OVERWINTER ECOLOGY OF WESTERN PAINTED TURTLES

(*CHRYSEMYS PICTA BELLII*)

IN A CANADIAN RESERVOIR

by

AMY DIANE DUNCAN

Technical Diploma in Recreation, Fish & Wildlife, Selkirk College, 2008
Bachelor of Natural Resource Science, Thompson Rivers University, 2010

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE

REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN

ENVIRONMENTAL SCIENCE

Thesis Examining Committee:

Karl Larsen (PhD), Professor and Thesis Supervisor
Natural Resource Sciences, Thompson Rivers University

Brian Heise (PhD), Associate Professor
Natural Resource Sciences, Thompson Rivers University

Leigh Anne Isaac (PhD), Senior Wildlife Biologist
VAST Resource Solutions, Cranbrook, BC
Adjunct Faculty Member, Natural Resource Sciences, Thompson Rivers University

Virgil Hawkes (MSc), Vice President and Senior Wildlife Biologist
LGL Limited, Sidney, BC

External Examiner:

Ron Brooks (PhD), Professor Emeritus
Department of Integrative Biology, University of Guelph

Thompson Rivers University

Kamloops, British Columbia, Canada

March 2016

Thesis Supervisor: Professor Karl Larsen (PhD)

ABSTRACT

Overwintering is a critical life-history stage for species inhabiting temperate environments characterized by harsh, cold winters lasting up to half the year or longer. Ectotherms, such as herpetofauna, display behavioural adaptations in order to survive winters. During this period of their life history, those animals occupying fluctuating environments, such as reservoirs, may face additional challenges. I studied the overwinter ecology of a population of Western Painted Turtles (*Chrysemys picta bellii*) in a hydroelectric reservoir in British Columbia, Canada. My study built upon a preliminary study on the general ecology and population demographics of this population. This preliminary study identified variation in overwintering tactics between two water bodies within the reservoir – congregated and dispersive hibernation. The specific goals of my research were to (1) document more closely the variation in overwintering tactics occurring across the different subpopulations, (2) determine if the turtles in the subpopulations were experiencing and/or selecting different conditions during hibernation, and (3) determine if changes in reservoir levels during winter elicited response(s) in the turtles, and if so, examine how this relationship contributed to the use of different tactics. I focused on two locations within the reservoir that supported the overwhelming majority of turtles, and also included a small, isolated pond not influenced by the reservoir. By using radio telemetry to track turtles over two winters, my research identified variation in the overwintering tactics used between water bodies and years, and determined that overwintering tactics did not vary with water depth, temperature or dissolved oxygen. Turtles in all locations buried in the reservoir substrate for winter. Turtles displayed longer movements in winter than those documented in similar studies; however, these movements could not be correlated to changes in water levels. Body condition was comparable between the reservoir and reference water bodies yet population density and turtle size were notably higher in the non-reservoir location. In conclusion, this population of turtles exhibited behavioural plasticity in their overwintering tactics, and did not appear to be impacted by water level fluctuations in winter.

Keywords: Western Painted Turtle, *Chrysemys picta bellii*, overwintering, behavioural plasticity, hibernation, northern, fluctuating, reservoir

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

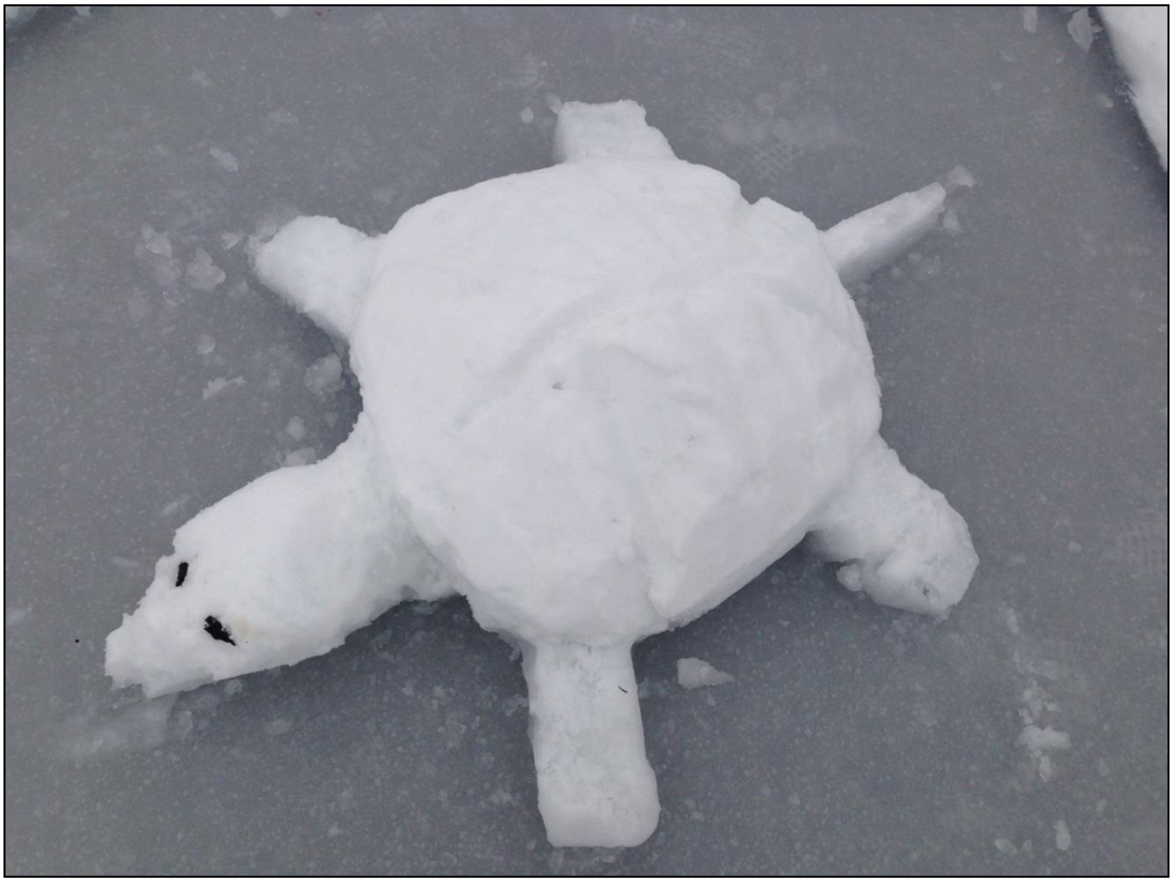
First and foremost, I'd like to thank my advisor, Dr. Karl Larsen, for his support, guidance, advice and for always making time for a beer with his grad students. Special thanks to Virgil Hawkes from LGL Limited, for partnering with me on this project and providing support, both advisory and financial. The Okanagan Nation Alliance also was a terrific organization to work with on this project, especially Dixon Terbasket, Natasha Audy and Al Peatt. Thanks as well to Doug Adama from LGL for his field time and expertise and to Janean Sharkey from LGL for volunteering on her day off. Thank you to my committee members, Dr. Brian Heise, Dr. Leigh Anne Isaac from VAST Resource Solutions, as well as Virgil Hawkes, I very much appreciate your time and encouragement.

Thank you for the great support from the faculty at Thompson Rivers University, including Jacqueline Sorenson, Shane Rollans, Lauchlan Fraser and Louis Gosselin. Thanks to field assistants Amber Merko and Eric Spilker and volunteer Mandy Ross for helping with turtling. Thanks to my fellow lab mates for the laughs, support and help when I needed it: Jo Anne Hales, Cheryl Blair, Sasindu Gunawardana, and Malcolm McAdie. I would also like to acknowledge the residents of Revelstoke who helped and supported this research: James Van Dam, Harvey Tarzwell and Steve Vandermeer. Innumerable thanks to my parents, John & Diane Leeming, for welcoming their daughter into their home once again to complete further studies.

Thanks to BC Hydro for making this project possible (Water Use Plan Project CLBMON-11B3). This research could not have been completed without their support and that of the Natural Sciences and Engineering Research Council of Canada. This work was completed in accordance with Thompson Rivers University Animal Care Committee Permit # 100451 and BC Ministry of Forests, Lands and Natural Resource Operations Wildlife Act Permit CB10-60676.

DEDICATION

This thesis is dedicated to my husband, Devon Duncan. This work would not be made possible without his unconditional love and support.



LIST OF FIGURES

LIST OF TABLES

Chapter One

INTRODUCTION TO THE THESIS

ANIMAL BEHAVIOUR IN RESPONSE TO ENVIRONMENTAL CHANGE

The study of animal ecology has long focused on the identification and evolutionary significance of various characteristics of species. In 1859, Charles Darwin put forth his hypothesis that variations within generations can become heritable if they contribute to the success of individuals (Darwin 1859). Variations can be seen both structurally and behaviourally and are considered *adaptive* in nature when a product of natural selection. The goal of studying variations in nature is to gain a better understanding of a species' ability or inability to respond to a given variable or condition, and to evaluate the efficiency of the response to the success of that species. Observations of variation over time can provide insight into a species' life history, which can enable ecologists to evaluate species and population health, identify impacts or challenges imposed upon an animal, and make decisions regarding species management and conservation. Recognition and exploration of such variations can be successful only with a clear understanding of the foundations of evolution, including common terminology.

Various terms have been used to refer to the evolution of animal behaviours; however some of these terms have come to be used almost synonymously in animal ecology, namely adaptation and plasticity, stability and canalization, strategy and tactic. As mentioned above, Darwin introduced the term *adaptation*, defined as "a modified part of an organism which performs a biological function for the organism and thus contributes to the organisms' state of adaptation" (Rose and Lauder 1996). An adaptation is relevant to more than just behaviour, and in terms of evolution, it involves a change in genotype (Ghalambor *et al.* 2007). *Plasticity* is a term easily confused with adaptation: it is "the ability of an organism to express different phenotypes depending on the biotic or abiotic environment (Agrawal 2001). The difference between plasticity and adaptation may be observed across environments: plasticity may appear through changes in phenotype across a variety of environments, whereas an adaptation is an evolutionary, genetic response to a change in environment. For example, species of arboreal snakes show differences in clutch size and in lateral ovary position compared to non-arboreal species (Pizzatto *et al.* 2007). Such differences can be considered an *adaptation* in response to environmental conditions because arboreal snakes tend to be more laterally compressed to enhance climbing ability and camouflaging in trees(Pizzatto *et al.* 2007). If, for example, these arboreal snakes exhibited an annual migration to a non-arboreal environment and their clutch size and morphology of the ovaries changed respective to the environmental change, these traits would then be considered plastic and not an adaptation. However, plasticity can *become* an adaptation, termed 'adaptive plasticity', when it "places populations close enough to a new phenotypic optimum for directional selection to act; plasticity that predictably enhances fitness and is most likely to facilitate adaptive evolution on ecological time-scales in new environments" (Ghalambor *et al.* 2007).

In contrast to the ideas of adaptation and plasticity are the terms 'canalization' and 'developmental stability'. The term canalization was coined by C.H. Waddington (1942) and *environmental canalization* is defined as "(the ability of) a genotype's phenotype to remain relatively invariant … when exposed to different environments" (Flatt 2005). Tantamount to the idea of canalization is *developmental stability*, which is known as the ability of an organism to successfully buffer itself against given environmental or genetic disturbances, or genotype-environment interactions (Markow 1995). 'Plasticity' is commonly understood to be the antithesis term to developmental stability (Bradshaw 1965).

