Five months under ice: biologging reveals behaviour patterns of overwintering freshwater turtles

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Abstract

Winter in temperate regions is characteristically the coldest period of the year. Species in these regions adapt to freezing temperatures with physiological or behavioural mechanisms to mitigate the threats of cold exposure. For aquatic species, taking refuge under the ice minimizes the risk of experiencing freeze injury. The northern map turtle (*Graptemys geographica* (LeSueur, 1817)) is one species that overwinters under the ice of lakes and rivers. Here, we observed the behaviour of free-ranging map turtles at a known overwintering site throughout an entire winter using biologgers equipped with tri-axial acceleration, temperature, and depth sensors. We observed that map turtles maintain localized locomotor activity at the overwintering site continuously during the winter. The extent and patterns of locomotor activity and habitat use varied between adult females, adult males, and juvenile females. Adult females were observed at the shallowest depths, coldest temperatures and moved the least, whereas juvenile females were observed at the deepest depths, warmest temperatures and moved the most. All groups remained at temperatures near freezing (0.98–1.39 °C) and at average depths ranging from 1.34 to 1.7 m. These behavioural patterns are consistent with a strategy to survive the winter while remaining aerobic and likely reflect differences in physiological demands.

Key words: biologging, freshwater, *Graptemys geographica*, northern map turtle, locomotor activity, overwintering

Introduction

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

Most species of freshwater turtles—including the eight species found in Canada—escape freezing winter temperatures by overwintering in liquid water under the ice (Ultsch

2006). Some species, including the painted turtle (*Chrysemys picta* (Schneider, 1783)) and the snapping turtle (*Chelydra serpentina* (Linnaeus, 1758)), can survive several months submerged in anoxic water (Ultsch 2006). Other species, however, cannot survive an entire winter without oxygen and rely on extrapulmonary routes to extract dissolved oxygen (DO) from the water (Ultsch and Cochran 1994; Reese et al. 2001, 2003). The northern map turtle (*Graptemys geographica* (LeSueur, 1817)) is such a species. Laboratory studies indicate that northern map turtles die from metabolic acidosis after 45 days in anoxic water at 3 °C, making normoxic conditions a requirement to support aerobic respiration throughout the winter (Crocker et al. 2000; Reese et al. 2001).

Northern map turtles overwinter communally (Ultsch et al. 2000; Fig. 1B) and show fidelity to their overwintering sites (Graham et al. 2000). This aggregation behaviour may indicate that overwintering sites with sufficient oxygen for this species are limited, and a similar suggestion has been made for other freshwater turtle species such as the spotted turtle (*Clemmys guttata* (Schneider, 1792)); Ultsch and Jackson 1995; Litzgus et al. 1999; Reese et al. 2001; Ultsch 2006; Rasmussen and Litzgus 2010). Northern map turtles appear to be responsive (i.e., conscious and actively moving) in winter and have been documented to show locomotor activity at temperatures near 0 °C (Evermann and Clark 1916; Graham and Graham 1992). This behaviour is uncommon for freshwa-

Fig. 1. Juvenile female northern map turtle (*Graptemys geographica*) with a tri-axial acceleration biologger and radio transmitter epoxied to her carapace following release (A). Northern map turtles with tri-axial acceleration biologgers and radio transmitter epoxied to their carapace (circled) under the ice in Feb 2021 at their known overwintering site at Lake Opinicon in Ontario (B). Image captured with an underwater Trident ROV (Sofar Ocean, San Francisco, CA).
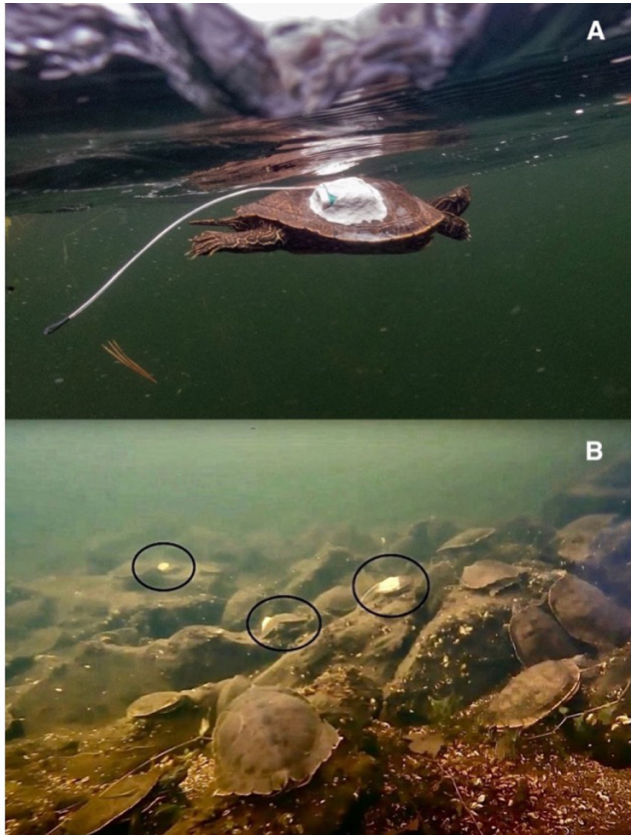
ter turtles at this time of year and has otherwise only been observed in the wood turtle (*Glyptemys insculpta* (LeConte, 1830)); Greaves and Litzgus 2007). The extent of northern map turtle winter activity and the reasons for it remain unknown; however, locomotor activity in the winter may be related to oxygen consumption. For instance, overwintering smooth softshell turtles (*Trionychidae* spp. (Fitzinger, 1826)) do "push-ups", which are hypothesized to help turtles shed the oxygen-depleted boundary layer and ventilate their skin surface (Plummer and O'Neil 2019). Winter activity may thus be necessary for anoxia-intolerant species to meet their demand for oxygen.

