IDENTIFYING SUITABLE TURTLE OVERWINTERING HABITAT THROUGH THE INTEGRATION OF THERMAL, CHEMICAL AND PHYSICAL WETLAND PROPERTIES

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Lay Abstract

Turtles are among one of the most vulnerable vertebrates on the planet and within Canada, all eight native species of freshwater turtle are designated as at-risk under the Committee on the Status of Endangered Wildlife in Canada . The primary extinction risk turtles face is the loss and degradation of their habitats. Turtles located in northern temperate regions use aquatic habitats that provide specific temperatures, dissolved oxygen, and water depths during overwintering. We characterized available overwintering habitat and combined aquatic temperatures, dissolved oxygen and water depth into a single metric, the resilience zone (RZ), to quantify overwintering habitat suitability. We found that Blanding's turtles actively selected specific overwintering conditions. This is the first study to quantify the turtle RZ and we found that overwintering habitat was already limited within a pristine landscape. Finally, our results show that the RZ can directly contribute to conservation and management strategies for at-risk turtle species.

Abstract

Turtles are among one of the most vulnerable vertebrates on the planet and within Canada, all eight native species of freshwater turtle are designated as at-risk under COSEWIC. The primary extinction risk turtles face is the loss of their habitats. At the northern limit of their range, turtles rely on the presence and persistence of aquatic habitats to survive the winter. In the face of unprecedented climate and land-use changes, identification of key overwintering habitat attributes is crucial because relatively little is known about how they interact to optimize overwintering habitat suitability. We measured *in-situ* thermal, chemical and physical conditions at Blanding's turtle overwintering sites within wetlands in the Georgian Bay Biosphere Mnidoo Gamii to (1) characterize available overwintering habitat, (2) test for overwintering habitat selection by Blanding's turtles and (3) quantify the turtle resilience zone (RZ). We measured water and sediment temperatures, dissolved oxygen (DO), water, ice and snow depth at 5 (2020-2021) and 19 (2021-2022) Blanding's turtle overwintering locations and four paired available microhabitats (floating mat, inflow, lagg, open water) across 5 wetlands. Blanding's turtles selected average daily temperatures that ranged from 3.58°C to 0.53°C with a mean of 1.08°C, minimum average DO observed was 1.10 mg/L and average water and ice depth ranged from 42cm to 79cm, 12cm to 38cm, respectively. Based on habitat use data, we quantified the RZ as present when aquatic habitats had temperatures between 0 and 4°C, DO ≥1 mg/L and minimum water depth of 40 cm. Using the RZ we identified suitable overwintering habitats within our study region and found that the RZ varied across wetlands and microhabitats. Overall, the RZ is a powerful tool that can be used to identify suitable overwintering sites which can directly contribute to conservation and management strategies for at-risk turtles.

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List of Abbreviations and Symbols

DO	Dissolved oxygen
GBB	Georgian Bay Biosphere Mnidoo Gamii
GLMM	Generalized linear mixed model
NE	North East
RZ	Resilience one
SE	Standard error
Spp.	Species
°C	Degrees Celsius
mg/L	Milligrams per Litre

Declaration of Academic Achievement

This thesis was written in a “sandwich thesis” format, where all written material was prepared solely by this author. Chapters one and three provide a general introduction and conclusion, respectively. Chapter two constitutes the main body of the thesis and is prepared as an individual manuscript. Dr. Mike Waddington and Dr. Chantel Markle contributed substantially to the research design and direction. Alexandra Clark, Grace Freeman, Lily Freeman, Alexander Furukawa, Taylor North, Emma Sherwood, Alexandra Tekatch, Gregory Verkaik, Sarah Wiebe, and Sophie Wilkinson assisted with radio-tracking of Blanding’s turtles and the collection of thermal, chemical and physical habitat data as it relates to freshwater turtle overwintering habitat.

Chapter 1: General Introduction

Reptiles are an extraordinarily diverse group, occupying a wide array of habitats and displaying a large variation in life-history traits (Taylor et al. 2020). They play key roles in ecosystems, contributing substantially through mineral cycling, predator-prey interactions, seed dispersal and act as bioindicators for environmental health (Böhm et al. 2013; Lovich et al. 2018; Taylor et al. 2020). Turtles are an ancient group of reptiles that have a body plan that has allowed them to persist for over 200 million years (Ernst and Lovich 2009) despite changing climates and predation threats (Buhlmann et al. 2009). However, climate mitigated and land-use changes, among many other factors, have resulted in turtles becoming arguably the most threatened of the major groups of vertebrae (IUCN 2008; Buhlmann et al. 2009; Hoffman et al. 2010; Lovich et al. 2018). The primary extinction risks turtles face is due to the loss, alteration and/or degradation of their habitats, road morality and poaching for the food and pet trade (Gibbons et al. 2000; Stanford 2020).

As ectotherms, turtles select habitats that help control body temperature due to the tight coupling between environmental temperature and metabolic regulation (Huey 1991). In North America freshwater turtles occur at latitudes as far north as 53 °N (Buhlmann et al. 2009) with climate most likely being the dominant abiotic force shaping the species distribution (IPCC 2014; Cunningham et al. 2016; McLaughlin 2017).The annual activity cycle of freshwater turtles located at northern latitudes and in seasonally cold climates is characterized by short summers and long winters, can be broken into two overarching categories: the active season (April to late October) and the overwintering season (late October to April) (Paterson, Steinberg and Litzgus 2012). The active season can be further subdivided into three periods; pre-nesting (April to late May), nesting (late May to July), and post-nesting (early July to late October) (Litzgus and Mousseau 2004; Millar and Blouin-Demers 2011). The seasonal shifts in behaviour requires species to use multiple critical habitat types, where critical habitat is defined as the habitat that is necessary for the survival or recovery of an at-risk species (SARA 2016). The habitat used by an individual species is dependent on their specific physiological adaptations. For example, some species of freshwater turtle can tolerate anoxic (less than 1 milligrams of dissolved oxygen [mg] per litre [L]) conditions while overwintering, while others cannot (Ultsch 1989; 2006). Therefore, to effectively discuss and outline critical habitats, the geographic range of interest, the corresponding species of freshwater turtles, and risk-factors must be fully understood .

In Canada, all eight native species of freshwater turtle are designated as at-risk under the Committee on the Status of Endangered Wildlife in Canada (COSEWIC 2002; 2008; 2012; 2014; 2016; 2018). The province of Ontario supports eight native species of freshwater turtle, many occurring at the most northern extent of their range. All native species of turtle found within Ontario are designated as at-risk provincially and/or federally (COSEWIC 2018). These species include the Blanding's turtle (*Emydoidea blandingii*), eastern musk turtle (*Sternotherus odoratus*), northern map turtle (*Graptemys geographica*), painted turtle (*Chrysemys picta*), snapping turtle (*Chelydra serpentina*), spiny softshell turtle (*Apalone spinifera*), spotted turtle (*Clemmys guttata*) and the wood turtle (*Glyptemys insculpta*). Throughout turtles' Canadian range, wetland and associated habitats are often fragmented and/or lost due to conversion of habitat for agriculture, hydropower projects and infrastructure development such as roads (Gibbons et al. 2000; Todd, Wilson and Gibbons 2010; KeeSvil et al. 2018; Stanford et al. 2020). In CanadaRoads also act as a dispersal barrier hindering turtles ability to move between habitats (Gibbons et al. 2000; Attum et al. 2008). A well-connected, heterogeneous landscape is key to freshwater turtles persistence because it allows turtles to access critical, but spatially distinct, habitats (Roe et al. 2009). For example, the Blanding's turtle is known to make extensive overland movements which results in an increased risk of mortality in fragmented landscapes due to roads and heavy machinery (Gibbons et al. 2000; Roe and Georges 2007; Roe et al. 2009; Böhm et al. 2013). Maintaining the integrity of wetlands and the surrounding uplands is essential because the reduction of habitats has greatly decreased the ability for aquatic and semi-aquatic turtle species to persist (Congdon et al. 2011).

One challenge to conserving a long-lived species like turtles is the perception of persistence. Turtles' life history traits such as high adult survivorship, low and variable juvenile recruitment and low reproductive success make them especially vulnerable to population decline (Gibbons 1987; Brooks, Brown and Galbraith 1991; Ramussen and Litzgus 2010; Keevil et al. 2018). For example, it may take upwards of 20 years before species such as the Blanding's turtle or snapping turtle reach sexual maturity making populations particularly vulnerable to adult female mortality (Congdon et al.1993; Keevil et al. 2018; Lovich et al. 2018). Yet, the persistence of adult turtles on a landscape does not necessarily mean that the population is stable. In fact, the population may be declining unless there is a high persistence of adults and juveniles and the rate of birth exceeds death, resulting in demographically declining populations (Congdon et al. 1993; Lovich et al. 2018). Specifically, a cohort of Blanding's turtles with a generation time of 37 years required a 72% annual survivorship of juvenile turtles (aged 1 - 13 years) (Congdon et al. 1993). Similarly, a population of snapping turtles experienced a mass mortality event in 1995 and 23 years later the population has failed to recover (Keevil et al. 2018). It cannot be understated that high adult survivorship, low and variable juvenile recruitment and low reproductive success do not act alone, it is the cumulative impacts of high adult survivorship, low and variable juvenile recruitment and low reproductive, among other factors such as road morality, that need the most attention to effectively conserve and manage freshwater turtle species and their habitat (Markle et al. 2020).

Freshwater turtles and their critical habitats

To protect habitat for a specific turtle species, it is key that we better understand how individual turtles use habitats and disperse themselves within and across habitats through space and time (Rasmussen and Litzgus 2010). Habitat selection can be defined as the use of habitats disproportionate to their availability (Johnson 1980) where habitat selection often mirrors seasonal shifts in behaviour (Rasmussen and Litzgus 2010). Furthermore, Freshwater turtles' narrow niche requirements make them especially susceptible to anthropogenic threats, strongly due to their low dispersal ability and strong dependence on multiple habitat types (Gibbons et al. 2000; Gibbons 2003; Böhm et al.

2013). At their northern limits, freshwater turtle species use multiple habitats including resident wetlands (wetland habitat where the majority of the active season is spent) (Markle and Chow-Fraser 2014), thermoregulation sites, mating sites, nest sites, travel corridors and overwintering sites. The selection of habitat types by freshwater turtles is strongly dictated by the time of year. An individual turtle may use multiple wetlands during the active season, where each wetland may meet a different need. For example, an ephemeral wetland may act as a rest stop during upland movements, providing opportunities for resource replenishment (Markle and Chow-Fraser 2014), whereas a large permanent wetland may be used as an overwintering site to provide protection from harsh winter conditions (Litzgus et al. 1999; Rollinson et al. 2008; Edge et al. 2009; Paterson et al. 2012; Markle and Chow-Fraser 2017). An individual turtle's resident wetland may be the same or different from its overwintering site, where northern freshwater turtle species primarily overwinter in aquatic ecosystems such as rivers, lakes, and several wetland types (Ultsch 1989; 2006; Markle et al. 2020).

Freshwater turtle overwintering habitat

Freshwater turtles located in northern temperate regions with seasonally cold climates, characterized by short summers are faced with harsh, long, cold winters. (Ultsch 1989; 2006). Most aquatic freshwater turtle species seek refuge from freezing conditions by reducing their metabolic activity and retreating underwater into some form of aquatic medium which acts as a thermal refuge (Ultsch 1989, Reese et al. 2002; Greaves and Litzgus 2007; Markle et al. 2020). This overwintering state may persist for a large portion of the turtle's annual activity cycle (Greaves and Litzgus 2008). Although it is an important component of the life history of turtle species, overwintering is a lengthy affair which may exceed over half the individual's lives (more than six months a year) (Ultsch 1989; 2006). Overwintering under water can be physiologically stressful and can result in death of a single individual or an mass mortality event due to predation, freezing or prolonged anoxia (Ultsch 1989; Hall and Cuthbert 2000; Ultsch 2006; Gasbarrini et al. 2021). Turtle populations are especially sensitive to adult morality and turtle populations take an extremely long time to recover from mass mortality events (Keevil et al. 2018). For example, a population of snapping turtles within Algonquin Park showed no signs of recovery 23 years after an overwintering mass mortality event (Keevil et al. 2018).

The majority of reptile species that use wetland habitats as overwintering sites occur from 40°–50°N and are centred on the Great Lakes in North America (Markle et al 2020). Overwintering presents an extreme challenge for freshwater turtles. A suitable overwintering site is defined as a habitat that provides the opportunity to access aerial or dissolved oxygen, cool, stable aquatic temperatures, vegetation cover and sediment for protection from predators during overwintering (Markle et al. 2020). It is hypothesized that some aspects of overwintering, including the availability of suitable sites, may limit the northern distribution and abundance of freshwater turtle species (Litzgus et al. 1999). Overwintering habitats must provide safe refuge under ice for extended periods of time and balance the risk of freezing, metabolic acidosis, and predation (Litzgus et. al. 1999; Litzgus and Brooks 2000; Edge et al. 2009; Markle and Chow-Fraser 2017; Markle et al. 2020). The availability of suitable overwintering sites and the ability to select appropriate sites may be the difference between survival and death for overwintering turtles (Paterson et al. 2012). Currently, there is limited field information that examines in-depth environmental conditions required to support overwintering survival beyond temperature selection (Litzgus et. al. 1999; Rollinson et al. 2008; Edge et al. 2009; Paterson et al. 2012; Marchand et al. 2018; Robichaud et al. 2022) and identification of habitat types occupied by overwintering turtles (Markle and Chow-Fraser 2017, Zagorski et al. 2019; Feng et al. 2020).

Overwintering sites selected by freshwater turtles include fens, bogs, beaver ponds, swamps, shrub swamps, spring-fed ponds, and streams (Ross and Anderson 1990; Lizgus et al. 1999; Sajwaj and Lang 2000; Joyal et al. 2001; Kiviat et al. 2004; Edge et al. 2009; Markle and Chow-Fraser 2014; 2017). Freshwater turtles have been observed to overwinter in groups (communal overwintering, Edge et al. 2009) and return to the same site each year (site fidelity, Switzer 1993). The combination of communal overwintering and overwintering site fidelity is hypothesized to increase access to mates and/or because of limited availability of suitable overwintering sites (Ross and Anderson 1990; Litzgus et al. 1999; Newton and Herman 2009; Markle and Chow-Fraser 2017). Successful overwintering may also lead to the establishment of communal overwintering since individuals may follow each other to overwintering sites (Litzgus et al. 1999). The degree of communal overwintering may also be related to population density, where in denser populations there is a greater likelihood of communal overwintering due to the increased number of individuals (Greaves and Litzgus 2007). However, communal overwintering can also be extremely detrimental to freshwater turtle populations. If overwintering conditions become unsuitable for extend periods, depending on the size of the overwintering group, anywhere from a few individuals to the entire population could experience overwintering mortality (Litzgus et al. 1999; Hall and Cuthbert 2000; Keevil et al. 2018; Gasbarrini et al. 2021).

There is a need to identify aquatic habitats that can provide thermal, physical and biogeochemical conditions necessary for successful turtle overwintering (Markle et al. 2020). There is a considerable amount of annual variation in the severity and length of winters and due to the dependence freshwater turtles have on the same overwintering habitats displayed through site fidelity, it is crucial that suitable overwintering conditions continue to persist year after year (Gibbons et al. 2000; Edge et al. 2009; Armstrong and Brooks 2014; Williams et al. 2015; Keevil et al. 2018; Markle et al. 2020). To ensure specific overwintering conditions persist, long-term changes in overwintering suitability should be placed within the context of wetland succession and spatial complexity of wetland habitat (Markle et al. 2020). Wetland infilling leads to the progression through multiple wetland classes, from shallow open water to fens and bogs, providing overwintering habitat for the majority of freshwater turtle species (Ross and Anderson 1990; Sajwaj and Lang 2000; Joyal et al. 2001; Kiviat et al. 2004; Edge et al. 2009; Markle et al. 2020). The process of infilling begins as a ring of floating vegetation (mat) that forms around the perimeter of a pond or shallow open water wetland, often supporting both painted and snapping turtles (Reese et al. 2002; Rollinson et al. 2008; Paterson et al. 2012; Markle et al. 2020). As vegetation continues to grow into the open water, it provides structure for *Sphagnum* moss and other sediments which can lead to the accumulation of peat and therefore the vertical thickening of the peat mat (Kratz and DeWitt 1986; Markle et al. 2020). The buoyancy of the peat mat allows it to expand both vertically and horizontally towards the deeper water in the middle of the water body and, as the peat mat develops, the surface and vertical spatial complexity of the wetland increases (Markle et al. 2020). Once the floating peat mat has accumulated enough peat to become grounded to the sediments on the bottom of the basin, the transition from lakes or open water wetlands to marshes or fens occur (see Markle et al. 2020), resulting in a mosaic of open water, floating peat and grounded peat providing key overwintering habitats for the Blanding's turtle (Markle and Chow-Fraser 2017) and the spotted turtle (Litzgus et al. 1999). The combination of microhabitat characteristics from multiple wetland classes allows spatially-complex wetlands to continually provide suitable overwintering sites for multiple freshwater turtle species (Markle et al. 2020).

To protect a population, it is critical that the population is provided with adequate and appropriate places for individuals to carry out their life processes, including suitable overwintering sites (Ramussen and Lizgus 2010). The dependence reptiles have on specific macro and microhabitats make them ideal for habitat selection studies (Böhm et al. 2013). However, habitat selection information gathered from a small number of individuals should be interpreted with caution (Ramussen and Lizgus 2010) because there is a heterogeneity among overwintering microenvironments in most habitats occupied by freshwater turtles (Ultsch 2006). In addition, just because there are no confirmed turtles using a macrohabitat as an overwintering site does not mean that the location is not suitable for overwintering (Markle and Chow-Fraser 2017). The combination of understanding habitats at both coarse (macrohabitat) and fine (microhabitat) scales can aid in improving management plans through the identification of suitable and unsuitable habitat that provide key resources needed for survival (Ramussen and Litzgus, 2010). Ultimately, without more detailed knowledge on suitable overwintering habitats, recovery strategies that rely on creation or restoration of overwintering habitats often do not contain the specific criteria necessary to ensure habitats are suitable (Markle and Chow-Fraser 2017). Measuring the physical characteristics of freshwater turtle overwintering sites can help more accurately identify and target suitable overwintering habitats for conservation and management plans (Paterson et al. 2012). With a predicted increase in temperature and decrease in precipitation as a result of climate change (Mothes et al. 2020), overwintering sites that were once suitable may become unsuitable overtime (Markle et al. 2020). Climate change poses a great risk to individuals that exhibit fidelity to overwintering habitats (Markle and Chow-Fraser 2017; McLaughlin et al. 2017). Since turtles living at the northern extent of their ranges may spend up to half of the year overwintering (Ultsh 1989; 2006) and with the cumulative effects of habitat loss and climate change, it is imperative that critical overwintering habitats are well understood to facilitate effective habitat management, protection, and restoration.

Overwintering site characteristics: Temperature

It has been hypothesized that thermal suitability is the most important factor affecting the overwintering success of freshwater turtle species (Greaves and Litzgus 2007, Rollinson et al. 2008; Edge et al. 2009; Paterson et al. 2012; Williams et al. 2015;

Markle and Chow-Fraser 2017). Freshwater turtles avoid mortality from freezing by overwintering in thermally stable microhabitats that buffer external temperatures, providing temperatures which hover around 0°C (Greaves and Litzgus 2007; 2008; Edge et al. 2009; Rasmussen and Litzgus 2010; Markle and Chow-Fraser 2017; Markle et al. 2020). When freshwater turtles select sites with lower temperatures, the probability of winter survival may be increased (Edge et al. 2009; Newton and Herman 2009; Paterson et al. 2012; Markle et al. 2020). Sufficiently cool temperatures allow turtles to reduce their metabolic rates and therefore conserve energy (Ultsch 1985; 1989). Energy conservation is key for turtle species at their northern limits since they are exposed to extremely long periods of dormancy (Greaves and Litzgus 2007; Markle and Chow-Fraser 2017).

Blanding’s turtles, snapping turtles, and spotted turtles have all been observed to overwinter in mean water temperatures ranging from 0.3°C – 4°C even with external air temperatures reaching -29°C (Litzgus et al. 1999; Rollinson et al. 2008; Edge et al. 2009; Paterson et al. 2012; Markle and Chow-Fraser 2017). When the depth of the water column allows it, turtles may move vertically within the water column to maintain a relatively constant body temperature (Robichaud et al. 2022). Another hypothesis is that turtles use thermally heterogeneous habitats to maintain constant body temperatures by selecting sites such as the inflow, which provides a near constant input of above-freezing water temperatures (Edge et al. 2009; Markle et al. 2020). Ideally, aquatic habitat temperatures would remain warm enough that a portion of the water column would remain unfrozen. In the event the entire water column does freeze, the sediment can provide temporary refuge from potentially lethal temperatures because substrate temperatures are often warmer and more stable than temperatures at mid-depth within the water column (Newton and Herman 2009; Markle and Chow-Fraser 2017; Markle et al. 2020). When turtles bury into the sediment, there is a trade-off between protection and access to dissolved oxygen since the sediment is under anoxic conditions (Ultsch 1989; Reese et al. 2003; Ultsch 2006). In addition to protection from potentially freezing temperatures, turtles may temporarily bury into the substrate for protection against predators (Taylor and Nol 1989; Edge et al. 2009; Markle and Chow-Fraser 2017; Markle et al. 2020). Low temperatures result in decreased mobility, which prohibits individuals from fleeing quickly from predators or relocating to a new overwintering site when needed (Litzgus et al. 1999; Greaves and Litzgus, 2007; Newton and Herman 2009; Edge et al. 2009). Moreover, temperature is thought to be the most important factor because the Blanding's turtle, snapping turtle, spotted turtle and painted turtle can tolerate overwintering in anoxic conditions (Greaves and Litzgus 2007, Paterson et al. 2012; Williams et al. 2015; Markle and Chow-Fraser 2017).