A *strategy* is defined as "a set of (behavioural, morphological, physiological) traits that optimize (the) success of an individual under given local conditions" (Nakadera and Koene 2013). Methods of foraging and reproduction are commonly recognized strategies within the ecological literature. The term strategy is commonly confused with *tactic*, which is "a trait or set of traits serving a particular function" (Oliveira and Taborsky 2008). A tactic can be behavioural, morphological or physiological traits that aid in creating different phenotypes within a species (Oliveira and Taborsky 2008). Essentially, a strategy is an evolved response to a given variable with a desired end result, and a tactic is an action used to conduct a strategy (Figure 1-1). For example, one study showed intraspecific differences in the mating strategies of melanistic and non-melanistic male Pond Sliders (*Trachemys scripta*); larger melanistic males utilized tactics such as biting and chasing to acquire females, whereas

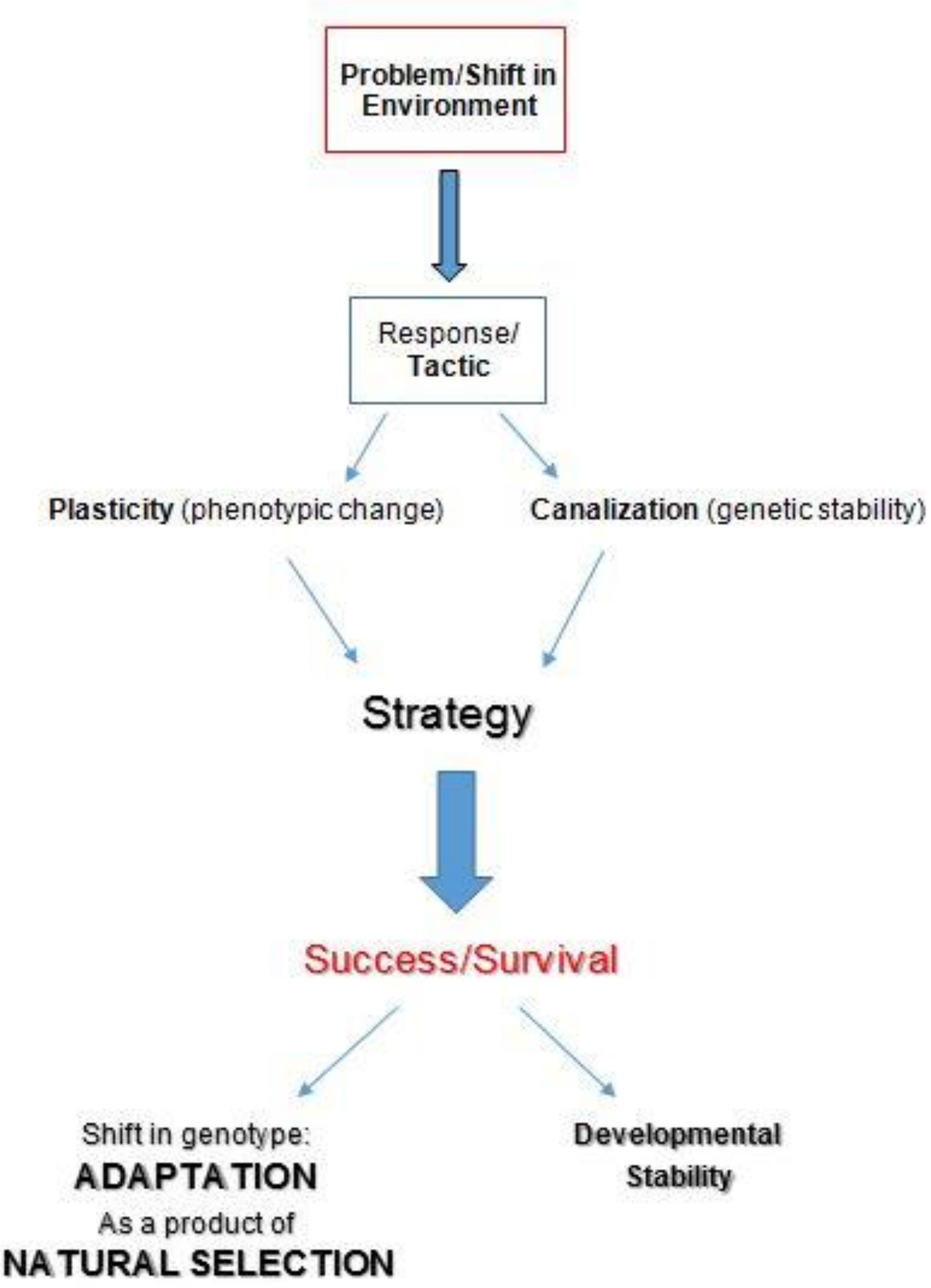


Figure 1-1: Flow chart showing the relationship between the terms tactic, plasticity, canalization, strategy and adaptation.

smaller non-melanistic males displayed titillation tactics (Thomas 2002). Thus, the end goal of the strategy is successful mating, and the tactics are the methods used to accomplish the goal.

Behavioural changes may be apparent across a range of species or populations in response to changing environmental pressures, both natural and anthropogenic (Foster and Endler 1999). Such changes challenge animal ecologists to understand whether animals can respond successfully to changes in their environment, through adaptation and/or plasticity, and whether tactics and strategies seen within a population are in fact allowing the animals to deal with perturbations or shifts in their environment. A good starting point is to document plasticity and whether it appears tenable to animals to cope with environmental changes; these situations can be seen among peripheral populations, where individuals occur at the limit of the species' range. These individuals tend to experience extreme conditions in comparison to conspecifics elsewhere and such situations offer a unique opportunity to investigate the causes for and results of shifts in behaviour.

OVERWINTERING IN REPTILES

Temperature is the main factor limiting the northern distribution of herpetofauna and influences many aspects of life, including hibernation and winter survival (review by Ultsch 1989, St. Clair and Gregory 1990). Availability of quality hibernacula is also thought to limit the northern extent of herpetofauna (Aleksiuk 1976, Prior and Weatherhead 1996, Greaves and Litzgus 2007, Paterson *et al.* 2012). Northern snakes, for example, are particular about site selection for hibernacula, and have preferences for site orientation, thermal properties, underground structure and microclimatic conditions (Prior and Weatherhead 1996, Gienger and Beck 2011). Freshwater turtles in northern environments are challenged by the duration and severity of winter;, adult turtles can survive hibernation for varying lengths of time (2-5 months, depending on species) (Reese *et al.* 2004, Ultsch *et al.* 1999, Newton and Herman 2009, Edge *et al.* 2009) and hatchling Painted Turtles can withstand limited bouts of freezing temperatures (St. Clair and Gregory 1990, Baker *et al.* 2003, Costanzo *et al.* 2008). Thus, herpetofauna in the north have adapted to prolonged periods of cold weather with a variety of behavioural and physiological adaptations (Aleksiuk 1976, Ultsch and Jackson 1982, review by Ultsch 1989, review by Jackson 2002, Reese *et al.* 2004, Costanzo *et al.* 2008, among others).

Hibernation (or overwintering) is a strategy used by herptiles to avoid unsuitable conditions (Storey and Storey 1990). Hibernation involves a reduction in metabolic activity, allowing the animal to be dormant for an extended period of time which maximizes survival time for optimal conditions. Some attribute this dormancy as a predator avoidance strategy during a sensitive period of the life cycle (Ultsch *et al.* 1985, Ultsch 2006). Animals in northern environments can experience longer hibernation periods because of the extended winter conditions at this latitude (Ultsch *et al.* 1985, Gienger and Beck 2011). Compared to endotherms, which are able to produce their own internal heat, ectotherms are restricted geographically by the limitations of their thermal physiology (St. Clair and Gregory 1990, Rosen 1991, Buckley *et al*. 2012) or their ability to tolerate freezing or anoxia in habitats that are iced over (review by Ultsch 1989).

Herpetofauna also possess a range of physiological adaptations that allow them to survive prolonged exposure to near- or below-freezing temperatures. Hibernation involves a 5-20 fold reduction in metabolism that reduces energy requirements and slows energy usage (Aleksiuk 1976, review by Storey 1996). Some herpetofauna, including species of hatchling turtles (Storey *et al*. 1988, Dinkelacker *et al.* 2005), adult turtles (Storey *et al.* 1993), frogs (Storey and Storey 1987, Layne and Kefauver 1997, Croes and Thomas 2000), toads (Swanson and Graves 1995), lizards (Halpern 1979) and snakes (Costanzo et al. 1988, Churchill and Storey 1992, Costanzo and Lee 1995), can supercool their body and thereby tolerate internal body temperatures below freezing for a limited amount of time. To do this, species utilize glucose and glycogen in their blood to synthesize cryprotectants that protect their cells and organs from damage from freezing (Croes and Thomas 2000). Other species also can withstand certain levels of internal freezing, termed freeze tolerance, where a portion of their body can freeze and recover upon thawing (Storey *et al.* 1988). Freeze-responsive genes aid reptiles in tolerating freezing in this state (review by Storey 2006). Finally, there are species that are able to employ both supercooling and freeze tolerance strategies, if required (review by Storey 2006). Those species that range across latitudes also may show physiological variation in hibernation between subspecies (Ultsch *et al.* 1985, Reese *et al.* 2004).

A key behavioural tactic to successful overwintering for northern herpetofauna is the selection of suitable hibernacula (review by Ultsch 1989, Rosen 1991, Taylor and Nol 1989, Swanson and Burdick 2010, among others). Herpetofauna can hibernate either terrestrially or aquatically, depending on the ecology and physiology of the species. Terrestrial hibernation involves either avoiding or tolerating a certain amount of body tissue freezing by selecting thermally buffered hibernation sites (Ultsch 1989, review by Storey 2006). For example, snake hibernacula are commonly found on rocky, south-facing slopes that maintain a warm ambient temperature through winter (Prior and Weatherhead 1996, Gienger and Beck 2011). Toads are known to burrow below the frost line to avoid freezing temperatures, and have been recorded as deep as 80 cm subsurface (in Swanson and Graves 1995). Aquatic hibernacula most often eliminate the chance of freezing, however a limiting factor is oxygen availability (review by Ultsch 1989), though some species can continue to utilize oxygen from the water column though winter (Greaves and Litzgus 2007).

Congregated hibernacula have been identified for a wide range of herpetofauna (Carpenter 1957, Brown and Parker 1976, review by Ultsch 1989, Meeks and Ultsch 1990, Lamoureux and Madison 1999, Litzgus *et al.* 1999, Newton and Herman 2009, Gienger and Beck 2011, among others), yet the reasons for this behaviour are not clear. Since herpetofauna are ectotherms, congregated hibernation will not provide body-heat benefits as endothermic hibernation does (see Czenze *et al.* 2013, for example). Research suggests congregated denning in ectotherms may be a response to limited or optimal denning site or a method of optimizing mating opportunities come spring (review by Ultsch 1989, Rollinson *et al.* 2008, Chen *et al.* 2012). Thus, congregated denning may be intentional in that the aggregations are brought about by selection for other traits more directly linked to fitness (i.e., fidelity to hibernacula, enhanced reproductive success etc.) (Gienger and Beck 2011).

Hibernation in Adult Canadian Freshwater Turtles

All adult freshwater turtles found in Canada hibernate aquatically, and can spend up to six months beneath the ice in an arrested state (review by Ultsch 1989). The Painted Turtle (*Chrysemys picta*), Map Turtle (*Graptemys geographica*), Blanding's Turtle (*Emydoidea* *blandingii*), Spotted Turtle (*Clemmys guttata*) and Wood Turtle (*Glyptemys insculpta*) are the freshwater turtle species found in Canada. Aquatically hibernating turtles can be separated into two groups: those that can withstand anoxia (anoxia-tolerant) and those who cannot (anoxia-intolerant). Anoxia tolerance is a factor limiting the northern range of freshwater turtles, as those species that can tolerate anoxia can be found relatively farther north (Reese *et al.* 2002). In these species, temperature is the main cue for submergence and emergence from hibernation for both adults and hatchling turtles (review by Ultsch 1989, Crawford 1991, Litzgus *et al.* 1999). Adults that overwinter aquatically utilize a variety of behavioural and physiological adaptations to withstand near freezing temperatures and anoxia.

