Northern map turtles overwinter communally (Ultsch et al. 2000; Fig 1b) and show fidelity to their overwintering sites (Graham et al. 2000). This aggregation behaviour may indicate that overwintering sites with sufficient oxygen for this species are limited, and a similar suggestion has been made for other freshwater turtle species like the Spotted turtle (*Clemmys guttata*; Ultsch and Jackson 1995; Litzgus et al. 1999; Reese et al. 2001; Ultsch 2006; Rasmussen and Litzgus 2010). Northern map turtles appear to be alert (i.e., conscious and actively moving) in winter and have been documented to show locomotor activity at temperatures near 0°C (Evermann and Clark 1916; Graham and Graham 1992). This behaviour is uncommon for freshwater turtles at this time of year and to date has not been continuously recorded in any other species, but has been observed in examples such as the Wood turtle (*Glyptemys insculpta*; Greaves and Litzgus 2007). The extent of Northern map turtle winter activity, as well as the reasons for it remain unknown; however, locomotor activity in the winter may be related to oxygen consumption. For instance, overwintering Smooth softshell turtles (*Trionychidae spp*.) do “push-ups” which are hypothesized to help turtles shed the oxygen depleted boundary layer and ventilate their skin surface (Plummer & O’Neil 2019). Winter activity may thus be necessary for anoxia intolerant species to meet their demand for oxygen.

Studying the behaviour of Northern map turtles under the ice is logistically challenging and our understanding of their overwintering behaviour thus comes from short term field observations and laboratory studies (Graham and Graham 1992; Crocker et al. 2000; Reese et al. 2001; Maginniss et al. 2004). Advancements in biologging technology allows researchers to record detailed activity patterns in free ranging animals (Wilson et al. 2006; Gleiss et al. 2011;

Fossette et al. 2012; Wright et al. 2014; Brownscombe et al. 2018) and thus offer opportunities to capture the behaviour of turtles overwintering in the wild. Here we used biologgers equipped with tri-axial acceleration, temperature, and pressure sensors to measure locomotor activity, as well as temperature and depth use of Northern map turtles overwintering in the wild. Tri-axial accelerometers produce recordings of acceleration in three spatial dimensions (see Halsey et al. 2009; Gleiss et al. 2011), which can then be used to calculate the overall dynamic body acceleration (ODBA) of animals, a proxy for locomotor activity and field metabolic rate (Wilson et al. 2006; Gleiss et al. 2011; Halsey et al. 2011).

Our first objective was to quantify locomotor activity, water temperature, and depth use of the Northern map turtle before, during, and after the period of forced submergence under the ice. Documenting locomotor activity, as well as temperature and depth use, provides information on how anoxia intolerant turtles meet their metabolic requirements during a significant part of their life. Our second objective was to test for differences among demographic classes (i.e., sex and age) in overwintering behaviour. The Northern map turtle shows pronounced sexual size dimorphism. In our study population, adult females weigh between 800 and 3880 g whereas males never exceed 400 g. We hypothesized that behaviours during the overwintering period would differ between demographic classes because of these marked size differences. Size differences between demographic classes have been observed to translate into inherent differences in metabolic rates and oxygen needs (Graham and Graham 1992). Graham and Graham (1992) measured oxygen consumption in one adult male and two adult females submerged at 3°C and found the male to consume 3.7 to 4 times less oxygen in absolute terms than the females. According to these measurements, adult females (i.e., larger turtles) should deplete DO more rapidly in their surroundings than males (i.e., smaller turtles), therefore we hypothesized that this would translate into reduced activity or a tendency for females to seek colder temperatures as a way to passively reduce metabolic and oxygen needs.

2.3 Materials and Methods

2.3.1 Study Site

We conducted this study in Lake Opinicon in Ontario, Canada (44°55’90” N, 76°32’80” W). Lake Opinicon is a medium-sized (7.9 km^2) shallow lake, averaging 2.5 m in depth, with a maximum depth of approximately 11 m (Feng et al. 2019). The surface of the lake typically remains frozen from late December to early April, apart from the lakes’ eastern end, where it connects to the Rideau Canal, and two creeks at the southeastern end of the lake (Feng et al. 2019). It is estimated that there are over 1500 Northern map turtles located within Lake Opinicon (1.9 turtles/ha; Bulté et al. 2010). Overwintering sites were previously identified along the shoreline of an island in Lake Opinicon using radiotelemetry (Carrière et al. 2009) and monitored as part of a mark-recapture study since 2004.

In this study, we defined the overwintering period as the time between ice-on and ice-off at the overwintering site because it corresponds to the period that atmospheric oxygen is unavailable to turtles. Using two time-lapse cameras (Timelaspecam, Winsgcape, Birmingham, Alabama, USA) to take daily pictures of the water surface in the vicinity of the communal hibernaculum we estimated this period to be 19-Dec-2020 to 27-Mar-2021. We defined two additional timeframes within our study period to delineate times without ice-coverage: pre-ice in the fall (24-Oct-2020 to 18-Dec-2020), and post-ice in the spring (28-Mar-2021 to 12-Apr-2021).

Between October 16th and 21st 2020, we captured 9 juvenile females, 17 adult females, and 14 adult males by snorkeling in the vicinity of the communal overwintering site. Juvenile females were included in the study because their body size is midway between adult females and adult males yielding three demographic classes for comparative purposes (i.e., adult males, adult females, and juvenile females). On each turtle, we fitted a biologger (22 x 45 x 8 mm, 11 g in air: Axy-5, TechnoSmArt, Guidonia Monticello, Italy) and a radio transmitter (15 x 8.2 mm, 1.5 g in air; NanoTag, Lotek, Newmarket, Ontario, Canada) to permit the recapture and retrieval of the biologgers. The biologgers and transmitters were taped together and epoxied to the right posterior edge of the carapace (Figure 2.1a, b).

The biologgers recorded acceleration (i.e., locomotor activity), water temperature (± 0.1 °C), and depth (± 5 cm). Acceleration was measured at sample rate of 10 Hz with an 8-bit resolution. Biologgers recorded on a schedule to preserve battery life in order to obtain measurements throughout the entire winter. Measurements were taken each day between 0:00 and 04:00 hr and between 07:00 and 17:00 hr. Turtles were relocated between the months of April and October 2021 using radiotelemetry and recaptured to remove the tags. Tags were removed within 12 hours of capture and turtles were released back to where they were captured. Thirty-seven of the 40 tagged turtles (92.5%) were recovered in the spring and summer following their overwintering. Three turtles were not recovered, and four faulty biologgers did not yield data leaving a sample size of 13 adult females (209 – 250 mm maximum carapace length; 1076 – 1675 g), 9 juvenile females (142 – 185 mm maximum carapace length; 357 – 1388 g), and 11 adult males (126 – 141 mm maximum carapace length; 222 – 302 g).

2.3.2 Environmental Monitoring

We installed six DO and temperature loggers (aquaMeasure DO, InnovaSea Systems Inc.) along a 500 m transect running parallel to the shoreline in the vicinity of the overwintering site. The loggers were stationed at depths ranging from 1.8 to 4.6 m and recorded DO and temperature every two hours between 07-Nov-2020 and 19-Apr-2021. Additionally, on three separate days (05-Dec-2020, 13-Feb-2021, 20-Feb-2021), we measured DO and temperature at 7 to 13 random locations using the same loggers. Measurements were used to create temperature and DO profiles of the overwintering site to observe how these parameters changed over time during the study period.

2.3.3 Locomotor Activity

Locomotor activity was calculated as ODBA following the methods described in Brownscombe et al. (2018). Tri-axial acceleration data consisted of acceleration (*g*) in three axes (A_x = surge, A_y = heave, A_z = sway with respect to the attachment location on the right posterior edge of the carapace) which was then corrected using a 2-second box smoother to remove static acceleration (i.e., gravity) from the dynamic acceleration (Shepard et al. 2008). The optimal smoothing interval was determined for 10 Hz based on the methods described in Shepard et al. (2008). Values of total daily ODBA were then obtained using these corrected values to produce the sum of absolute dynamic acceleration from all three axes (A_x, A_y, A_z). These values were then observed over time throughout the study period to determine if total daily ODBA during the overwintering period (19-Dec-2020 to 27-Mar-2021) was greater than 0 *g*, which would confirm locomotor activity occurred. This was done using *ggplot2* package to create graphs in R Studio (Wickham 2016). A background acceleration measurement was taken with an accelerometer left untouched for a 24-hour period under the same configuration as the deployed biologgers. This produced a total ODBA of 0.02 *g*, confirming that when no movement occurred ODBA is near 0 *g*. Plots of locomotor activity, as well as depth use and temperature use, for a randomly selected turtle over one randomly selected day from each period (26-Oct-2020, 13-Feb-2021, 09-

Apr-2021) has been included as supplementary material to visualize how behaviour changes across one day of recording (Appendix A).

2.3.4 Statistical Methods

To assess external influences on locomotor activity during the ice-on period, a linear mixed-effects model was fitted using the *lmer* function from the *nlme* package in R (Bates et al. 2015) with ODBA as the response variable where demographic class, water temperature, depth, and DO were considered as predictor variables, and turtle ID was used as a random variable. Water temperature and depth from the biologgers were included as measurements that are generally considered to influence locomotory activity (i.e., ODBA) and were measured simultaneously by the biologgers. Demographic class was included because of our hypothesis this difference in size and age would influence locomotor activity. Measurements from the DO loggers were also initially included because it was expected locomotor activity might be driven by this species trying to meet their oxygen requirements under the ice. Model selection using Akaike Information Criterion (AIC) was used to produce a final model; the best model was selected based on an AIC difference greater than 2. This model was then followed up with an ANOVA to explore the differences among the significant predictor factors, and finally a Tukey post-hoc test using the *glht* function from the *multcomp* package.

Linear models were used to test the hypothesis that behaviour during the overwintering period differs between demographic classes. Model assumptions were checked following those described in Zuur et al. (2010), utilizing the *ggdensity* and *ggqqplot* functions in R to confirm normality and heterogeneity of variances. A linear model was fit using water temperature as the response variable and demographic class as the predictor variable to test our hypothesis that behaviours would differ between these classes. Similarly, a second linear model was fit with depth use as the response variable to determine if depth use differed between the three demographic classes. Both linear models were fit using the *aov* function. Using the *glht* function from the *multcomp* package, Tukey post-hoc tests were used to further explore the significant differences among the sexes (Hothorn et al. 2009). This same process was repeated for all three periods to allow us to also test for behavioural differences in our demographic classes within each time frame. P values < 0.05 were deemed statistically significant. All values have been reported as mean values +/- standard deviation unless otherwise indicated. All analyses were conducted in R (4.1.2) via R Studio (2021.09.1).