Overwintering site characteristics: Dissolved oxygen

The availability of dissolved oxygen is another important contributor to overwintering habitat suitability (Ultsch 2006). During the overwintering period, depending on the microhabitat selected, turtles may or may not have access to dissolved oxygen (Reese et al. 2003). Access to aerial oxygen is often severely limited because persistent ice cover can prevent turtles from surfacing to breathe for months at a time (Ultsch 1989; Litzgus et al. 1999; Markle and Chow-Fraser 2014). Without access to aerial oxygen, turtles may be forced to overwinter in an anoxic microenvironment or within the water column, which may become oxygen depleted overtime (Ultsch 1989). An anoxic aquatic environment is defined as an aquatic habitat that is virtually depleted of dissolved oxygen (Demaison and Moore 1980). Turtles can overwinter for up to seven months which means selecting a microhabitat which is anoxic, or prone to becoming anoxic, may result in severe physiological stress (Ultsch 1989; 2006). Generally speaking, there are two distinct physiological groups of turtles, those that tolerate severely hypoxia (< 2 – 3 mg/L of DO) or anoxia (< 1 mg/L of DO) and those that are relatively anoxia intolerant and must select normoxic microhabitats (Ultsch 1989; 2006). Both snapping and painted turtles are confirmed to be anoxia tolerant (Ultsch 1989; Reese et al. 2002). Blanding's turtles and spotted turtles are considered to be anoxia tolerant because they are have been found overwintering with anoxia-tolerant species, or select microhabitats that are prone to becoming severely hypoxic or anoxic throughout the winter (e.g., swamps, fens and bogs) (Ultsch 1989; Litzgus et al. 1999; Reese et al. 2002; Ultsch 2006; Newton and Herman 2009; Edge et al. 2009; Markle and Chow-Fraser 2017). Turtles that are anoxia tolerant have specific adaptations to successfully overwinter under extended periods of low or non-existent dissolved oxygen (Ultsch 1989; 2006). Turtle species rely on extrapulmonary uptake of dissolved oxygen and although they are adapted for low oxygen conditions, normoxic microhabitats put far less physiological stress on overwintering turtles which can result in longer winter survival (Ultsch 1989). Under conditions that warrant anaerobic respiration, turtles are at risk of respiration acidosis due to excessive accumulation of lactic acid (Ultsch 1989). An overwintering site with both dissolved oxygen and specific aquatic temperatures can improve the success of overwintering turtles. As the temperature within the water column decreases, metabolic demands decrease which can delay the onset of anaerobic respiration, further delaying the accumulation of lactic acid and therefore potentially extending the amount of time turtles can stay submerged (Ultsch 1989). This is important because even if oxygen is available in very small amounts, it can reduce the level of anaerobic by-products (Ultsch 1989; 2006).

Across overwintering sites, dissolved oxygen availability is often heterogeneous (Ultsch 2006). Freshwater turtles may select microhabitats with relatively high dissolved oxygen or move to areas that have higher dissolved oxygen availability (Ultsch 2006). The variability in dissolved oxygen among the overwintering microhabitats could be due to exposure to aerial oxygen, decaying vegetation, and/or flowing water (Newton and Herman 2009; Markle et al. 2020). During overwintering, turtles may select or move to areas that open to aerial oxygen faster than others (i.e., melting of ice). As an example, if a turtle were to overwinter along the wetland edge, since ice tends to melt from the shore toward the centre of the waterbody, turtles may be exposed to aerial oxygen earlier than those who select microhabitats closer to the centre of the waterbody (Ultsch 1989). Similarly, turtles that select microhabitats which receive inputs of highly oxygenated unfrozen water (i.e. points of inflow) from connected waterbodies or uplands will have access to higher levels of dissolved oxygen than turtles thats elect microhabitats in stagnant sections of the waterbody (Newton and Herman 2009; Edge et al. 2009; Markle et al. 2020). Increased inputs of water from connected waterbodies and surface runoff can lead to increased water movement which can reduce the probability of ice cover formation and, therefore, potentially provide turtles with access to aerial oxygen (Markle et al. 2020). Temporary melting of ice cover caused by rainfall or above-freezing temperatures could also temporarily provide turtles access to aerial oxygen and allow for increased dissolved oxygen availability through mixing with air (Greaves and Litzgus 2008; Edge et al. 2009; Markle et al. 2020). Although freeze-thaw cycles contribute to dissolved oxygen availability and/or allow for access to aerial oxygen (Greaves and Litzgus 2007; Markle et al. 2020), it may lead to increased risk of newly emerged turtles becoming stranded and exposed to freezing external air temperatures. Furthermore, rapid oscillations in external temperatures resulting in early emergence could have negative energetic consequences (Williams et al. 2015) on overwintering turtles.

Overwintering site characteristics: Waterlevel

For wetlands that develop in bedrock depressions, the extent of erosion and weathering of bedrock influences both the depth and shape of the depression and thus the wetland (Anusa et al. 2012). Stability of the water table within the wetland is predominantly controlled by fill-and-spill water storage (Spence and Woo 2002; 2003; 2006). The fill-and-spill model can be defined as once a depression (or wetland) has reached its capacity (i.e., fills) any additional water (rainfall or overland flow) spills out of the depression onto the landscape (Spence and Woo 2002; 2003; 2006). The storage capacity of the wetland depression is continually being filled via inputs from rainfall, snowmelt, watershed surface water, and potentially ground water when wetland water levels are low (Oswald et al. 2011). Thus, as the water level within the wetlands increases, the potential for runoff increases (Spence et al. 2009). The runoff or “spill” may contribute to the filling of other hydrologically connected depressions, eventually discharging out of the catchment outlet (Spence and Woo 2002; 2003; 2006). The shape and depth of the wetland depression, amount of water present, regional climate conditions (i.e., temperature, precipitation) and hydrophysical properties of the material within the wetland depression influence the wetland hydroperiod, its ability to store water and the rate at which the wetland fills and spills (Brooks and Hayashi 2002; Altermatt et al. 2009; Vanschoenwinkel et al. 2009).

Freshwater turtle survival is strongly linked to water table dynamics especially during the overwintering period, where turtles require a sufficiently deep water column to minimize the likelihood of freezing (Markle et al. 2020). If the microhabitat does not provide a sufficient amount of free water, a turtle may be forced to bury into the sediment for extended periods of time, risking metabolic acidosis (Taylor and Nol 1989; Edge et al. 2009; Paterson et al. 2012; Markle and Chow-Fraser 2017; Markle et al. 2020). On the other hand, a large column of unfrozen water between the ice and sediment increases the turtle's risk of predation by predators such as river otters (Paterson et al. 2012). Furthermore, It is critical that water depths are stable during the overwintering period because both low and/or rapid declines in water levels have been linked to mass mortalities (Hall and Cuthbert 2000). If water table depth is shallow during the overwintering season, turtles will often congregate into areas where sufficiently deep water is present. This congregation increases the risk of mass mortality due to predation, and/or disease based on their proximity to one another (Allender 2011; Gasbarrini et al.

2021). If water levels were to rapidly drop at any point in the overwintering season, the lethargic turtles may be unable to relocate to an overwintering site that offers deeper water, greatly increasing the risk of mass mortality due to freezing and/or desiccation (Hall and Cuthbert 2000; Newton and Herman 2009; Gasbarrini et al. 2021).

Although water table stability is important for overwintering habitat suitability, natural disturbances , such as beaver dams, directly influence the hydrological behaviour of the wetland and watershed. Beaver dams result in the creation of ponds, alter drainage patterns, enhance water storage and evaporation losses, and change stream flow regimes (Woo and Waddington 1990). The stabilization of stream flow and creation of deep open water pools can serve as habitat for freshwater turtles, including overwintering sites (Johnston and Naiman 1987; Joyal et al. 2001; Yagi and Litzgus 2012). The presence of newly flooded areas caused by the creation of ponds, diversion channels, and multiple-surface flow paths because of beaver dams (Woo and Waddington 1990) can also increase both habitat availability and hydrological connectivity between areas, making it easier for turtles to travel within and among sites (Yagi and Litzgus 2012). Most beaver dams will collapse gradually after several years of decay, most likely due to beaver abandonment (Stock and Schlosser 1991). Beaver dams may also fail due to a variety of reasons such as high intensity rainfall, spring runoff associated with rapid snowmelt, human destruction, or the collapse of upstream dams (Butler and Malanson 2005). Abrupt beaver dam failures can result in the rapid draining of the upstream pond and possibly lead to catastrophic downstream floods (Stock and Schlosser 1991). If a beaver dam were to collapse during the overwintering period resulting in the drainage of an overwintering site, any turtles present would most likely perish due to exposure of freezing temperatures and/or desiccation based on their inability to relocate to another suitable site in a timely manner (Edge et al. 2009; Newton and Herman 2009).

Anthropogenic land use changes can also have detrimental impacts on freshwater turtle habitats through altered water table dynamics (Sirois et al. 2014). At the watershed scale, the spatial distribution and cumulative storage capacity of wetlands can regulate overall connectivity, yet anthropogenic influences such as the development of roads and infrastructure can lead to variation and/or loss of wetlands (Todd et al. 2010; Evenson et al. 2018). Having wetlands spatially distributed throughout the landscape is key for many freshwater turtle species because highly connected habitat patches (e.g., wetlands, forested valleys, rock barrens) allow individuals to access and move between habitats easily (Attum et al. 2008). The development of roads can result in habitat fragmentation, alteration of wetland vegetation communities and wetland temperature (Trombulak and Frissell 2000). Roads have been observed to alter growing season temperatures in an adjacent bog and fens by 1°C and 1.2°C, respectively, over a two-year period (Saraswati and Strack 2019; Williams-Mounsey et al. 2021). Roads also have the potential to alter (sub)surface flow and divert natural drainage patterns acting as dams, resulting in upslope flooding and downslope drying (Megahan 1972; Wemple 1996; Trombulak and Frissell 2000; Chimner et al. 2016; Williams-Mounsey et al. 2021). This can be detrimental because alteration of flow can change the timing and magnitude of runoff, possibly destroying wetland habitat (Trombulak and Frissell 2000). Culverts are often used to mitigate the impacts of roads on (sub)surface flow but unless installed correctly, culverts can be ineffective at mitigating the hydrologic impacts of roads on wetlands (Saraswati et al. 2020). In the context of turtle overwintering habitat, if a wetland is intersected by a road and alteration of flow occurs, the downslope portion may no longer have enough water to support an overwintering turtle. Impacts of land use change and insufficient mitigation techniques may result in loss of habitat and negatives impact to local turtle populations

*Impacts of climate change on overwintering habita*t

The earth is undergoing unprecedented rapid climatic change, resulting in an increase in temperatures, evapotranspiration, rainfall, storm severity, and storm frequency (IPCC 2012; 2022). This increase in environmental variability creates the potential for an increase in the frequency of extreme weather-related events such as wildfires, droughts, and floods (IPCC 2014). Winter air temperatures are predicted to increase, with decreasing snow cover and increasing precipitation in northern temperate regions such as Canada (Williams et al. 2015). The average winter in North America is predicted to be shorter with an increase in the frequency of both extreme high temperatures and longer mid-winter warm spells (Williams et al. 2015). Weather whiplash, defined as a rapid shift in weather conditions (Casson et al. 2019) is becoming more prevalent under changing climates (IPCC 2013) yet there is currently a lack of understanding of the causes and consequences of winter weather whiplash events (Casson et al. 2019) and their impacts on turtle overwintering habitat is not well understood. Increased winter rainfall and therefore increased inputs into the wetland via surface runoff may increase the amount of oxygenated water entering the system potentially discouraging ice formation (Markle et al. 2020). Although this may decrease the risk of exposure to anoxic conditions, it may increase overwintering turtles' risk of predation (Markle et al. 2020). Furthermore, increases in weather whiplash events leading to freeze-thaw cycles within overwintering habitats may have negative energetic consequences on overwintering turtles (Williams et al. 2018; Markle et al. 2020). Ongoing impacts of climate change on suitable turtle habitat is expected to cause shifts in species geographic range and increase extinction rates (Hanson et al. 2001; Thomas et al. 2004; Jetz et al. 2007). To accurately guide mitigation efforts, species that benefit, and those that are vulnerable to global climate change must be identified (Williams et al. 2015). As ectotherms, increased average temperature will likely increase rates of metabolism in overwintering turtles, possibly increasing the rate of use of stored energy reserves, and risking metabolic acidosis earlier on in the overwintering period (Newton and Herman 2009; Williams et al. 2015; Markle and Chow-Fraser 2017; Ritchie 2017; Markle et al. 2020). The shift in timing in temperature patterns may have consequences for the long-term viability of turtle populations because freshwater turtles often associate seasonal patterns, such as nesting, and overwintering with weather related cues (Bowen et al. 2005; Gibbons et al. 2000). Each federally listed turtle species has a federal recovery strategy that sets the strategic direction to stop and/or reverse the decline of the specified species (Environment Canada 2016). Under each recovery strategy, climate change is identified as a current, widespread and continuous threat with minimal understanding of the severity of its impacts (Environment Canada 2016; 2018). Climate change is expected to affect the availability of suitable habitats across turtles Canadian range (Environment Canada 2016; 2018). Specifically, Blanding's turtles are considered to be one of the most sensitive reptiles within the Great Lakes region to the impacts of climate change (Environment Canada 2016). Under current climate scenarios and land conversion rates, Blanding's turtle populations (especially those in southern Ontario) seem to be at higher risk of extinction with little potential for gene flow across populations than other turtle species (Millar and Boulin-Demers 2012). The interaction between turtle species and the changes in the abiotic environment will ultimately determine if they survive critical activities such as overwintering, under changing climatic conditions (Williams et al. 2015). Climatic microrefugia, such as the microhabitats used as overwintering sites by freshwater turtles, are locations on the landscape that support the species while the surrounding climatic conditions become unsuitable (Rull 2009). Furthermore, due to turtles limited dispersal ability turtles will most likely be unable to adjust to shifts in their range as needed for population persistence (Hamilton et al. 2018). With this in mind, it is critical that as climatic microrefugia become limited, they are identified and protected so they can continue to provide opportunities for species persistence in the face of regionally deteriorating conditions (McLaughlin et al. 2017).

Turtle resilience zone

Used turtle overwintering habitat and available overwintering habitat often share the same abiotic characteristics (e.g., specific aquatic temperatures) suggesting that specific abiotic factors are deliberately selected by overwintering turtles (Rollinson et al. 2008). Suitable overwintering sites are typically characterized by basic thermal, physical and biogeochemical microhabitat characteristics including dissolved oxygen content, substrate type and depth, vegetation cover, and/or water temperature (Greaves and Litzgus 2007; Edge et al. 2009; Jackson and Ultsch 2010; Millar and Blouin-Demers 2011; Markle and Chow-Fraser 2017; Markle et al. 2020). A suitable overwintering site provides temperatures that allow for the reduction in the turtle's metabolism but prohibits the entire water column from freezing (Herbert and Jackson 1985; Ultsch 1989; Edge et al. 2009; Markle and Chow-Fraser, 2017). Specifically, laboratory (Ultsch 1989) and *in-situ* overwintering data from Blanding's turtles (Edge et al. 2009), painted turtles (Rollinson et al. 2008), snapping turtles (Brown and Brooks 1994) and spotted turtles (Litzgus et al. 1999) indicate overwintering turtles select stable temperatures near 0°C which contribute to successful overwintering (Ultsch 1989; 2006). When access to aerial oxygen is limited the risk of metabolic acidosis can be reduced by selecting cooler water temperatures, which further emphasizes the importance of low overwintering water temperatures (Herbert and Jackson 1985; Markle et al. 2020). The lack of key abiotic factors (aquatic temperature, dissolved oxygen, water depth) individually or combined at certain overwintering habitats may result in overwintering habitats being unsuitable. The term resilience zone can be used to define the space that optimizes overwintering habitat suitability (Smolarz et al. 2018) where the turtle resilience zone is the space that provides stable, cool water temperatures near 0°C, sufficient dissolved oxygen, and substrate for burying (Markle et al. 2020). The resilience zone quantifies environmental conditions that affect overwintering turtles, further described as physiological tolerances, but excludes biotic interactions, where changes in the resilience zone size can be used as a metric to identify vulnerable overwintering habitat (Markle et al. 2020). The resilience zone has important applications in the conservation and management of turtle species at-risk and their habitats since it can be used to identify resilient and/or vulnerable overwintering habitat (Markle et al. 2020).

Eastern Georgian Bay: Georgian Bay Biosphere Mnidoo Gamii

Along the eastern coast of Georgian Bay is an UNESCO biosphere known as the Georgian Bay Biosphere Mnidoo Gamii (GBB). The GBB stretches approximately 175 km from Port Severn in the south to the French River in the north and inland from the Georgian Bay coast to the highway 400/69 corridor. The GBB is situated within the Robinson-Huron Treaty of 1850 and Williams Treaty of 1923, located on Anishinabek territory and is considered a region of global ecological significance. The GBB supports a high diversity of flora and fauna, dominated by mixed, deciduous, coniferous forest, wetlands, and peatlands with the bedrock exposed at the surface (Crins et al. 2009). Out of the eight native turtle species in Ontario, six species (excluding the spiny softshell and wood turtle) are found within the GBB. The abundance of rock barrens provides shallow, lichen-covered, soil-filled depressions which are key nesting habitats for turtle species within Georgian Bay (Litzgus and Brooks 1998; Markle and Chow-Fraser 2014; Markle et al. 2021). The aquatic habitats provide resources such as thermoregulation sites, protection from predators and access to mates (Roe and Georges, 2007; Congdon et al. 2011). Furthermore, the abundance of wetlands and peatlands provide overwintering environments which maximize the winter survival of turtle species (Markle et al. 2020). Most of the wetland habitats within the GBB are still in relatively pristine condition with minimal loss when compared to southern Ontario (Cvetkovic and Chow-Fraser 2011). Relatively minimal habitat losses present a pressing need to identify and protect critical habitat areas within the GBB before additional habitat loss takes place (Crins et al. 2009; Millar and Blouin-Demers 2011). Further, this will allow for the preparation of accurate conservation and management strategies for freshwater turtle populations within the GBB. Freshwater turtles are of particular interest in landscape-scale conservation studies due to their long-lived nature and dependence on both aquatic and terrestrial habitats, and at-risk status (Gibbs and Shriver 2002; Markle and Chow-Fraser 2014). Additionally, habitat selection varies across their range which highlights the importance of site-specific habitat studies since findings may not be identical in all regions (Markle and Chow-Fraser 2014).

Freshwater turtles within the Georgian Bay Biosphere

Out of the six freshwater turtle species found within the GBB, four turtle species including the Blanding's turtle, painted turtle, snapping turtle and spotted turtle are known to be associated with various inland wetland types such as bogs, fens, lakes rivers and beaver ponds throughout the GBB (Obbard and Brooks 1981; Litzgus et al. 1999; Rollinson et al. 2008; Edge et al. 2010; Markle and Chow-Fraser 2014) and within our primary study area 20 km inland from Georgian Bay. The other two species, musk turtles and northern map turtles will not be discussed due to their lack of association with inland wetlands such as bogs and fens (Laverty et al. 2016; Robichaud et al. 2022).

The Blanding's turtle is a medium sized semi-aquatic freshwater turtle species with a distinctive bright yellow chin and highly domed black to brown shell (Ernst and Lovich 2009; COSEWIC 2016). The Blanding's turtle range is centred around the Great Lakes, with two populations in Canada, the Great Lakes/St. Lawrence population, and the Nova Scotia population. The Great Lakes/St. Lawrence population is designated as Endangered by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) due to population decline (COSEWIC 2016). Furthermore, the Blanding's turtle is listed as Threatened federally under the Species at Risk Act (SARA) (Environment Canada 2016) and provincially within both Ontario and Quebec under the Endangered Species Act and Act Respecting Threatened or Vulnerable Species, respectively (ARTVS) (ESA 2007; ARTVS 2009). Within Ontario, Blanding's turtle populations occur from extreme southwestern Ontario, east to Ottawa, north to North Bay and Sudbury, and northwest to Sault-St. Marie. The sub-population within the Georgian Bay region accounts for 48% of the Blanding's turtle Canadian range (COSEWIC 2016). The total population of Blanding's turtles within Ontario is estimated to be > 50,000 mature individuals with subpopulations ranging from a few dozen to approximately 100 individuals (COSEWIC 2016). The population size is projected to continue to decline by 40% over the next two generations (80 years) and by 50% in just under three generations mainly due to large numbers of road mortality (COSEWIC 2016).