Aquatic Hibernation

In addition to responding to near- or below-freezing temperatures, freshwater turtles that hibernate aquatically also must deal with reduced or depleted oxygen levels. Some turtles hibernate aquatically but remain within the water column and continue to utilize dissolved oxygen in the water as required; these turtles are known as ‘anoxia intolerant’. Although these animals do not have direct access to air, they are still able to utilize oxygen from water and complete aerobic glycolysis to maintain metabolism and function (Crocker *et al.* 2000). Other turtles bury into the mud or bottom substrate and settle themselves into an environment void of oxygen; being referred to as ‘anoxia tolerant’ species (Belkin 1963, review by Ultsch 1989). These turtles have different physiological adaptations that allow them to withstand the absence of oxygen to body tissues (hypoxia). These adaptations allow turtles to overwinter underwater and avoid harsh conditions and terrestrial predators.

Turtles that overwinter in oxygenated waters (normoxic) are able to conduct oxygen exchange via aerobic metabolism in the cells (Graham and Forsberg 1991, Crocker *et al.* 2000, Maginniss *et al*. 2004). Under these conditions, the primary avenue for oxygen uptake is through the integument, and also to a lesser extent through the cloaca and the buccopharyngeal cavity (Jackson *et al*. 2004). Reese *et al.* (2001) credited metabolic depression with allowing Map Turtles (*Graptemys geographica*) to stay submerged in normoxic water for five months while showing no signs of lactate accumulation or a decrease in blood pH. Additionally, an increase in blood hemoglobin concentrations in Map Turtles submerged in normoxic water correlated with an increase in the blood oxygen (O_2) carrying capacity (Maginniss *et al*. 2004). In this species, extrapulmonary oxygen uptake is sufficient to supply resting metabolic rates and elimination of carbon dioxide (CO_2) over winter (Reese *et al*. 2001). Wood Turtles also have been observed surfacing during winter, and it is thought that extrapulmonary gas exchange supplemented with aerial uptake further aids the animal in avoiding anaerobiosis (Graham and Forsberg 1991).

Anoxia-tolerant turtles rely on anaerobic glycolysis for energy while overwintering in anoxic (non-oxygenated) environments, that results in an accumulation of acid metabolites within the body and a depletion of stored substrates (Jackson 2000, Jackson 2002, Reese *et al.* 2002). In order to survive the resulting effects, turtles depress their metabolism to a substantially reduced rate that delays resource depletion and slows acid build-up in the blood. Cellular responses to this metabolic depression show a coordinated down-regulation in ATP production and utilization rates that is thought to be made possible by a mechanism known as channel arrest. Channel arrest slows the passive flux of ions through membrane channels, yet still allows required ions to reach the brain (review by Jackson 2002). A large amount of glycogen or glucose is required to supply energy during anoxia that turtles store within their liver and muscle prior to hibernation (Jackson 2002).

Lactic acid is a byproduct of ATP hydrolysis, and after prolonged submergence in an anoxic environment, turtles can show very high lactate levels in their blood (Jackson 2000, Reese *et al.* 2002). The resulting amount exceeds the normal buffering ability of the body, and methods of supplemental buffering are required. Painted Turtles have very high concentrations of bicarbonate ions (HCO_3-) in extracellular fluids that are the initial buffer in response to acid accumulation. In addition, anoxia-tolerant freshwater turtles have the ability to mobilize calcium (Ca^{2+}), potassium (K^+) and magnesium (Mg^{2+}) ions from their shell and skeleton to buffer plasma lactate accumulation; the elevated calcium levels have also shown a small effect in protecting the heart muscle from severe acidosis (Jackson *et al*. 2000, review by Jackson 2002, Reese *et al.* 2002, Reese *et al*. 2004). The species best known for withstanding anoxia is the Western Painted Turtle (*Chrysemys picta belli*). This species is able to withstand hypoxia for up to 5 months at 3°C in controlled lab conditions, the longest duration of all northern freshwater turtles (Ultsch and Jackson 1982).

THE PAINTED TURTLE (*CHRYSEMYS PICTA*) – ECOLOGY AND CONSERVATION

Three of the four subspecies of *Chrysemys* are found within Canada: the Eastern Painted Turtle (*C. p. picta*), the Midland Painted Turtle (*C. p. marginata*), and the Western Painted Turtle (*C. p. bellii*) (Figure 1-2). The Southern Painted Turtle (*C. p. dorsalis*) is found only within the United States. The *Chrysemys* species is the only Northern American turtle whose range spans across the continent (Ernst and Barbour 1989, Ernst *et al*. 1994). Painted Turtles are easily identified by their olive carapace, the orange-red coloring on their plastron and the yellow lines along their head and tail (COSEWIC 2006) (Figure 1-3). This species exhibits sexual dimorphism where the female is larger in size than the male (COSEWIC 2006).

Painted Turtles prefer shallow, slow-moving aquatic habitats with soft bottoms including ponds, marshes, sloughs, lakes, oxbows and streams (Ernst *et al.* 1994, COSEWIC 2006). Terrestrial habitat is also essential for female Painted Turtles that may travel up to 100 m inland to find suitable nesting sites (Baldwin *et al.* 2004). Painted Turtles rely on submerged logs, rocks, mud banks, and shoreline for basking (COSEWIC 2006, Schiller and Larsen 2012).

Painted Turtles court in spring and fall, and it is thought that effective mating occurs in fall (Gibbons 1968). Nesting takes place from May into early July (Mahmoud 1968). Females are considered sexually mature in northern climates between 12 to 15 years of age, whereas males are considered mature between eight to ten years (COSEWIC 2006). Female Painted Turtles have the ability to store sperm for up to three years, and are also reported to be promiscuous, resulting in mixed-paternity clutches in the same and between years (Pearce and Avise 2001). Females can lie between 4 – 20 eggs per clutch, averaging 12 (Ernst and Barbour 1989, Ernst *et al*. 1994). Turtle growth can vary by year, as growth rates are dependent upon air and water temperature, amount of rainfall, and food availability (Ernst *et al.* 1994). In the north, Painted Turtles can hibernate up to seven months, which results in a very short growing season (approx. 90 days) (Ernst *et al.* 1994).

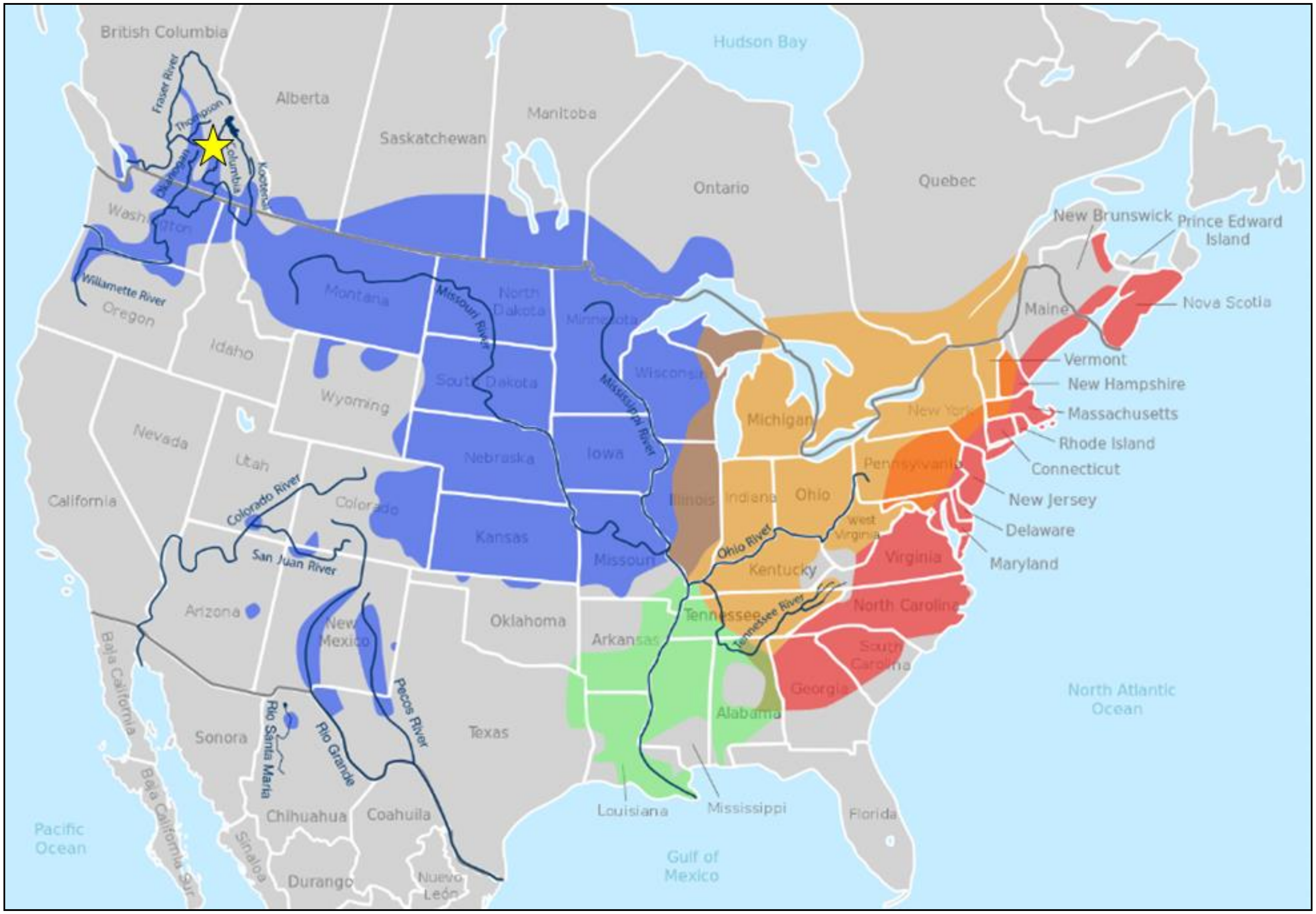


Figure 1-2: Range map for Painted Turtles. Blue indicates Western Painted Turtle (*C.p. bellii*) range, orange indicates ranges of Midland Painted Turtle (*C.p. marginata*), red indicates range of Eastern Painted Turtle (*C.p. picta*) and green indicates range of Southern Painted Turtle (*C.p. dorsalis*). The yellow star indicates the approximate location of the study population. Map adapted from Wikipedia (2016).