2.4 Results

2.4.1 Habitat Characteristics

Average daily DO and water temperature at the overwintering site varied throughout the study period (Figure 2.2). The average daily DO observed at the overwintering site during the pre-ice period remained between 92% and 100% dissolved oxygen saturation before ice formation occurred. The average daily temperature during this period was 9.1°C at the highest, which steadily declined to a low of 0.3°C when ice formation occurred. During the ice-on period, average daily DO saturation steadily decreased throughout, declining from 94% to 9% at its lowest point (Figure 2.2). Conversely, the daily average temperature steadily increased between 0.7°C and 5.1°C before ice melt occurred (Figure 2.2). After ice melt, DO saturation rapidly increased, with daily averages increasing from 33% back to the 100% saturation seen in the pre-ice period. Daily average temperatures ranged between 4.7°C and 9.2°C. Random site samples of DO and temperature on December 5 ranged from 88.5 to 90.6% and 2.5 – 3.3°C. On February

13, values ranged from 48.6 to 78.9%, and -0.5 and 2.4°C. On February 20, values ranged from 47.1 to 85.6% and 1.9 to 4.6°C.

2.4.2 Locomotor Activity

Total daily ODBA for each individual Northern map turtles was greater than 0 *g* each day throughout the study period (Figure 2.3a). Model selection with AIC resulted in a final model with a fixed slope where demographic class, water temperature, and depth were the independent predictor variables. We hoped to include our DO measurements in our model but they were eliminated from the final model given their inclusion produced higher AIC scores. The best-fit model did not include interactions between independent variables, and turtle ID was included as a random effect to account for the repeated measures across individuals. The final linear mixed-effects model suggested that water temperature ($F_{1,\,3081} = 8.771, p = 0.003$) and demographic class ($F_{2,\,33} = 3.480, p = 0.042$) both had a significant effect on locomotor activity (ODBA) during the ice-on overwintering period whereas depth did not ($F_{1,\,2645} = 2.517, p = 0.113$). Adult females showed the least amount of locomotor activity (578 ± 79 *g*) compared to adult males (603 ± 98; $z_2 = 2.097, p = 0.09$) and juvenile females (608 ± 94 *g*; $z_2 = 2.355, p = 0.049$) during the ice-on period (Figure 2.3a). There was no statistically significant difference in total daily ODBA between juvenile females and adult males ($z_2 = -0.371, p = 0.927$; Figure 2.4a). There was a weak but statistically significant positive relationship between total daily ODBA and water temperature ($F_{1,\,3081} = 8.771, p = 0.003;$ Figure 2.5). In the last week before the ice completely melted, the mean water temperature around the Northern map turtles increased from 2.7 to 4.3 °C in a single week. We suspected this rapid change in temperature had an overly strong influence on the association between ODBA and temperature. We thus ran the model again after removing that week of data (~8% of the total period) and the relationship between ODBA and temperature become statistically non-significant, indicating the relationship we observed was primarily driven by the increasing temperatures during the last week of ice-on (Figure 2.5).

Total daily ODBA for each group was lower during the ice-on period relative to the pre-ice and post-ice period (Figure 2.3a). Adult females had an average daily ODBA of 597 ± 74 *g* during pre-ice and 634 ± 80 *g* during the post-ice period, making ODBA 3 and 9 % higher than during the ice- on period. Adult males had an average daily ODBA of 674 ± 124 *g* during pre-ice and 868 ± 219 *g* during post-ice, making ODBA 10 and 30% higher than during ice-on. Finally, juvenile females had an average daily ODBA of 615 ± 71 *g* during the pre-ice period and 644 ± 74 *g* during the post-ice period, which was 1 and 6 % higher than the ice-on period. Total daily ODBA differed between demographic classes in the pre-ice period ($F_{2, 1836} = 223.9, p < 0.001$) and post-ice period ($F_{2, 472} = 139.5, p < 0.001$; Figure 2.4a). During the pre-ice period, adult male locomotor activity was greater than adult females ($t_2 = 20.01, p < 0.001$) but less than juvenile females ($t_2 = 15.85, p < 0.001$). Juvenile female locomotor activity was higher than adult female ($t_2 = 2.377, p = 0.046$; Figure 2.4a). During the post-ice period, adult males had greater locomotor activity than adult females ($t_2 = 15.17, p < 0.001$) and juvenile females ($t_2 = 16.99, p < 0.001$), while no difference in locomotor activity was detected for adult and juvenile females ($t_2 = 16.36, p = 0.594$).

2.4.3 Depth and Temperature Use

Daily average water temperature use differed between demographic classes during the ice-on period ($F_{2, 3218} = 43.72, p < 0.001$) (Figure 2.3b). Adult females were observed at 1.0 ± 1.0°C lower than both adult males (1.15 ± 0.98°C; $t_2 = 4.242, p < 0.001$) and juvenile females (1.4 ± 1.1°C; $t_2 = 9.242, p < 0.001$). Adult males were observed at lower temperatures than juvenile females ($t_2 =-5.069, p <0.001$). The daily average temperature each demographic class was observed at during the ice-on period was lower than those seen in the pre-ice and post-ice periods (Figure 2.4b). Adult females were observed at an average daily temperature of 4.7 ± 2.6 °C during the pre-ice period and 7.5 ± 3.3 °C during the post-ice period, adult males at 4.7 ± 2.6 °C pre-ice and 7.6 ± 3.2 °C post-ice, and finally juvenile females at 5.0 ± 2.5 °C pre-ice and 7.5 ± 2.7 °C post-ice. We did not detect any statistically significant differences in daily average temperature use during the pre-ice period when compared between demographic classes ($F_{2,\ 1836} = 0.717, p = 0.488$) or post-ice period ($F_{2,\ 472} = 0.115, p = 0.891$; Figure 2.4b).

The daily average depths recorded during the ice-on period were shallower than those seen in the pre-ice and post-ice period and differed between demographic classes during the ice-on period $F_{2,\ 3218} = 79.95, p < 0.001$; Figure 2.3c). Adult females were observed shallower (1.34 ± 0.63 m) on average when compared to both adult males (1.52 ± 0.61 m; $t_2 = 6.494, p < 0.001$) and juvenile females (1.7 ± 0.69 m; $t_2 = 12.59, p < 0.001$; Figure 2.4c). Adult males were observed at shallower depths than juvenile females ($t_2 = -6.257, p < 0.001$; Figure 2.4c). Adult females were observed at an average depth of 2.72 ± 0.92 m during the pre-ice season and 2.46 ± 1.16 m during post-ice. Adult males were observed at an average depth of 2.24 ± 0.74 m pre-ice and 1.91 ± 0.88 m post-ice. Finally, juvenile females were observed at 2.63 ± 0.61 m pre-ice and 2.65 ± 0.97 m post-ice. Depth use differed between sexes in both the pre-ice ($F_{2,\ 1836} = 80.22, p < 0.001$) and post-ice periods ($F_{2,\ 472} = 21, p < 0.001;$ Figure 2.4c). In the pre-ice season, adult males were shallower than adult females ($t_2 = -12.135, p < 0.001$) and juvenile females ($t_2 = -9.124, p < 0.001$), while there was no difference between adult and juvenile females ($t_2 = 1.950, p = 0.125$: Figure 2.4c). In the post-ice season, adult males were once again shallower than adult ($t_2 = -4.993, p < 0.001$) and juvenile females ($t_2 = -6.039, p < 0.001$), while no difference was observed between adult and juvenile females ($t_2 = 1.642, p = 0.228$) (Figure 2.4c).

2.5 Discussion

In temperate areas, freshwater turtles primarily overwinter in water and can spend a third of their life trapped below ice. Despite the substantial amount of time some turtles spend under the ice, very little is known about their behaviour during this part of their annual cycle. To address this gap, we used biologgers to quantify winter locomotor activity, water temperature use, and depth use of overwintering Northern map turtles near the northern edge of their range where they experience 119 days of ice cover per year on average. Locomotor activity under the ice was successfully recorded in 33 turtles throughout the entire overwintering period. Locomotor activity was lower during the ice-on period, relative to pre-ice and post-ice periods, but Northern map turtles remained active during the entire duration of their forced winter submergence. Video recordings show that overwintering Northern map turtles are active enough to change their location on a regular basis in the winter (Bulté, unpublished data). Some of the locomotor activity measured with the loggers thus corresponds to movement between locations within the overwintering site. While some aquatic ectotherms (i.e., fish, amphibians, turtles) become dormant in winter (Ultsch 1989; Speers-Roesch et al. 2018), our results suggest that Northern map turtle do not become completely dormant and immobile. Instead, we suggest that the observed locomotor activity is part of the winter survival strategy of Northern map turtles.

Northern map turtles are anoxia intolerant and will die after 45 days when submerged in anoxic water at 3°C (Ultsch and Jackson 1995; Reese et al. 2001). Previous studies have proposed several mechanisms by which Northern map turtles remain aerobic and prevent acid-base or ionic disturbances in the blood stream, which can result in mortality (Maginniss et al. 2004). These include an improved efficiency of extrapulmonary gas exchange across the body relative to other species, and adaptations of the blood oxygen transport system (Jackson et al. 2001; Reese et al. 2001, Maginniss et al. 2004). Moreover, independent video recordings of overwintering Northern map turtles in Lake Opinicon, suggest that they may also use buccopharyngeal gas exchange to remain aerobic (Bulté, unpublished data).

It has been proposed that Northern map turtles physiologically depress their metabolism to some extent, but this idea is only supported by observations of reduced activity (Crocker et al. 2000; Maginniss et al. 2004). Given the amount of locomotor activity we observed, metabolic depression may only occur under anoxic or hypoxic conditions. Under such conditions, Northern map turtles may rely on anaerobic metabolism so depressing metabolism can slow down the build-up of lactic acid and changes in blood pH which can lead to death (Reese et al. 2001; Jackson et al. 2007). Alternatively, Northern map turtles may not engage in an active metabolic depression, but rather may be taking advantage of cold-water temperatures to passively reduce their metabolic processes as well as their oxygen demand. This could afford them energy savings over extended dormancy, as well as reduce their oxygen needs during a time when such a resource becomes increasingly limited as the season goes on. By maintaining locomotor activity, Northern map turtles may be able to move into microclimates that support their oxygen needs and avoid anoxic conditions, therefore removing the need to resort to metabolic depression. Further, locomotion may also support oxygen needs by allowing Northern map turtles the chance to escape the oxygen depleted boundary layer and ventilate their skin surface, hence favouring cutaneous gas exchange (Plummer & O’Neil 2019).

Locomotory activity was 1-10% higher in the pre-ice period and 6-30% higher in the post-ice period relative to the ice-on period for all Northern map turtles (Figure 2.3a), which coincided with warmer water temperatures and greater DO availability (Figure 2.2, Figure 2.3b).

Locomotor activity for adult females was 4% lower than adult males and 5% lower than juvenile females. These small differences in activity may be linked to the marked sexual size dimorphism present in this species. Adult males and juvenile females (i.e., smaller individuals) may move more, simply because smaller individuals have a lower absolute need for oxygen as well as a greater relative surface area to obtain it from the water (Graham and Graham 1992). Thus, it may be easier for smaller turtles to meet their oxygen demand in winter. Locomotor activity may be riskier for adult females. The metabolism of submerged females at 2°C is 3.7 to 4 times that of males (Graham and Graham 1992). The oxygen demand cost of locomotion combined with the risk of entering a microhabitat with less DO may constrain locomotory activity of females compared to males.

Males may also have a reproductive incentive to move in winter. Northern map turtles mate just before and after the winter (Vogt et al. 2018), and in lake Opinicon mating occurs at communal overwintering sites within days of the spring ice melt (Bulté et al. 2018; Bulté et al. 2021). Male Northern map turtles prefer larger females over smaller ones (Bulté et al. 2018) and also appear to consider the potential for sperm competition when selecting mates (Bulté et al. 2021). Males may use the winter to position themselves near preferred mates to be at an advantage when the spring mating season resumes.