The spotted turtle is a small freshwater turtle species with a black shell speckled with yellow-orange spots with a distinctive orange spot found behind each eye (Ernst and Lovich 2009; COSEWIC 2014). The spotted turtle populations within Canada occur within southern Ontario and southern Quebec, from extreme southwestern to southeastern Ontario and north to Georgian Bay (COSEWIC, 2014). Spotted turtles are listed as Endangered under both SARA and the ESA and Threatened under the ARTVS (ESA 2007; ARTVS 2009; Environment Canada 2014; COSEWIC 2014). Approximately 17% of the spotted turtles global range occurs within Ontario, with the provincial total population estimated to be > 3,000 individuals (COSEWIC 2014). There is a projected > 40% decline in the Canadian population of spotted turtles over the next three generations (123 years) based on annual mortality rates in Southwestern Ontario and Georgian Bay (Litzgus 2006; Enneson and Litzgus 2009; COSEWIC 2014).

The snapping turtle is Canada's largest freshwater turtle. Snapping turtles have black, olive, or brown shells typically covered in algae due to the extensive time spent in aquatic habitats (Ernst and Lovich 2009). Approximately 10% of the snapping turtle's range occurs in Canada and they are found throughout central and southern Ontario (COSEWIC 2008). Snapping turtles remain relatively abundant in eastern Canada, but very little is known about their population trends in Canada (COSEWIC 2008). Like the Blanding's and spotted turtles, snapping turtles are vulnerable to adult mortality, where local population declines are expected to occur wherever anthropogenic threats such as road mortality, habitat loss and subsidized predators are most prevalent (COSEWIC 2008). The snapping is currently designated as a species of special Special Concern (ESA 2007; COSEWIC 2008).

The midland painted turtle is a small to medium sized freshwater turtle widespread across North America, distributed throughout southern, central, eastern, and northern-western Ontario. The painted turtle has an olive to blacks smooth and relatively flat carapace with red or dark orange markings on the marginal scutes as well as red and yellow stripes on the head and neck. Road mortality, habitat degradation and invasive species are among the factors leading to the decline in midland painted turtle populations, the midland painted turtle is currently listed as a species of special Special Concern, unfortunately the midland painted turtle recently received its at-risk status in 2018 (COSEWIC 2018).

Thesis objectives

To effectively protect habitat for a species, it is necessary to understand how individuals choose to disperse themselves throughout time and space. A simple delineation of habitats without determining how or why individuals are using those areas may not fully protect a species from decline (Rasmussen and Litzgus 2010). It is critical to determine how pristine macro- and microhabitats vary both spatially and temporally to provide a better reference for habitats that are being restored after being heavily affected by human developments (Roe et al. 2009). Furthermore, when protecting habitats, it is critical that the surrounding terrestrial zone is included because this zone is fundamentally linked to the function of wetlands (Gibbons et al. 2000). For successful conservation, adaptation, and management of wetlands, the larger landscape matrix must be included to ensure that wetland conservation is not limited to just the wetlands themselves (Murray 2019). This is especially important since the associated global climate changes may influence the usage of wet habitat and dry habitats among freshwater turtle species (Markle and Chow-Fraser 2014). Furthermore, long-term monitoring of turtle populations is essential because they are a long-lived species. By developing long-term monitoring programs from long-lived species, it provides a better understanding of the long-term risks associated with short-term and long-term perturbations needed to inform management decisions (Gibbons et al. 2000; Keevil et al. 2018). The importance of both long-term and short-term monitoring cannot be understated because the combination of both can provide information on population size and status and can reveal population trends and/or causes of those trends (Todd et al. 2010).

Given that wetlands are critical overwintering sites for turtle species at-risk and little is known about the spatial and temporal variability of the chemical, thermal and physical attributes in these habitats, our objective was to provide a detailed spatiotemporal quantification of the space that optimizes overwintering habitat suitability, also known as the turtle resilience zone (Smolarz et al. 2018; Markle et al. 2020). First, we used *in-situ* thermal (sediment and water temperature), chemical (dissolved oxygen content) and physical (water, ice and snow depth) data from four different available microhabitat types (i.e., floating mat, inflow, lagg, open water) within 5 confirmed overwintering wetlands in the Georgian Bay Biosphere Mnidoo Gamii to characterize abiotic factors that contribute to overwintering habitat suitability. We predicted that the abiotic factors would be consistent across available habitats at the landscape scale (wetlands) but differ across the microhabitat scale. Next, we characterized overwintering habitat use by adult Blanding's turtles through the investigation of *in-situ* thermal (aquatic temperatures), chemical (dissolved oxygen content) and physical (water, ice and snow depth) data from 5 (2020) and 19 (2021) adult Blanding's turtles. Following, we tested for evidence of overwintering habitat selection by Blanding's turtles by comparing abiotic factors (thermal and chemical) at Blanding's turtle overwintering locations to abiotic factors at available overwintering habitats (wetlands and microhabitats). We hypothesized that overwintering conditions at Blanding's turtle locations would be more stable because freshwater turtles need specific overwintering conditions (e.g., temperature) to survive. Therefore, we predicted that Blanding's turtles would actively select habitats that buffer changes to thermal, physical and geochemical characteristics. Specifically, we hypothesized that Blanding's turtles would select overwintering habitats with aquatic temperatures between 0 — 3°C, dissolved oxygen contents above anoxic conditions (≥ 2 mg/L) and water depths of at least 50 cm. Next, we used both *in-situ* turtle overwintering data and literature values to complete our fourth objective. We defined suitable thermal, chemical, and physical properties for overwintering Blanding's turtles and combined the variables into a measurement of resilience zone presence, size and duration. We predicted that dissolved oxygen would have the greatest control on resilience zone size and that dissolved oxygen would remain limited within overwintering macrohabitats. Lastly, we predicted that resilience zone size would differ within wetlands as opposed to across wetlands.

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Chapter 2: Identifying suitable overwintering habitat through the integration of thermal, chemical and physical wetland properties

Abstract

Turtles are among one of the most vulnerable vertebrates on the planet and within Canada, all eight native species of freshwater turtle are designated as at-risk under COSEWIC. The primary extinction risk turtles face is the loss of their habitats. At the northern limit of their range, turtles rely on aquatic habitats to survive the winter. For up to six months of the year turtles retreat underwater in habitats such as bogs and fens to take refuge from freezing air temperatures. Suitable overwintering habitats are characterized by the availability of thermal, chemical and physical habitat characteristics and the term resilience zone (RZ) can be used to define the space that optimizes habitat suitability. With that in mind we 1) characterized both available and used Blanding's turtle overwintering habitat, 2) tested for habitat selection by Blanding's turtles and 3) combined selected abiotic factors into a single measurement of RZ. We measured aquatic temperatures, dissolved oxygen (DO), water, ice and snow depth at 5 (2020-2021) and 19 (2021-2022) Blanding's turtle overwintering locations and four paired available microhabitats (floating mat, inflow, lagg, open water) across 5 wetlands. We found that Blanding's turtles selected average daily temperatures that ranged from 3.58°C to 0.53°C with a mean of 1.08°C, minimum average DO contents of 1.10 mg/L and average water and ice depth ranged from 42cm to 79cm, 12cm to 38cm, respectively. Based on habitat use data, we identified the RZ as present when aquatic temperatures were between 0 — 4°C, dissolved oxygen contents ≥1 mg/L and water depths were ≥ 40 cm and found that

the RZ varied across wetlands and microhabitats. Overall, the RZ can be used to monitor changes in abiotic variables to identify overwintering habitats at-risk of becoming unsuitable in the face of climate and land-use changes.

Introduction

The earth is undergoing unprecedented rapid climatic change, resulting in an increase in temperatures, evapotranspiration, rainfall, storm severity, and storm frequency (IPCC 2012; 2022). This increase in environmental variability creates the potential for an increase in the frequency of extreme weather-related events such as wildfires, droughts, and floods (IPCC 2014). Furthermore, winter air temperatures are predicted to increase, with decreasing snow cover and increasing precipitation in northern temperate regions such as Canada (Williams et al. 2015). The average winter in North America is predicted to be shorter with an increase in the frequency of both extreme high temperatures and longer mid-winter warm spells (Williams et al. 2015). Weather whiplash, defined as a rapid shift in weather conditions (Casson et al. 2019) is becoming more prevalent under changing climates (IPCC 2013) yet there is currently a lack of understanding of the causes and consequences of winter weather whiplash events (Casson et al. 2019). Identifying species and habitats most threatened by the impacts of climate change is key for prioritizing habitat protection and implementing accurate mitigation strategies (West et al. 2009; He et al. 2019; Powers and Jetz 2019; Weiskopf et al. 2020).

Climate mitigated and land-use changes, among many other factors including road morality and poaching, have resulted in turtles becoming arguably the most threatened of the major groups of vertebrae (IUCN 2008; Buhlmann et al. 2009; Hoffman et al. 2010; Lovich et al. 2018). As ectotherms, turtles select habitats that help control body temperature due to the tight coupling between environmental temperature and metabolic regulation (Huey 1991). In North America freshwater turtles occur at latitudes as far north as 53 °N (Buhlmann et al. 2009) with climate most likely being the dominant abiotic force shaping the species distribution (IPCC 2014; Cunningham et al. 2016; McLaughlin 2017). Furthermore, turtles located in northern temperate regions with seasonally cold climates, characterized by short summers are faced with harsh, long, cold winters. (Ultsch 1989; 2006). Most aquatic freshwater turtle species seek refuge from freezing conditions by reducing their metabolic activity and retreating underwater into habitats including various wetland types (e.g., bog, fen, marsh) which acts as a thermal refuge (Ultsch 1989, Reese et al. 2002; Greaves and Litzgus 2007; Rollinson et al. 2008; Edge et al. 2009; Paterson et al. 2012; Markle and Chow-Fraser 2017; Markle et al. 2020) (Figure A1).

Overwintering presents an extreme challenge for freshwater turtles. A suitable overwintering site is defined as a habitat that provides the opportunity to access aerial or dissolved oxygen, cool, stable aquatic temperatures near 0°C, vegetation cover and sediment for protection from predators during overwintering (Markle et al. 2020). It is hypothesized that some aspects of overwintering, including the availability of suitable sites, may limit the northern distribution and abundance of freshwater turtle species (Litzgus et al. 1999). Overwintering habitats must provide safe refuge under ice for extended periods of time and balance the risk of freezing, metabolic acidosis, and predation (Litzgus et. al. 1999; Litzgus and Brooks 2000; Edge et al. 2009; Markle and

Chow-Fraser 2017; Markle et al. 2020). The availability of suitable overwintering sites and a turtle's ability to select appropriate sites may be the difference between survival and death for overwintering turtles (Paterson et al. 2012). Currently, there is limited field information that examines in-depth environmental conditions required to support overwintering survival beyond temperature selection (Litzgus et. al. 1999; Rollinson et al. 2008; Edge et al. 2009; Paterson et al. 2012; Marchand et al. 2018; Robichaud et al. 2022) and identification of habitat types occupied by overwintering turtles (Markle and Chow-Fraser 2017, Zagorski et al. 2019; Feng et al. 2020). Specifically, overwintering DO data is limited in the literature, on average average in wetland habitats DO is collected 1 — 4 times per month (January - March) during the overwintering season (Rollinson et al. 2008; Edge et al. 2009; Paterson et al. 2012; Gasbarinni et al. 2021).

Used turtle overwintering habitat and available overwintering habitat often share the same abiotic characteristics (e.g., specific aquatic temperatures) suggesting that specific abiotic factors are deliberately selected by overwintering turtles (Rollinson et al. 2008). Suitable overwintering sites are typically characterized by basic thermal, physical and biogeochemical microhabitat characteristics including dissolved oxygen content, substrate type and depth, vegetation cover, and/or water temperature (Greaves and Litzgus 2007; Edge et al. 2009; Jackson and Ultsch 2010; Millar and Blouin-Demers 2011; Markle and Chow-Fraser 2017; Markle et al. 2020). A suitable overwintering site provides temperatures that allow for the reduction in the turtle's metabolism but prohibits the entire water column from freezing (Herbert and Jackson 1985; Ultsch 1989; Edge et al. 2009; Markle and Chow-Fraser, 2017). Specifically, laboratory (Ultsch 1989) and *in-situ* overwintering data from Blanding's turtles (Edge et al. 2009), painted turtles (Rollinson et al. 2008), snapping turtles (Brown and Brooks 1994) and spotted turtles (Litzgus et al. 1999) indicate overwintering turtles select stable temperatures near 0°C which contribute to successful overwintering (Ultsch 1989; 2006). When access to aerial oxygen is limited the risk of metabolic acidosis can be reduced by selecting cooler water temperatures, which further emphasizes the importance of low overwintering water temperatures (Herbert and Jackson 1985; Markle et al. 2020). The lack of key abiotic factors (aquatic temperature, dissolved oxygen, water depth) individually or combined may result in overwintering habitats being unsuitable. The term resilience zone can be used to define the space that optimizes overwintering habitat suitability (Smolarz et al. 2018; Markle et al. 2020) where the turtle resilience zone is the space that provides stable, cool water temperatures near 0°C, sufficient dissolved oxygen, and substrate for burying (Markle et al. 2020). The resilience zone quantifies environmental conditions that affect overwintering turtles, further described as physiological tolerances, but excludes biotic interactions, where changes in the resilience zone size can be used as a metric to identify vulnerable overwintering habitat (Markle et al. 2020). The resilience zone has important applications in the conservation and management of turtle species (Markle et al. 2020). The resilience zone is a powerful tool that can be used to identify suitable overwintering habitat through the integration of critical habitat factors. Further, the resilience zone can be used to monitor changes in abiotic variables to identify habitats across northern temperate regions that are at-risk of becoming unsuitable in the face of climate and land-use changes.

Given that wetlands are critical overwintering sites for turtle species at-risk and little is known about the spatial and temporal variability of the chemical, thermal and physical attributes in these habitats, our objective was to provide a detailed spatiotemporal quantification of the space that optimizes overwintering habitat suitability, also known as the turtle resilience zone (Smolarz et al. 2018; Markle et al. 2020). First, we used *in-situ* thermal (sediment and water temperature), chemical (dissolved oxygen content) and physical (water, ice and snow depth) data from four different available microhabitat types (i.e., floating mat, inflow, lagg, open water) within 5 confirmed overwintering wetlands in the Georgian Bay Biosphere Mnidoo Gamii to characterize abiotic factors that contribute to overwintering habitat suitability. We predicted that the abiotic factors would be consistent across available habitats at the landscape scale (wetlands) but differ across the microhabitat scale. Next, we characterized overwintering habitat use by adult Blanding's turtles through the investigation of *in-situ* thermal (aquatic temperatures), chemical (dissolved oxygen content) and physical (water, ice and snow depth) data from 5 (2020) and 19 (2021) adult Blanding's turtles. Following, we tested for evidence of overwintering habitat selection by Blanding's turtles by comparing abiotic factors (thermal and chemical) at Blanding's turtle overwintering locations to abiotic factors at available overwintering habitats (wetlands and microhabitats). We hypothesized that overwintering conditions at Blanding's turtle locations would be more stable because freshwater turtles need specific overwintering conditions (e.g., temperature) to survive. Therefore, we predicted that Blanding's turtles would actively select habitats that buffer changes to thermal, physical and geochemical characteristics.

Specifically, we hypothesized that Blanding's turtles would select overwintering habitats with aquatic temperatures between 0 — 3°C, dissolved oxygen contents above anoxic conditions (≥ 2 mg/L) and water depths of at least 50 cm. Next, we used both *in-situ* turtle overwintering data and literature values to complete our fourth objective. We defined suitable thermal, chemical, and physical properties for overwintering Blanding's turtles and combined the variables into a measurement of resilience zone presence, size and duration. We predicted that dissolved oxygen would have the greatest control on resilience zone size and that dissolved oxygen would remain limited within overwintering macrohabitats. Lastly, we predicted that resilience zone size would differ within wetlands as opposed to across wetlands.

Methods

Study area

The Georgian Bay ecoregion is situated on the southern portion of the Precambrian Shield with a landscape characterized by a mosaic of wetlands, forests, and open rock barrens (Crins et al. 2009). This ecoregion is known for its ridge and trough topography (Wester et al. 2018) where uplands are dominated by bedrock ridges that are often exposed at the surface or covered by a very thin, discontinuous, mineral material overlain with moss and lichen mats (Wester et al. 2018). Valleys and troughs contain wetland communities such as large complexes of marshes, bogs, fens, and beaver ponds (Wester et al. 2018). Individual wetlands may progress through multiple wetland classes from shallow open water to fens and bogs providing habitats for many diverse flora and fauna.

The Georgian Bay Biosphere Mnidoo Gamii (GBB) located in the eastern Georgian Bay region, spans 175 km from Severn River to the French River and is situated on traditional Anishinabek territory. The GBB supports the highest concentration of reptiles and amphibians in Canada (State of the Bay 2018) and there are numerous species of at-risk turtles found within the GBB including the Blanding's turtle (*Emydoidea blandingii*), snapping turtle (*Chelydra serpentina*), and midland painted turtle (*Chrysemys picta)*. The Great Lakes/St. Lawrence population of Blanding's turtles is designated as Endangered by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC 2016) and listed as Threatened within Ontario under the Endangered Species Act (ESA 2007). The snapping turtle is listed as Special Concern (SARA, 2002; ESA, 2007) and the midland painted turtle is designated as a species of Special Concern (COSEWIC 2018). The range in aquatic habitats provide a winter aquatic refuge for turtle species found within the GBB (Litzgus et al. 1999; Markle and Chow-Fraser 2014; Markle and Chow-Fraser 2017; Markle et al. 2020; Gassbarini et al. 2021). The wetlands are often dominated by *Sphagnum* spp. and various vegetation species, including but not limited to, sedges (*Carex* spp.), cranberry (*Vaccinium oxycoccos*), tamarack (*Larix laricina*), and water lilies (*Nymphaeaceae spp*.).

The climate of the GBB region is cool temperate and humid (Crins et al. 2009). The 30-year (1981-2010 climate normal) daily average maximum and minimum air temperatures are 25.5°C and -16.8 °C, respectively (Dunchurch ~45 km NE from study site, Environment Canada, 2022). Over the 2020-2021 overwintering season, the air temperature from October 2020 – May 2021 reached a maximum of 24.7 °C and a minimum of -25.9 °C. The 2021-2022 overwintering season saw a maximum air temperature of 25.5 °C and a minimum of -30.2°C. The 30-year (1981-2010 climate normal) annual precipitation in the region is 1118.2 mm with the majority of precipitation occurring from October – January (Dunchurch ~45 km NE from site, Environment Canada, 2022). The 30-year (1981-2010 climate normal) precipitation is dominated by 95% rainfall in October and 68% rainfall in November and then the pattern shifts towards snowfall in December through February (72-78% snowfall) (Environment Canada 2022). As the overwintering season progresses, March is dominated by almost equal parts of rainfall (55%) and snowfall (45%). April is dominated by rainfall (83%) which generally coincides with spring emergence (Environment Canada 2022; Obbard and Brooks 1981; Taylor and Nol 1989; Litzgus et al. 1999).

Assessing overwintering habitat availability

To assess the ecohydrological controls on turtle overwintering habitat, we monitored winter conditions at five wetlands in the Parry Sound District that were confirmed to support overwintering turtles (Figure 1; Figure 2; Figure A2). We selected these five wetlands because they contained a variety of different aquatic habitat types occupied by turtle species including Blanding's turtles, painted turtles and snapping turtles. Each wetland varied in size and spatial complexity, with four out of the five wetlands belonging to relatively large, continuous complexes (Table 1).

Table 1: Definitions used to classify habitat types in the NOBEL study region (Wetlands Working Group 1997; Edge et al. 2009; Markle et al. 2014; Markle et al. 2020) and the extent (ha) of each wetland.

Wetland name	ID	Brief Description	Area (ha)	Instrumentation
Bog A, Bog B	DIN 104, DIN 810	Water table is at or near the surface, high in spatial complexity with a mixture of open water, floating and grounded *Sphagnum* peat mats, vegetation such as leather leaf, sedges and grasses are present. Some sections of the wetland are covered in a thick layer of *Sphagnum* moss. Main source of water inputs is through precipitation, snowmelt and overland flow.	Bog A - 11 Bog B - 7.6	Temperature profile(s) at microhabitats (floating mat, inflow, open water. A single ground water well. DO, water, snow and ice depth at microhabitat locations. Bog A only:

				radio-tracked overwintering Blanding's turtles and collected temperature, DO, water, snow and ice depth at each turtle's location.
Open Water Wetland A (OWW A), Open Water Wetland B (OWW B)	DIN 418, DIN 447	Relatively small body of standing water with water depths around 2 m in the summer. Surface cover is dominated by open water patches, some dense, of leatherleaf are present, some small floating *Sphagnum* peat mats are present. Dead birch and poplar trees are located along the edges of the	OWW A - 2.1 OWW B - 1.2	Temperature profile(s) at microhabitats (floating mat, inflow, open water. A single ground water well. DO, water, snow and ice depth at microhabitat

		wetland. Main source of water inputs is through precipitation, snowmelt and overland flow. Represents transition between bog, fens, marshes and swamps.		locations.
Lake A, Lake B	DIN 732, DIN 109	Relatively large open body of water when compared to other aquatic habitats within the NOBEL study. Water depths far exceed 2 ms in depth in the centre. Surface cover is confined to bays, which contain areas of high spatial complexity with a mixture of open water, floating and grounded *Sphagnum* peat mats,	Lake A - 41.2 Lake B - 3.7	Lake A only: Temperature profile(s) at microhabitats (floating mat, inflow, open water. A single ground water well. DO, water, snow and ice depth at microhabitat

		vegetation such as leather leaf, sedges and grasses are present. Main source of water inputs is through precipitation, snowmelt and overland flow.		locations. Lake B only: Radio-tracked an overwintering Blanding’s turtle and collected temperature, DO, water, snow and ice depth at the turtle's location.