Studying turtles under the ice is logistically challenging and our understanding of their overwintering activity in nature largely comes from direct but short-term field observations (e.g., Graham and Graham 1992; Crocker et al. 2000) and radio-telemetry studies conducted from the surface of the ice without direct observations (Litzgus et al. 1999; Greaves and Litzgus 2007). These approaches, while insightful, have a limited potential for documenting activity throughout the winter. In radio-telemetry studies, activ-

ity is inferred from changes in location so locomotor activity not resulting in a measurable change in location is not documented. Moreover, the frequency of movement captured by radio-telemetry studies is limited by the frequency at which animals can be tracked. Finally, manual radio-tracking is limited to the portion of the winter during which the ice is safe to walk on potentially excluding important shifts in activity during the shoulder seasons.

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

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

Materials and methods

Study site

This research was approved and conducted in accordance with the Canadian Council on Animal Care Guidelines as administered by Carleton University, Protocol No. 113287, Permit No. 1096574. We conducted this study in Lake Opinicon

in Ontario, Canada (44°55′90″N, 76°32′80″W). Lake Opinicon is a medium-sized (7.9 km²) shallow lake, averaging 2.5 m in depth, with a maximum depth of approximately 11 m (Feng et al. 2019). It does not stratify during the winter months. The surface of the lake typically remains frozen from late December to early April, apart from the lake's eastern end, where it connects to the Rideau Canal, and two creeks at the southeastern end of the lake (Feng et al. 2019). It is estimated that there are over 1500 northern map turtles located within Lake Opinicon (1.9 turtles/ha; Bulté et al. 2010). Overwintering sites were previously identified along the shoreline of an island in Lake Opinicon using radio-telemetry (Carrière et al. 2009) and monitored as part of a mark-recapture study since 2004.

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

Between October 16th and 21st 2020, we captured 9 juvenile females, 17 adult females, and 14 adult males by snorkeling in the vicinity of the communal overwintering site. Juvenile females were included in the study because their body size is midway between adult females and adult males yielding three demographic classes for comparative purposes (i.e., adult males, adult females, and juvenile females). On each turtle, we fitted a biologger (22 mm × 45 mm × 8 mm, 11 g in air; Axy-5, TechnoSmArt, Guidonia Monticello, Italy) and a radio transmitter (15 × 8.2 mm, 1.5 g in air; NanoTag, Lotek, Newmarket, Ontario, Canada) to permit the recapture and retrieval of the biologgers. The biologgers and transmitters were taped together and epoxied to the right posterior edge of the carapace (Figs. 1A and 1B).

The biologgers recorded acceleration (i.e., locomotor activity), water temperature (±2 °C), and depth (±5 cm). Acceleration was measured at sample rate of 10 Hz with an 8-bit resolution. Biologgers recorded on a schedule to preserve battery life to obtain measurements throughout the entire winter. Measurements were taken each day between 0:00 and 04:00 h and between 07:00 and 17:00 h. Turtles were relocated between the months of April and October 2021 using radio-telemetry and recaptured to remove the tags. Tags were removed within 12 h of capture and turtles were released back to where they were captured. Thirty-seven of the 40 tagged turtles (92.5%) were recovered in the spring and summer following their overwintering. Three turtles were not recovered, and four faulty biologgers did not yield data leaving a sample size of 13 adult females (209–250 mm maximum carapace length; 1076–1675 g), 9 juvenile females (142–185 mm maximum carapace length; 357–1388 g), and 11 adult males (126–141 mm maximum carapace length; 222–302 g).

Under-ice videography

The accelerometry data indicated important locomotor activity during the ice on period. To qualitatively assess the extent of activity during this period, we installed an underwater camera (Eyoyo Underwater Fishing Camera) in January 2022 at the hibernation site. The camera was attached to a 1.1 kg diving weight and sat at approximately 15 cm above the substrate. The camera cables were fed through a 2.5 cm diameter, 30 cm long galvanized steel pipe. We visited the camera 11 times between 22-Jan-2022 and 13-Mar-2022 and made recordings on each occasion.

Environmental monitoring

We installed six DO and temperature loggers (aquaMeasure DO, InnovaSea Systems Inc.) along a 500 m transect running parallel to the shoreline in the vicinity of the overwintering site. Each logger was stationed individually for a total of six measurement sites at water depths ranging from 1.8 to 4.6 m and recorded DO saturation (±5%) and temperature (±0.2 °C) every 2 h between 7-Nov-2020 and 19-Apr-2021. Each logger was approximately 1.5 m off the bottom of the lake. Additionally, on three separate days (5-Dec-2020, 13-Feb-2021, 20-Feb-2021), we measured DO saturation and temperature at 7–13 random locations using the same loggers. These measurements were also taken near the bottom of the lake, at depths ranging from 2.1 to 7.6 m. Measurements were used to create temperature and DO profiles of the overwintering site to observe how these parameters changed over time during the study period.