Northern map turtles were all observed at lower temperatures during the ice-on period relative to the pre- and post-ice periods (Figure 2.3b). This observation can in part be attributed to the fact that temperatures are generally colder during this period of the year. More interestingly, all three demographic classes were observed at average daily temperatures near 1°C during the ice-on period. Adult females were observed at marginally lower water temperatures relative to adult males and juvenile females. Like locomotor activity observations, these subtle differences in water temperature use may be attributed to the difference in body size between demographic classes and therefore metabolic needs. Although water temperature use was statistically different between the observed groups, the biological significance of these differences is unclear because the absolute differences in temperature were small (< 0.5°C). Winter water temperature selection has been studied in many freshwater turtles (Brown and Brooks 1994; Litzgus et al. 1999; Crocker et al. 2000; Greaves and Litzgus 2007; Rollinson et al. 2008; Edge et al. 2009) but rarely in relation to sex. Due to the dramatic difference in body size seen in Northern map turtles, small differences in temperature selection may translate to biologically meaningful differences in metabolism over several months. Confirming temperature selection in this species and further quantifying metabolic rates at near-freezing temperatures would allow us to assess the metabolic consequences of the temperature use differences we observed in the field.

Regardless of the differences in water temperature use among demographic classes, all turtles remained at water temperatures near 1°C. Other species of turtles have been reported to select water temperatures near 0°C in winter (Brown and Brooks 1994; Litzgus et al. 1999; Crocker et al. 2000; Greaves and Litzgus 2007; Rollinson et al. 2008; Edge et al. 2009). Selecting colder water has the advantages of passively reducing oxygen demand through the effects of temperatures on metabolism and increasing oxygen availability due to the inverse relationship between DO and water temperature. While some species of freshwater turtles engage in metabolic depression in winter (Hebert and Jackson 1985; Jackson et al. 2001), this mechanism has not been directly demonstrated in Northern map turtles. Given the level of activity we observed, we suggest that under normoxic conditions such as those at our study site, Northern map turtles do not physiologically depress their metabolic rate further than what is predicted from the effects of temperature alone.

Along with locomotor activity and water temperature use, depth use also differed among groups (Figure 2.3c). Adult females were observed at the shallowest depths followed by adult males and juvenile females. Depth use for all groups was shallower during the ice-on period relative to the pre- and post-ice periods. Depth use may be influenced by several factors, and the shift to shallower depths likely reflects a preference for lower temperatures and higher DO concentrations, given cold water can hold more oxygen. The observed depth use may also be linked to substrate preferencs. Observations of Northern map turtles at overwintering sites indicate they often lay on rocks and logs or open sand/gravel substrates (Graham and Graham 1992; Fig 2.1b). By laying atop hard substrates rather than burying themselves or settling in loose sediment, Northern map turtles may keep their skin exposed and free of debris which may be essential for extrapulmonary gas exchange.

Although measurements of DO were taken, we ultimately removed DO from our model because it was not a significant predictor of ODBA. This, however, should not be taken as unequivocal evidence that DO does not influence the behaviour of overwintering Northern map turtles. We believe DO may play an important role at scales that we did not measure. In our study, the overall trend in DO was measured only in the vicinity of the communal overwintering site. Individual turtles may not respond to the average lake DO but may respond to small, localized variations that we were incapable of measuring. The concentration of DO may also play a role in how overwintering sites are chosen. Studies on Painted turtles (*Chrysemys picta*) and Blanding's turtles (*Emydoidea blandingii*) have suggested that when aerobic metabolism is maintained, DO is a driving force in winter habitat selection (Rollinson et al. 2008; Edge et al.

2009). We did not measure the availability of DO in the whole lake so we cannot assess if Northern map turtles select this particular overwintering site because of its relatively high DO availability in winter. At least half of the population of Northern map turtles in Lake Opinicon uses our study site to overwinter (Bulté, unpublished) suggesting that suitable overwintering sites are limited. As such, DO may well be an important limiting factor for overwintering survival.

Overall, this study revealed that Northern map turtles remain active throughout their overwintering period. To our knowledge, this is the first direct demonstration that a turtle overwintering under the ice can remain active all winter using this type of biologging technology. Given a schedule was used to prolong the life of the biologgers batteries, we captured 14 hour of behaviour a day for this species which, for the scope of this project, was sufficient confirm movement occurs continuously under the ice. Future studies that are able to record 24-hour periods may provide further insight into how behaviours change throughout the day and/or identify patterns in behaviour related to the diurnal cycle of map turtles that our study did not address. We also identified intraspecific behavioural differences across demographic classes, suggesting overwintering behaviour is influenced by sex and potentially driven by the sexual dimorphism observed in this species. The results of this study should be used to inform future studies related to ectotherm overwintering behaviour. Such information may be useful in identifying critical overwintering habitat and will continue to provide insight into the life-history and fitness of freshwater turtles in the wild.

2.6 Figures

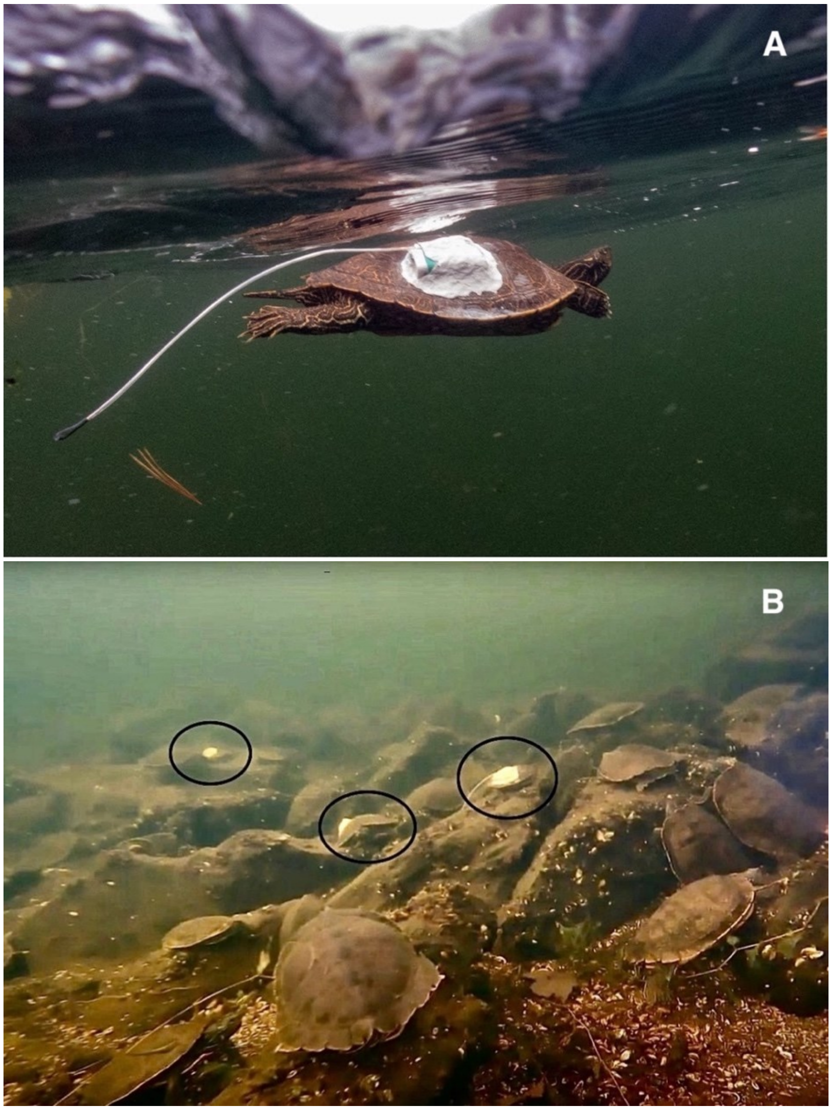

Figure 2.1 Juvenile female Northern map turtle (*Graptemys geographica*) with a tri-axial acceleration biologger and radio transmitter epoxied to her carapace following release (A). Northern map turtles with tri-axial acceleration biologgers and radio transmitters epoxied to their carapace (circled) under the ice in Feb-2021 at their known overwintering site at Lake Opinicon in Ontario (B). Image captured with an underwater Trident ROV (Sofar Ocean, San Francisco, CA).

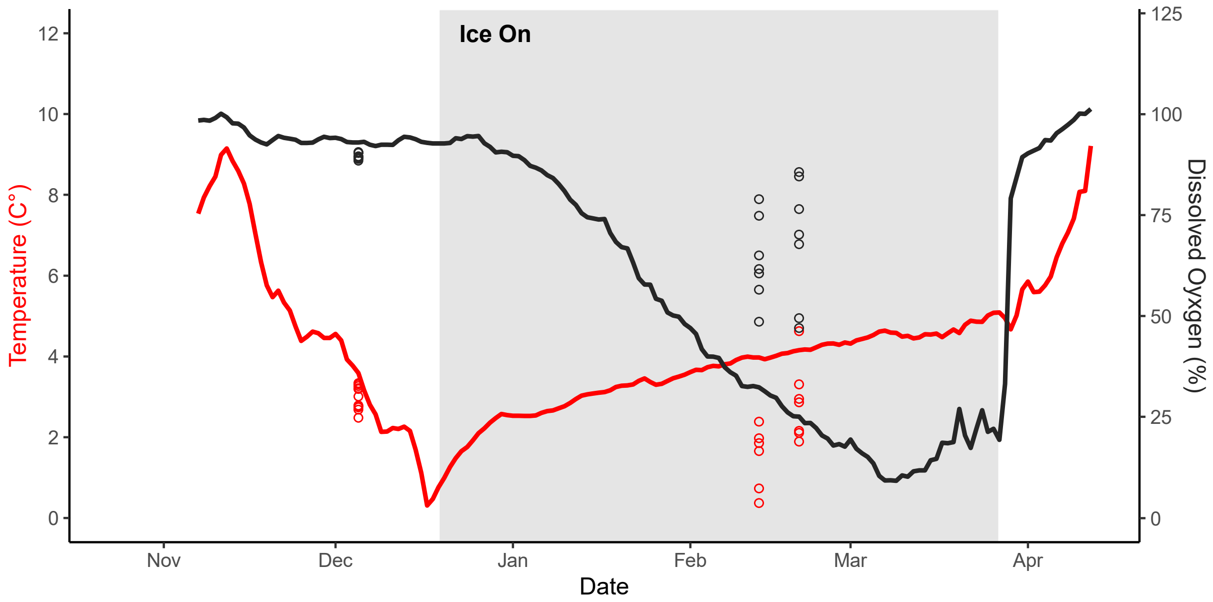

Figure 2.2 Water temperature (°C) and dissolved oxygen (%) measurements from six loggers at known Northern map turtle (*Graptemys geographica*) overwintering site in Lake Opinicon, Ontario from 07-Nov-2020 to 12-Apr-2021. Average water temperature (red) and dissolved oxygen (black) are indicated by solid lines; range of each measurement shown with shading around line. Shaded background area indicates the period of ice-on. Additional points included from random site samples of temperature and dissolved oxygen taken on the lake on 05-Dec-2020, 13-Feb-2021, and 20-Feb-2021.