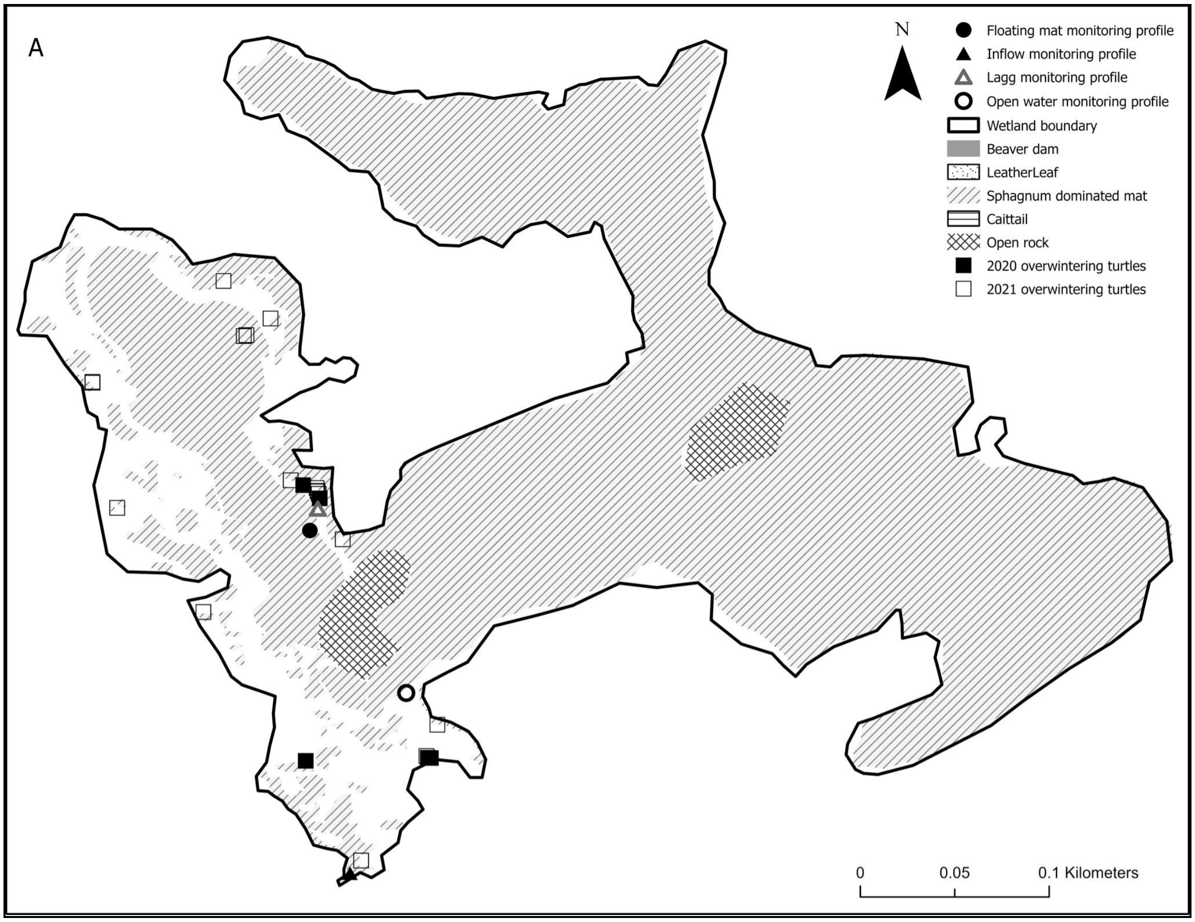

Figure 1: Map of Bog A with locations of instrumented available habitats within the NOBEL study region. Map includes overwintering locations of Blanding's turtles. Legend is applicable for Figure 2.

Figure 2: Map of Bog A within the NOBEL study region, B) represents open water wetland A (OWW A), C) represents open water wetland B, D) represents the portion of Lake A that was instrumented, E) represents Bog B. Please refer to the legend within Figure 1.

Due to the habitat complexity of each wetland, we monitored specific microhabitat locations to capture the variability of overwintering conditions within a wetland. Therefore, we selected four different microhabitats within each wetland to assess how abiotic resilience zone factors (i.e., water and sediment temperature, water and sediment depth, and DO content) varied both spatially and temporally. The microhabitats assessed included (1) under a floating mat, an accumulation of peat that is slightly buoyant and floats in deeper water, often dominated by *Sphagnum* mosses (see Markle et al. 2020), (2) in the lagg, the transitional area found between the wetland centre and surrounding uplands, within two metres from surrounding mineral land (Howie and Meerveld 2011), (3) in open water, two-metre radius of no surrounding vegetation and (4) at the inflow, point of entry for surface water from an upstream location, surface water is almost continuously entering the wetland at this point (Figure A3). While there are multiple microhabitat locations within a single wetland, we selected specific monitoring locations that had a minimum of 45 cm of water and sediment at the time of microhabitat selection (28th September 2020). For large wetlands (Bog A, Bog B, and Lake A) we targeted areas of the wetland for instrumentation based on turtle emergence survey data and ease of access in the winter to optimize safety during winter data collection.

At each of the 4 microhabitats across 5 wetlands (20 monitoring locations), we installed temperature profiles to quantify available water and sediment temperatures in the 2020-2021 and 2021-2022 overwintering seasons. Each profile recorded continuous water temperature every 30 minutes from October 1 to May 1 in both winters (HOBO Pendant MX Water Temperature Data Logger, Onset Computer Corporation, Bourne, MA, USA). Each profile was equipped with four data loggers to capture the approximate vertical zone where turtles may overwinter, including 7 and 50 cm above and below the sediment-water interface (Figure A4). Previous studies (e.g. Edge et al. 2009; Markle and Chow-Fraser 2017; Gasbarrini et al. 2021) placed temperature loggers 7 cm above the sediment to approximate the location of an overwintering turtle if they were sitting on the wetland bottom. We attached each logger to a ¾ inch PVC pipe and inserted the profile at the microhabitat monitoring location. We inserted a two-inch slotted PVC pipe overtop of the profile to help protect the profile and allow us to easily identify its location. To retrieve the temperature data without disturbing the microhabitat, we temporarily removed the PVC pipe with the temperature loggers and left the outer, slotted PVC pipe in place to ensure the temperature loggers were placed in the exact same locations both overwintering seasons.

At each of the five wetlands, we recorded water table depth at 15-minute intervals over both overwintering seasons using Solinst Levelogger pressure transducers located in 2-inch PVC wells. To monitor the duration of ice cover during the two overwintering seasons, we set up a Stealth Cam (QS20 Trail Camera) that was programmed to take photos hourly between 10:00 and 15:00 every day from 1 October to 1 May during both winters. Lastly, air temperature was recorded every 15 minutes at a micrometeorological tower located 150 m to 1500 m from all sites (using either a HMP35C (Vaisala Oyj, Helsinki, Finland) or HC2S3 relative humidity probe with an adjacent thermocouple wire; Smolarz 2018).

We characterized the thermal (sediment and water temperature), chemical (DO) and physical (water table dynamics, ice cover, ice thickness, sediment depth) properties from the available microhabitats within each of the five wetlands. First, we plotted measured sediment and water temperatures across the entire overwintering period (1 October – 1 May). We then completed a one-centimetre linear interpolation to estimate aquatic temperatures across the entire one-metre profile at 1-cm depth increments. We also used a 5 cm linear interpolation to estimate dissolved oxygen at 5-cm depth increments from the top of the water column to the sediment. We averaged the continuous (15- minute) water level data on a daily time scale and adjusted it to accurately represent the water depth at each of the available microhabitats within the wetlands. Finally, we calculated the average snow depth and ice thickness depth for each of the sample weeks.

Assessing overwintering habitat use

To assess overwintering habitat use we affixed iButtons (Alpha Mach, Sainte-Julie, Quebec) to the rear marginal scutes of 5 (2020-2021) and 19 (2021-2022) adult Blanding's turtles and radio tracked individuals within a single wetland (Bog A) over two winters (2020-2021 and 2021-2022). As part of another concurrent study, turtles were caught opportunistically by hand in fall 2020 and spring 2021. We identified the sex of each turtle using morphological characteristics such as concavity of the plastron and position of the cloacal opening (Hamernick 2000; Innes et al. 2008; Markle and Chow-Fraser 2014). We weighed each turtle (Pesola) to ensure the turtles were large enough to carry the AI-2F radio transmitter (Holohil Systems Ltd., Carp, ON, Canada, weight = 19 g) and an iButton (iBwetland iButton, Alpha Mach, Sainte-Julie, Quebec). We notched the scutes of each turtle with a unique code for later identification if one was not already present (Cagle 1939). Using quick dry and plumbers epoxy, we attached a radio tag and iButton to the rear marginal scutes (Markle and Chow-Fraser 2014). Five turtles were initially captured and tagged in September 2020 (delay in spring 2020 field work due to COVID-19) with the remaining individuals tagged in spring 2021.

During the 2020-2021 overwintering season, 5 Blanding's turtles (4 males, 1 female) were tracked twice a week from 1 October until 25 October and every 10-14 days until 16 December 2020. Due to COVID-19 restrictions imposed by McMaster University, we were unable to access the site from 21 December 2020 to 8 March 2021, prohibiting in-person data collection. We resumed weekly tracking on 9 March 2021, until emergence (mid-April) when each turtle was tracked a minimum of twice per week. During the 2021-2022 overwinter season, 19 Blanding's turtles (11 males, 8 females) were tracked twice a month from 1 October 2021 until April 19th, 2021.

When each turtle was radio tracked, we collected water temperature, dissolved oxygen, ice thickness and sediment, snow, and water depth at each turtle location and all 20 of the microhabitat profiles to facilitate habitat selection analyses. We used an ice auger (1.5 inches diameter) to manually drill a hole in the ice approximately one metre to the north (or south if wetland edge/dense vegetation was present) at each tracked turtles' location and microhabitat profile. We ensured auguring was completed slowly to avoid any additional unnecessary mixing of the water column. As soon as resistance caused by the ice surface was released, the auger was slowly removed, and ice depth was measured. Following auguring, we recorded water temperature (°C) and DO (mg/L) content every 10 cm from the top of the water surface until the sediment was reached using a YSI ProSolo ODO Optical Dissolved Oxygen Meter (YSI Incorporated, Yellow Springs, Ohio, USA). In the instance of communal overwintering, to minimize disturbance, the same data was used for multiple individuals (i.e., only one hole was drilled).

We calculated the average maximum and minimum overwintering temperatures and DO content for all tagged Blanding's turtles to quantify the thermal and chemical properties at overwintering turtle locations. We also calculated the average water, snow and ice depth for all overwintering Blanding's turtles across each of the sample weeks.

To estimate each Blanding's turtle's vertical position within the overwintering profile, we selected a paired microhabitat that best represented the overwintering habitat type used. Next, we compared the Blanding's turtle iButton temperature with the 1-cm interpolated available microhabitat temperatures and estimated the turtle's vertical position based on the closest matching microhabitat temperature. Finally, we calculated the turtle's average daily vertical position.

Assessing overwintering habitat selection

We conducted all analyses in R 4.2.1 (R Core Team, 2022). We used a breakpoint analysis of mean daily Blanding's turtle overwintering temperatures (from affixed iButtons) to define the winter 2020-2021 and 2021-2022 overwintering periods to identify when turtle temperatures become stable (Markle and Chow-Fraser 2017). Using this approach, we defined the 2020-2021 overwintering season as 12 December 2020 to 21 March 2021 and the 2021-2022 season as 28 November 2021 to 9 April 2022.

We characterized habitat use at three scales; landscape, microhabitat and profile (Figure 3). At the profile scale, we investigated each microhabitat within each wetland independently whereas for the microhabitat scale we separated habitat data by microhabitat. Comparatively, at the landscape scale we separated habitat data by wetland. Water and sediment temperatures and DO data were compared at all three scales where water, snow and ice depth was investigated at the landscape scale. Similarly, we calculated the resilience zone at all three scales. We tested for temperature selection by Blanding's turtles at the microhabitat and profile scale and for evidence of DO selection at the microhabitat scale. DO selection was only tested at the microhabitat scale due the limited dataset, we first compared all available depths at both turtle and available habitats then compared turtle selected depths at both turtle and available habitats. Finally, we tested the effect of temperature and dissolved oxygen on Blanding's turtle overwintering site selection at the microhabitat scale.

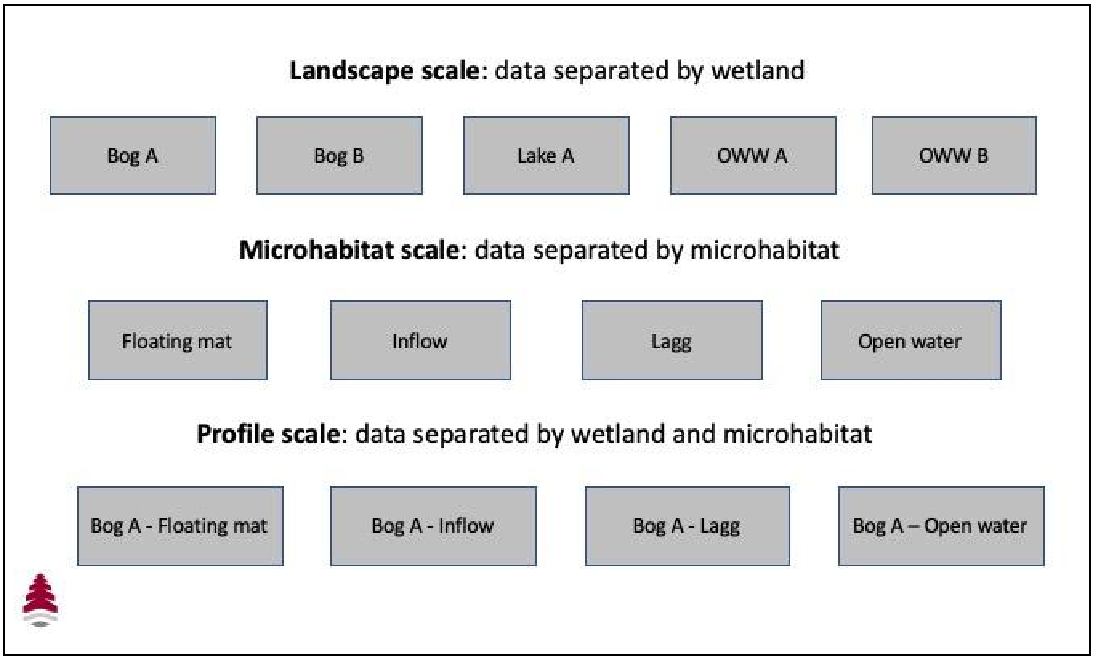

Figure 3: Conceptual diagram of characterization of habitat scales.

To test for temperature habitat selection at the microhabitat scale, we modeled differences in temperature at Blanding's turtle overwintering locations and available overwintering using a generalized linear mixed effects model (lme4, Bates et al. 2015) using a Gamma distribution (inverse). The first model compared used vs. available aquatic temperatures from 7 cm only at the profile scale. This model included site type (turtle, inflow, open water, lagg, floating mat) and sample week as fixed effects and wetland and profile ID as nested random effects to account for repeated measures. This was to compare our model results with similar studies within the literature (e.g., Edge et al. 2009; Markle and Chow-Fraser 2017; Gasbarinni et al. 2021). We ran this model a second time but compared used vs. available aquatic temperatures at hypothesized turtle depths at the profile scale. To test for evidence of habitat selection based on dissolved oxygen we fit a generalized linear mixed effects model (lme4, Bates et al. 2015) using a Gamma distribution (log link) to model differences in DO content at used Blanding's turtle locations and available habitats at the landscape scale. We included site type (turtle, inflow, open water, lagg, floating mat) and sample month as fixed effects and profile ID as random effects to account for repeated measures. For both temperature and DO we set values ≥ 0 mg/L to effectively 0 mg/L (0.0000000001).

Finally, we used a generalized linear mixed effects model (GLMM) (lme4, Bates et al. 2015) with a binomial distribution (log link) to determine the effect of temperature and dissolved oxygen on Blanding's turtle overwintering site selection at the landscape scale. We modeled the site type (1 for a site occupied by a Blanding's turtle and 0 for available habitat) and included temperature and dissolved oxygen as fixed effects and wetland and profile ID as nested random effects. For all models that included DO data, we were limited to winter 2021-2022 due to the lack of DO data collected in winter 2020-2021 (due to COVID-19 access restrictions).

Quantifying the freshwater turtle resilience zone

To quantify the freshwater turtle resilience zone, thresholds for defining suitability are required for each variable (temperature and DO and water depth). Therefore, we gathered temperature, DO and water depth values from the literature (Greaves and Litzgus 2007; 2008; Rollinson et al. 2008; Edge et al. 2009; Rasmussen and Litzgus 2010; Paterson et al. 2012; Gasbarrini et al. 2021) and used *in-situ* Blanding's turtle habitat use data from this study to determine the thresholds for the freshwater turtle resilience zone. Based on *in-situ* Blanding's turtle habitat use data, we defined the requirements for the turtle resilience zone to be between 0 — 4 °C, ≥1 mg/L DO, and ≥40 cm water depth. We then calculated when and how long the resilience zone was present, and the size of the resilience zone throughout the 2021-2022 overwintering season. We also conducted a sensitivity analysis to investigate how the resilience zone changed under temperature thresholds to 0 — 2 °C and DO thresholds of ≥ 0 mg/L, in both scenarios we left all other variables as described above. This was completed to test what variable had the greatest control on the resilience zone and test temperature thresholds more in line with literature values (Edge et al. 2009; Gasbarrini 2021).

Results

Overwintering habitat use and availability

During the 2020-2021 overwintering season, all turtles overwintered in Bog A, four of five turtles were in the lagg with two individuals within 2 — 5 m from the lagg continuous monitoring profile (Figure 1). The other two turtles were located within 2 m of each other, in an open water patch located < 2 m from the wetland edge. The 5th turtle was located at the edge of a small floating mat, surrounded by a mosaic of open water patches and floating mats (Figure 1). Of the 19 turtles radio tracked in winter 2021-2022, 18 turtles overwintered in Bog A and a single female overwintered along the edge of a lake (Lake B) in open water. The majority of the turtles (16/18), overwintered in the lagg and two turtles overwintered amongst floating mats. Three of the four turtles that were tracked both winters showed fidelity to their overwintering site (<5 m) (Figure 1). We observed multiple cases of communal overwintering (two or more individuals within 2 m of each other) across both winters. In 2020-2021, we recorded two overwintering pairs, one within the lagg (2 males) and one in an open water patch located < 2 m from the wetland edge (2 males). However, we suspect there were additional un-tagged turtles overwintering with the pair of males in the lagg because there were multiple captures of non-tagged turtles at that site in spring 2021. In 2021-2022, we recorded three overwintering groups, one within the lagg (same location as winter 2020-2021, 2 males and 3 females), one amongst floating mats (2 males), one within the lagg (1 male, 1 female). During winter 2021-2022, following ice formation no turtles moved >1m between subsequent radiolocations. Due to limited site access we were only able to radio-track immediately following ice on (12 December 2020) and again two weeks before ice off (9 March 2021) but no turtles moved >1m between visits.

The overwintering seasons lasted 99 days (December 2020 – March 2021) and 132 days (November 2021 – April 2022). Onset and duration of ice cover varied depending on the location within each wetland. The longest duration of continuous ice cover the turtles experienced was 136 days. Ice thickness reached a maximum of 38 ± 5 cm in late February 2022 and stayed relatively stable (within 7 cm) until reaching a minimum depth of 12 ± 9 cm right before emergence. Similar to ice depth, snow depth reached a maximum of 36 ± 7 cm in mid-January 2022 and stayed relatively stable until there was no snow present on the final sample week.