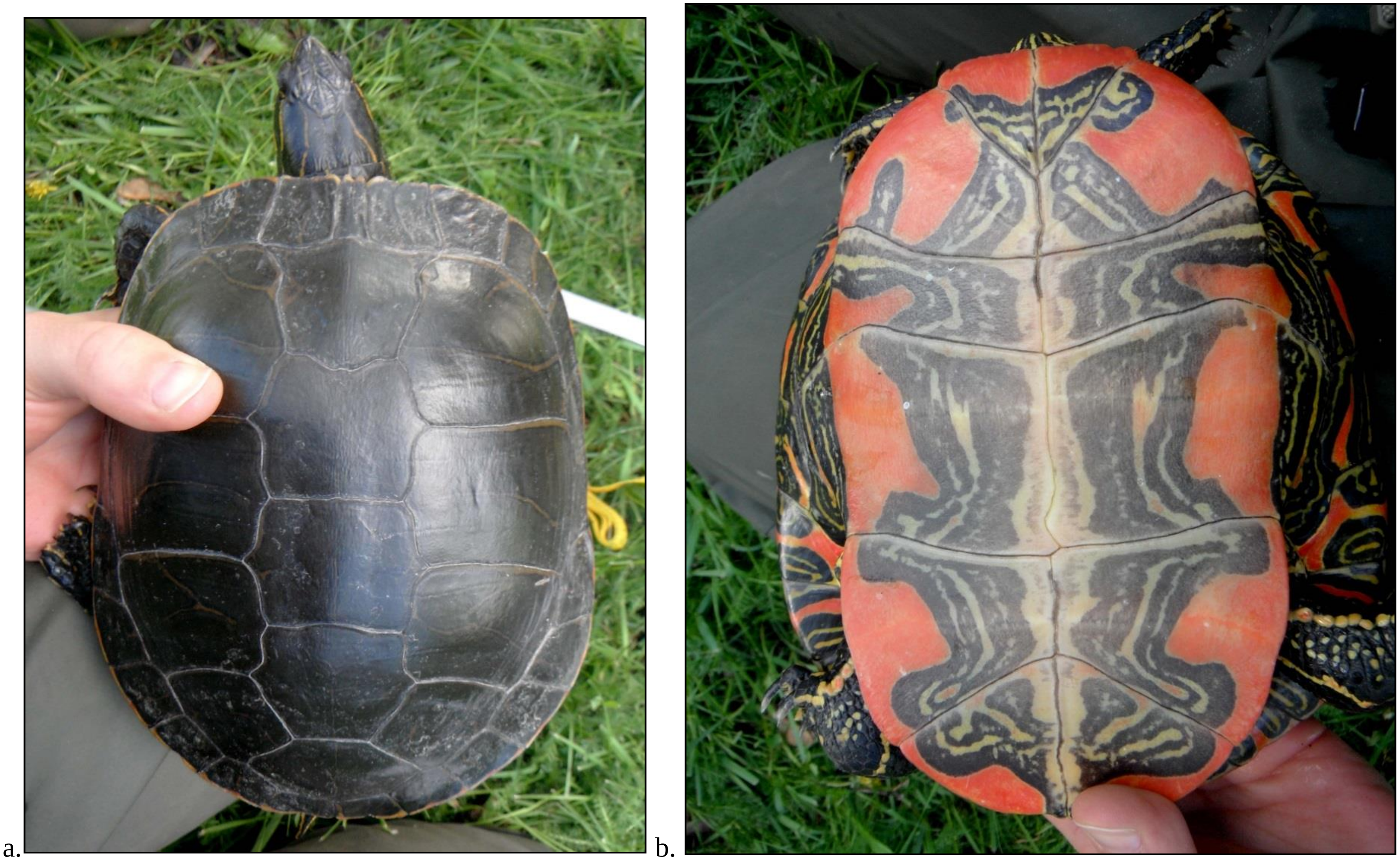


Figure 1-3: Morphology of the Western Painted Turtle (*Chrysemys picta bellii*) showing (a.) carapace and (b.) plastron (photos by author).

Painted Turtles are diurnal, meaning they are active in the day and rest at the bottom of a water body at night (Ernst *et al*. 1994). In the morning, Painted Turtles will bask to raise their body temperature before foraging. Painted Turtles tend to forage at temperatures between 15-30°C, otherwise they spend their time basking or in estivation (Ernst 1972, Ernst *et al*. 1994). They are considered omnivorous generalists and most commonly feed on algae, fish, frogs and invertebrates. Juveniles are more carnivorous than adults, as Painted Turtles tend to become more herbivorous as they age (Ernst *et al.* 1994).

A number of threats oppose the Painted Turtle including wetland loss and degradation due to anthropogenic influence, resulting in water pollution, riparian vegetation loss, bank erosion, and habitat fragmentation (COSEWIC 2006). Road traffic also poses a threat to Painted Turtles, especially nesting females (Schiller and Larsen 2012; COSEWIC 2006). Painted Turtles of all ages can be predated by a number of species, including, but not limited to: otters, raccoons, badgers, coyote, bears, herons, skunks, crows, ravens and raptors (Ernst *et al.* 1994, COSEWIC 2006, Duncan pers. obs.) Painted Turtles are especially sensitive to drought which can reduce population sizes through mortality and emigration (Lindeman and Rabe 1990). Winterkill can result from ponds drying in winter (Christiansen and Bickham 1989). Additional threats to winter survival include the potential of freezing and/or lactic acidosis from prolonged submergence in anoxic water (Ultsch 2006).

Painted Turtle Adaptations for Overwintering

Physiological

Painted Turtles possess a suite of characteristics that enable them to survive in northern environments (Ultsch and Jackson 1982, Ultsch *et al.* 1999, Reese *et al.* 2000, review by Ultsch 1989). Three main physiological strategies are utilized by Painted Turtles to survive overwintering within an anoxic environment: (1) a depression in metabolic processes, (2) mobilization of buffering ions from the shell and skeleton to neutralize lactic acid accumulation in the blood, and (3) the lactic acid “sink” effect of the turtles’ shell and bones (Jackson 2002). All species of Painted Turtle have shown the ability to survive in normoxic (oxygenated) conditions for up to 150 days of submergence (Reese *et al*. 2004). When overwintering in normoxic environments, Western Painted Turtles have the ability to utilize both anaerobiosis and aerobiosis which further helps reduce plasma lactate concentrations (Ultsch *et al*. 1985; Reese *et al*. 2004).

In addition to metabolic depression and plasma lactate buffering, Painted Turtles can also store excess lactic acid in the bones and shell (Jackson *et al.* 2000, Jackson 2002). Prolonged anoxia results in increased lactate concentrations in the shell and bones, reaching similar levels to those measured in plasma. It is estimated that 40-45% of total body lactate resides within the bone and shell during winter, and during recovery from anoxia in spring, lactate levels drop in the shell and bones, and in plasma (Jackson *et al.* 2000, Jackson 2002). It is suggested that other species of anoxia tolerant freshwater turtles may also have this ability (Reese *et al*. 2002).

Of all *Chrysemys* subspecies, the Western Painted Turtle exhibits the greatest ability to buffer lactate accumulation and can survive the longest duration of submergence in anoxic waters (Reese *et al*. 2001, Reese *et al.* 2004, Jackson 2002). When Western Painted Turtles are placed in normoxic conditions to simulate hibernation, plasma lactate concentrations remain lower than when the animals are submerged in anoxic environments. This suggests that Western Painted Turtles have the ability to combine anaerobic and aerobic metabolism mechanisms under contrasting environments (Ultsch and Jackson 1982; Reese *et al*. 2004).

Behavioural

Painted Turtles use a wide variety of sites for hibernation. They overwinter in small lakes and ponds that freeze over entirely in winter, but can also be found in streams (COSEWIC 2006). The total water depth of Painted Turtle overwintering spots tends to be 20-100 cm depth beneath the ice (Taylor and Nol 1988, St. Clair and Gregory 1990). Painted Turtles have been recorded to bury into anoxic substrate up to 45 cm depth, but also have been observed overwintering atop the substrate within a normoxic water column (Taylor and Nol 1988, St. Clair and Gregory 1990, Crocker *et al.* 2000, Rollinson *et al.* 2008). It is thought that Painted Turtles bury into substrate to avoid predators such as otters and muskrats that remain active during winter (Ultsch *et al.* 1985, Ultsch 2006). When buried, the turtles remain at a slightly higher and more stable temperature than they would in the water column, which is preferable to maintain dormancy. Temperatures at overwintering spots measure between 3°C and 6.3°C (Peterson 1987, Taylor and Nol 1989, Crawford 1991, Rollinson *et al.* 2008), and dissolved oxygen levels can vary from normoxic (13.6 mg/L) to anoxic (0.1 mg/L) during hibernation (Rollinson *et al.* 2008).

The hibernating behaviours of Painted Turtles have primarily been studied in natural water bodies, whereas anthropogenic environments (especially those subject to change) may pose additional challenges. Hydroelectric operations that are managed for power generation tend to follow a general trend in water level manipulation, and can range up to 20 m difference throughout the annual cycle. Populations of Painted Turtles have established in locations that have been manipulated for human-use, such as hydroelectric reservoirs, but the animals' methods of dealing with these additional pressures in winter have not been studied.

RESERVOIR ENVIRONMENTS

Fluctuating, anthropogenic environments such as hydroelectric reservoirs provide an interesting backdrop to study how species respond behaviourally to changes in habitat. Hydroelectric reservoirs can display characteristics similar to both lotic and lentic environments, and can often be intermediate of the two (Nogueira *et al.* 2012). Reservoirs are characterized by three different water level phases dictated by hydroelectric operations as a result of anthropogenic demand and seasonal precipitation regimes. The three water level phases reservoirs typically exhibit are designated as the maximum level phase, emptying phase and minimum level phase (Geraldes and Boavida 2005). These phases generally follow the same annual cycles, and depending on their location, can reach peak water levels in summer (July/August), emptying through the winter and into spring, reaching the lowest levels in March/April when spring freshet begins to refill the reservoir once again. Ecological processes within reservoirs are dependent upon and/or at the mercy of these varying phases, and species that inhabit reservoir environments must be adaptable to such conditions.

A primary threat identified for freshwater turtle populations residing in reservoirs is the reduction or elimination of nesting sites and basking habitats during the active season (Gardner and Oberdorster 2010). In winter, however, most lakes and reservoirs at northern latitudes are covered with a thick sheet of ice. Though many reservoirs maintain a certain level of water flow beneath the ice, this is restricted by hydroelectric operations, and many sections of a reservoir can become stagnant during this period. This results in a decreased concentration of dissolved oxygen, termination of gas exchange between water and the atmosphere, and a restriction of light penetration (Puklakov *et al.* 2002, Prowse *et al.* 2007). Additional water level fluctuations in winter can alter the reservoir environment and pose unique challenges to inhabiting animals, including freshwater turtles. Studying turtles in these environments will help us understand both the propensity of these animals to deal with a suite of overwinter conditions, and the implications for conservation and resource management.

THESIS OBJECTIVES

For my thesis, I examined in detail the overwintering ecology of Western Painted Turtles (*Chrysemys picta bellii*) in the Revelstoke Reach Reservoir near Revelstoke, British Columbia, Canada (50.9981° N, 118.1956° W) in the winters of 2012-13 and 2013-14. This population is faced with unique circumstances for overwintering; not only are they one of the most northerly populations of turtles in Canada, but they inhabit a fluctuating water reservoir controlled by upstream hydroelectric operations.

The goal of my research was to closely examine the habitats used by overwintering Western Painted Turtles within this fluctuating, northern environment. A key component of my research was to investigate the apparent variation in overwintering tactics used by these animals (congregated and dispersed) within the reservoir, and to determine if this variation was out of necessity caused by local environmental conditions (lack of overwintering habitat, hydroelectric influence), or ‘selection’ for optimal overwintering habitat characteristics. From an applied standpoint, my work also sought to determine the potential impacts to overwintering turtles as a result of hydroelectric operation and provide recommendations for managing fluctuating reservoir environments.

These animals are of particular interest because the species is recognized in British Columbia as ‘blue-listed’ (vulnerable) and this population occurs within an anthropogenic environment near the northernmost extent of the species’ range (COSEWIC 2006). These conditions likely act to constrain a number of viable life-history strategies (including overwintering) occurring at this location. This provided a unique opportunity to examine causes and effect of different overwintering behaviours in this species.