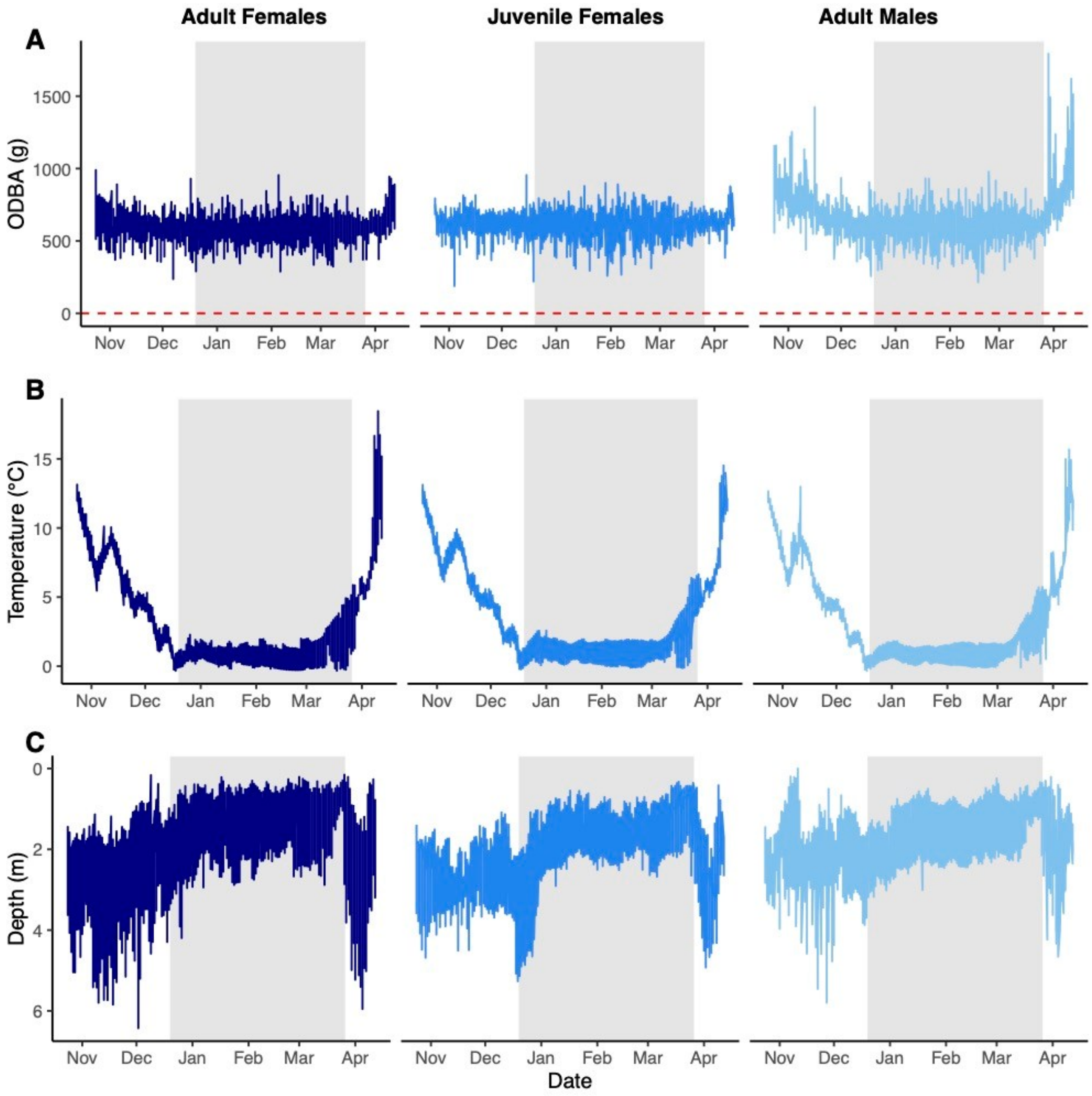

Figure 2.3 Observations of overall dynamic body acceleration (ODBA; A), water temperature use (B) and depth use (C) for Northern map turtle (*Graptemys geographica*) during the study period (24-Oct-2020, to 12-Apr-2021) at Lake Opinicon in Ontario. Results are separated as a reflection of demographic classes: adult females (n=13), juvenile females (n=9), and adult males (n=11). Ice-on overwintering period for Lake Opinicon is represented with the shaded area between 19-Dec-2020 and 27-Mar-2020. No locomotor activity (ODBA = 0.02 *g*) is indicated with a dashed line.

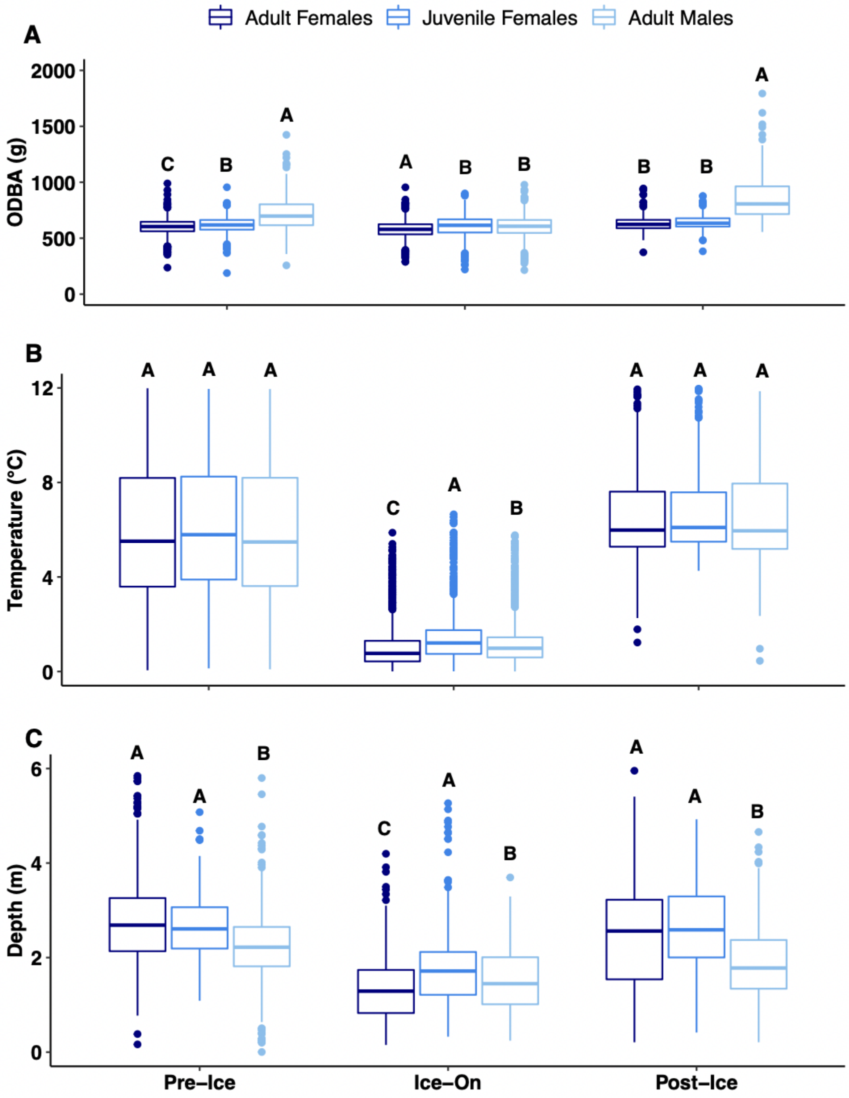

Figure 2.4 Seasonal differences in behavioural observations of overall dynamic body acceleration (ODBA; A), water temperature use (B) and depth use (C) of Northern map turtles (*Graptemys geographica*) across the study period (24-Oct-2020 to 12-Apr-2021) at Lake Opinicon in Ontario. Results are separated as a reflection of demographic classes: adult females (n=13), juvenile females (n=9), and adult males (n=11). Similar letters indicate there are no differences in behaviour between the respective groups for the given season, while differing letters indicate significant dissimilarities.

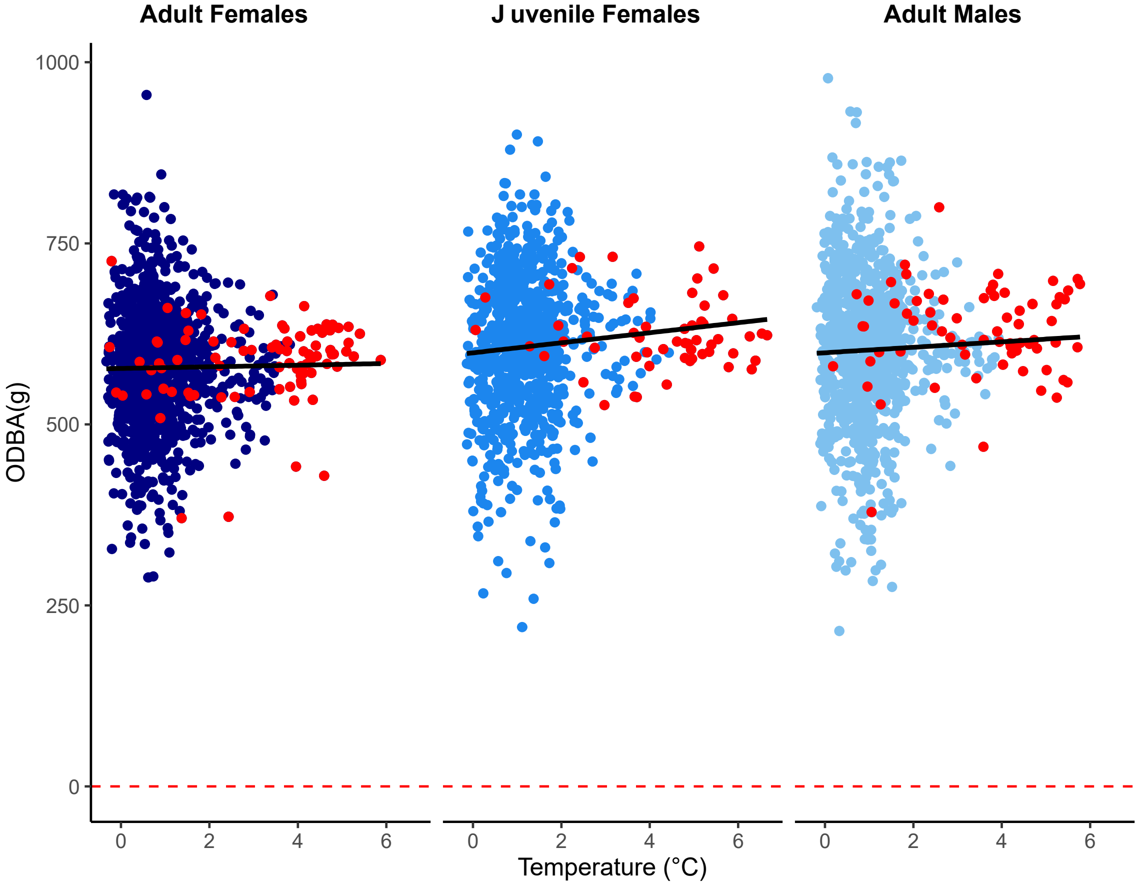

Figure 2.5 Northern map turtle (*Graptemys geographica*) overall dynamic body acceleration (ODBA) and temperature (°C) during the ice-on period (19-Dec-2020 to 27-Mar-2021) at Lake Opinicon, Ontario. Solid lines indicate local regression for adult females (n=13), juvenile females (n=9), and adult males (n=11). Red points represent water temperature values from the last week of the ice-on period which made the relationship with ODBA statistically significant. No locomotor activity (ODBA = 0.02 g) is indicated with a dashed line.