At turtle overwintering locations in 2021-2022, the average manual water depth ranged 79 cm ± 22 cm to 42 ± 14 cm (above the sediment) and was fairly stable at individual turtle overwintering sites, only varying by 6 cm (Figure 4). However, water depths varied greatly depending on the location within the wetland. For example, although each of the instrumented available habitat locations had a minimum water depth of 50 cm, depths greater than 160 cm were present within all five wetlands. Some locations throughout the wetlands had water depths less than 50 cm, some as low as 10 – 20 cm of free-standing water. Across both overwintering seasons, water depth slowly declined by 20 cm until mid-March when snowmelt and precipitation events resulted in an increase in water depths (Figure 4). At open water wetland A, the beaver dam failed in early October 2021 resulting in a rapid drop in water depth (Figure 4) resulting in most microhabitats having less than 50 cm of free-standing water during the 2021-2022 overwintering season. The relative change in water depth at turtle sites followed the same pattern as the available habitat profiles within Bog A (Figure 4).

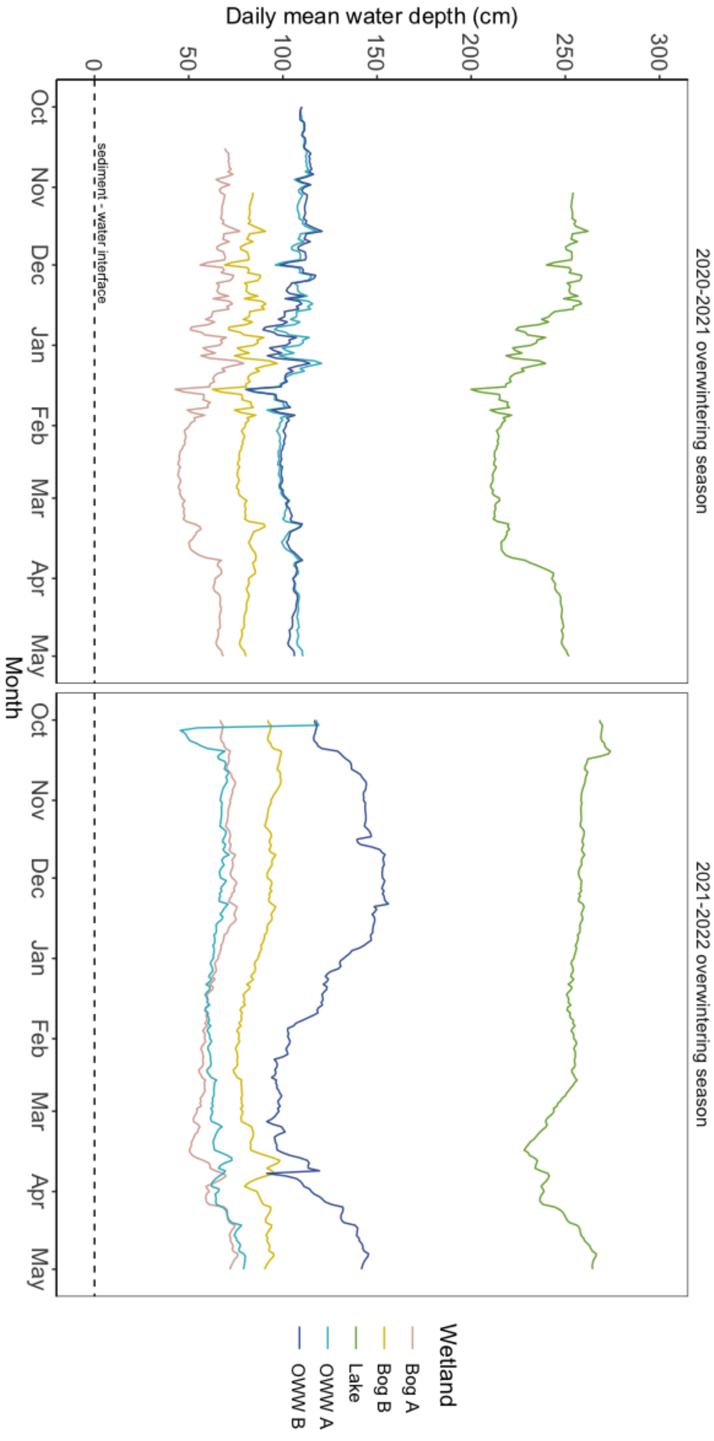

Figure 4: Mean daily water depth across both overwintering seasons (2020-2021 and 2021-2022).

Overwintering habitat use: temperature and estimated vertical movements

Over both winters, the sediment and water temperatures across all microhabitats declined steadily from October (2020: 10.46 ± 1.95 °C, 2021: 12.5 ± 2.72 °C) to early March 2021 (1.98 ± 1.49 °C) and late March 2022 (2.06 ± 1.44 °C). Temperatures began to increase from late March 2021 (4.3 ± 1.74 °C) and early April 2022 (5.19 ± 0.95 °C) to May (2021: 8.59 ± 2.12 °C, 2022: 12.5 ± 5.7 °C) (Figure 5). Temperatures were 2 °C warmer in the sediment (2020-2021: 6.55 ± 3.16 °C and 2021-2022: 6.69 ± 3.52 °C) than the water column (2020-2021: 4.70 ± 4.04 °C and 2021-2022: 4.79 ± 4.52 °C) (Figure 5). The open water had the highest overall average daily temperature, 9.92 ± 1.70 °C where the lagg (2020-2021) and the inflow (2021-2022) had the lowest daily temperatures, -0.95 ± 1.83°C and -0.60 ± 0.26 °C, respectively. The floating mat was the only habitat that did not have temperatures below 0°C across both overwintering seasons.

Wetland water temperatures were nearly identical to microhabitats (2020-2021: 4.71 ± 4.05 °C and 2021-2022: 4.82 ± 4.48 °C) (Figure 6). There was nearly a four degree difference in OWW A maximum average daily temperatures between winter 2020-2021 (7.04 ± 1.04 °C) to winter 2021-2022 (2.95 ± 3.38 °C) (Figure 6). OWW A had the coldest maximum average daily temperature of 2021-2022 with nearly a nine degree difference between maximum average daily temperatures between Lake A and OWW A (Figure 6). Bog A, where the majority of the tagged Blanding's turtles were overwintering, had comparable average daily temperatures to Bog B (Figure 6).

Table 2: Maximum and minimum daily mean temperatures at each depth relative to the sediment – water interface (cm) during both overwintering seasons (2020, 2021).

Depth (cm) relative to sediment	Maximum Temperature (°C)	Standard Deviation	Minimum Temperature (°C)	Standard Deviation
50 (winter 2020)	1.84	1.79	0.36	0.31
7	4.54	1.43	1.22	0.77
-7	5.69	1.33	1.98	1.03
-50	8.66	1.25	3.83	0.95
50 (winter 2021)	3.21	2.12	-0.04	0.77
7	5.54	1.80	1.36	1.36
-7	6.61	2.41	1.98	1.37
-50	8.86	2.38	3.56	1.99

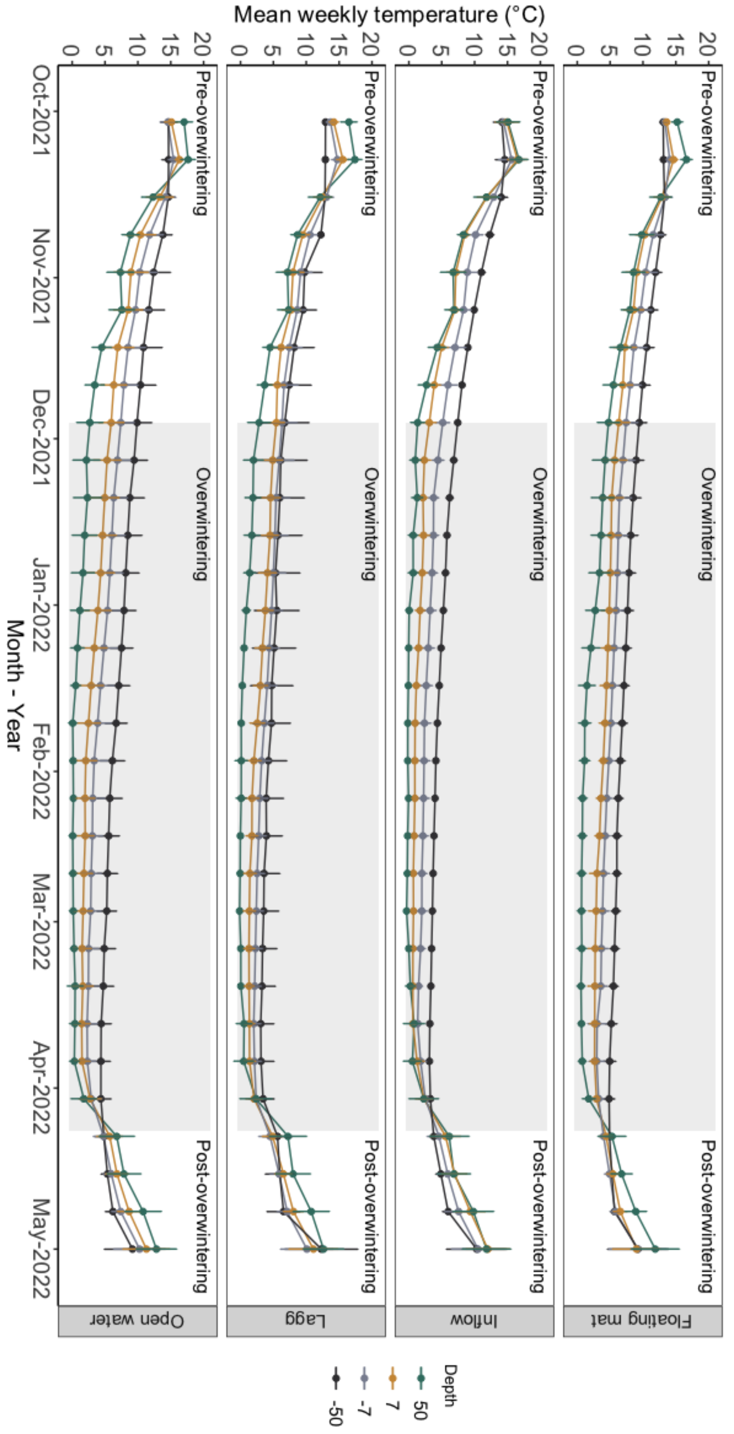

Figure 5: Mean weekly (standard deviation) water temperature at each of the four microhabitats over the 2021-2022 overwintering season.

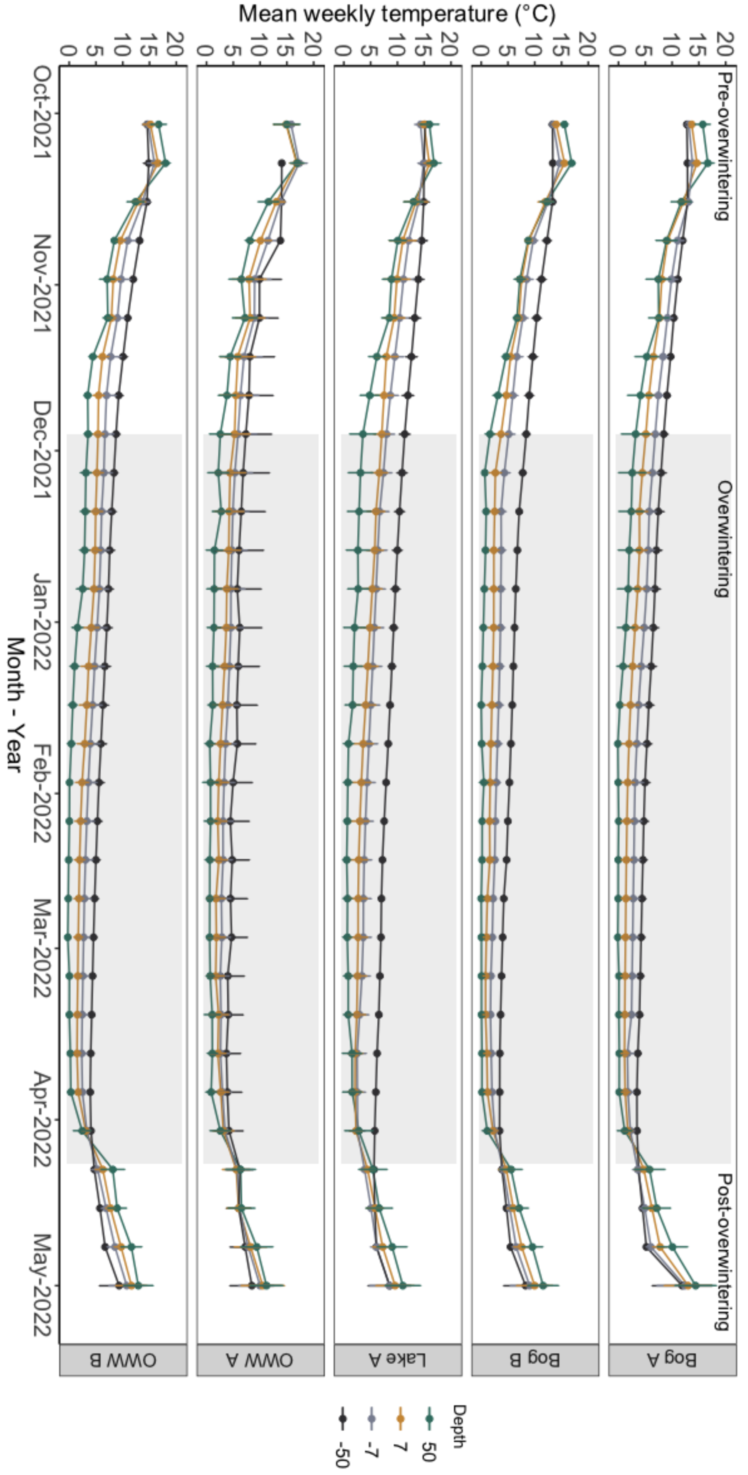

Figure 6: Mean weekly (standard deviation) water temperature at each of the five wetlands over the 2021-2022 overwintering season.

When comparing the turtle iButton temperatures to temperatures at the microhabitat most representative of their overwintering location, the turtle's hypothetical vertical position changed temporally. The two turtles overwintering in an open water habitat had a hypothetical average daily vertical position below the sediment from mid-January to emergence, making up 52% (2020-2021) and 63% (2021-2022) of overwintering days. Comparatively, the one (2020-201) and two (2021-2022) turtles overwintering in and around floating mats were hypothetically located in the sediment for < 7% of the overwintering season, all occurring within less than two weeks of emergence. The majority of turtles had a hypothetical average daily depth consistently between 50 – 30 cm above the sediment with the lowest average daily depth reaching 9 ± 31 cm above the sediment. The hypothetical maximum vertical position for turtles overwintering in the lagg and floating mat was 12 cm higher than those overwintering in an open water habitat. Even if Blanding’s turtle iButton temperatures are compared to other profile temperatures, the turtles are moving vertically within the water column. As an example, on 15 February 2021 the hypothetical average daily depth was 21 ± 4 cm above the sediment when Blanding’s turtle iButton temperatures were compared to the inflow habitat.

Overwintering habitat use: Dissolved oxygen

Across all wetlands, the highest DO recorded during the 2021-2022 overwintering season was 12.64 mg/L on 15 December 2021 during a period of ice on, at an inflow location (Figure 7). The lowest DO recorded was 0 mg/L, making up about 8.7% of the 2021-2022 overwintering season (Figure 7). Average DO ranged from 5.76 mg/L ± 4.23

mg/L to 1.68 ± 2.05 mg/L mg/L on the week of 3 April 2022 and 6 February 2022, respectively (Figure 7). Average DO was above hypoxic conditions for 60% of the 2021-2021 winter, in December, late January, late February and mid March - early April (Figure 7). Overwintering Blanding's turtles selected for higher DO when compared to habitat data mainly when average habitat DO was considered hypoxic, early January, late February to early March. The average DO across all 19 turtles ranged from 5.94 ± 2.30 mg/L to 1.16 ± 1.74 mg/L.The individual overwintering in Lake B was responsible for the highest DO value, 13.34 mg/L observed at the start of overwintering. Average DO was above hypoxic conditions for 40% of the time, December, late February and April. Over half of the sample weeks (~55%) had an average DO considered as hypoxic or anoxic.

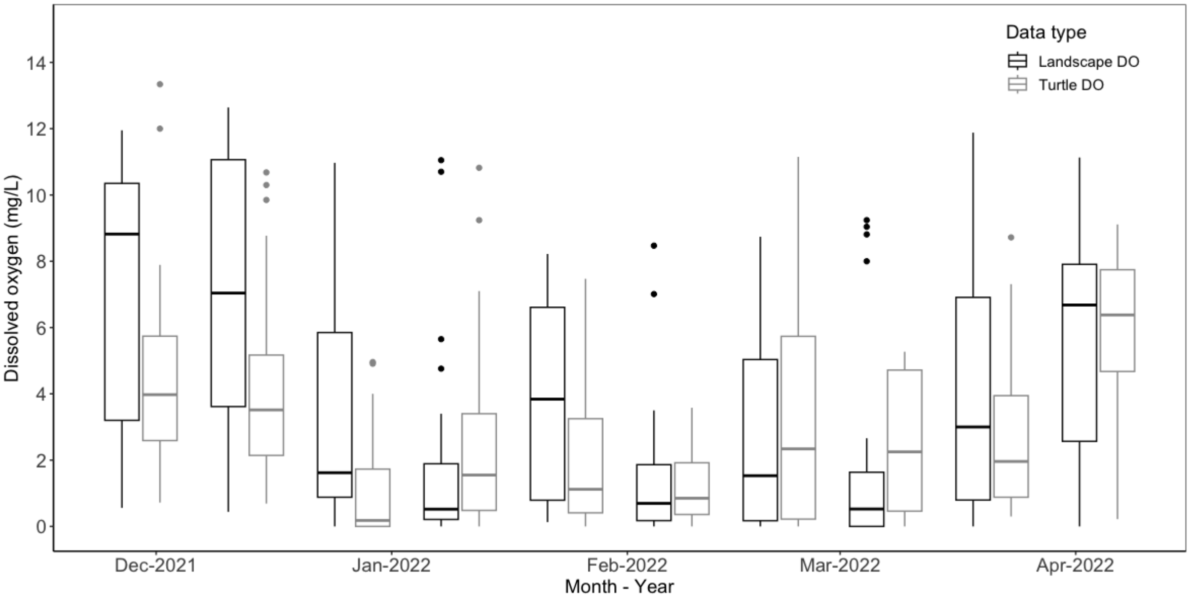

Figure 7: Weekly DO (mg/L) at the landscape scale (averaging microhabitat data across all wetlands) compared to DO at turtle occupied locations. Boxplot shows mean weekly DO with tails and outliers.

The floating mat had the highest proportion of anoxic (0-1 mg/L DO) observations (68%) with only one sample week above hypoxic conditions (Figure 8). Comparatively, the majority of sample weeks at both the lagg (60%) and open water (50%) were above hypoxic ranging conditions with not a single average DO falling below 1 mg/L (Figure 8). Whereas the inflow only had one week during the 2021-2022 overwintering season that had values considered below hypoxic with average DO values ranging from 8.97 ± 3.11 mg/L to 2.52 ± 2.15 mg/L (Figure 8).

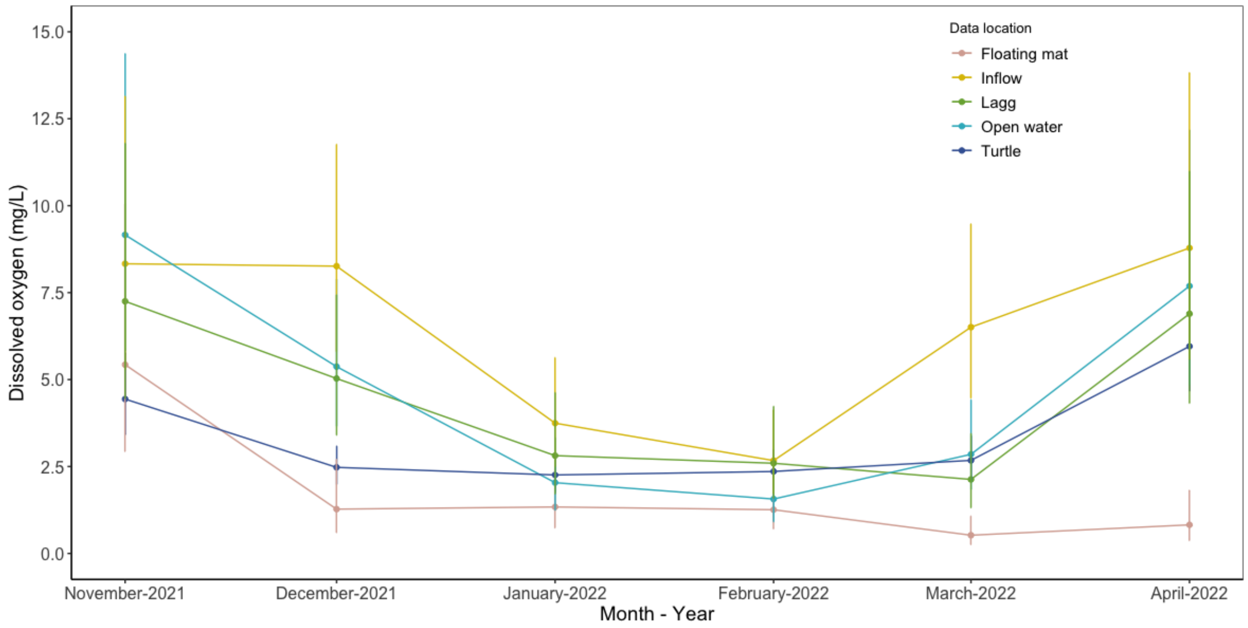

Figure 8: DO (mg/L) at the landscape scale, comparing average monthly (SE) available habitat dissolved oxygen contents at turtle depths to average monthly (SE) Blanding's turtle selected dissolved oxygen contents.