STUDY AREA AND PREVIOUS RESEARCH

I conducted my work within the reservoir on the Columbia River near Revelstoke, British Columbia, Canada (50.9981° N, 118.1956° W). The Columbia River is the largest river in the Pacific Northwest and the fourth largest river in North America (Osbourne 2012). The hydraulic head of the Columbia River drops 820 m from its headwaters at Columbia Lake to its entrance into the ocean, making it a desirable river for hydropower generation. Beginning in the late 1960s the Columbia River was fragmented first with the implementation of the Hugh Keenleyside Dam (1968) and later with the Revelstoke Dam (1984), which then created the Arrow Lakes Reservoir (BC Hydro 2007). This reservoir lies between the Monashee and Selkirk Mountains spanning approximately 240 km from Revelstoke, British Columbia (BC), Canada south to Castlegar, BC. The Arrow Lakes Reservoir is separated into the Upper and Lower Arrow Lakes and is licensed to operate between 418.64 MASL (meters above sea-level) and 440.1 MASL with a maximum allowable level as approved by the Comptroller of Water Rights of 440.75 MASL (BC Hydro 2007). Revelstoke Reach, where my study sites were located, is a subsection of the Upper Arrow Lakes section of the Columbia River that is located immediately south of the town of Revelstoke (50.9981° N, 118.1956° W), and approximately 20 km south of the upstream Revelstoke Dam.

The climate of the Columbia Basin is variable by year and by season and can also vary geographically due to the terrain and elevational gradients throughout the Basin. The Revelstoke area is characterized by a humid continental climate with no dry season. Precipitation falls most often as snow during the cold months from approximately mid-November to early March, though Revelstoke does receive a fair amount of rain in the warm season as well that can contribute to small peaks in run-off. Snow packs provide the dominant source of run-off into the Columbia Basin, and peak run-off occurs from May to early July (BC Hydro 2007). The reservoir is characterized by rapid infill in spring with a drop in water levels through August that is dependent by year and may be held back for fall storage (Hawkes *et al.* 2013). The Revelstoke Reach Reservoir reaches its lowest water levels in winter/early spring, and begins to fill again come spring freshet (Hawkes *et al.* 2013).

Mean monthly air temperature (°C) followed the same trend as the 30-year mean in both study years, except the lows recorded in February 2014 (Figure 1-4). The winter of 2013 saw less precipitation than the 30-year mean, though did receive more precipitation than the long-term mean in the months of March and June (Figure 1-4). Similarly, the months of 2014 received less precipitation than the long-term mean, except for March. The reservoir followed a predictable trend over both winters compared to the 10-year mean, and reached its lowest point in February of both years (Figure 1-5).

Previous to the present study, Basaraba (2014) conducted a two-year pilot study on the same population of turtles, primarily focusing on summer ecology. This provided a foundation for my work in terms of habitat use, seasonal movement, nesting success and demographics. Basaraba estimated a population size within the reservoir of 242 adult and juvenile turtles. She identified six nesting sites located a maximum of 80 m from shoreline, and observed females lay only one clutch per year. Basaraba determined that individual turtles occupied an average of 72 ha of habitat space and moved an average of 59 m per day. Although Basaraba observed a change in basking substrates as water levels rose during summer, she did not determine any adverse effects from the fluctuating water table (reservoir operations) on this population. Basaraba did monitor a small number of turtles over winter and detected variations in the spatial orientation of overwintering sites used by reservoir subpopulations; in one location (Montana Slough) turtles congregated in a floating mass of vegetation to overwinter, and in a separate location (Airport Marsh) turtles overwintered scattered across a large area.

In keeping with the Basaraba study, I focused my work on the two main subpopulations of turtles in the reservoir (Airport Marsh and Montana Slough - see Figure 1-6). To provide comparative data, I also focused on Turtle Pond (TP), a small isolated body of water (approximately 2.74 ha in size and 1 km from the reservoir) that supports a relatively large number of turtles (>100 turtles, Tarswell 2013, pers. comm.) (Figure 1-6). The Airport Marsh and Montana Slough sites are both easily influenced by changes in water elevation, whereas Turtle Pond is an isolated pond that is unaffected by changes in the reservoir. Cartier Bay is an additional location within the reservoir (Figure 1-6) where Basaraba detected a

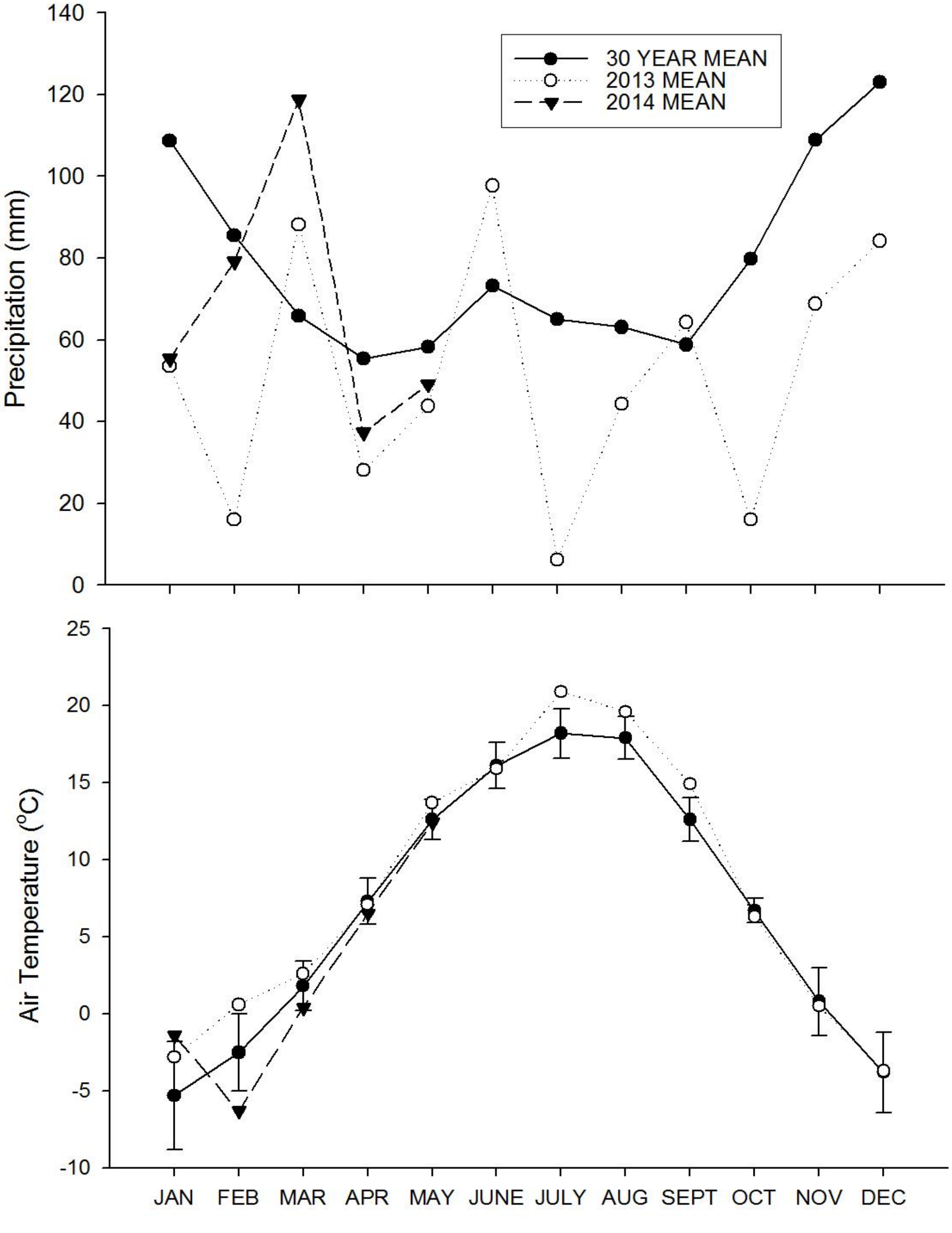


Figure 1-4: Mean monthly temperature (°C) and precipitation (mm) for Revelstoke, BC for study periods Jan - Dec 2013 and Jan - May 2014 compared to the 30-year mean (1971-2000, Environment Canada 2014). Standard deviation error bars available for 30-year mean temperatures only.

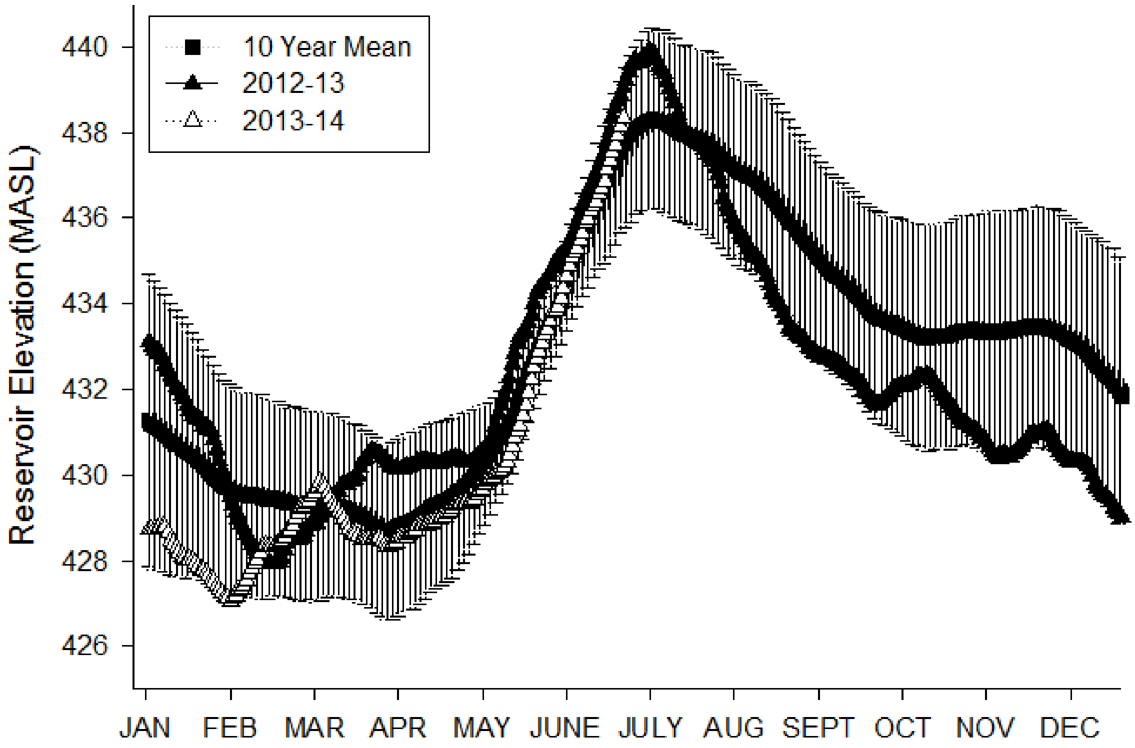


Figure 1-5: Annual reservoir elevations (MASL) in both study years compared against the 10-year mean (±SD) (BC Hydro data retrieved from LGL Ltd.).

Figure 1-6: Revelstoke Reach showing study locations Airport Marsh, Montana Slough, Williamson Lake and Turtle Pond, also showing non-overwintering location Cartier Bay, Revelstoke Reach, BC, Canada.

small number of turtles (6 sightings) during summer. To date, there is no evidence of animals overwintering in this water body and it was therefore excluded from my study. Additionally, Williamson Lake supports a small number of turtles; however sufficient data were not collected to include this location in my study.