Locomotor activity

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

Statistical methods

To assess external influences on locomotor activity during the ice-on period, a linear mixed-effects model was fitted using the *lmer* function from the *nlme* package in R (Bates

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

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

Results

Habitat characteristics

Average daily DO and water temperature at the overwintering site varied throughout the study period (Fig. 2). These measurements are useful in providing insights into the conditions experienced at the site but were not used further in our statistical analysis. The average daily DO observed at the overwintering site during the pre-ice period remained between 92% and 100% DO saturation before ice formation occurred. The average daily temperature during this period was 9.1 °C at the highest, which steadily declined to a low of 0.3 °C when ice formation occurred. During the ice-on period, average daily DO saturation steadily decreased throughout, declining from 94% to 9% at its lowest point (Fig. 2). Conversely, the daily average temperature steadily increased between 0.7 and 5.1 °C before ice melt occurred (Fig. 2). After ice melt, DO saturation rapidly increased, with daily averages increasing from

33% back to the 100% saturation seen in the pre-ice period. Daily average temperatures ranged between 4.7 and 9.2 °C. Random site samples of DO and temperature on December 5 ranged from 88.5% to 90.6% and 2.5 to 3.3 °C, respectively. On February 13, the values ranged from 48.6% to 78.9% and −0.5 to 2.4 °C. On February 20, the values ranged from 47.1% to 85.6% and 1.9 to 4.6 °C.

Locomotor activity

Total daily ODBA for each individual northern map turtle was greater than 0*g* each day throughout the study period (Fig. 3A). Model selection with AIC resulted in a final model with a fixed slope where demographic class, water temperature, and depth were the independent predictor variables The best-fit model did not include interactions between independent variables, and turtle ID was included as a random effect to account for the repeated measures across individuals. The final linear mixed-effects model suggested that water temperature ($F_{[1,3081]} = 8.771$, $p = 0.003$, $\eta^2 = 0.003$) and demographic class ($F_{[2,33]} = 3.480$, $p = 0.042$, $\eta^2 = 0.17$) both had a significant effect on locomotor activity (ODBA) during the ice-on overwintering period, whereas depth did not ($F_{[1,2645]} = 2.517$, $p = 0.113$, $\eta^2 = 0.001$). Adult females showed the least amount of locomotor activity ($578 \pm 79g$) compared with adult males (603 ± 98; $z_{[2]} = 2.097$, $p = 0.09$) and juvenile females ($608 \pm 94g$; $z_{[2]} = 2.355$, $p = 0.049$) during the ice-on period (Fig. 3A). There was no statistically significant difference in total daily ODBA between juvenile females and adult males ($z_{[2]} = -0.371$, $p = 0.927$; Fig. 4A). There was a weak but statistically significant positive relationship between total daily ODBA and water temperature ($F_{[1,3081]} = 8.771$, $p = 0.003$, $\eta^2 = 0.004$; Fig. 5). In the last week before the ice completely melted, the mean water temperature around the northern map turtles increased from 2.7 to 4.3 °C in a single week. We suspected this rapid change in temperature had an overly strong influence on the association between ODBA and temperature. We thus ran the model again after removing the data for that week (∼8% of the total period) and the relationship between ODBA and temperature became statistically nonsignificant, indicating the relationship we observed was primarily driven by the increasing temperatures during the last week of ice-on (Fig. 5).

Total daily ODBA for each group was lower during the ice-on period relative to the pre-ice and post-ice periods (Fig. 3A). Adult females had an average daily ODBA of $597 \pm 74g$ during the pre-ice period and $634 \pm 80g$ during the post-ice period, making ODBA 3% and 9% higher than during the ice-on period. Adult males had an average daily ODBA of $674 \pm 124g$ during the pre-ice period and $868 \pm 219g$ during the post-ice period, making ODBA 10% and 30% higher than during the on-ice period. Finally, juvenile females had an average daily ODBA of $615 \pm 71g$ during the pre-ice period and $644 \pm 74g$ during the post-ice period, which was 1% and 6% higher than the ice-on period. Total daily ODBA differed between demographic classes in the pre-ice period ($F_{[2,1836]} = 223.9$, $p < 0.001$, $\eta^2 = 0.2$) and the post-ice period ($F_{[2,472]} = 139.5$, $p < 0.001$, $\eta^2 = 0.37$; Fig. 4A). During the pre-ice period, adult male locomotor activity was greater than that of adult fe-