Chapter 3: The effect of temperature on the standard metabolic rate of Northern map turtles (*Graptemys geographica*) in simulated under-ice conditions

3.1 Abstract

Aquatic ectotherms, like certain species of frogs and freshwater turtles, spend up to half of the year submerged under ice as they overwinter in bodies of water. While this behaviour can buffer animals against the subfreezing temperatures on land, it also restricts them from atmospheric oxygen. Freshwater turtles have been broadly categorized into two groups: anoxia tolerant and intolerant. For both groups, reducing metabolism during the winter may be critical to survival, as it helps both reduce oxygen demand and conserve energy stores. Notably, many species of freshwater turtles select habitats with low temperatures (~1°C) which would passively reduce metabolism. Here, the standard metabolic rate (SMR) of the anoxia intolerant Northern map turtle (*Graptemys geographica*) was measured to test the extent to which temperature affects SMR at the range which is available to this species in the winter. We simulated under-ice conditions to estimate SMR at 1°C and 4°C, and found SMR to be 2.1 times lower on average at 1°C. Given that Northern map turtles are anoxia intolerant, selecting for overwintering sites that remain near 1°C would decrease oxygen demand. We suspect that reducing oxygen demand, is a mechanism that allows Northern map turtles to extend how long they can survive submerged under ice when oxygen is a critical but limited resource.

Key Words: metabolic rate, freshwater, *Graptemys geographica*, respirometry, overwintering

3.2 Introduction

At mid- to high- latitudes in the Northern hemisphere, aquatic ectotherms may spend up to half of the year submerged under the ice that forms on bodies of water (Williams et al. 2015). The ice provides these organisms with a buffer against temperatures below 0°C that occur in the terrestrial habitats above (Jackson and Ultsch 2010). However, the formation of ice restricts gas exchange between the water and air. When combined with high levels of under-ice biological oxygen demand from microbes involved with decomposition (Bengtsson and Ali-Maher 2020), declines in oxygen availability are common which can lead to hypoxic or anoxic conditions in the water (Kauko et al. 2017; Studd et al. 2021). Understandably, few lunged vertebrates overwinter completely submerged under water, but northern-ranging frogs and freshwater turtles are notable exceptions, with many species overwintering exclusively under the ice (Jackson and Ultsch 2010).

Physiologists have divided freshwater turtles into two broad categories with respect to aquatic overwintering: those who can survive the entire winter in anoxic water and those who cannot (Ultsch and Jackson 1995; Jackson and Ultsch 2010). The former are generally referred to as anoxia tolerant, whereas the latter are referred to as anoxia intolerant. Anoxia intolerant species may only survive a few weeks under such conditions (Ultsch and Jackson 1982; Reese et al. 2001; Reese et al. 2003). Consequently, anoxia intolerant species depend on habitats with sufficient dissolved oxygen concentrations to survive the winter and use extra-pulmonary gas-exchange to uptake dissolved oxygen from the water (Hebert and Jackson 1985; Yokosuka et al. 2000; Reese et al. 2002; Heiss et al. 2010). Regardless of the degree of anoxia tolerance, it is believed that reducing metabolism during the winter is paramount to freshwater turtle winter survival. Under anoxic conditions, a low metabolism limits the build-up of lactic acid which can lead to lethal acidosis (Rollinson et al. 2008). Under normoxic conditions, a low metabolism helps reduce oxygen consumption and energy use and may extend survival (Jackson and Ultsch 2010).

Some species actively and substantially depress their metabolism during the winter through physiological mechanisms (i.e., metabolic depression). For example, the Painted turtle (*Chrysemys picta*) engages in metabolic depression in response to anoxic conditions (Hebert and Jackson 1985; Jackson et al. 2001). Habitat selection can also help species reduce their metabolism during the winter. These animals may capitalize on the passive effect temperature has on metabolism by selecting habitats with colder temperatures that can reduce oxygen demand (Brown et al. 2004). The temperature of water under the ice generally remains between 0 and 4°C throughout the winter due to the relationship between water density and temperature (McMeans et al. 2020). Interestingly, many studies on freshwater turtle overwintering have found various species to selection for temperatures near 1°C in their environment, which has been proposed as a strategy to reduce metabolism (Brown and Brooks 1994; Litzgus et al. 1999; Crocker et al. 2000; Greaves and Litzgus 2007; Rollinson et al. 2008; Robichaud et al. in preparation). Although the temperature differential between 1°C and 4°C is small, the change in metabolism over this range may be significant, especially when considered over extended overwintering periods (Gregory 1982). Moreover, previous studies have reported strong Q_{10} (i.e., temperature coefficient measure of thermal sensitivity) effects over low temperatures in turtles, such as those observed by Herbet and Jackson (1985) in Painted turtles, suggesting Q_{10} can be quite large over small temperature ranges (i.e., $Q_{10} = 13.3$ between 3°C and 10°C).

The Northern map turtle (*Graptemys geographica*) is an anoxia intolerant species that appears to select overwintering sites in rivers and lakes with sufficient dissolved oxygen to sustain aerobic metabolism during the winter (Crocker et al. 2000; Robichaud et al. in preparation). This species has been observed to aggregate and show fidelity to their overwinter sites, suggesting that habitats with suitable overwintering conditions are limited (Graham and Graham 1992; Ultsch et al. 2000). We recently used biologging technology to study the overwintering behaviour of this species near the northern edge of its range, where they experience an average of 119 days of ice coverage per year. During the ice-on period at a known hibernation site, Northern map turtles were observed at daily average temperatures around 1°C and located at depths between 1 and 2 m (Robichaud et al. in preparation; Figure 2.3b,c). We also showed that this species engages in localized locomotor activity throughout the entire winter, a behaviour that has not been observed and recorded in many species thus far apart from the Wood turtle (*Glyptemys inscuplta*) for example (Greaves and Litzgus 2008). Based on these observations, we hypothesized that the temperatures and depths Northern map turtles select may reduce metabolism and oxygen demands during this critical stage of their annual cycle.

We investigated the effect of temperature on the metabolism of overwintering Northern map turtles at a range of temperatures available to this species during the winter. Our objective was to estimate standard metabolic rate (SMR) for submerged adult females Northern map turtle at 1°C and 4°C and to explore the possible respiratory and energetic benefits of selecting cooler temperatures during the winter. The experimental temperatures are ecologically relevant because temperatures under the ice generally do not exceed 4°C (McMeans et al. 2020), and Northern map turtle have been observed to spend their winter near 1°C (Robichaud et al. in preparation). While Graham and Graham (1992) estimated the SMR of submerged Northern map turtle at 2°C, how much winter temperatures effects metabolism in this species is unknown. By estimating

SMR across this wider range of temperatures, to provide more insight into how this species can survive extended submergence during the winter.

3.3 Methods

3.3.1 Study Species

On November 11, 2021, prior to surface ice formation, we captured Northern map turtles by snorkelling at a known overwintering site in Lake Opinicon, in Ontario, Canada (44°55’90” N, 76°32’80” W). We initially captured four adult female turtles (1839 ± 481 g) and four adult males (261 ± 38 g) that were transported back to the lakeside research facility and immediately placed in a 378-L Rubbermaid stock tank. The males were not used further in the study as the experimental set up was not appropriate for their body size (i.e., the turtles were too small relative to our setup) and could not capture accurate measurements. The holding facility was in a boat house which was open air and provided overhead shelter. To keep holding conditions as natural as possible, the tank was filled with water directly from Lake Opinicon to approximately 80% capacity to give turtles adequate space to swim freely and surface for air as would occur at this time of year. Water was replaced every other day with fresh lake water. Temperature was kept at lake temperature which was between 5°C and 8°C during the study period. Turtles were not fed during their holding period given they do not feed during winter, and to ensure that were in a post-absorptive state. Natural light entered the holding area and was used as the main light source to maintain the circadian rhythm of the turtles. Turtles were held for a total of 17 days and were released at their site of capture. Turtles were returned before ice formed on the surface of the lake to ensure they could return to their natural habitat and proceed with this part of their yearly cycle without further disturbance.

3.3.2 Data Analysis

Oxygen consumption (MO_2) without access to air was measured in a closed-flow respirometry system over the course of 24 hours. Our respirometry protocol was designed with consideration of methods described elsewhere (Steffensen 1989, Svenden et al. 2016, Chabot 2016, Mochnacz et al. 2017). A 378-L Rubbermaid stock tank was filled with water from Lake Opinicon, pumped into the tank using an aquarium pump fitted with a screen filter to prevent excess biomass from entering the system. Water in the stock tank was then chilled to the appropriate experimental temperature (VWR Refrigerated Circulating Baths, Avantor, Pennsylvania, USA). Approximately 15% of the water (60-L) was replaced between trials to prevent any build-up of waste produced by the turtles and to minimize any background bacteria growth. Trials were completed with all four turtles at 4°C first, then 1°C. The respirometry system was composed of two separate measurement chambers set in parallel, connected to the same stock tank primary water source. These two respirometry chambers (32-L plastic bins) were partially submerged in a second larger holding plastic bin (102-L) to help maintain the desired temperature for the trial. Each respirometry chamber was fitted with a contactless oxygen sensor spot (OXSPS, Pyroscience, Aachen, Germany) on the inner wall, as well as a temperature logger (RBRsolo^3 , RBR, Ottawa, Ontario, Canada) to obtain accurate temperature measurements throughout the trial. External sensor cables (PyroScience; SPFIB-BARE-CL2, Pyroscience, Aachen, Germany) were connected to the outside of the chambers to record sensor spot measurements the change in oxygen saturation (%) using the respective manufactures measuring device and software (FireString-O2/Pyro Oxygen Logger, Pyroscience, Aachen, Germany). Equipment was calibrated prior to each trial following PyroScience calibration recommendations for a one-point calibration in air-saturated water.

For each trial, turtles were weighed then fitted with a biologger (22 x 45 x 8 mm, 11 g in air: Axy-5, TechnoSmArt, Guidonia Monticello, Italy) secured with hot glue to the right posterior edge of the carapace to record locomotor activity. These data were used to ensure no excessive activity occurred during the measurement period. Each turtle was placed into its own respirometry chamber, which was then sealed and filled fully with oxygenated water from the primary holding tank. Once full, chambers were sealed and MO_2 was measured for 24 hours. All trials began between 20:00 and 23:00 and ran until the following day at the same time. Following the trial period, accelerometers were removed, and turtles were reweighed before being returned to the holding stock tank. The same methods were used for all turtles at both temperatures. Turtles were tested in the same order for both trials, however each was placed in the opposite respirometry chamber for their second trial to account for any effect a given chamber may have had. Background measurements were also taken at 4°C and 1°C in each of the two respirometry chambers to account for background metabolic activity from microorganisms in the water.