Bog B and Lake A had similar proportions of anoxic DO values, 35% and 25%, respectively. Comparatively, at Bog A and OWW B nearly 50% of DO values were considered anoxic with average DO values ranging from 5.16 ± 3.94 mg/L to 0.71 ± 1.17 mg/L. Within Bog A, the floating mat had the highest proportion of sample weeks under anoxic conditions (90%) with average DO values ranging from 3.66 mg/L ± 1.28 mg/L to 0 ± 0 mg/L. The maximum average DO value at the open water (8.21 ± 3.48 mg/L) was nearly double the value at the lagg (4.2 mg/L ± 2.01 mg/L). The inflow had the highest average DO value out of the four available habitat types with values ranging from 9.59 ± 1.63 mg/L to 0.29 ± 0.36 mg/L. All minimum DO values at microhabitats within Bog A were considered anoxic, ranging from 0 mg/L (floating mat) to 0.53 ± 0.33 mg/L (open water). DO values at turtle depths (Bog A), ranged from 11.57 mg/L to 0 mg/L with hypoxic conditions occurring over 70% of sample weeks.

Overwintering habitat selection by Blanding's turtles: Temperature

Overwintering Blanding's turtle temperatures (2020-2021: 0.87 ± 0.46 °C, 2021-2022: 1.30 ± 0.74 °C) declined from the start of overwintering until approximately one week before emergence, when they slowly began to increase (Figure 9). Across both winters, average daily temperatures ranged from 1.64 ± 0.34 °C to 0.53 ± 0.26 °C (2020-2021) and 3.58 ± 1.43 °C to 0.73 ± 0.55 °C (2021-2022) (Figure 9). Weekly Blanding's turtle temperatures and weekly available temperatures at a depth of 7 cm at Bog A were different over both winters. Specifically, in winter 2020-2021, three (floating mat: Estimate ± SE [Est.] = 1.38, Z = 4.06, P = 4.82e-05, inflow: [Est.] = 0.61, Z = 1.49, P = 0.14, lagg: [Est.] = 1.42, Z = 4.18, P = 2.89e-05, open water: [Est.] = 0.94, Z = 2.52, P = 0.012) of the four microhabitats were overall warmer than Blanding's turtle temperatures. In winter 2021-2022, the 7 cm data logger at the floating mat failed but all three (inflow: [Est.] = 0.49, Z = 2.60, P = 0.0094, lagg: [Est.] = 0.99, Z = 5.24, P = 1.61e-07, open water: [Est.] = 0.66, Z = 2.47, P = 0.00053) of the other available habitats were overall warmer than weekly turtle temperatures. Blanding's turtle temperatures were also colder than available habitats most representative of their overwintering microhabitat at similar depths. Over both winters, turtles overwintering in the lagg and floating mat were colder than available temperatures at the lagg continuous profile within Bog A (2020-2021: [Est.] = 0.53, Z = 2.49, P = 0.013, 2021-2022: [Est.] = 0.66, Z = 7.12, P = 0.11e-12) and floating mat continuous profile (2020-2021: [Est.] = 1.10, Z = 4.10, P = 4.07e-05, 2021-2022: [Est.] = 0.93, Z = 5.51, P = 3.65e-08). There were no differences between the turtles overwintering in the open water and the available temperatures at the open water continuous profile (2020-2021: [Est.] = 0.0956, Z = 0.44, P = 0.66, 2021-2022: [Est.] = 0.16, Z = 0.55, P = 0.59) during either winter.

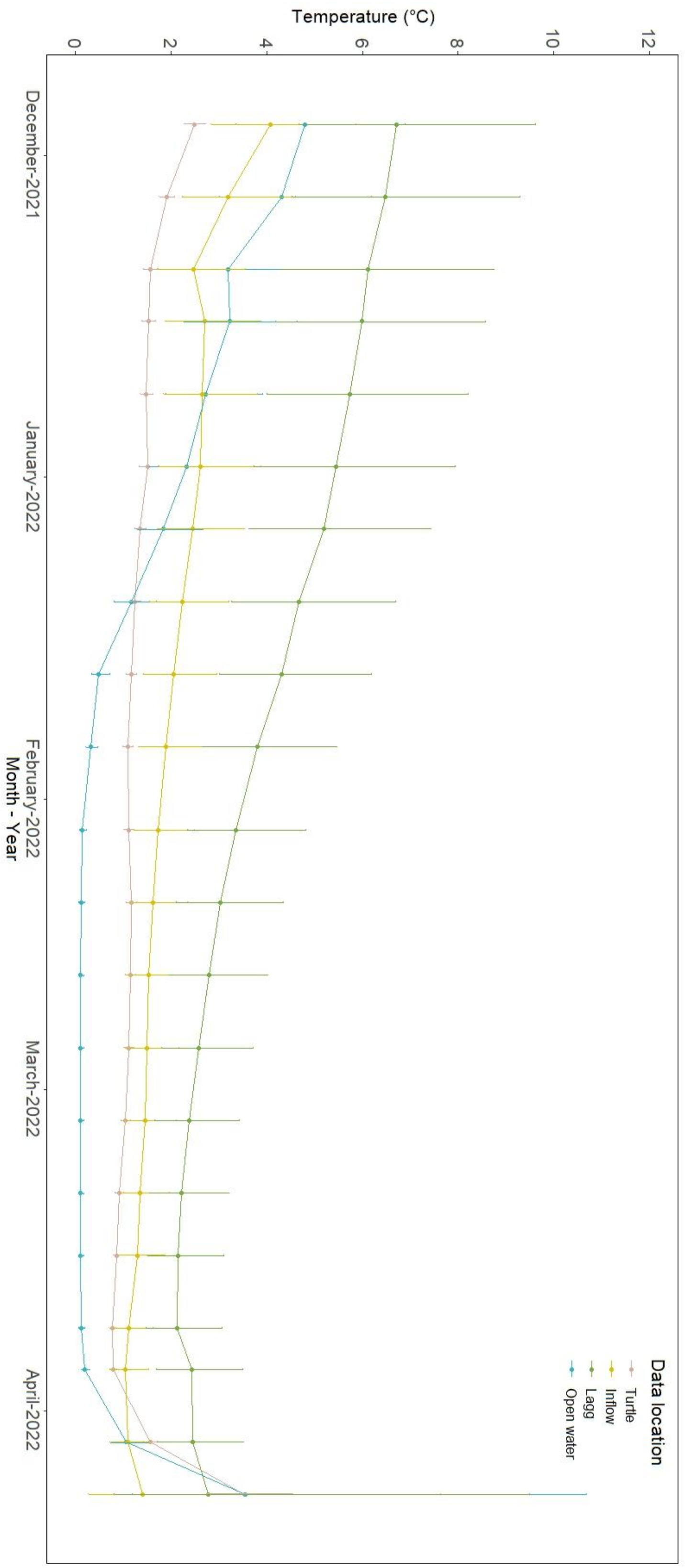
Temperature (°C)
0
2
4
6
8
10
12
December-2021
January-2022
February-2022
March-2022
April-2022
Month - Year
Data location
Turtle
Inflow
Lagg
Open water

Figure 9: Temperature (°C) at the profile scale (Bog A), comparing average weekly (SE) available habitat temperatures at a depth of 7 cm to (SE) Blanding's turtle selected temperatures.

Overwintering habitat selection by Blanding's turtles: Dissolved oxygen

Dissolved oxygen (Estimate ± SE [Est.] = 1.36, Z = 13.1, P = < 2e-16) at selected turtle locations (all depths) was different from three of the four available habitats (all depths) during winter 2021-2022 (Figure 10). Both the inflow ([Est.] = 0.75, Z = 0.22 , P = 0.0008) and lagg ([Est.] = 0.44, Z = 0.22 , P = 0.046) had mean monthly DO values higher whereas the floating mat had mean monthly DO values lower than Blanding's turtle locations ([Est.] = -0.60, Z = 0.25 , P = 0.16) (Figure 10). There were no differences in mean monthly DO between Blanding's sites and available habitats within the first month of the 2021-2022 overwintering season (Floating mat: [Est.] = 0.32, Z = 0.23 , P = 0.15, Inflow: [Est.] = 0.093, Z = 0.19 , P = 0.62, Lagg: [Est.] = -0.16, Z = 0.18 , P = 0.38, Open water: [Est.] = 0.025 , Z = 0.21 , P = 0.91). The lagg had mean monthly DO values lower than Blanding's turtle locations during a period of substantial ice cover, January to March (Jan: [Est.] = -0.45 , Z = 0.21 , P =0.036, Feb: [Est.] = -0.98, Z = 0.22 , P =6.99e-06, March: [Est.] = -0.67, Z = 0.21 , P =0.0012). Comparatively, in February when ice thickness reached maximum the floating mat was the only available that didn't have a mean monthly DO value lower ([Est.] = -0.22, Z = 0.25 , P = 0.39) than those at Blanding's turtle locations. February was the only month where the open water had mean monthly values different ([Est.] = -0.80, Z = 0.23 , P =0.00062) than values at Blanding's locations. In addition to the lagg, the floating mat had mean monthly DO values lower than values at turtle locations within the full final month of the 2021-2022 overwintering season ([Est.] = -1.10, Z = 0.27 , P =3.98e-05).

When comparing DO (Estimate ± SE [Est.] = 1.49, Z = 11.2, P = < 2e-16) at selected turtle locations (turtle selected depths) two of the four available habitats (turtle selected depths) were different than selected turtle locations during winter 2021-2022 (Figure 8). Both the inflow ([Est.] = 0.63, Z = 0.27 , P = 0.019) and open water ([Est.] = 0.72, Z = 0.27 , P = 0.0063) had mean monthly DO values higher than Blanding's turtle locations. The open water was the only available habitat that had monthly DO values different than turtle locations in January ([Est.] = -0.83, Z = 0.35 , P = 0.017) and February ([Est.] = -1.13, Z = 0.36 , P = 0.0018). In March, the inflow ([Est.] = 0.26, Z = 0.1 , P = 0.40) was the only available habitat that didn't have mean monthly DO values different than turtle locations. There was not a single month where the inflow had a mean monthly DO significantly different from Blanding's turtle locations.

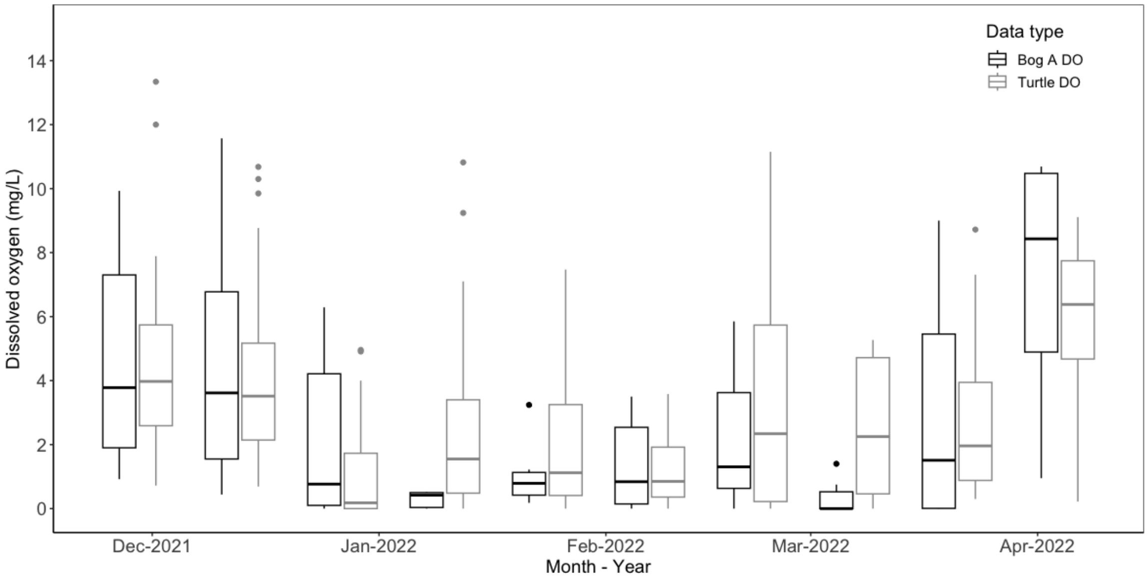

Figure 10: Weekly DO (mg/L) at the profile scale (Bog A) at the same depths

overwintering turtles compared to DO at turtle occupied locations. Boxplot shows mean weekly DO with tails and outliers.

Quantifying the freshwater turtle resilience zone

Based on the habitat use and selection data, we defined the requirements for the freshwater turtle resilience zone to be water and sediment temperatures between 0 – 4 °C, DO contents greater ≥ 1 mg/L and a water depth ≥40 cm above the sediment. There were no recorded temperatures at overwintering turtle locations below 0°C during both overwintering periods, the lowest average daily temperature was 0.53 ± 0.26 °C (1 March 2021) and 0.73 ± 0.55 °C (25 March 2022). The highest average daily temperature across all 19 turtles was 3.58 ± 1.43 °C, occurring the day before 2022 emergence. During winter 2020-2021 the maximum temperature at a turtle location was 3.96 °C. Over winter 2021-2022, within the first month of overwintering and two days of emergence, 2 and 11 turtles had temperatures above 4°C, respectively. Dissolved oxygen and water depth was selected based on minimum average selected values. The average DO (2021-2022) 5.94 ± 2.30 mg/L to 1.16 ± 1.74 mg/L and the average manual water depth (2021-2022) ranged 79 cm ± 22 cm to 42 ± 14 cm (above the sediment) across all 19 turtles.

Spatiotemporal variability of the freshwater turtle resilience zone

Bog A and Bog B had the most stable resilience zone, present 100% of the 2021-2022 overwintering season and providing a maximum average daily size of 45 cm and 43 cm (Figure 11). On the other hand, OWW A did not have a resilience zone at any point during winter 2021-2022 (Figure 11). Lake A and OWW B had relatively stable resilience zones, only losing it for two days in March (OWW B: 5 March 2022 and 20 March 2022, Lake A: 14 and 15 March 2022) (Figure11). We identified when the average daily resilience zone size was less than 7 cm because that is the approximate height of an overwintering turtle (Edge et al. 2009) and anything less can longer support an entire overwintering turtle. There were only 6 instances where the resilience zone was present at a size of than 7 cm. The majority of these instances occurred at Lake A within the first 5 days of the overwintering season (28 November 2021) and there was only one day where the resilience zone size was less than 7 cm at Bog A, 15 January 2022.

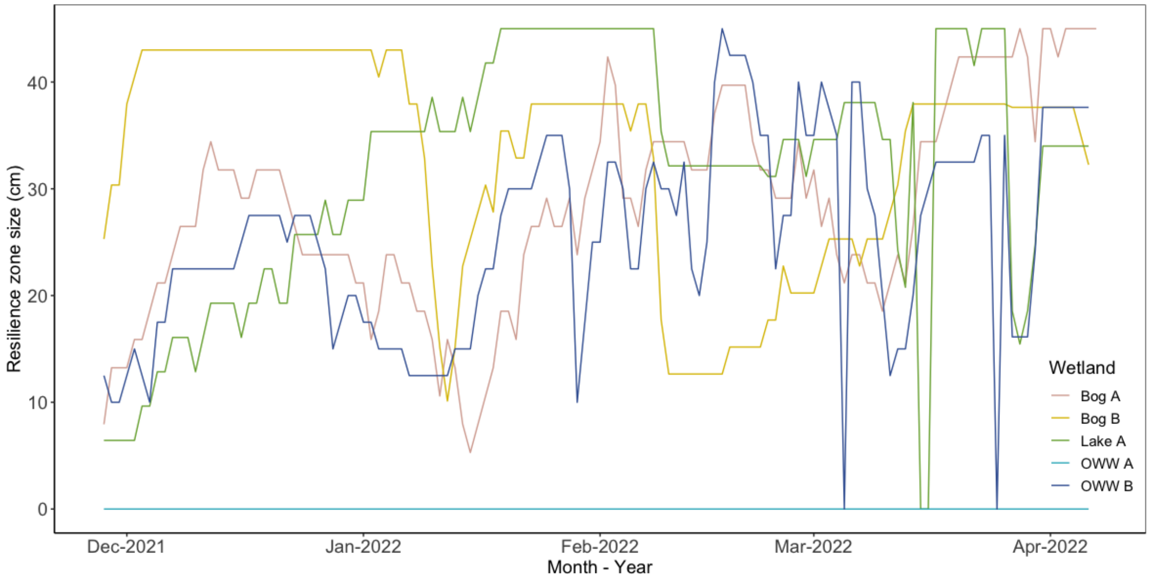

Figure 11: Mean daily resilience zone size across all five wetlands during the 2021-2022 overwintering season.

Similar to the landscape scale, both the lagg and inflow had the most stable resilience zone, present 100% of the 2021-2022 overwintering season (Figure 12). The open water had the most variable resilience zone, with nearly double the overwintering days without a resilience zone (38 days) as the floating mat (18 days) (Figure 12). The majority of days where the floating mat was without a resilience zone occurred within the first two weeks of December (28 November 2021 - 15 December 2021) and a few days before emergence (7 April 2022). The open water lost the resilience zone over multiple continuous occurrences from early January to late March, the longest consecutive period without the resilience zone was from 23 February 2022 to 13 March 2022. Although the lagg and inflow never lost the resilience zone size, both habitats had a resilience zone less than 7 cm on one (16 February 2022) and two (12 - 13 March 2022) occasions, respectively. All available habitats provided a maximum average daily size of 45 cm.

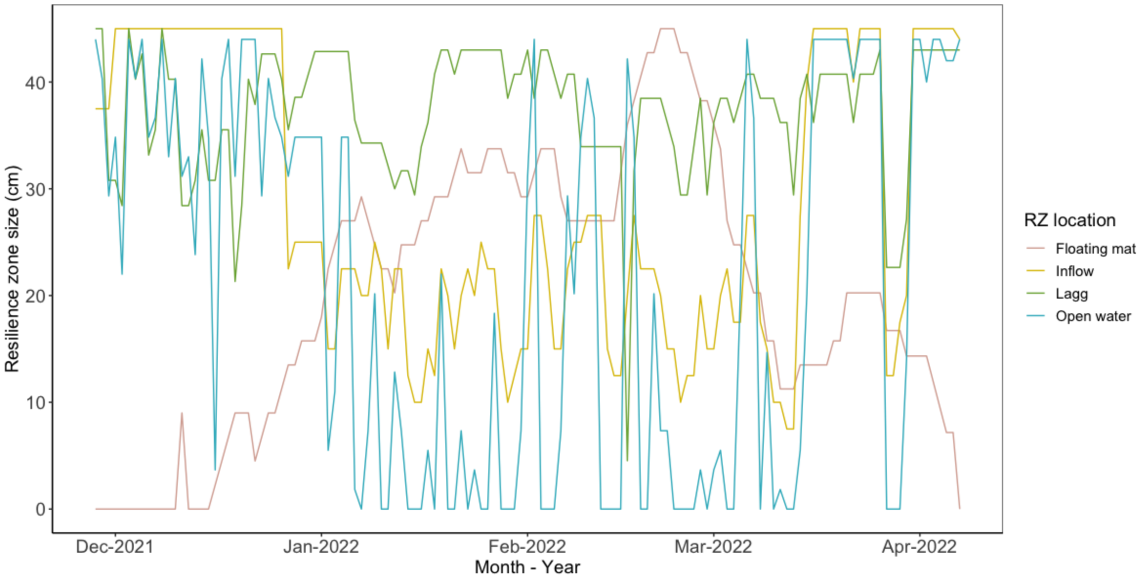

Figure 12: Mean daily resilience zone size across all four available habitats during the 2021-2022 overwintering season.

At Bog A, the floating mat was without a resilience zone for nearly two consecutive months (28 November 2021 to 18 January 2022) and therefore had a resilience zone for less than half (45%) of the 2021-2022 overwintering season (Figure

13). Similarly, the lagg, the microhabitat type where the majority of turtles overwintered, had a relatively variable resilience zone, present for 65% of the 2021-2022 overwintering season (Figure 13). The loss of resilience occurred over two large consecutive timeframes (28 November 2021 - 7 January 2022 and 21 February 2022 - 13 March 2022). During periods of substantial ice cover (January - March), when the resilience zone was present, the resilience zone size was no greater than 20 cm until 10 days before emergence when it increased until reaching a maximum daily size of 43 cm. Both the open water and inflow had the most stable resilience zone, present for 75% and 77% of the 2021-2022 overwintering season, reaching a maximum daily size of 44 cm, respectively.