Fig. 2. Water temperature (°C) and dissolved oxygen (%) measurements from six loggers at known northern map turtle (*Graptemys geographica*) overwintering site in Lake Opinicon, Ontario, from 7-Nov-2020 to 12-Apr-2021. Average water temperature and dissolved oxygen are indicated by solid lines; range of each measurement is shown with shading around line. Shaded area indicates the period of ice-on. Additional points included from random site samples of temperature and dissolved oxygen taken on the lake on 5-Dec-2020, 13-Feb-2021, and 20-Feb-2021.
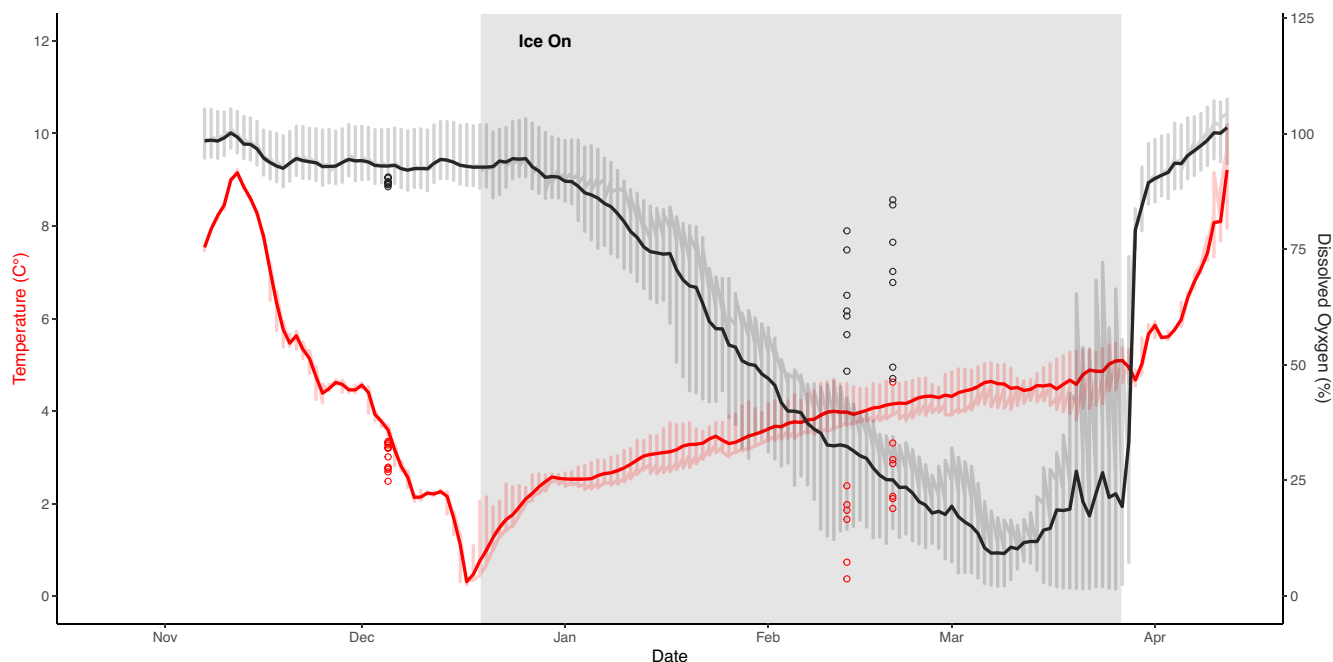
males ($t_{[2]} = 20.01$, $p < 0.001$) and less than that of juvenile females ($t_{[2]} = 15.85$, $p < 0.001$), while juvenile female locomotor activity was higher than that of adult female ($t_{[2]} = 2.377$, $p = 0.046$; Fig. 4A). During the post-ice period, adult males had greater locomotor activity than adult females ($t_{[2]} = 15.17$, $p < 0.001$) and juvenile females ($t_{[2]} = 16.99$, $p < 0.001$), while no difference in locomotor activity was detected for adult and juvenile females ($t_{[2]} = 16.36$, $p = 0.594$).

The videos gathered with the underwater camera confirmed that turtles are capable of substantial locomotor activity under the ice. On February 3, we recorded two active adult females (Video S1). One turtle entered the field of view during the recording and moved through half or the field of view before setting on a log. The other turtle was stationary but displayed repeated head and leg movements over the duration of the 10 min recording. The sequence of 11 recordings gathered over 50 days also show that northern map turtles move regularly under the ice (Fig. 6; Video S2). The number of turtles in the frame varied between zero and four with both adult females and males captured on camera in various combinations.

Depth and temperature use

Daily average water temperature use differed between demographic classes during the ice-on period ($F_{[2,3218]} = 43.72$, $p < 0.001$, $\eta^2 = 0.03$) (Fig. 3B). Adult females were observed at 1.0 ± 1.0 °C lower than both adult males (1.15 ± 0.98 °C; $t_{[2]} = 4.242$, $p < 0.001$) and juvenile females (1.4 ± 1.1 °C;

$t_{[2]} = 9.242$, $p < 0.001$). Adult males were observed at lower temperatures than juvenile females ($t_{[2]} = -5.069$, $p < 0.001$). The daily average temperature each demographic class was observed at during the ice-on period was lower than those seen in the pre-ice and post-ice periods (Fig. 4B). Adult females were observed at an average daily temperature of 4.7 ± 2.6 °C during the pre-ice period and 7.5 ± 3.3 °C during the post-ice period, adult males at 4.7 ± 2.6 °C pre-ice and 7.6 ± 3.2 °C post-ice, and finally juvenile females at 5.0 ± 2.5 °C pre-ice and 7.5 ± 2.7 °C post-ice. We did not detect any statistically significant differences in daily average temperature use during the pre-ice period when compared between demographic classes ($F_{[2,1836]} = 0.717$, $p = 0.488$; $\eta^2 = 0.0008$) or post-ice period ($F_{[2,472]} = 0.115$, $p = 0.891$, $\eta^2 = 0.0005$; Fig. 4B).