Mass-specific SMR (mg O_2 kg^{-1} h^{-1}) was calculated following the equation:

$$SMR = (\Delta O_2 - BG) \cdot (V_{Chamber} - V_{NMT}) / m_{NMT}$$

where ΔO_2 (mg O_2 l^{-1} h^{-1}) is the slope of MO_2 in the respirometry chamber, BG (mg O_2 l^{-1} h^{-1}) is the background rate of oxygen decline at the respective trial temperature, $V_{Chamber}$ (L) is the total volume of the respirometry chamber, V_{NMT} (L) is the volume of the Northern map turtle in the given trial, and m_{NMT} (kg) is the mass of the respective turtle. Oxygen saturation was converted to mg O_2 l^{-2} prior to the calculation of SMR. The first four hours of the measurements were removed from the 24 hours of data to account for an acclimation period as the turtles adjusted to their experimental environment. The last two hours of measurements were also removed from calculations as we noted MO_2 fluctuated randomly when turtles were disturbed by our preparation activities which usually occurred during this time. This resulted in 18 hours of continuous MO_2 measurements to derive SMR from. Total respirometry chamber volume accounted for the size of the chamber as well as the tubing immediately connected to the chamber which held water during the trial. Turtle volume was calculated using the change volume (L) when a turtle was added to a bucket of water.

To test our hypotheses that SMR would be lower at 1°C than 4°C, a paired Student's t-test was conducted in R using the *t.test* function. Using the SMR values, Q_{10} was calculated for each individual. We performed additional calculations based on our SMR results examine how much energy stores at 1°C and 4°C differ. The total energy expenditure of two different macromolecules was estimated over the minimum (i.e., 99 days), average (i.e., 119 days), and maximum (i.e., 143 days) duration of the ice-on period at our study site. The energy storage composition of turtles in terms of macromolecules is unclear, thus we included both sources for this exercise (Jackson and Ultsch 2010), so this was done first by assuming energy expenditure from 100% glycogen as an energy source, then 100% triglycerides. All analysis were performed in R (4.1.2) via R Studio (2021.09.1), and graphs created with the *ggplot* package (Wickham 2016). P values < 0.05 were deemed statistically significant.

3.4 Results

Aquatic oxygen consumption and subsequently mass-specific metabolic rate was successfully recorded for all four adult females (Table 3.1). Our biologger recordings produced values that were all less than 0.05 *g* of acceleration, indicating little to no movement occurred that would have interfered with SMR estimation. Our calculations indicated that the SMR was lower at 1°C than 4°C, with a statistically significant difference between these values ($t_3 = -2.97,$

p = 0.03; Figure 3.1). The Q_{10} values for all four individuals ranged from 6.75 to 27.93 (Table 3.1).

Energy expenditure was lower at 1°C for both sources across all durations of ice-on (Figure 3.2). Triglyceride values were lower than glucose values for all durations (Figure 3.2). The average total amount of triglyceride that would be metabolized at 1°C was estimated to be 5.40 g after 97 days, 6.62 g after 119 days, and 7.95 g after 143 days. At 4°C, we estimated these values to be 11.76 g, 14.28 g, and 17.34 g for 97, 119, and 143 days respectively. Average total glucose values at 1°C were 14.54 g after 97 days, 17.83 g after 119 days, and 21.43 g after 143 days. Finally, glucose at 4°C was estimated to be metabolized in the highest amounts at 31.6 g after 97 days, 38.86 g after 119 days, and 46.70 g after 143 days.

3.5 Discussion

Many freshwater turtle species spend the winter at water temperatures near 1°C, presumably as a strategy to lower metabolism (Brown and Brooks 1994; Litzgus et al. 1999; Crocker et al. 2000; Greaves and Litzgus 2007; Rollinson et al. 2008). Here, we estimated the metabolic consequence of selecting such low temperature by comparing the SMR of Northern map turtles at 1°C and 4°C. We found SMR in females at 1°C tended to be about half of the SMR at 4°C (Table 3.1; Figure 3.1). These values demonstrate that selecting the coolest temperature available during the winter may reduce the absolute demand for oxygen by between 37 and 56% thereby reducing the risk of running out of this limited resource. The values calculated for Q_{10} are fairly large relative to the usual biological values of 2 to 3, showing temperature does have a strong effect on metabolism during an acute temperature change (Table 3.1). This points to temperature having a strong influence on metabolism at low temperatures for

Northern map turtles which has similarly been observed in other freshwater turtle species such as the Painted turtle (Herbert and Jackson 1985). Herbert and Jackson (1985) attributed high Q_{10} in Painted turtles to physiological metabolic depression. Active metabolic depression has been suggested for Northern map turtles (Reese et al. 2001; Maginniss et al. 2004) although never formally quantified. In our study, change in temperature was likely too fast to allow for this physiological change to occur. The change in metabolic rate then is likely a result of a more passive reduction in metabolism as a result of cold temperatures. Future studies over a longer period of time and across a wider temperature range may be able to confirm if this reduction is passive or an active metabolic depression.

Reduced SMR at 1°C would reduce oxygen demand, but it may also yield substantial energy savings. Energy expenditure was lower at 1°C for both glucose and triglyceride based on our estimates (Figure 3.2). However, when considering these values relative to the body mass of these four turtles, it is evident that proportionally the savings between temperatures are small. For instance, for the average ice duration, the amount of glucose burned at 1°C versus 4°C account for 0.98 and 2.11% of body mass, respectively. Similarly, if triglycerides are the main substrate, 0.43% and 0.94% of body mass is being used at 1°C and 4°C respectively. From this modelling exercise, we see that energy savings at these low temperatures are minimal and presumably trivial. Instead, temperature selection and reduced metabolism may be more beneficial to managing oxygen needs rather than energy supplies. Indeed, previous research has suggested that oxygen rather than energy is the primary limiting factor for the survival of overwintering turtles (Jackson and Ultsch 2010).

Recently, we showed that Northern map turtles are active continuously throughout the winter (Robichaud et al. in preparation). It remains unknown what role this activity plays in winter survival, but it may relate to oxygen dependence as has been proposed for species who demonstrate similar behaviours (Greaves and Litzgus 2008). Observations made by Ultsch suggest that multiple freshwater turtle species could survive over 200 days of submergence without extra food beforehand, if conditions remained normoxic for the duration of submergence (Jackson and Ultsch 2010). This emphasizes the likely importance of oxygen in the locations freshwater turtles choose to overwinter. For Northern map turtles specifically, Crocker at el. (2000) concluded that individuals in the wild experienced insignificant changes to blood acid-base or ionic status when in oxygenated water at 1°C. Reese et al. (2001) concluded that this species experienced lethal changes in blood chemistry when subjected to prolonged anoxic conditions at 3°C. Together, this information emphasizes the importance of normoxic conditions and therefore we suspect the localized movement that occurs during the winter may allow individuals to seek out microhabitats with higher dissolved oxygen concentrations. This would make meeting energy demands easier, allowing them to extend survival time during submergence under ice.

The movement we previously observed may also help Northern map turtles select for spaces within their overwintering site that remain at 1°C. The range of temperatures under ice in freshwater systems remains between 0°C and 4°C (McMeans et al. 2020). We have now observed that there is considerable metabolic reduction at 1°C relative to 4°C and this species is thermally sensitive. Northern map turtles may move to locate spaces within their overwintering site that are cooler as temperatures fluctuate throughout the year. In doing so, this species may be able to maintain the reduced metabolic rates we observed for the duration of the overwintering period. The oxygen consumption we observed between 1°C and 4°C is biologically relevant, therefore maintaining a position at 1°C may be critical to survival when the length of winter is unknown. As noted in our energy estimates, ice duration at our study site has lasted between 97 and 143 days. By remaining at 1°C conditions, Northern map turtles may be able to minimize and maintain a reduce oxygen demand which could be considerably beneficial given the duration of winter can vary by several weeks from year to year.

Our study showed that winter temperature selection by turtles can gave a strong effect on metabolism. Under-ice temperatures should be considered when attempting to identify and conserve overwintering habitats, along with oxygen availability. Future studies should assess the thermal sensitivity of SMR in male Northern map turtles. Male map turtles weigh approximately ten times less than adult females and may not have the same response to temperature change. Estimating male SMR would allow for comparison between the sexes to determine if one group is more thermally sensitive, potentially because of this size difference. Studies could also be done with more individuals and across a wider range of temperatures to assess the thermal dependence of this species’ metabolism more accurately. Further, assessing how oxygen demand and metabolism changes throughout the duration of the ice-on period could provide insight into how this relationship changes over time, as temperature changes and oxygen supplies decline through the season.

Table 3.1 Mass-specific standard metabolic rates (mg O2 kg-1 h−1), mass (g), and Q10 values for four female Northern map turtles (*Graptemys geographica*).

Individual	**Mass (kg)**	**SMR at 1°C (mg O_2 kg^{-1} h^{-1})**	**SMR at 4°C (mg O_2 kg^{-1} h^{-1})**	**Q_{10}**
Female 1	2.17	1.97	3.50	6.75
Female 2	2.33	1.48	2.84	8.82
Female 3	1.46	1.93	5.00	23.87
Female 4	1.39	1.97	5.35	27.93

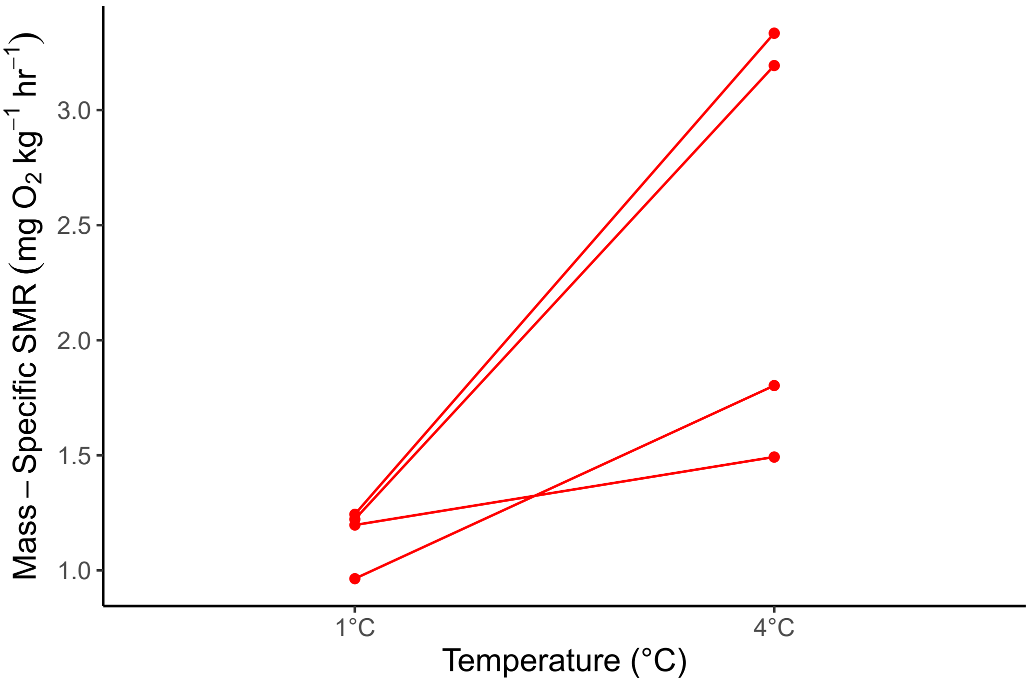

Figure 3.1 Interaction between mass-specific metabolic rate (mg O_2 kg^{-1} h^{-1}) and temperature (1°C, 4°C) for four female Northern map turtles (*Graptemys geographica*; 1839 ± 481 g).