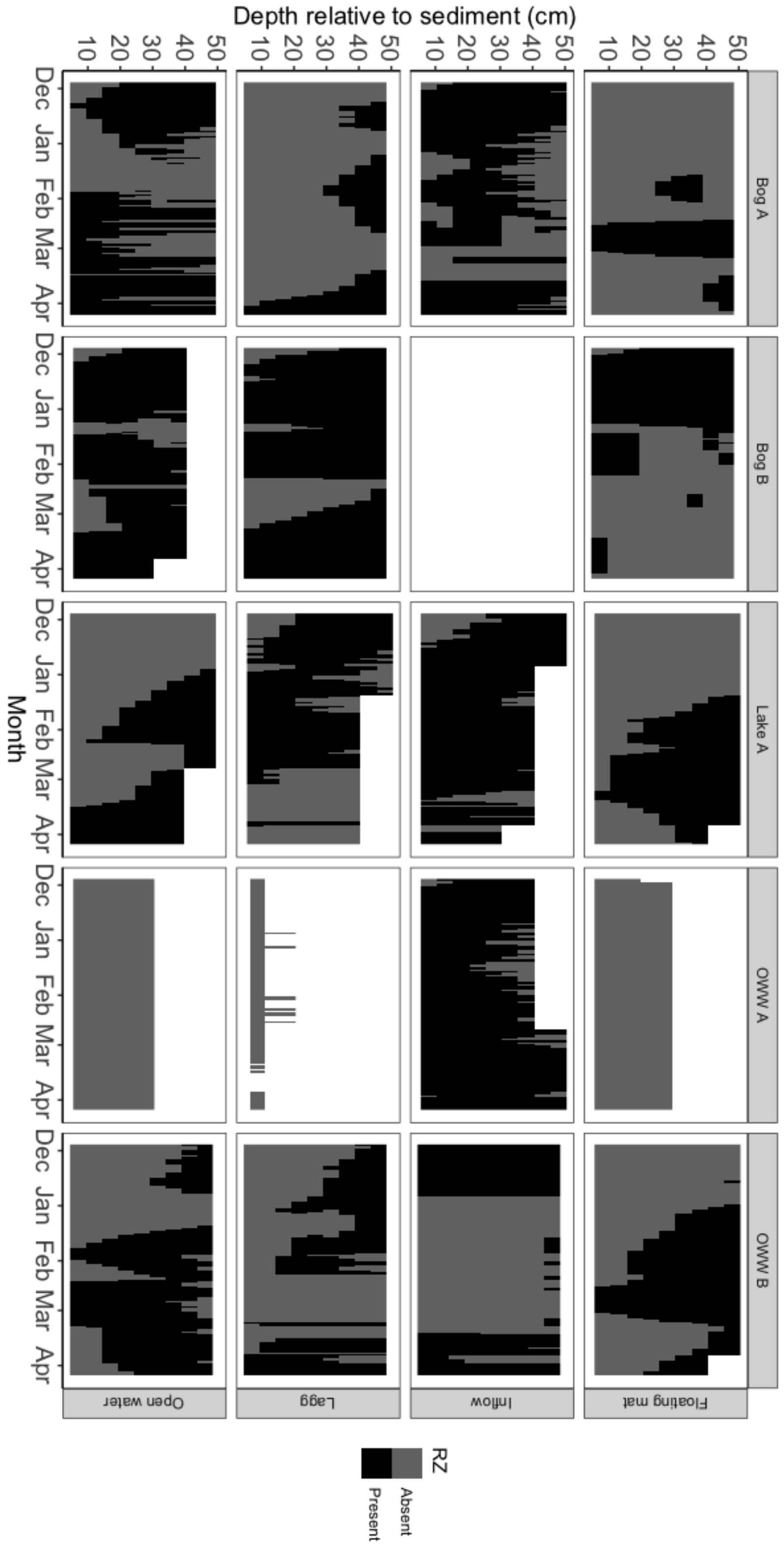
Depth relative to sediment (cm)
10
20
30
40
50
Month
Dec
Jan
Feb
Mar
Apr
Bog A
Bog B
Lake A
OWW A
OWW B
Open water
Lagg
Inflow
Floating mat
RZ
Absent
Present

Figure 13: Resilience zone presence and absence across all four available habitats within all five wetlands during the 2021-2022 overwintering season.

We conducted a sensitivity analysis on the requirements for the freshwater turtle resilience zone by removing DO as a requirement, testing completely anoxic conditions. There were 132 more days (100% present) with a resilience zone when DO was not a factor in the resilience zone calculation. OWW A provided the smallest average daily resilience zone ranging from 1.83 ± 4.22 cm to 28 ± 21.7 cm compared to 0 cm under the resilience zone with at least 1 mg/L of DO. At the microhabitat scale there were an additional 56 days where the resilience zone was present. There was only a single day where the resilience zone reached a minimum of 6.93 ± 11.6 cm (floating mat), supporting an entire overwintering turtle for the entire 2021-2022 winter.

We conducted a second sensitivity analysis on the requirements for the freshwater turtle resilience zone by adjusting the temperature threshold to 0 - 2 °C, testing under cooler conditions. Under these conditions Bog A no longer had a resilience zone for 100% of the 2021-2022 winter, instead 94% of overwintering days had a resilience zone. Similarly, Bog B and Lake A and OWW B had 2 — 5 additional days without a resilience zone under temperatures between 0 - 2°C. OWW A had 131 more days with a resilience zone, with resilience zone size ranging from 0.55 ± 1.62 cm to 28 ± 21.37 cm. At the microhabitat scale, both the floating mat and open water went from the resilience zone being present 86% and 71% of the the 2021-2022 winter to 100% of the time, respectively. The resilience zone size decreased at the floating mat from a maximum daily average resilience zone size of 45 ± 0 cm to 22.8 ± 17.9 cm. The lagg and inflow available habitats number of days with a resilience zone remained relatively unchanged, both losing the resilience zone for only a single day (7 April 2022). The greatest impact to the inflow and the lagg was the change in resilience zone size, 28% and 17% of 2021-2022 overwintering days had a resilience zone size below 7 cm. Comparing this to the calculation with 0 - 4 °C, there was only one day with a resilience zone size below 7 cm at the lagg, and not a single day at the inflow.

Discussion

The turtle resilience zone: Water depth

Our study is the first to estimate the spatiotemporal dynamics of the turtle resilience zone. Stable and sufficient water depths are a critical part of the turtle resilience zone. Blanding's turtles selected habitats with average water depths between 79 cm and 42 cm, similar to other studies within the surrounding eastern Georgian Bay area (Edge et al. 2009). We suggest that Blanding's turtles select overwintering habitats with water depths of at least 40 cm to maximize the amount of liquid water while overwintering. Although we did not test for differences in the amount of liquid water at turtle locations compared to available habitats, Edge et al. (2009) found that overwintering Blanding's turtles used the only location that was known to have liquid water, showing possible selection for specific water depths. Further, we suggest that when deeper water depths are available Blanding's turtles do not select the deepest water depths (Edge et al. 2009) since a relatively thick layer of ice and small buffer of liquid water may deter and act as a barrier against winter predators (Taylor and Nol 1989; Greaves and Litzgus 2007). A study population of snapping turtles in Algonquin Provincial Park experienced overwintering predation by river otters that resulted in approximately 50% of the adult population being killed with limited evidence of population recovery after 23 years (Brooks et al. 1991; Keevil et al. 2018). Removal of half of an at-risk species population can have a disproportionately large impact on population persistence and may be the dominant process in causing local extirpation or even extinction (Mangel and Tier 1994; Keevil et al. 2018). Therefore prevention of overwintering predation is key to maintaining turtle populations. However, there is a minimum amount of liquid water required for successful overwintering since overwintering sites that fully freeze can cause mortality (Clair and Gregory 1990; Brown and Brooks 1994; Crocker et al. 2000; Platt et al. 2008; Keevil et al. 2018). During winter 2021-2022 maximum ice depths (38 cm) would leave <4 cm of liquid water at the lowest turtle selected depth (42 cm). This would force a turtle partially or fully bury into the sediment (Edge et al. 2009; Markle et al. 2020). The minimal amounts of unfrozen water would still allow the turtle to expose its head into the water column and uptake any available DO.

A single wetland (OWW A) within our study experienced a beaver dam failure early in the 2021-2022 overwintering season resulting in relatively low water depths. Although beaver dams can create new wetlands that can act as turtle overwintering habitat (Johnson and Naiman 1987; Collen and Gibson 2001; Rollinson et al. 2008; Yagi and Litzgus 2012), beaver dams can collapse leading to disastrous consequences (Hillman 1998). A beaver dam may fail due to water surges from intense rainstorms or high spring runoff (Hillman 1998) and/or in the absence of regular maintenance (Butler and Manson 2005). Furthermore, beaver caused flooding is not always desirable in areas near infrastructure since it can cause damage to roads, railways, crop lands and residences (Collen and Gibson 2001; Swinnen et al. 2019) therefore, beaver dams that threaten infrastructure are often forcibly removed (Hillman 1998). Regardless of the cause of failure, a collapsing beaver dam will release the water held behind it almost instantaneously causing large and rapid water table declines (Hillman 1998; Butler and Manson 2005). Although beaver dam failures can have disastrous impacts, few have been studied (Westbrook et al. 2020) and there is an overall lack of documentation on beaver dam failure and its impact on turtle overwintering habitat. Beaver dams have been reported to fail during the winter (Taylor et al. 2010; Keevil et al. 2018; Munir and Westbrook 2020) leading to the mortality of overwintering turtles (Keevil et al. 2018). Our results show that beaver dam failure at any point in the late fall and winter can lead to substantial loss of suitable overwintering habitat and therefore high potential for winterkill (Keevil et al. 2018). In our study, the beaver dam collapsed within a month and a half of overwintering, providing time for turtles to flee the wetland or move to locations within the wetland that offered deeper water depths. A beaver dam failure between late November - late March would likely lead to a mass mortality event due to low water conditions and exposure to freezing air temperatures (Platt et al. 2008; Keevil et al. 2018; Gasbarinni et al. 2021). Our study supports that the presence of beavers and their dams are important for maintaining suitable overwintering habitat (Rollinson et al. 2008; Yagi and Litzgus 2012) and we suggest that beaver dam management plans should consider the impacts removal and alteration of beaver dams have on species at-risk turtles.

In addition to beaver dam failure, low seasonal water levels and droughts can greatly increase the potential for resilience zone loss which could ultimately lead to winterkill and mass mortality events (Christiansen and Bickham 1989; Gasbarrini et al. 2021). Resilient wetlands habitats that help to reduce the impacts of drought (Hubbard and Linder 1986) are important for maintaining the resilience zone because the vertical overwintering profile requires a deep water column to minimize the likelihood of freezing (Markle et al. 2020). Although identifying and protecting resilient wetlands is important for the persistence of critical overwintering habitat, our results show that even a resilient wetland would be unlikely to recover from a rapid and large-scale winter water table decline. Furthermore, shallow lakes with wetland bays are particularly sensitive to any rapid change in water-level (Coops et al. 2003). Winter water level drawdowns are a common lake and reservoir management practice to achieve specific water depths (Carmignani and Roy 2017). Yet, research on winter water drawdown effects is limited on semi-aquatic species, such as turtles (Carmignani and Roy 2017). Based on our results, drawdowns are likely to have a negative impact on the resilience zone. We suggest that any unavoidable manipulation to water levels in turtle overwintering habitat should be an increase in water levels. As an example, the beaver dam failure at OWW A moved water across the landscape eventually reaching OWW B, increasing the water table depth by approximately 50 cm. Although we were unable to calculate resilience zone size in winter 2020-2021, based on the primary feedbacks and interactions that influence the turtle resilience zone size (Figure 5; see Markle et al. 2020) an increase in water depth would increase the turtle resilience zone size. Management of water levels within snake overwintering habitat has been suggested to ensure water levels maintain stability (Johnson et al. 2000; Shine and Mason 2004). Although expensive, this approach may be possible for turtle species but, conserving existing suitable overwintering habitat should be the primary approach (Smolarz et al. 2018) to ensure the presence of the resilience zone.

The turtle resilience zone: Temperature

The wetlands within our study acted as a thermal refuge from external air temperatures, reaching as low as -30°C, consistent with other studies (Litzgus et al. 1999; Newton and Herman 2009; Markle and Chow-Fraser 2017). We found that Blanding's turtles selected weekly temperatures that were overall lower and more stable than water temperatures in available habitats at 7 cm above the sediment. Our observation is in line with Edge et al. (2009) and Gasbarinni et al. (2021) but opposite to that by Markle and Chow-Fraser (2017) where Blanding's turtles overwintered in water bodies that were warmer than unoccupied habitats. In addition, our results show that Blanding's turtles overwinter in habitats that offer a range of aquatic temperatures but maintain specific temperatures (0 – 4°C). In both winters, following ice formation no turtles moved >1m between subsequent radiolocations, similar to other radio-tagged overwintering Blanding's turtles in similar habitat types (Edge et al. 2009). Instead, our results show that overwintering Blanding's turtles move vertically within the water column to maintain specific thermal conditions as suggested by Edge et al. (2009). As an example, in early February 2022, an individual had a 2 °C increase in mean daily temperature. Substantial ice cover and low external air temperatures would prevent the turtle from aerial basking indicating vertical movement within the overwintering profile, most likely from the water column into the sediment. A recent study by Robichaud et al. (2022) indicates that Northern map turtles also move vertically within the overwintering profile. The majority of 2020-2021 *in-situ* Blanding's turtle overwintering temperatures were values of 2°C or less, compared to 2021-2022 which were of 4°C or less. However, the 2021-2022 results were primarily driven by one turtle who used warmer temperatures and winter temperatures that were slightly warmer than 2020-2021. Although we did not investigate if there were significant differences between winters, other studies, such as Gasbarrini et al. (2021) observed variation in habitat temperatures from year to year.

Our results are comparable with overwintering turtle temperatures in the other parts of Ontario where turtles selected temperatures near 0°C to maintain a low metabolic state (Litzgus et al. 1999; Greaves and Litzgus 2007; 2008; Rollinson et al. 2008; Edge et al. 2009; Rasmussen and Litzgus 2010; Paterson et al. 2012; Gasbarrini et al. 2021). Furthermore, this study is among at least 7 others that use iButtons to estimate overwintering turtle body temperature (Grayson and Dorcas 2004; Greaves and Litzgus 2007; 2008; Rollinson et al. 2008; Edge et al. 2009; Rasmussen and Litzgus 2010; Gasbarrini et al. 2021). Similar to other Blanding's turtle overwintering studies, there was minimal variation in mean winter turtle temperatures, 0.87 ± 0.46°C (2020-2021) and 1.30 ± 0.74°C (2021-2022) (Edge et al. 2009). Our results support the hypothesis that overwintering turtles select overwintering habitats based on specific thermal conditions (Edge et al. 2009; Markle and Chow-Fraser 2017). Although Markle and Chow-Fraser (2017) compared unconfirmed habitat types to confirmed habitats, our study was the first to compare overwintering turtle temperatures to different available habitats within wetlands. We found that at the microhabitat scale, mean weekly turtle temperatures were overall colder than that of three (floating mat, lagg and open water) of the four available habitats at a depth of 7 cm. During winter 2021-2022 at the profile scale (Bog A), the 7 cm data logger at the floating mat failed but all three of the other available habitats were overall warmer than weekly turtle temperatures. A floating mat available habitat was often adjacent and in relatively close proximity (<2m) to the lagg where the majority of turtles overwintered (2020-2022: 5/5 and 2021-2022: 18/19). Our results suggest that Blanding's turtles select microhabitats that allow them to make small horizontal (<1m) and vertical movements between different microhabitat types (lagg and floating mat) to actively select specific thermal conditions. Furthermore, the adjacent floating mat may provide structure for the turtles to latch onto to help maintain a specific vertical position. Our results are inline with Markle and Chow-Fraser (2017) and indicate that aquatic temperatures are not uniform throughout the wetland. We suggest that the floating mat that lines either side of the lagg acts as a thermal buffer to slow the full freezing of the lagg. Further, we hypothesize that the floating mat helps prevent the freezing of the water beneath it. Temperature and ice data, as well as *in-situ* observations indicated that the floating mat remained unfrozen for longer periods than other microhabitats. This is likely due to the presence of entrapped air within the floating mat as well as the dark colour of the mat itself relative to other available habitats. We suggest that the lack of floating mat and surrounding vegetation within open water sections can result in deeper freezing of the water column. Snow depth may also play an important factor in preventing the water column from freezing fully since snow depth acts as an insulator that can reduce heat loss (Zhang 2005).

The turtle resilience zone: Dissolved oxygen

During the overwintering season Blanding's turtles experienced 99 (winter 2020-2021) and 132 (winter 2021-2022) days of continuous ice cover. This is similar to other Blanding's turtle studies located within our study region such as Algonquin Provincial Park (101 – 136 days, Edge et al. 2009) and the southeastern shore of Georgian Bay (99 days, Markle and Chow-Fraser 2017). During extended periods of consistent ice cover, overwintering turtles had no access to aerial oxygen resulting in the need for extrapulmonary uptake of dissolved oxygen and/or anoxia tolerance (Ultsch 1989; 2006). Similar to other studies, there was a range of DO (0 — 16 mg/L) across all wetlands and microhabitats (Rollinsion et al. 2008; Edge et al. 2009; Paterson et al. 2012; Gasbarrini et al. 2021). The variability in dissolved oxygen among the overwintering microhabitats could be due to exposure to aerial oxygen, decaying vegetation, and/or flowing water (Newton and Herman 2009; Markle et al. 2020). Furthermore, overwintering turtles may select or move to areas that open to aerial oxygen faster than others (i.e., melting of ice). As an example, turtles overwintering along the wetland edge turtles may be exposed to aerial oxygen earlier than those who select microhabitats closer to the centre of the waterbody since ice tends to melt from the shore toward the centre of the waterbody (Ultsch 1989). Historically, overwintering turtle studies collected DO data 1 — 4 times per winter (Crawford 1991; Greaves and Litzgus 2007; 2008; Rollinson et al. 2008; Edge et al. 2009; Paterson et al. 2012; Gasbarinni et al. 2021). Comparatively, we collected DO 10 times over the 2021-2022 winter. We found that overwintering Blanding's turtles chose microhabitats with higher levels of DO than surrounding available habitats and our study was the first to show evidence of selection for DO by Blanding's turtles in confirmed habitats. Edge et al. (2009) found that Blanding's turtles selected habitats that offered different amounts of DO than creek and lake habitats, where turtles did not overwinter. Similarly, Rollinson et al. (2008) saw that overwintering painted turtles selected lower temperatures when overwintering in a severely hypoxic habitat, suggesting that while maintaining an aerobic metabolism, painted turtles were selecting a microhabitats based DO. During periods of substantial continuous ice cover there was a greater range of DO at Blanding's turtle locations than in the available habitats within Bog A. Although Blanding's turtles select for high levels of DO, our results support the hypothesis that Blanding's turtles are anoxia tolerant (Kofron and Schreiber 1985; Ross and Anderson 1990; Ultsch 2006; Edge et al. 2009) since the minimum mean DO across all the sample weeks was approximately 1 mg/L. Furthermore, the preference to available habitats (lagg) with lower levels of available DO than the inflow support the hypothesis that temperature is the driving factor in overwintering site selection by Blanding's turtles, not DO (Edge et al. 2009; Markle and Chow-Fraser 2017). If DO was the main factor in Blanding's turtles overwintering site selection we would expect Blanding's turtles to overwinter in the inflow (Markle et al. 2020). Although, despite the ability to overwinter in anoxic environments and the preferences towards temperature, we suggest that normoxic and hypoxic overwintering conditions optimizes overwintering habitat suitability (Markle et al. 2020) because oxygen available in even very small amounts will reduce the level of anaerobic by-products in extracellular tissues (Bagatto and Henry 2000; Crocker et al. 2000). Further, laboratory studies have indicated that overwintering turtles survive longer when submerged in oxygenated water (Ultsch and Jackson 1982). Therefore, DO availability is an important component of the resilience zone.

The turtle resilience zone: Integration of abiotic factors

Our results support our hypothesis that DO has the greatest control on the resilience zone. When the sensitivity analysis was conducted under fully anoxic conditions, the resilience zone was present for 100% of the 2021-2022 winter, indicating that temperatures between 0 — 4°C were not limited in the water column at the landscape and microhabitat scale. Comparatively, when the sensitivity analysis was conducted under temperatures between 0 — 2°C the days without a resilience zone increased, especially at the floating mat and open water habitat indicating that DO within specific microhabitats may be more limited at temperatures between 0 — 2°C than 0 — 4°C. The integration of both DO and temperature is important considering temperature plays a key role in controlling the rates of biogeochemical processes in peats (Fenner et al. 2005). Temperatures between 0 — 2°C were often available higher in the water column which may lead to lower DO levels (Andres et al. 2020). Our results show that DO is not homogenous down the overwintering profile which is inline with other studies, where vertical gradients of DO caused micro-scale (1 m) heterogeneity of the water column (Vad et al. 2013). DO is often quickly removed in anoxic peat (Walpen et al. 2018) further limiting the availability of DO in floating mat available habitats. Furthermore, we suggest that the floating mat may act as a barrier to aerial oxygen preventing mixing with the free water directly beneath the floating mat and instead, near the sediment where temperatures were warmer DO may have been able to mix with the free oxygenated water in the lagg.