The daily average depths recorded during the ice-on period were shallower than those seen in the pre-ice and post-ice periods and differed between demographic classes during the ice-on period ($F_{[2,3218]} = 79.95$, $p < 0.001$, $\eta^2 = 0.05$; Fig. 3C). Adult females were observed shallower (1.34 ± 0.63 m) on average when compared with both adult males (1.52 ± 0.61 m; $t_{[2]} = 6.494$, $p < 0.001$) and juvenile females (1.7 ± 0.69 m; $t_{[2]} = 12.59$, $p < 0.001$; Fig. 4C). Adult males were observed at shallower depths than juvenile females ($t_{[2]} = -6.257$, $p < 0.001$; Fig. 4C). Adult females were observed at an average depth of 2.72 ± 0.92 m during the pre-ice season and 2.46 ± 1.16 m during post-ice. Adult males were observed at an average depth of 2.24 ± 0.74 m pre-ice and

Fig. 3. Observations of overall dynamic body acceleration (ODBA) (A), water temperature use (B), and depth use (C) for northern map turtle (*Graptemys geographica*) during the study period (24-Oct-2020 to 12-Apr-2021) at Lake Opinicon in Ontario. Results are separated as a reflection of demographic classes: adult females (*n* = 13), juvenile females (*n* = 9), and adult males (*n* = 11). Ice-on overwintering period for Lake Opinicon is represented with the shaded area between 19-Dec-2020 and 27-Mar 2020. No locomotor activity (ODBA = 0.02*g*) is indicated with a dashed line.
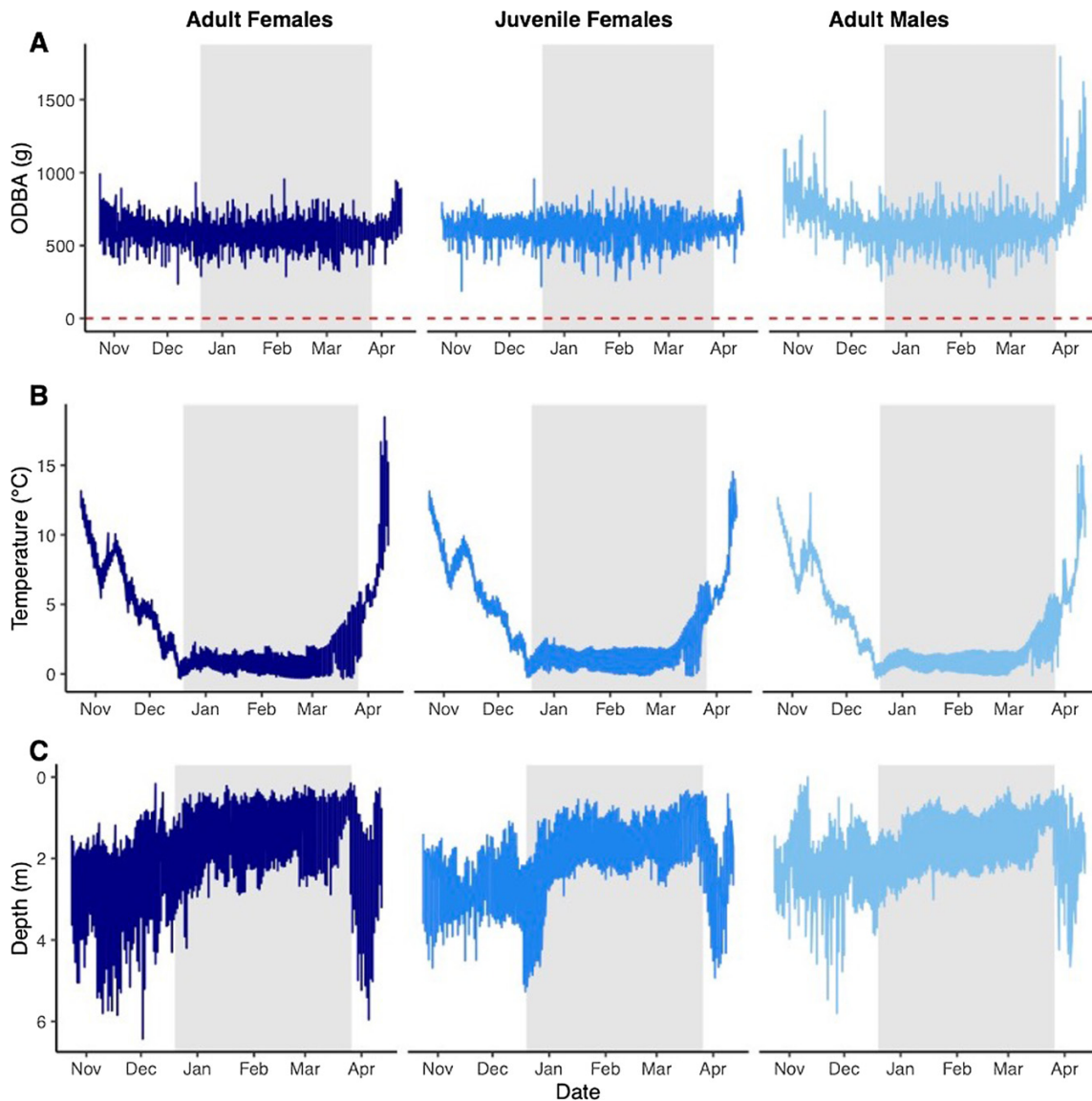
1.91 ± 0.88 m post-ice. Finally, juvenile females were observed at 2.63 ± 0.61 m pre-ice and 2.65 ± 0.97 m post-ice. Depth use differed between sexes in both the pre-ice ($F_{[2,1836]} = 80.22$, $p < 0.001$, $\eta^2 = 0.08$) and post-ice periods ($F_{[2,472]} = 21$, $p < 0.001$, $\eta^2 = 0.08$; Fig. 4C). In the pre-ice season, adult males were shallower than adult females ($t_{[2]} = -12.135$, $p < 0.001$) and juvenile females ($t_{[2]} = -9.124$, $p < 0.001$), while there was no difference between adult and juvenile females ($t_{[2]} = 1.950$, $p = 0.125$; Fig. 4C). In the post-ice season, adult males were once again shallower than adult ($t_{[2]} = -4.993$, $p < 0.001$) and juvenile females ($t_{[2]} = -6.039$, $p < 0.001$), while no difference was observed between adult and juvenile females ($t_{[2]} = 1.642$, $p = 0.228$) (Fig. 4C).