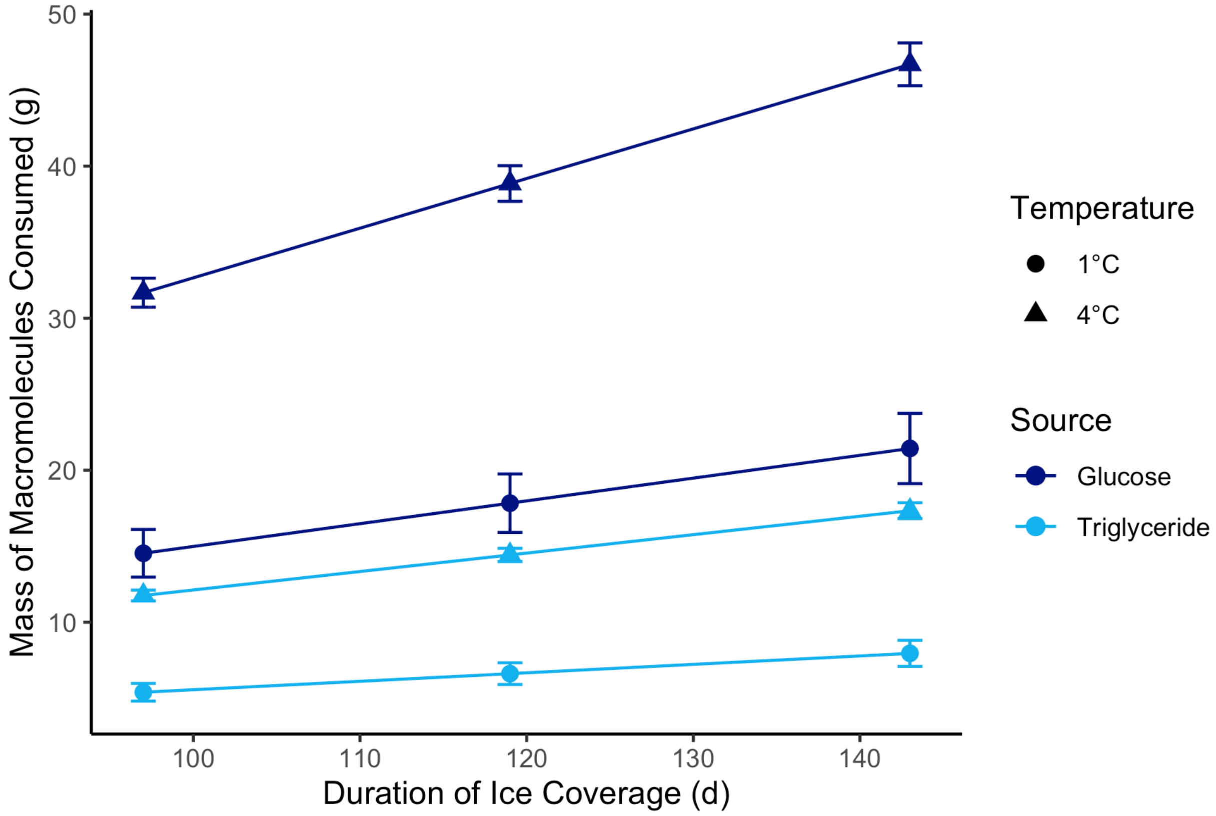

Figure 3.2 Estimates of the average total amount of glucose and triglyceride that would be metabolized by Northern map turtles (*Graptemys geographica*; 1839 ± 481 g) at constant temperatures of 1°C (circles) and 4°C (triangles) for the minimum (97 days), average (119 days), and maximum (143 days) duration of ice coverage at a known overwintering site in Eastern Ontario. Error bars denote standard deviation for values.

Chapter 4: General Discussion

4.1 Overall Conclusions

In this thesis, I addressed key knowledge gaps about Northern map turtle overwintering behaviour and physiology. Northern map turtles are widely distributed across Eastern and Central North America (Bulté and Blouin-Demers 2010), making winter a large part of their annual cycle. By making use of recent advancements in biologging technology and the longstanding practice of respirometry, I provided insight into how this species survives the coldest part of their year. Although previous studies on Northern map turtle overwintering ecology exist (e.g., Graham and Graham 1992; Crocker et al. 2000; Reese et al. 2003; Maginniss et al. 2004), my work is the first to monitor behaviour continuously across the entire winter season in the wild using biologging technology, and the first to calculate metabolism at specific ecologically relevant temperatures to better understand their winter behaviour. The findings from both of my data chapters complement each other to deepen our understanding of why Northern map turtles engage in various overwintering behaviours (i.e., amount of locomotor activity, temperature use, and depth use), and how this relates to their physiological demands (i.e., oxygen, energy) throughout their overwintering period.

In Chapter 2, I investigated Northern map turtle overwintering behaviour by observing their locomotor activity, water temperature use, and depth use. My objective was to monitor these behaviours before, during, and after the period of forced submergence under ice that Northern map turtles experience during the winter. Further, I tested for differences in overwintering behaviour across three demographic classes: adult females, juvenile females, and adult males. Through this, I discovered Northern map turtles move continuously throughout winter and I detected statistically significant differences in the amount of locomotor activity, water temperature used, and depth used by each group. All three groups of turtles moved less during the ice-on period, where adult males moved the most followed by juvenile females and finally adult females. During the ice-on period, all three groups were seen at daily average temperature near 1°C, with juvenile females and adult males observed at an average temperature slightly above 1°C (1.4°C and 1.2°C respectively), and adult females at 1°C. All three groups moved into shallower depths as the winter went on, where juvenile females were observed at the deepest average daily depths (1.7 m), followed by adult males (1.5 m) and finally adult females (1.3 m). Notably, the least movement, coldest temperatures, and shallowest depths for all three groups were observed during the ice-on period relative to the pre-ice and post-ice periods. Although significant differences in behaviour were detected between the demographic groups, the biological relevance of these differences remains unclear, as well as the purpose for continuous movement. Ultimately, I concluded that these observed behaviours are likely attributed to energy and oxygen requirements, which set the basis for Chapter 3.

In Chapter 3, I looked to understand the temperature dependence of Northern map turtle metabolism at winter water temperatures. I estimated the mass specific standard metabolic rate of four adult females at 1°C and 4°C, then compared SMR between these two temperatures. This allowed me to test the extent to which temperatures in this range affect SMR. I determined that 1°C was relevant to test at based on the data collected in Chapter 2, as Northern map turtles were observed at daily average temperatures near 1°C during the ice-on period. In freshwater systems, temperatures under the ice generally do not exceed 4°C (McMeans et al. 2020), making 4°C the likely highest temperature they would experience during this time of year. I found a statistically significant difference in SMR at 1°C versus 4°C, and found it to be 2.1 times lower on average at 1°C. Using SMR estimates I then estimated the total energy expenditure over the minimum (i.e., 99 days), average (i.e., 119 days), and maximum (i.e., 143 days) duration of the ice-on period at Lake Opinicon. First by assuming winter energy came from metabolizing 100% glucose then 100% triglycerides. When considering these values relative to the body mass of my four turtles, the energy savings between temperatures are very small, approximately 2% body mass at most. From this it can be said that energy savings between these temperatures are presumably trivial. Other researchers have speculated that overwintering is not energetically demanding for freshwater turtles under the ice, meaning oxygen, rather than energy, is likely to be a limiting factor for overwintering survival (Litzgus et al. 1999; Jackson and Ultsch 2010). As such, I concluded that the reduced SMR values seen at 1°C are more important for reducing oxygen consumption during the winter than maintaining energy stores.

The findings in my two data chapters have allowed me to hypothesize how winter behaviours relate to changes in metabolism and therefore oxygen consumption. From my previous observations (Chapter 2) and those from previous studies (Crocker et al. 2000), Northern map turtles use temperatures near 1°C during the overwintering period. Further, other freshwater turtle species who overwinter under the ice (i.e., Snapping turtles, Brown and Brooks 1994; Spotted turtles, Litzgus et al. 1999; Wood turtles, Greaves and Litzgus 2007; Painted turtles, Rollinson et al. 2008; Blanding's turtles. Edge et al. 2009) demonstrate a similar preference for water temperatures during the winter and it is thought that this might be a critical temperature for overwintering freshwater turtles. Other studies on anoxia intolerant freshwater turtles have speculated about why 1°C is critical to freshwater turtle winter survival. Some have suggested this helps prevent ionic disturbances (i.e., Na^+, Cl^-, K^+) that can lead to metabolic acidosis, or that perhaps 1°C helps manage oxygen demands by suppressing metabolic costs (Crocker et al. 2000; Reese et al. 2001; Greaves and Litzgus 2007; Greaves and Litzgus 2008).

Due to the anoxia intolerant nature of Northern map turtles, it is highly likely that an active selection of 1°C minimizes their oxygen demands during the overwintering period (Reese et al. 2001). For a normoxic species, the decrease in oxygen consumption at 1°C relative to 4°C observed may be enough of an oxygen saving to minimize the risk of running out of this limited resource under the ice. Alternatively, the selection of 1°C may be more so driven by the fact that colder water holds more DO, making the benefit of seeking colder water temperatures twofold.

The depth use observed in Chapter 2 can also be connected to the metabolic rates quantified in Chapter 3. Similar to the notion that Northern map turtles select for 1°C as a result of metabolic and oxygen needs, depth may be an environmental variable they select for as well. The shift of turtles into shallower depths when ice-on occurs may be the result of them moving into colder spaces, possibly reducing metabolic and oxygen needs, and/or having higher oxygen concentrations, to meet oxygen demands. As suggested in Chapter 2, depth use may also reflect substrate preference which can also be related to physiological needs during winter. Graham and Graham (1992) observed Northern map turtles laying on rocks and logs, or open sand/gravel substrates in a river under the ice of a river in Vermont. Video recordings of my study population under the ice indicate the Lake Opinicon population share a similar preference for logs and open conditions during their overwintering period (Bulté, unpublished data). These substrates may allow Northern map turtles to keep their skin free of debris, improving their ability to engage in extrapulmonary gas exchange in an environment that becomes increasingly limited in oxygen as the season goes on.