Additional Details on Water Bodies in Study

Airport Marsh is approximately 130 ha in size and sits at an elevation of 437 MASL, the highest of all the study sites (Figures 1-7 and 1-8). My research area in AP spanned 1.9 km N-S and approximately 900 m E-W at the widest point. This area is bordered by the Revelstoke Airport runway (Figure 1-6), and my sampling area did not exceed this boundary. This site is characterized by bulrush (*Schoenoplectus tabernaemontani*), common cattail (*Typha latifolia*), milfoil (*Myriophyllum spp*.), pondweed (*Potamogeton spp*.) and reed canary grass (*Phalaris arundinacea*) (Basaraba 2014).

Montana Slough is the farthest south study site (approximately 2.5 km south of Airport Marsh) and lies at an elevation of approximately 432 MASL (Figures 1-9 and 1-10). This site is characterized by a massive, floating vegetated island that changes size relative to reservoir water levels. My research area at this location was approximately 43 ha in size. At full pool (July/August), only small sections of the island remain above water, and in low water the land mass extends far west toward the main channel of the Columbia River. The floating island is typified by moss (*Sphagnum spp*.), willows (*Salix spp*.), sedge (*Carex spp*.) and reed canary grass (Basaraba 2014), with some rooted coniferous trees (*Pinus spp*.) surviving as well.

Turtle Pond is a small isolated pond nestled within a residential area just off of Airport Way (Figure 1-11 and 1-12). It is surrounded by private residential property and resort land owned and managed by Revelstoke Mountain Resort. Turtle Pond is approximately 6.5 ha in size, spanning 460 m length (N-S) and 175 m at its greatest width (E-W). Turtle Pond is at a higher elevation than both Airport Marsh and Montana Slough, at 443 MASL. In summer, the dominant vegetation at Turtle Pond is Yellow Pond Lily (*Nuphar luteum*), with willows (Salix spp.) and cottonwoods (*Populus spp*.) bordering the water body.

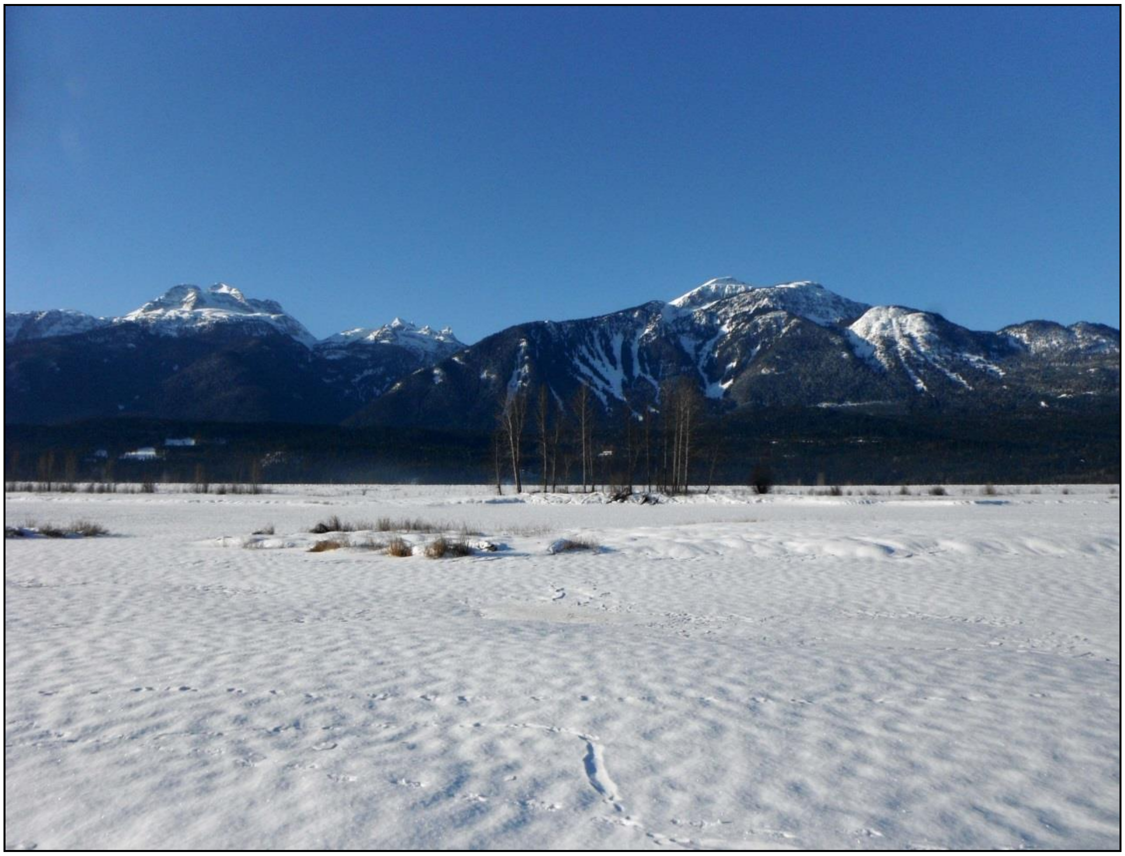


Figure 1-7: Airport Marsh, February 2014. Photo by author.

Figure 1-8: Airport Marsh, May 2014. Photo by author.

Figure 1-9: Montana Slough, February 2014. Photo by K. Larsen.

Figure 1-10: Montana Slough, June 2013. Photo by author.

Figure 1-11: Turtle Pond, February 2013. Photo by N. Audy.

Figure 1-12: Turtle Pond, May 2014. Photo by author.

LITERATURE CITED

Agrawal, A.A. 2001. Phenotypic Plasticity in the Interactions and Evolution of Species. Science. 294(5541): 321-326.

Aleksiuk, M. 1976. Reptilian hibernation: evidence of adaptive strategies in *Thamnophis sirtalis patietalis*. Copeia. 1976(1): 170-178.

Baldwin, E. A., Marchand, M.N. and Litvaitis, J.A. 2004. Terrestrial habitat used by nesting painted turtles in landscapes with different levels of fragmentation. Northeastern Naturalist. 11(1): 41-48.

Basaraba, N. 2014. The Ecology of Western Painted Turtles (*Chrysemys picta bellii*) in a Northern Canadian Reservoir. MSc Thesis. Thompson Rivers University. 110 p.

Baker, P.J., Costanzo, J.P., Iverson, J.B. and Lee Jr., R.E. 2003. Adaptations to terrestrial overwintering of hatchling northern map turtles, *Graptemys geographica*. Journal of Comparative Physiology B. 173(8): 643-651.

BC Hydro. 2007. Columbia River Project Water Use Plan. Revised for Acceptance by the Comptroller of Water Rights. BC Hydro Power Corporation. Burnaby, BC. 65 p.

Belkin, D.A. 1963. Anoxia: Tolerance in Reptiles. Science. 139(3554): 492-493.

Bradshaw AD. 1965. Evolutionary significance of phenotypic plasticity in plants. Advances in Genetics. 13:115-155.

Brown, W.S. and Parker, W.S. 1976. Movement ecology of *Coluber constrictor* near communal hibernacula. Copeia. 1976(2): 225-242.

Buckley, L.B., Hurlbert, A.G. and Jetz, W. 2012. Broad-scale ecological implications of ectothermy and endothermy in changing environments. Global Ecology and Biogeography. 21: 873-885.

Bury, R.B. and Germano, D.J. 2003. Differences in habitat use by Blanding's Turtles, *Emydoidea blandingii*, and Painted Turtles, *Chrysemys picta*, in the Nebraska Sandhills. American Midland Naturalist. 149:241-244.

Carpenter, C.C. 1957. Hibernation, hibernacula and associated behaviour of the Three-Toed Box Turtle (*Terrapene Carolina triunguis*). Copeia. 1957(4): 278-282.

Chen, W., Zhang, L., and Lu, X. 2012. Pairs during hibernation in a temperate frog: an unusual male mating strategy among anurans. Journal of Ethology. 30(1): 15-21.

Christiansen, J.L. and Bickham, J.W. 1989. Possible historic effects of pond drying and winterkill on the behaviour of *Kinosternon flavescens* and *Chrysemys picta*. Journal of Herpetology. 23(1): 91-94.

Churchill, T.A., and Storey, K.B. 1992. Freezing survival of the garter snake *Thamnophis sitralis parietalis*. Canadian Journal of Zoology. 70(1): 99-105.

Costanzo, J.P., Claussen, D.L., Lee Jr., R.E. 1988. Natural freeze tolerance in a reptile. Cryo-Letters. 9: 380-385.

Costanzo, J.P., and Lee Jr., R.E. 1995. Supercooling and ice nucleation in vertebrate ectotherms. Chapter 12 from Biological Ice Nucleation and its Applications. APS Press. St. Paul. 370 p.

Costanzo, J.P., Lee Jr., R.E. and Ultsch, 2008. Physiological ecology of overwintering in hatchling turtles. Journal of Experimental Zoology. 309A(6): 297-379.

COSEWIC. 2006. COSEWIC assessment and status report on the Western Painted Turtle *Chrysemys picta bellii* (Pacific Coast population, Intermountain-Rocky Mountain population and Prairie/Western Boreal - Canadian Shield population) in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. vii + 40 pp.

Crawford, K.M. 1991. The winter environment of painted turtles, *Chrysemys picta*: temperature, dissolved oxygen, and potential cues for emergence. Canadian Journal of Zoology. 69: 2493-2498.

Crocker, C.E., Feldman, R.A., Ultsch, G.R. and Jackson, D.C. 2000. Overwintering behaviour and physiology of eastern painted turtles (*Chrysemys picta picta*) in Rhode Island. Canadian Journal of Zoology. 78(6): 936-942.

Croes, S.A., and Thomas, R.E. 2000. Freeze tolerance and cryoprotectant synthesis of the Pacific Tree Frog (*Hyla regilla*). Copeia. 3: 863-868.

Czenze, Z.J., Park, A.D. and Willis, C.K.R. 2013. Staying cold through dinner: cold-climate bats rewarm with conspecifics but not sunset during hibernation. Journal of Comparative Physiology B. 30(1): 15-21.

Darwin, C. 1859. The Origin of Species By Means of Natural Selection or the Preservation of Favored Races in the Struggle for Life. 1998 Modern Library Paperback Edition. New York. 689 p.

Dinkelacker, S.A., Costanzo, J.P. and Lee Jr., R.E. 2005. Anoxia tolerance and freeze tolerance in hatchling turtles. Journal of Comparative Physiology B., 175(3): 209-217.

Edge, C.B., Steinberg, B.E., Brooks, R.J. and Litzgus, J.D. 2009. Temperature and site selection by Blanding’s Turtles (*Emydoidea blandingii*) during hibernation near the species’ northern range limit. Canadian Journal of Zoology. 87: 825-834.

Environment Canada. 2014. Canadian Climate Normals Station Data 1971-2000. [Web accessed June 2014. http://climate.weather.gc.ca/climate_normals/results_e.html?stnID=1372&prov=&lan

g=e&dCode=4&dispBack=1&StationName=revelstoke&SearchType=Contains&province=ALL&provBut=&month1=0&month2=12].

Ernst, C.H. 1972. Temperature-activity relationship in the Painted Turtle, *Chrysemys picta*. Copeia. 1972(2): 217-222.

Ernst, C.H. and Barbour, R.W. 1989. Turtles of the World. Smithsonian Institution Press. Washington, DC. United States. 313 p.