Discussion

In temperate areas, freshwater turtles primarily overwinter in water and can spend a third of their life trapped below ice. Despite the substantial amount of time some turtles spend under the ice, comparatively little is known about their behaviour during this part of their annual cycle. To address this gap, we used biologgers and under-ice videography to document winter locomotor activity, water temperature use, and depth use of overwintering northern map turtles near the northern edge of their range where they experience 119 days of ice cover per year on average. Locomotor activity was lower during the ice-on period, relative to pre-ice and post-ice periods, but northern map turtles remained active during the

Fig. 4. Seasonal differences in behavioural observations of overall dynamic body acceleration (ODBA) (A), water temperature use (B), and depth use (C) of northern map turtles (*Graptemys geographica*) across the study period (24-Oct-2020 to 12-Apr-2021) at Lake Opinicon in Ontario. Results are separated as a reflection of demographic classes: adult females ($n = 13$), juvenile females ($n = 9$), and adult males ($n = 11$). Similar letters indicate that there are no differences in behaviour between the respective groups for the given season, while differing letters indicate significant dissimilarities.
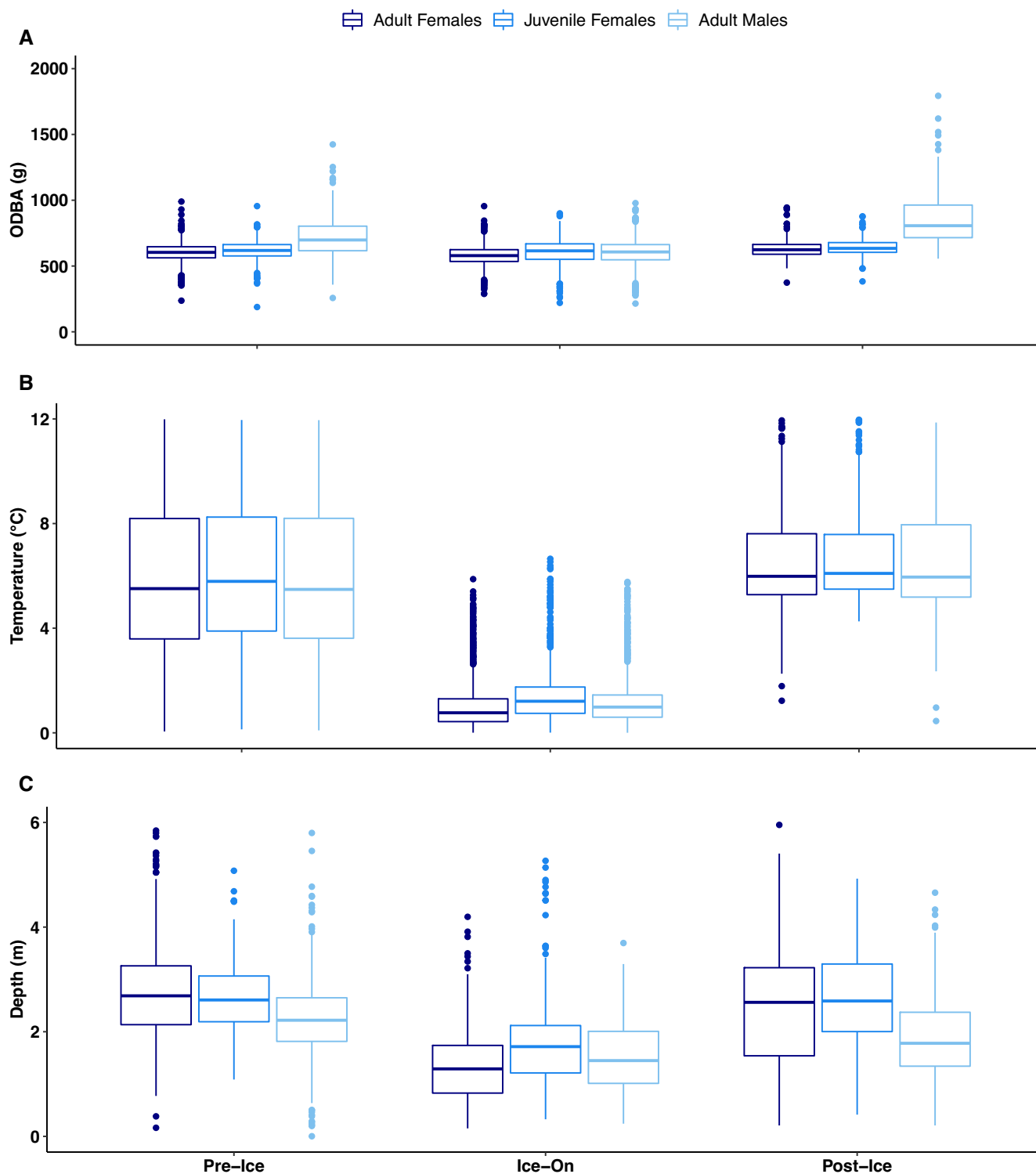
entire duration of their forced winter submergence. Video recordings show that overwintering northern map turtles are active enough to change their location on a regular basis in the winter. Some of the locomotor activity measured with the loggers thus likely corresponds to movement between locations within the overwintering site. While some aquatic ec-