It is possible that the continuous locomotor activity observed in Chapter 2 is the result of Northern map turtles moving to better meet physiological demands. As suggested in Chapter 2, movement may be useful to promote extrapulmonary gas exchange. This reasoning has also been suggested in Wood turtles, which also have been observed to engage in small amounts of movement during the winter (Greaves and Litzgus 2008). Movement may allow this species to flush their skin with fresh oxygenated water. Softshell turtles are highly anoxia intolerant and have been observed engaging in "push-ups" during the winter to create currents around their body to replenish the oxygen-depleted boundary layer around their skin (Plummer and O'Neil 2019). Movement may allow Northern map turtles to engage in something similar, allowing them to flush their skin with fresh oxygenated water. Northern map turtles may also in engage in movement to seek microhabitats within the overwintering site that have more favourable conditions. Here, favourable conditions are those that better meet their physiological requirements (i.e., higher concentrations of DO to meet oxygen demands or cooler temperatures to reduce metabolic rate and oxygen needs).

4.2 Future Research Directions

In Chapter 2, significant differences in movement were observed between the demographic classes. An individual's amount of locomotor activity may be linked to the physiological demands associated with their demographic class, specifically body size. Smaller animals have higher mass-specific metabolic rates (Brown et al. 2004), and Graham and Graham (1992) observed this in a single male Northern map turtle at 2°C, noting their higher mass-specific oxygen need as well. Male map turtles having a higher mass-specific demand for oxygen may explain why a statistically significant difference in locomotor activity was detected in Chapter 2. Males may either be flushing their skin more frequently or perhaps seeking areas of higher oxygen. Only females were tested in Chapter 3 and the sample size in Graham and Graham (1992) was limited to one male, therefore future studies of male Northern map turtles could provide helpful comparisons for metabolic temperature dependence. Larger sample sizes could further be used to comment on how metabolic temperature dependence differs across sexes and age classes which may help explain the biological significance in the statistically significant differences detected in behaviour in Chapter 2. Regardless of demographic class, there is likely a trade-off occurring between energy spent on movement, and the benefit of moving into more favourable conditions. Alternatively, it could be that energy spent on movement is not a limiting factor and greatly outweighed by the benefit of avoiding potential anoxic conditions. Energy acquisition and storage has been noted as critical for other animals who overwinter under-ice such as freshwater fish (Fernandes and McMeans 2019) and frogs, however for freshwater turtles it has been suggested oxygen is a greater concern (Jackson and Ultsch 2010).

Due to the intolerance Northern map turtles have to anoxic conditions, it is plausible much of the behaviours observed in Chapter 2 are largely a result of oxygen need, however it remains uncertain for this species whether oxygen or energy is the limiting factor in overwintering. Based on the results of Chapter 3 and suggestions from other freshwater turtle studies, I believe the overwintering behaviour I recorded is primarily driven by oxygen demand. A study done on the anoxic intolerant Spotted turtle, found little change in body mass over the winter, suggesting overwintering is not energetically demanding (Litzgus et al. 1999). Jackson and Ultsch (2010) also suggested that in studies of turtle submergence, extra food beforehand did not affect survival, indicating energy is likely not a limiting factor in overwintering. Additionally, Crocker et al. (2000) and Reese et al. (2001) both indicated that without adequate oxygen supplies, Northern map turtles will not survive more than a few weeks. Estimates of SMR from Chapter 3 suggest that total energy consumption and the energy burned at 1°C and 4°C accounts for only a small percent of total body mass. Therefore, it can be speculated that

Northern map turtle overwintering is largely driven by oxygen demands rather than energetic needs, as oxygen may be the more limited resource. If true, this could be useful in explaining temperature and depth use (i.e., habitats with higher oxygen saturations or spaces to minimize oxygen demand) as well as the constant locomotor activity observed (i.e., energy expenditure is not a grave concern, movement can take individuals to better oxygenated microhabitats). However, given that the behaviours observed in Chapter 2 could be driven by temperature selection as well, further studies on these factors should be considered. Future studies on Northern map turtle overwintering temperature selection in the wild could be used to confirm that 1°C is the environmental variable being selected for rather than, for example, a substrate or oxygen preference. Alternatively, studies could test to see if oxygen availability drives habitat selection regardless of the water temperature. Previous studies on Painted turtle overwintering found they selected for dissolved oxygen rather than temperature when maintaining aerobic metabolism was possible (Rollinson et al. 2008). Northern map turtles may engage in a similar behaviour, selecting for microclimates with higher dissolved oxygen instead of specifically seeking 1°C water. By determining whether oxygen or temperature drives overwintering habitat selection, we may determine how Northern map turtles select overwintering sites.

Broadly, my findings can be applied to future research on ectothermic species in the winter. As previously mentioned, winter can be a challenging period to study animals, thus a knowledge gap exists with respect to what many ectotherms do in the wild. Much of the literature surrounding this topic and cited in my thesis was based on results in a lab setting or anecdotal information. Having successfully recorded behaviours in the wild, my study outlined in Chapter 2 can serve as a framework for future studies on the overwintering behaviour of ectothermic species. To date, no other studies have used these technologies to record freshwater turtle behaviour continuously throughout the winter, therefore limiting our knowledge of which species may be active during this season. Gaining an understanding of overwintering ecology is becoming increasingly important under the growing concerns of climate change (Studd et al. 2021). Organisms at mid- to high latitudes in the Northern hemisphere are expected to be the most affected by climate change relative to organisms at lower latitudes (Dillion et al. 2010). Given the gap in knowledge on how organisms survive winter, it is hard to predict how these species will respond to changes in this part of their annual cycle as the pressures of climate change continue to grow. By conducting further research on overwintering, specifically as it relates to yearly energy budgets and metabolism, researchers may be able to better predict responses to climate change (Dillion et al. 2010).

These findings also may also inform management of critical species habitats and species populations. With specific respect to the Northern map turtle, further research on overwintering ecology may be useful in determining the preferred overwintering conditions. This can help identify critical overwintering sites which, as noted previously, appear to be limited. Overwintering sites offer species refuge from sub-freezing temperatures, predation, and in some instances anthropogenetic activities such as prescribed fires (Brown and Brooks 1994; Roe and Bayles 2021). Identifying these sites can help inform appropriate species management in the future. Communal overwintering is suggested to be the result of suitable overwintering sites being limited (Gregory 1982; Ultsch 1989). Aggregation behaviour has been observed in Northern map turtles (Graham and Graham 1992), as well as other aquatic ectotherms like leopard frogs (*Rana pipiens*; Ultsch et al. 2000), Snapping turtles (Brooks and Brown 1994), and Spotted turtles (Litzgus et al. 1999). These and other animals can spend up to half of their annual cycle overwintering, therefore in cases of habitat protection or restoration, critical overwintering habitat should be taken into consideration. The identification of overwintering habitats may also benefit researchers as well. If these sites are successfully identified, they may make efforts to monitor populations easier. As turtles return to overwintering sites for the season, researchers may be able to conduct population monitoring studies quicker relative to the summer months when individuals are more widely scattered across bodies of water.

Collectively, this thesis describes how Northern map turtles respond to winter in the wild, and these findings are important in the continued study of ectothermic winter behaviour and physiology. Winter is arguably one of the most physiologically challenging parts of an ectotherms annual cycle. Future studies should further explore both areas and how they relate to one another to enrich our understanding of how Northern map turtles and all ectotherms survive winter. It is my hope that this information presented in this thesis can also be used to inform management and conservation efforts to ensure species such as the Northern map turtle persist well into the future.

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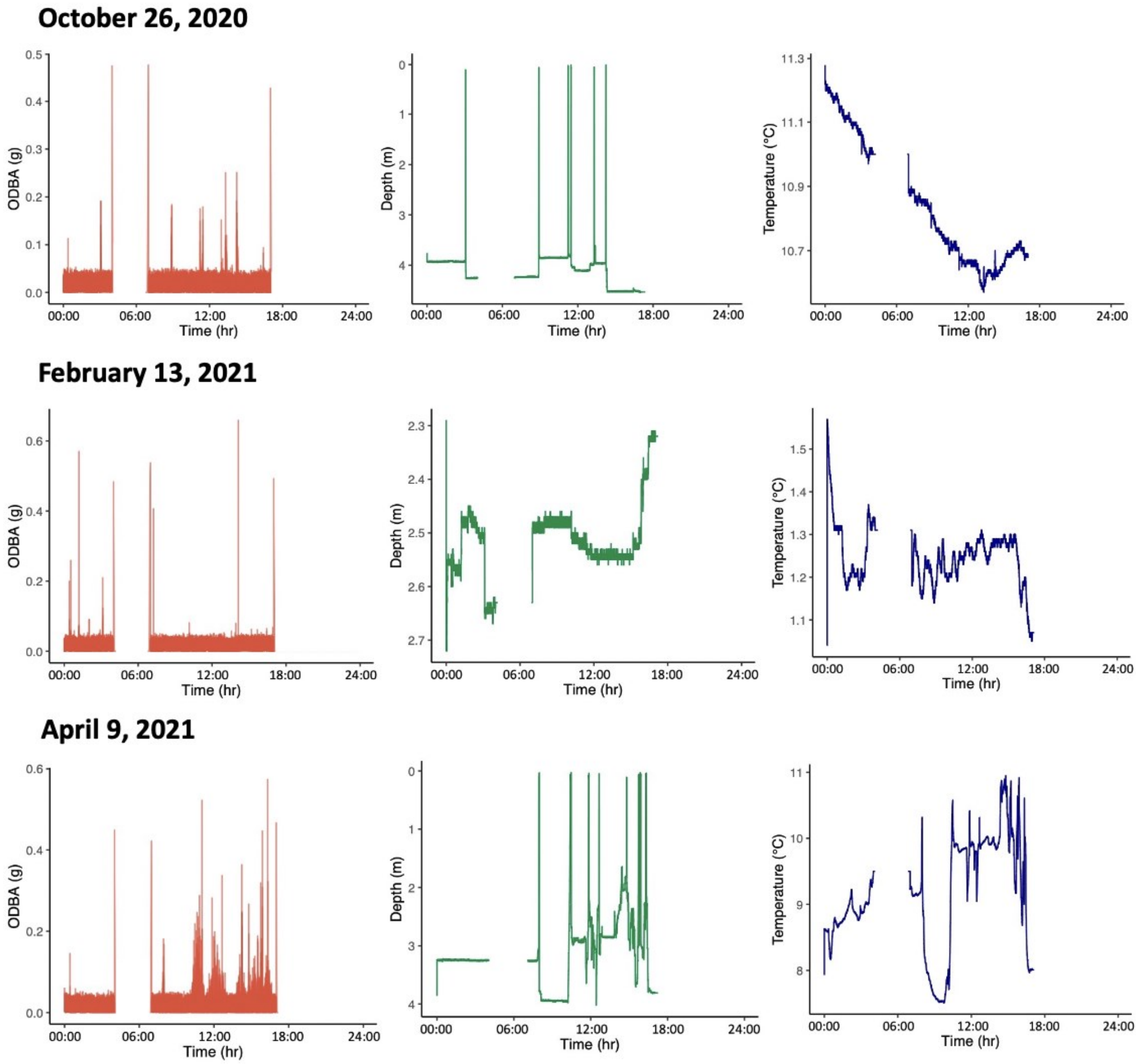
Appendix A



A.1 Locomotor activity (ODBA), depth use (m), and temperature use (°C) across one day of study from each study period (i.e., pre-ice 26-Oct-2020, ice-on 13-Feb-2021, post-ice 09-Apr-2021) for one randomly selected turtle (i.e., juvenile female). Recording occurred from 00:00 to 04:00 then again from 07:00 to 17:00 each day.