Ernst, C.H., Lovich, J.E. and Barbour, R.W. 1994. Turtles of the United States and Canada. Smithsonian Institution. United States. 578 p.

Flatt, T. 2005. The evolutionary genetics of canalization. The Quarterly Review of Biology. 80(3): 287-316.

Foster, S.A., and Endler, J.E. 1999. Geographic variation in behaviour: perspectives on evolutionary mechanisms. Oxford University Press. New York. 337p.

Gardner, S.C.M., and Oberdorster, E. 2005. Toxicology of Reptiles. CRC Press. 328 p.

Geraldes, A.M., and Boavida, M-J. 2005. Seasonal water level fluctuations: Implications for reservoir limnology and management. Lakes and Reservoirs: Research and Management. 10: 59-69.

Ghalambor, C.K., McKay J. K., Carroll S. P. and D. N. Reznick. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Functional Ecology. 21(3): 394-407.

Gibbons, J.W. 1968. Reproductive potential, activity and cycles in the painted turtle, *Chrysemys picta*. Ecology. 49(3): 399-409.

Gienger, C.M., and Beck, D.D. 2011. Northern Pacific Rattlesnakes (*Crotalus oreganus*) use thermal and structural cues to choose overwintering hibernacula. Canadian Journal of Zoology. 89: 1084-1090.

Graham, T.E. and Forsberg, J.E. 1991. Aquatic oxygen uptake by naturally wintering wood turtles *Clemmys insculpta*. Copeia. 1991(3): 836-838.

Greaves, W.F. and Litzgus, J.D. 2007. Overwintering ecology of wood turtles (*Glyptemys insculpta*) at the species' northern range limit. Journal of Herpetology. 41(1): 32-40.

Halpern, E.A. 1979. Supercooling of the Iguanid Lizard *Sceloporus jarrovi*. Physiological Zoology. 52(2): 190-204.

Hawkes, V., Adama, D., and Leeming, A. 2013. BC Hydro and Power Authority CLBMON11B3 Revelstoke Reach Painted Turtle Monitoring Program. Monitoring Year 4 (2012) Annual Report. LGL report EA 3414. Unpublished report by LGL Limited environmental research associates, Sidney, BC, for BC Hydro Generations, Water License Requirements, Burnaby, BC. 42 pp.

Jackson, D.C. 2000. Living without oxygen: lessons from the freshwater turtle. Comparative Biochemistry and Physiology. 125(2000): 299-315.

Jackson, D.C., Ramsey, A.L., Paulson, J.M., Crocker, C.E. and Ultsch, G.R. 2000. Lactic acid buffering by bone and shell in anoxic softshell and painted turtles. Physiological and Biochemical Zoology. 73(3): 290-297.

Jackson, D.C. 2002. Hibernating without oxygen: physiological adaptation of the painted turtle. Journal of Physiology. 543(3): 731-737.

Jackson, D.C., Rauer, E.M., Feldman, R.A. and Reese, S.A. 2004. Avenues of extrapulmonary oxygen uptake in western painted turtles (*Chrysemys picta bellii*) at 10°C. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology. 139(2): 221-227.

Lamoureux, V.W., and Madison, D.M. 1999. Overwintering habitats of radio-implanted green frogs, *Rana clamitans*. Journal of Herpetology. 33(3): 430-435.

Layne Jr., J.R., Kefauver, J. 1997. Freeze tolerance and postfreeze recovery in the frog *Pseudacris crucifer*. Copeia. 2: 260-264.

Lindeman, P.V., and Rabe, F.W. 1990. Effect of drought on the Western Painted Turtle, *Chrysemys picta bellii*, in a small wetland ecosystem. Journal of Freshwater Ecology. 5(3): 359-364.

Litzgus, J.D., Costanzo, J.P., Brooks, R.J., Lee, R.E. 1999. Phenology and ecology of hibernation in spotted turtles (*Clemmys guttata*) near the northern limit of their range. Journal of Zoology. 77(9): 1348-1357.

Maginnis, L.A., Ekelund, S.A. and Ultsch, G.R. 2004. Blood oxygen transport in common map turtles during simulated hibernation. Physiological and Biochemical Zoology. 77(2): 232-241.

Mahmoud, I.Y. 1968. Nesting behavior in the western painted turtle, *Chrysemys picta bellii*. Herpetologica. 24(2): 158-162.

Markow, T.A. 1995. Evolutionary ecology and developmental instability. Annual Review of Entomology. 10:105-120.

Meeks, R.W. and Ultsch, R.G. 1990. Overwintering behavior of Snapping Turtles. Copeia. 1990(3): 880-884.

Nakadera, U., and Koene, J.M. 2013. Reproductive strategies in hermaphroditic gastropods: conceptual and empirical approaches. Canadian Journal of Zoology. 91(6): 367-381.

Newton, E.J. and Herman, T.B. 2009. Habitat, movements and behaviour of overwintering Blanding's turtles (*Emydoidea blandingii*) in Nova Scotia. Canadian Jounral of Zoology. 87(4): 299-309.

Nogueira, M.G., Perbiche-Neves, G., and Danilo A. O. Naliato. 2012. Limnology of Two Contrasting Hydroelectric Reservoirs (Storage and Run-of-River) in Southeast Brazil.

Accessed online 05-15-2013. Available: http://www.intechopen.com/books/hydropower-practice-and-application/limnology-of-two-contrasting-hydroelectric-reservoirs-storage-and-run-of-river-in-southeast-brazil

Oliveira, R.F. and Taborsky, M. 2008. Alternative Reproductive Tactics: an Integrative Approach. Cambridge University Press. 507 p.

Osbourne, R.P. 2012. Climate Change and the Columbia River Treaty. Washington Journal of Environmental Law and Policy. 49. Accessed 05-08-2014. Available: https://digital.law.washington.edu/dspace-law/bitstream/handle/1773.1/1148/2WJELP075.pdf?sequence=1

Paterson, J.E., Steinberg, B.D. and Litzgus, J.D. 2012. Generally specialized or especially general? Habitat selection by Snapping Turtles (*Chelydra serpentina*) in central Ontario. Canadian Journal of Zoology. 90: 139-149.

Peterson, C.C. 1987. Thermal relations of hibernating Painted Turtles, *Chrysemys picta*. Journal of Herpetology. 21(1): 16-20.

Pizzatto, L., Almeida-Santos, S.M., and R. Shine. 2007. Life history adaptations to arboreality in snakes. Ecology. 88(2): 359-366.

Prior, K.A. and Weatherhead, P.J. 1996. Habitat features of Black Rat Snake hibernacula in Ontario. Journal of Herpetology. 30(2): 211-218.

Prowse, T.D., Bonsal, B.R., Duguay, C.R., Hessen, D.O., and Vuglinsky, V.S. 2007, Global Outlook for Ice and Snow. Chapter 8: River and Lake Ice. 14 p. Accessed online 04-06-2014. Available: http://www.unep.org/geo/geo_ice/PDF/GEO_C8_LowRes.pdf

Puklakov, V.V., Edel'shtein, K.K., Kremenetskaya, E.R., and Gashkina, N.A. 2002. Water self-purification in the Mozhaisk Reservoir in winter. Water Resources. 26(6): 655-664.

Reese, S.A., Crocker, C.E., Jackson, D.C. and Ultsch, G.R. 2000. The physiology of hibernation among painted turtles: the midland painted turtle (*Chrysemys picta marginata*). Respiration Physiology. 124(2000): 43-50.

Reese, S.A., Crocker, C.E., Carwile, M.E., Jackson, D.C., Ultsch, G.R. 2001. The physiology of hibernation in common map turtles (*Graptemys geographica*). Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology. 130(2): 331-340.

Reese, S.A., Jackson, D.C., and Ultsch, G.R. 2002. The physiology of overwintering in a turtle that occupies multiple habitats, the Common Snapping Turtle (*Chelydra serpentina*). Physiological and Biochemical Zoology. 75(5): 432-438.

Reese, S.A., Stewart, E.R., Crocker, C.E., Jackson, D.C. and Ultsch, G.R. 2004. Geographic variation of the physiological response to overwintering in the painted turtle (*Chrysemys picta*). Physiological and Biological Zoology. 77(4): 619-630.

Rollinson, N., Tatersall, G.J., and Brooks, R.J. 2008. Overwintering habits of a northern population of painted turtles (*Chrysemys picta*): winter temperature selection and dissolved oxygen concentrations. Journal of Herpetology. 42(2): 312-321.

Rosen, P.C. 1991. Comparative ecology and life history of the Racer (*Coluber constrictor*) in Michigan. Copeia. 4: 897-909.

Rose, M.R. and Lauder, G.V. 1996. Adaptation. Academic Press Limited, California, USA.

Schiller, N. and Larsen, K.W. 2012. Columbia River Project Water Use Plan. Reference: CLBMON11B3 Arrow Lakes Reservoir. Annual Report 2011: Revelstoke Reach Western Painted Turtle (*Chrysemys picta bellii*) Monitoring Program. Prepared for BC Hydro. Kamloops, BC.

St. Clair, R.C., and Gregory, P.T. 1990. Factors affecting the northern range limit of Painted Turtles (*Chrysemys picta*): Winter acidosis or freezing? Copeia. 1990(4): 1083-1089.

Storey, K.B., and Storey, J.M. 1987. Persistence of freeze tolerance in terrestrially hibernating frogs after spring emergence. Copeia. 1987(3): 720-726.

Storey, K.B., Storey, J.M., Brooks, S.P., Churchill, T.A. and Brooks, R.J. 1988. Hatchling turtles survive freezing during winter hibernation. Proceedings of the National Academy of Sciences of the United States of America. 85(21): 8350-8354.

Storey, K.B. and Storey, J.M. 1990. Metabolic rate depression and biochemical adaptation in anaerobiosis, hibernation and estivation. The Quarterly Review of Biology. 65(2): 145-174.

Storey, K.B., Layne, J.R., Cutwa, M.M., Churchill, T.A. and Storey, J.M. 1993. Freezing survival and metabolism of box turtles, *Terrapene carolina*. Copeia. 1993(3): 628-634.

Storey, K.B. 1996. Metabolic adaptations supporting anoxia tolerance in reptiles: Recent advances. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology. 113(1): 23-35.

Storey, K.B. 2006. Reptile freeze tolerance: Metabolism and gene expression. Cryobiology. 52: 1-16.

Swanson, D.L., and Graves, B.M. 1995. Supercooling and freeze intolerance in overwintering juvenile Spadefoot Toads (*Scaphiopus bombifrons*). Journal of Herpetology. 20(2): 280-285.

Swanson, D.L. and Burdick, S.L. 2010. Overwintering physiology and hibernacula microclimates of Blanchard’s Cricket Frogs at their northwestern range boundary. Copeia. 2010(2): 247-253.

Taylor, G.M., and Nol, E. 1988. Movements and hibernation sites of overwintering painted turtles in southern Ontario. Canadian Journal of Zoology. 67: 1877-1881.

Thomas, R.B. 2002. Conditional mating strategy in a long-lived vertebrate: Ontogenetic shifts in the mating tactics of male slider turtles (*Trachemys scripta*). Copeia. 2002(2): 456-461.

Ultsch, G.R. and Jackson, D.C. 1982. Long-term submergence at 3°C of the turtle, *Chrysemys picta belli*, in normoxic and severely hypoxic water. Journal of Experimental Biology. 96: 11-28.

Ultsch, G. R., Hanley, R. W., and Bauman, T. R. 1985. Responses to anoxia during simulated hibernation in northern and southern painted turtles. Ecology. 66(2): 388-395.

Ultsch, G.R. 1989. Ecology and physiology of hibernation and overwintering among freshwater fishes, turtles and snakes. Biological Review. 1989(64): 435-516.