Fig. 5. Northern map turtle (*Graptemys geographica*) overall dynamic body acceleration (ODBA) and temperature (°C) during the ice-on period (19-Dec-2020 to 27-Mar-2021) at Lake Opinicon, Ontario. Solid lines indicate local regression for adult females (*n* = 13), juvenile females (*n* = 9), and adult males (*n* = 11). Red points represent water temperature values from the last week of the ice-on period, which made the relationship with ODBA statistically significant. Zero locomotor activity (ODBA = 0.02*g*) is indicated with a dashed line.
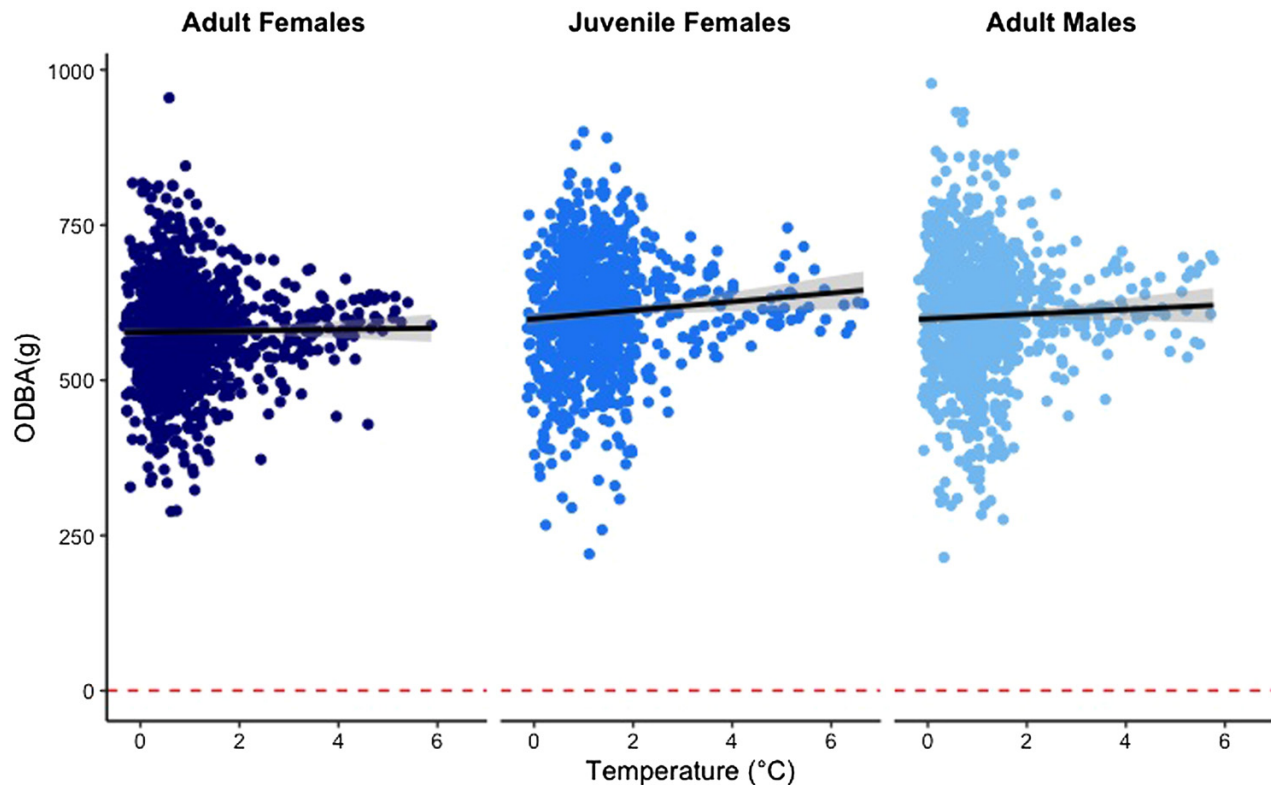
totherms (i.e., fish, amphibians, turtles) become dormant in winter (Ultsch 1989; Speers-Roesch et al. 2018), our results indicate that northern map turtles remain active throughout the winter. We thus suggest that locomotor activity is part of the winter survival strategy of northern map turtles.

Northern map turtles are considered anoxia intolerant relative to other freshwater turtle species (i.e., snapping turtles, painted turtles) and will die after 45 days when submerged in anoxic water at 3 °C (Ultsch and Jackson 1995; Reese et al. 2001). Previous studies have proposed several mechanisms by which northern map turtles remain aerobic and prevent acid-base or ionic disturbances in the blood stream, which can result in mortality (Maginniss et al. 2004). These include an improved efficiency of extrapulmonary gas exchange across the body relative to other species, and adaptations of the blood oxygen transport system (Jackson et al. 2001; Reese et al. 2001; Maginniss et al. 2004). Moreover, in one of our video recordings (Video S1), the turtle in the forefront depresses its lower jaw at a regular interval suggesting that they may also use buccopharyngeal gas exchange to remain aerobic.