In addition to temperature, we suggest that DO influences the availability of the resilience zone over water depth since overwintering turtles within our study and others (Rollinson et al. 2008; Edge et al. 2009; Newton and Herman 2009; Paterson et al. 2012) have shown to select overwintering habitats that are sufficiently deep. Although dramatic drops in water depths can lead to exposure to freezing air temperatures and therefore winterkill, DO is more important on a day-to-day basis since large scale water declines are often infrequent one time catastrophic occurrences. (Platt et al. 2008; Keevil et al. 2018; Gasbarinni et al. 2021). Therefore any overwintering habitats without 40 cm of water at the start of the overwintering season does not optimize overwintering habitat suitability and therefore are automatically deemed unsuitable under the resilience zone criteria. This is not to say that turtles cannot successfully overwintering in habitats with less than 40 cm (Litzgus et al. 1999; Rasmussen and Litzgus 2010; Paterson et al. 2012), instead the resilience zone is about optimizing overwintering habitat suitability and water depths greater than 40 cm will provide some access to free water (Edge et al. 2009).

The first quantification of the resilience zone indicated that the spatiotemporal variability in the resilience zone is high, showing variability among microhabitats and wetlands. With this in mind, microhabitat availability alone may not be a reliable factor to identify wetlands that provide suitable overwintering habitat. Our results indicate that overwintering Blanding's turtles moved <1m during the winter (Edge et al. 2009) but move vertically within the water column potentially tracking specific resilience zone conditions, when available. However, overwintering Blanding's turtles appear to be fairly robust to resilience zone loss. Based on our observations, Blanding's turtles withstood overwintering in habitats without the resilience zone for periods up to 49 (floating mat), 21 (lagg), and 14 (open water) days, suggesting that Blanding's turtles could survive nearly 2 months in habitats without the resilience zone. This goes against the suggestion that the complete loss of the resilience zone would not permit winter survival (Markle et al. 2020). Although, we agree with Markle et al. (2020) that additional data would be required before attempting to predict survival probability (Kearney 2006; Kearney and Porter 2009), especially since the ability to tolerate extended periods outside the resilience zone may be critical in the face of climate and land-use changes.

Although our work did not provide a blanket approach to identifying suitable overwintering habitat, the resilience zone allowed us to identify suitable overwintering habitat within our study sites. Additionally, our work is a major step towards the identification of habitat factors and how they contribute to resilient ecosystems (Markle et al. 2020). At this point, we highly recommend those working to identify suitable overwintering habitats to use the resilience zone as a guideline rather than a hard and fast solution. We suggest that the combination of *in-situ* microhabitat use by overwintering turtles (Litzgus et al. 1999; Rollinson et al. 2008; Edge et al. 2009; Newton and Herman 2009; Paterson et al. 2012; Marchand 2018; Robichaud 2022) paired with the resilience zone is the currently best way to identify suitable overwintering habitat. Further work is needed to confirm a reliable factor to identify wetlands and microhabitats that will provide suitable overwintering habitat.

Impacts of climate and land-use changes on the turtle resilience zone

Suitable turtle overwintering habitat is at-risk in the face of climate-use changes. The resilience zone is a powerful tool that can be used to identify at-risk overwintering habitats. This is especially critical considering an increasing amount of habitat will become unsuitable due to rising temperatures and increased frequency of climatic events (Keith et al. 2014; IPPC 2019; Mothes et al. 2020). In response to climate change turtles may need to move further to reach suitable habitats as the number of suitable habitats decreases (Hamilton et al. 2018). Furthermore, turtles may continue to select habitats that become unsuitable due to a fidelity to previously suitable overwintering sites. This is of great importance due to the increased pressure of infrastructure development within the northern limit of the species range (e.g., expansion of the 400 series highway). Removal of suitable overwintering habitat and the further fragmentation of the landscape can decrease ecosystem resilience which is key in ensuring species at-risk persistence (Griffith et al. 2009; Mori et al. 2013; Hamilton et al. 2018). Based on our findings, we suggest that decreased precipitation and overall drier wetland conditions (Flato and Boer 2001; Colombo et al. 2007) will have the greatest impact on turtle overwintering habitat. Decreased water depths showed an increased risk of exposing an overwintering to freezing temperatures through encroaching ice on the anoxic sediment. Although this may be minimized by warmer winter temperatures and therefore decreased ice cover, rapid reversal in extreme weather known as weather whiplash (Casson et al. 2019) may counterbalance overall increased winter temperatures. The rapid back and forth changes in winter weather may cause rapid oscillations in the resilience zone which could have energetic consequences (Williams et al. 2015) on overwintering turtles. Increased overwintering temperatures and the persistence of weather whiplash events (i.e., winter heat waves) may lead to quicker use of overwintering turtles metabolic stores putting them at greater risk of overwintering mortality (Ultsch 1989; 2006). We suggest that false springs (warm spell followed by cold snap) can also have an impact on overwintering turtles. Following emergence, turtles increase their body temperature and therefore metabolic rate through basking (Jackson 1971), if turtles metabolic rates were increased before being able to fully replenish energy stores, rapidly forced back into an overwintering may have detrimental consequences. Further research on the impacts of weather whiplash on overwintering turtles is needed to understand the consequences of these events, especially since weather whiplash events are expected to increase in frequency (Casson et al. 2019).

The decline and fragmentation of freshwater habitats due to the demand for infrastructure development directly contributes to the loss of already limited suitable turtle overwintering habitat (Markle and Chow-Fraser 2017). Wetland habitats are patchily dispersed throughout the landscape (Euliss et al. 2004) and roads can act as isolating barriers to accessing these habitats (Roe et al. 2006). Over time, turtles may be forced to select unsuitable overwintering habitats due to inaccessibility and increased lack of suitable habitat (Vaner and Dearing 2014). The development of roads have the potential to change local hydrology which can lead to upslope flooding and downslope drying in relation to the road (Cochand et al. 2020). This can greatly impact overwintering turtles that display site fidelity to the (now) downslope portion of the road. If water depths are no longer sufficiently deep, the turtle(s) are at an increased risk of being exposed to freezing temperatures and/or the anoxic sediment and although the upslope portion of the road may provide deeper water depths that may come with increased risk of predation (Paterson et al. 2012). Changes in wetland hydrology due to infrastructure development may result in the change in wetland vegetation (Sheng et al. 2012) and potentially wetland state which may minimize the diversity in available microhabitats (Markle et al 2020). Persistence of specific microhabitat types, such as the lagg, is important for maintaining the resilience zone. Overall, the loss of suitable overwintering sites can greatly impact freshwater turtle populations (Gibbons et al. 2000). Our results show that the resilience zone can be used to identify remaining suitable habitat in need of protection against land-use changes. Furthermore, when the resilience zone is coupled with fall habitat movement data it could act as a champion to protect travel corridors used to access overwintering sites. Our results provide a deeper understanding of suitable overwintering habitat and once paired with active management, it can contribute to future viability of turtle populations (Mothes et al. 2022). Based on our findings, we suggest that the resilience zone is implemented in conservation and management plans for at-risk turtle species to ensure suitable overwintering habitat is protected, actively managed and accurately restored.

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Chapter 3: General Conclusion

Freshwater turtles at the northern limit of their range are faced with harsh, long, cold winters. Most aquatic freshwater turtle species seek refuge from freezing conditions by reducing their metabolic activity and retreating underwater into some form of aquatic medium that acts as a thermal refuge (Ultsch 1989, Reese et al. 2002; Greaves and Litzgus 2007; Markle et al. 2020). The abundance of wetlands and peatlands within the Georgian Bay Biosphere Mnidoo Gamii provide overwintering environments that maximize the winter survival of turtle species (Markle et al. 2020). Overwintering habitats must provide safe refuge under ice for extended periods of time and balance the risk of freezing, metabolic acidosis, and predation (Litzgus et. al. 1999; Litzgus and Brooks 2000; Edge et al. 2009; Markle and Chow-Fraser 2017; Markle et al. 2020). The availability of suitable overwintering sites may be the difference between survival and death for overwintering turtles (Paterson et al. 2012). Currently, there is limited field information that examines in-depth critical environmental variables needed for suitable overwintering beyond temperature selection (Litzgus et al. 1999; Rollinson et al 2008; Edge et al. 2009; Paterson et al. 2012) and identification of habitat types occupied by overwintering turtles (Markle and Chow-Fraser 2017). Due to the dependence turtles at their northern range limit have on overwintering habitats, there is a need to identify aquatic habitats that can provide thermal, physical and biogeochemical conditions necessary for successful turtle overwintering (Markle et al. 2020). Identifying the factors that contribute to suitable overwintering habitat is key for the conservation and management of turtle species at-risk populations. Furthermore, with the cumulative effects of habitat loss and climate change, it is imperative that critical turtle overwintering habitats are well understood to facilitate effective habitat management, protection, restoration and creation.

In chapter two we determined that overwintering Blanding's turtles move vertically within the overwintering profile to maintain specific thermal and chemical within selected microhabitats. Furthermore, we determined that Blanding's turtle temperatures were significantly different than available habitats at a depth of 7 cm above the sediment. This result suggests that the past approach of assuming Blanding's turtles overwinter within a small vertical space (i.e., directly on the sediment or buried beneath it) (Edge et al. 2009) is no longer the most accurate approach (Robichaud et al. 2022). While some individuals may choose to overwinter directly on or beneath the sediment it may be a result of the conditions provided by the selected microhabitat since thermal, chemical and physical properties varied across microhabitat types within and among wetlands. Through the use of *in-situ* overwintering abiotic data we provided the first quantification of the turtle resilience zone. We determined that dissolved oxygen had the greatest control on the resilience zone and found that based on the resilience zone, suitable overwintering habitat was limited across our study wetlands. Further, evidence of both site fidelity and communal overwintering by Blanding's turtles supports the hypothesis that suitable overwintering sites are limited (Edge et al. 2009; Markle and Chow-Fraser 2017). We found Blanding's turtles showed preference to specific microhabitat types (i.e. lagg) likely due to the range of habitat characteristics available due to variation in ecohydrological drivers. Our results have important implications when considering both climate and land-use changes on aquatic overwintering habitat. The amount of suitable overwintering habitat is likely going to decrease due to reduced precipitation and overall drier wetland conditions as a result of climate change (Flato and Boer 2001; Colombo et al. 2007) and infrastructure development, which can lead to the alteration of landscape hydrology (Cochand et al. 2020). Weather whiplash is also an important factor to consider since rapid oscillations in the resilience zone caused by weather whiplash could have energetic consequences (Williams et al. 2015). These winter weather whiplash events may lead to faster use of overwintering turtles metabolic stores putting them at greater risk of overwintering mortality (Ultsch 1989; 2006).

In summary, this thesis has expanded the knowledge of freshwater turtle overwintering habitat near the northern limit of multiple species range. We identified the range of abiotic (thermal, chemical and physical) factors available within overwintering habitats. We then applied that knowledge, as well as both *in-situ* Blanding's turtle overwintering and literature data to provide the first quantification of the turtle resilience zone. Through this we determined that specific habitat factors are required to allow for successful overwintering and if these factors are lost temporarily, turtles can still overwinter successfully. In addition, we found that turtle overwintering habitat is already limited within a relatively pristine region of the northern limit of the species range. Further, we found that the resilience zone is an extremely efficient tool in identifying resilient and vulnerable overwintering habitat when abiotic habitat data is available. We believe our findings would be relevant to other overwintering habitats across the species range and highly suggest the use of the resilience zone in identifying suitable overwintering habitats in conservation and management strategies. In terms of future work, additional available habitat profiles should be added to each microhabitat type to determine variation within microhabitats within each wetland. In addition, we suggest collecting a continuous dissolved oxygen profile at each turtle and available habitat location. Further, overwintering conditions at Blanding's turtle locations should be collected from multiple wetlands across the study area, if populations permit. Collecting overwintering data across multiple winters would allow for between winter differences and would provide results that may be better equipped to inform managers in the face of climate and land use change. We suggest completing this same study at overwintering habitats throughout northern temperate regions that are both unimpacted and that have greatly been impacted by land-use change. For example, large amounts of fragmentation and degradation due to infrastructure development or impacted by wildfire. Investigating the resilience zone in impacted overwintering habitats can provide an idea of how the resilience zone changes and how impacted overwintering habitats can be better managed to optimize the resilience zone. Finally, we suggest adding a physiological component to the resilience zone, as suggested by Markle et al. (2020). This will allow for greater understanding of the fitness impacts of overwintering when the resilience zone is present or absent. Further, a physiological component will aid in understanding how impacts of climate and land use changes will affect overwintering turtles. Overall, this thesis has greatly contributed to the knowledge and understanding of turtle overwintering habitat and all the findings can be directly applied to future conservation and management plans for protecting, restoring and building turtle species at-risk overwintering habitat.

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Appendix

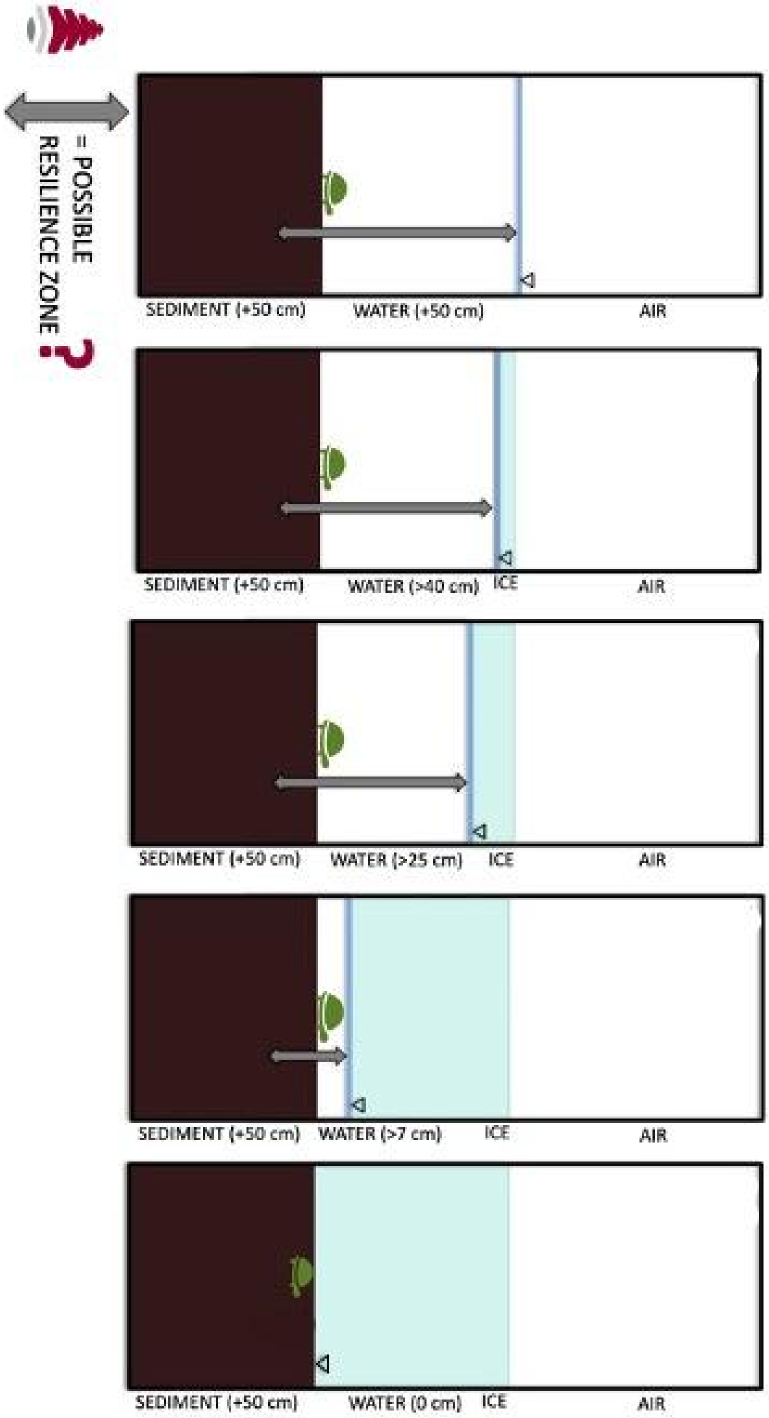
= POSSIBLE RESILIENCE ZONE ?
SEDIMENT (+50 cm)
WATER (+50 cm)
AIR
SEDIMENT (+50 cm)
WATER (>40 cm)
ICE
AIR
SEDIMENT (+50 cm)
WATER (>25 cm)
ICE
AIR
SEDIMENT (+50 cm)
WATER (>7 cm)
ICE
AIR
SEDIMENT (+50 cm)
WATER (0 cm)
ICE
AIR

Figure A1: Conceptual model of the vertical overwintering profile as the winter progresses. The available resilience zone may differ depending on winter weather and ecohydrological conditions.

Figure A2: Five wetlands instrumented within this study, figure provides an example of wetland state. Surface complexity, cover and microhabitat type varies depending on the location within each wetland.

Figure A3: Four microhabitat types within each wetland instrumented within this study.

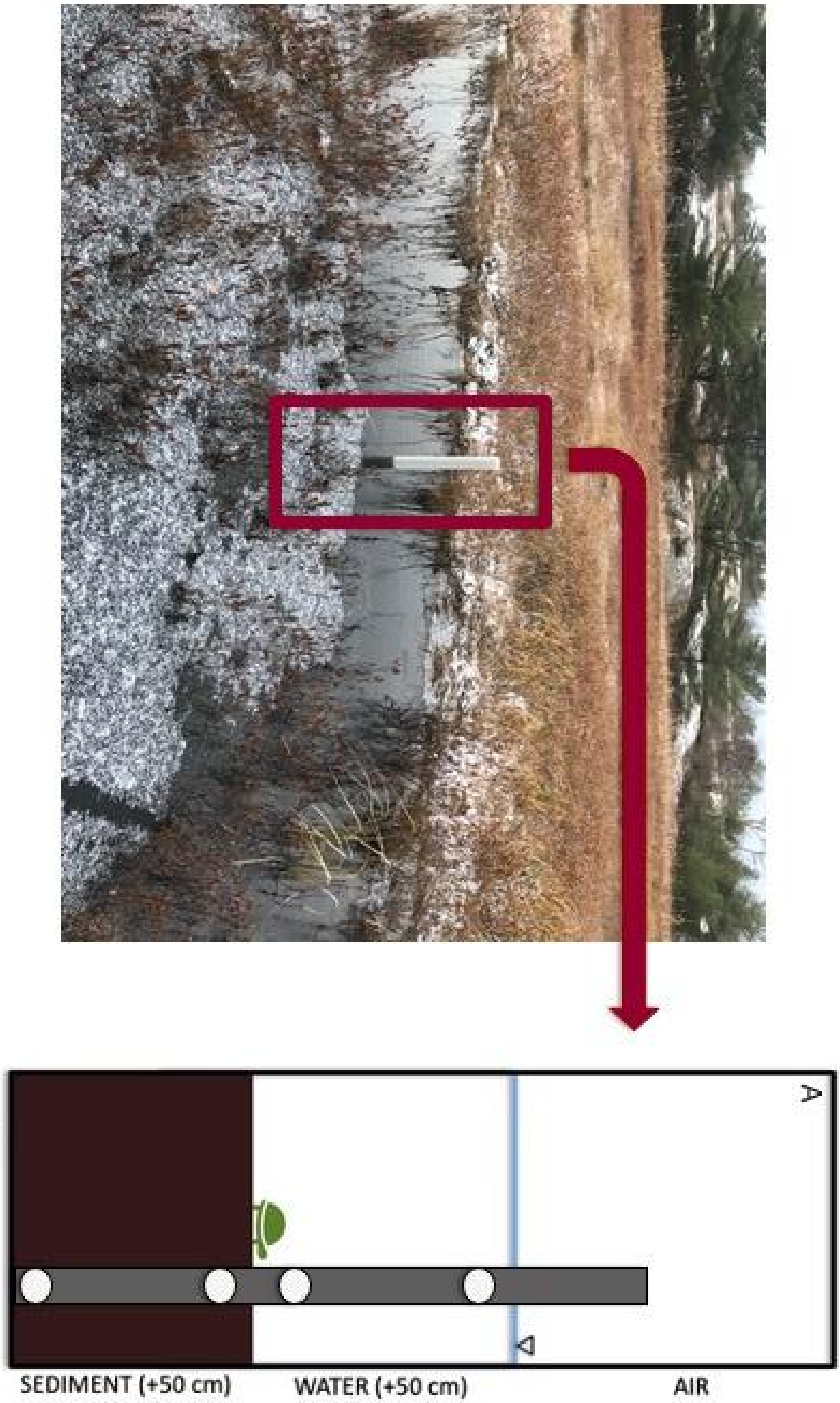

Figure A4: Conceptual model of what each monitoring profile looks like at each of the four microhabitats across the five wetlands. Overwintering turtle represents the hypothesized location (directly on or buried into the sediment) based on previous studies.