In a laboratory study, Maginniss et al. (2004) observed reduced activity of northern map turtles under force submergence in hypoxic water (equivalent to 31% DO) at 3 °C. They suggested that hypoxic conditions trigger metabolic depression and thus reduced activity. Interestingly, we did not observe such a shift in activity with declining DO. The concentration of DO in the vicinity of the overwintering site declined throughout the ice cover period reaching hypoxic levels (<20%) by February 9 with 46 days of ice remaining. Yet the activity level of the turtles remained the same throughout the ice-cover period. The difference in response to hypoxia may be attributable to differences in body temperature. We found map turtles to overwinter at around 1 °C. The passive effect of temperature on metabolism and thus oxygen demand between 1 and 3 °C may be enough to allow map turtles to remain active even under hypoxic conditions. By maintaining locomotor activity, northern map turtles may be able to prevent the build-up of anoxic water around them, and therefore removing the need to resort to metabolic depression.

Locomotory activity was 1%–10% higher in the pre-ice period and 6%–30% higher in the post-ice period relative to the ice-on period for all northern map turtles (Fig. 3A), which coincided with warmer water temperatures and greater DO availability (Figs. 2 and 3B). Adult female locomotor activity was 4% lower than that of adult males and 5% lower than that of juvenile females. These small differences in activity may be linked to the marked sexual size dimorphism present in this species. Adult males and juvenile females (i.e., smaller individuals) may move more, simply because smaller individuals have a lower absolute need for oxygen as well as a greater relative surface area to obtain it from the water (Graham and Graham 1992). Thus, it may be easier for smaller turtles

Fig. 6. Northern map turtles (*Graptemys geographica*) under the ice between Jan 2022 and Mar 2022 at a known hibernation site in Lake Opinicon in Ontario captured on an underwater camera (Eyoyo Underwater Fishing Camera).

to meet their oxygen demand in winter. Locomotor activity may be riskier for adult females as the metabolism of submerged females at 2 °C is 3.7–4 times that of males (Graham and Graham 1992). The oxygen demand cost of locomotion combined with the risk of entering a microhabitat with less DO may constrain locomotory activity of females compared with males.

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

Northern map turtles were all observed at lower temperatures during the ice-on period relative to the pre- and post-ice periods (Fig. 3B). This observation can in part be attributed to the fact that temperatures are generally colder during this period of the year. More interestingly, all three demographic classes were observed at average daily temperatures near 1 °C during the ice-on period. Adult females were observed at marginally lower water temperatures relative to adult males and juvenile females. Like locomotor activity observations, these subtle differences in water temperature selection may be attributed to the difference in body size between demo-

graphic classes and therefore metabolic needs. Although water temperature use was statistically different between the observed groups, the biological significance of these differences is unclear because the absolute differences in temperature were small (<0.5 °C). Winter water temperature selection has been studied in many freshwater turtles (Brown and Brooks 1994; Litzgus et al. 1999; Crocker et al. 2000; Greaves and Litzgus 2007; Rollinson et al. 2008; Edge et al. 2009) but rarely in relation to sex. Due to the dramatic difference in body size seen in northern map turtles, small differences in temperature selection may translate in biologically meaningful differences in metabolism over several months. Quantifying metabolic rates at near-freezing temperatures would allow to assess the metabolic consequences of the temperature differences we observed in the field.

Regardless of the differences in water temperature use among demographic classes, all turtles remained at water temperatures near 1 °C. Other species of turtles have been reported to select water temperatures near 0 °C in winter (Brown and Brooks 1994; Litzgus et al. 1999; Crocker et al. 2000; Greaves and Litzgus 2007; Rollinson et al. 2008; Edge et al. 2009). Selecting colder water has the advantages of passively reducing oxygen demand through the effects of temperatures on metabolism and increasing oxygen availability due to the inverse relationship between DO and water temperature.

Along with locomotor activity and water temperature use, depth use also differed among groups (Fig. 3C). Adult fe-

males were observed at the shallowest depths followed by adult males and juvenile females. Depth use for all groups was shallower during the ice-on period relative to the pre- and post-ice periods. Depth use may be influenced by several factors, and the shift to shallower depths likely reflects a preference for lower temperatures and higher DO concentrations, given the cold water can hold more oxygen. The observed depth use may also be linked to substrate selection. Observations of northern map turtles at overwintering sites indicate that they often lie on rocks and logs or open sand/gravel substrates (Graham and Graham 1992; Fig. 1B). By lying atop hard substrates rather than burying themselves or settling in loose sediment, northern map turtles may keep their skin exposed and free of debris, which may be essential for extrapulmonary gas exchange.

Overall, this study revealed that northern map turtles remain active throughout their overwintering period. To our knowledge, this is the first direct demonstration that a turtle overwintering under the ice can remain active all winter using biologging technology. We also identified intraspecific behavioural differences across demographic classes, suggesting overwintering behaviour is influenced by sex and potentially driven by the sexual dimorphism observed in this species. The results of this study should be used to inform future studies related to ectotherm overwintering behaviour. Such information may be useful in identifying critical overwintering habitat and will continue to provide insights into the life history and fitness of freshwater turtles in the wild.

Acknowledgements

Article information

History dates

Copyright

Data availability
Summary data are available in the supplementary material. Full datasets and raw data are available upon request.

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Competing interests
The authors declare no conflict of interest.

Funding information

Supplementary material

Supplementary data are available with the article at https://doi.org/10.1139/cjz-2022-0100.

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