Ultsch, G.R., Carwile, M.E., Crocker, C.E. and Jackson, D.C. 1999. The physiology of hibernation among painted turtles: the eastern painted turtle *Chrysemys picta picta*. Physiological and Biochemical Zoology. 72(4): 493-501.

Ultsch, G.R. 2006. The ecology of overwintering among turtles: where turtles overwinter and its consequences. Biological Reviews. 81(3): 339-367.

Waddington, C.H. 1942. Canalization of development and the inheritance of acquired characters. Nature. 150(3811): 563–565.

Wikipedia. 2016. “Painted Turtle” search. The free encyclopedia. Accessed 24-03-2016. Available: https://en.wikipedia.org/wiki/Painted_turtle.

Chapter Two

OVERWINTERING ECOLOGY OF A NORTHERN POPULATION OF WESTERN PAINTED TURTLES (*CHRYSEMYS PICTA BELLII*) IN A CANADIAN RESERVOIR

INTRODUCTION

Overwintering is a critical life-history stage for species inhabiting temperate and more polar conditions. These extreme environments are characterized by harsh, cold conditions that can last up to half the year or longer. Many species' ranges are limited by these conditions, especially ectothermic animals that have internal body temperatures strongly tied to their immediate environment. Ectotherms such as herpetofauna, therefore display both behavioural and physiological adaptations in order to survive winters (Ultsch 1989, Crocker *et al.* 2000, Muir *et al.* 2014). Physiological adaptations include freeze tolerance, supercooling, metabolic depression and other methods of buffering acidosis (Jackson 1968, Ultsch and Jackson 1982, Ultsch *et al.* 1985, Voituron *et al.* 2000, Jackson *et al.* 2000, Jackson 2002, Packard and Packard 2003, Jackson *et al*. 2004, Reese *et al.* 2004, Storey 2006, review by Bickler and Buck 2007, Tattersall 2008, Costanzo and Lee 2013, Costanzo *et al.* 2013, among others). Behavioural responses include using overwintering sites that afford appropriate micro-habitat conditions (Aleksiuk 1976, Taylor and Nol 1989, Harvey and Weatherhead 2006, Mathies and Martin 2008, Newton and Herman 2009, Rollinson *et al.* 2008, Edge *et al.* 2009, Gienger and Beck 2011, among others). Important in this regard is protection from lethal temperatures, predators, and desiccation. For example, using aquatic habitats as hibernacula should, in theory provide refuge from subzero temperatures (Meeks and Ultsch 1990, Strain *et al*. 2012), yet not all aquatic environments will automatically exclude this threat, while other factors such as predation and acidosis may still be present (Ultsch 1989, Edge *et al*. 2009).

Northern adult freshwater turtles almost exclusively hibernate underwater (but see Claussen *et al.* 1991), and the species best known for tolerating aquatic hibernation for extended periods is the Painted Turtle (*Chrysemys picta*) (Jackson 1968, Ultsch and Jackson 1982, Ultsch *et al.* 1985, Herbert and Jackson 1985a, 1985b, Ultsch *et al.* 1999, Reese *et al.* 2000, Crocker *et al.* 2000, Reese *et al.* 2004, review by Storey 2007). In particular, the westernmost subspecies of this turtle (*C.p. bellii*) reaches latitudes of 52°N or greater in Canada, where it is capable of tolerating relatively long periods of anoxic conditions due to an advanced ability to regulate and store lactic acid during hypoxia (Ultsch *et al.* 1985, Jackson 1997, Jackson 2000a, 2000b, Reese *et al.* 2004).

The overwintering physiology of Painted Turtles has been relatively well studied compared to the ecology and behaviour of these animals in their natural environment. Still, a number of studies have reported the animals overwintering in ponds and small lakes at shallow depths (20-100 cm) and either nestle within the bottom substrates to a depth as low as 45 cm (Ernst 1972, Peterson 1987, Taylor and Nol 1989) or lie atop the substrate within the oxygenated water column (St. Clair and Gregory 1990, Crocker *et al.* 2000, Rollinson *et al.* 2008). Use of specific habitat characteristics for overwintering sites also has been documented in other species of northern freshwater turtles (Greaves and Litzgus 2008). Some freshwater turtles also may possess the ability to select overwinter sites based on pre-hibernation habitat conditions; Rollinson *et al.* (2008) found that female Painted Turtles in Algonquin Park, Ontario hibernating in oxygenated waters used sites based on dissolved oxygen (DO) concentrations, whereas those using anoxic conditions selected sites based on temperatures. Edge *et al.* (2009) found variation in hibernacula site selection based on oxygen availability as a result of ice cover in a northern population of Blanding's Turtles (*Emydoidea blandingii*). These trends indicate that northern freshwater turtles can be specific in their overwinter habitat use, but also may display behavioural plasticity based on the characteristics of their environment. However, most studies have looked at turtles inhabiting natural environments; few have investigated the overwinter effects of an anthropogenic, manipulated environment.

Intuitively, reservoir environments may pose challenges to turtles, particularly if they are locations in areas where surface ice forms. Such environments may be characterized by fluctuating water tables that pose additional threats to the animals, including the potential for winterkill. Overwinter mortalities have been reported as a result of being embedded into the ice, for both small and large numbers of turtles (St. Clair and Gregory 1990, Murphy and Corn 1977 cited by Christiansen and Bickham 1989). Fluctuations in water levels also may affect water quality parameters, including temperature, dissolved oxygen, pH and conductivity; Rollinson *et al.* (2008) identified temperature and dissolved oxygen as the primary factors influencing overwinter site selection for northern Painted Turtles. Given these observations, it is prudent to investigate further the relationship between turtles, winter, and fluctuating environments such as reservoirs.

I studied the overwinter ecology of a population of Western Painted Turtles (*Chrysemys picta bellii*) (WPT hereafter) in a hydroelectric reservoir in British Columbia, Canada. My study built upon a preliminary study by Basaraba (2014) on the general ecology and population demographics of this population. Although Basaraba (2014) did not focus on the overwintering stage of the animals' life history, her work with telemetered animals suggested that overwintering in the reservoir occurred in two primary locations, and moreover, the animals in these two locations appeared to be displaying different tactics. She reported that all the turtles in one subpopulation within the reservoir overwintered congregated whereas others were dispersed. Following on this, the specific goals of my research were to (1) examine more closely the variation in overwintering tactics occurring across the different subpopulations of turtles in this area, (2) determine if the variation is out of necessity due to lack of suitable overwintering habitat, and (3) document if changes in reservoir levels during winter elicit response(s) in the turtles, and if so, examine if this phenomenon contributes to the use of different tactics.

METHODS

Study Area

My field research took place from January 2013 – May 2014 in a section of the Arrow Lakes Reservoir referred to as 'Revelstoke Reach', lying immediately south of the town of Revelstoke, British Columbia, Canada (50.9981° N, 118.1956° W). The reservoir lies within the Upper Arrow Lakes portion of the Columbia River and is regulated by two hydroelectric dams. This section of the river has existed as a reservoir since the implementation of the downstream Hugh Keenleyside Dam in 1968, and was further affected by the upstream Revelstoke Dam starting in 1984. The operator (BC Hydro) currently is licensed to manage up to a 20-m fluctuation in water levels, from 418.64 – 440.1 MASL. The reservoir typically reaches full pool between July-August, decreasing throughout fall and winter until reaching its lowest levels in March-April (BC Hydro 2007). During the two winters of this study, the fluctuations recorded were within this normal range, and the lowest levels occurred in February of each winter (see Figure 1-5, Chapter 1).

Average total precipitation at this location is approximately 94.6 cm per year, half of which falls as snow (based on 30-year averages 1971-2000, Environment Canada 2014). Revelstoke experiences below-zero temperatures from November to March and can have snow depths reaching a maximum of 173 cm in February (Environment Canada 2014). Ice develops on local water bodies in November, and persists from approximately December to early April (pers. obs.)

My research focused on two locations within the reservoir that support the overwhelming majority of turtles, identified as Airport Marsh (AP) and Montana Slough (MS) (Basaraba 2014). Both locations lie on the eastern side of the reservoir, being approximately 2.5 km straight-line distance from one another. The more northern site (AP) sits at an elevation of 437 MASL, is approximately 130 ha in size and is sheltered from the main channel of the Columbia River by an airport runway that buffers sudden increases in water level. Submerged areas of AP can reach depths up to ≈2.5 m at high water (mid-summer), and the site is characterized by a soft, muddy substrate that can be up to 0.5 m thick.

Montana Slough (MS) is slightly lower in elevation (432 MASL) and is also smaller in size as measured by maximum area during high water (43 ha). Montana Slough is relatively sensitive to water level fluctuations from reservoir operations and can nearly flood entirely during full pool. A unique feature of the MS site is a vegetated island (approx. 1-2 ha in area at high water) that features a small pool of water designated as ‘Winter Pond’ (approx. 2 m deep, 0.13 ha in size). At high water, MS can reach depths of approx. 3 m with substrate depths of approx. 10 cm in the main body and 30-40 cm in Winter Pond. In her study, Basaraba (2014) documented 8 telemetered turtles overwintering in Winter Pond in the winter of 2011-2012.

To compare effects from the reservoir, I also studied a nearby isolated water body [Turtle Pond (TP)] that contained turtles. Turtle Pond is ≈0.5 km from the reservoir and is 6.5 ha in size. It is ≈2.5 m at its deepest point, and substrate depth ranges from 15-40 cm.

Trapping and Equipment

In spring (May 2013/2014) and late summer (August-October 2013) I captured adult turtles using hoop traps baited with sardines and/or cat food. Traps were checked at a minimum every 12 hours. I incorporated 19 adult turtles still carrying transmitters from the Basaraba (2014) study, recapturing these animals when necessary to remove and replace aging transmitters. I targeted equal numbers of male and female adult turtles for telemetry and distinguished the two sexes by secondary sexual characteristics (Harless and Morlock 1979).

Turtles were selected to ensure the radio-transmitters (models SI-2FT, SB-2F, or AI-2FT from Holohil Systems Ltd., Ontario CA) and all attachment material did not exceed 5% of body weight. Forty-one of 44 transmitters used were temperature sensitive (pulse rate proportional to temperature, Models SI-2FT and AI-2FT), and I affixed them to the turtles by drilling small holes at the posterior of the carapace and securing them using stainless steel wire (Grayson and Dorcas 2004, Basaraba 2014) (Figure 2-1) to reduce potential loss due to shedding of the scutes (CCAC *n.d.*, Grayson and Dorcas 2004). Marine epoxy (Amazing GOOP® Marine Epoxy Paste, eclecticproducts.com) was placed around the wire to prevent snagging and unwinding.

In the first winter, temperature-sensitive transmitters were relied on to determine carapace temperatures, with iButtons and depth/temperature loggers introduced in the second winter to provide corroborating data. Ultimately, the iButtons and depth/temperature recorders produced the most reliable and precise temperature data (depth recorders accuracy 0.01°C, iButtons 0.5°C) and these data were used exclusively for turtles in the second winter. Two telemetered turtles from each of the three study sites carried temperature and depth recorders (DST-milli-TD and DST-milli-F depth recorders, Star-Oddi, Iceland). These recorders were affixed to the side of the carapace opposite the radio transmitter (Figure 2-1). An additional five telemetered turtles each carried a Thermochron iButton® temperature datalogger (Maxim Integrated, USA) waterproofed in Plasti-Dip (Performix Brand® - see Grayson and Dorcas 2004) and bonded to radio transmitters with marine epoxy (as described above).

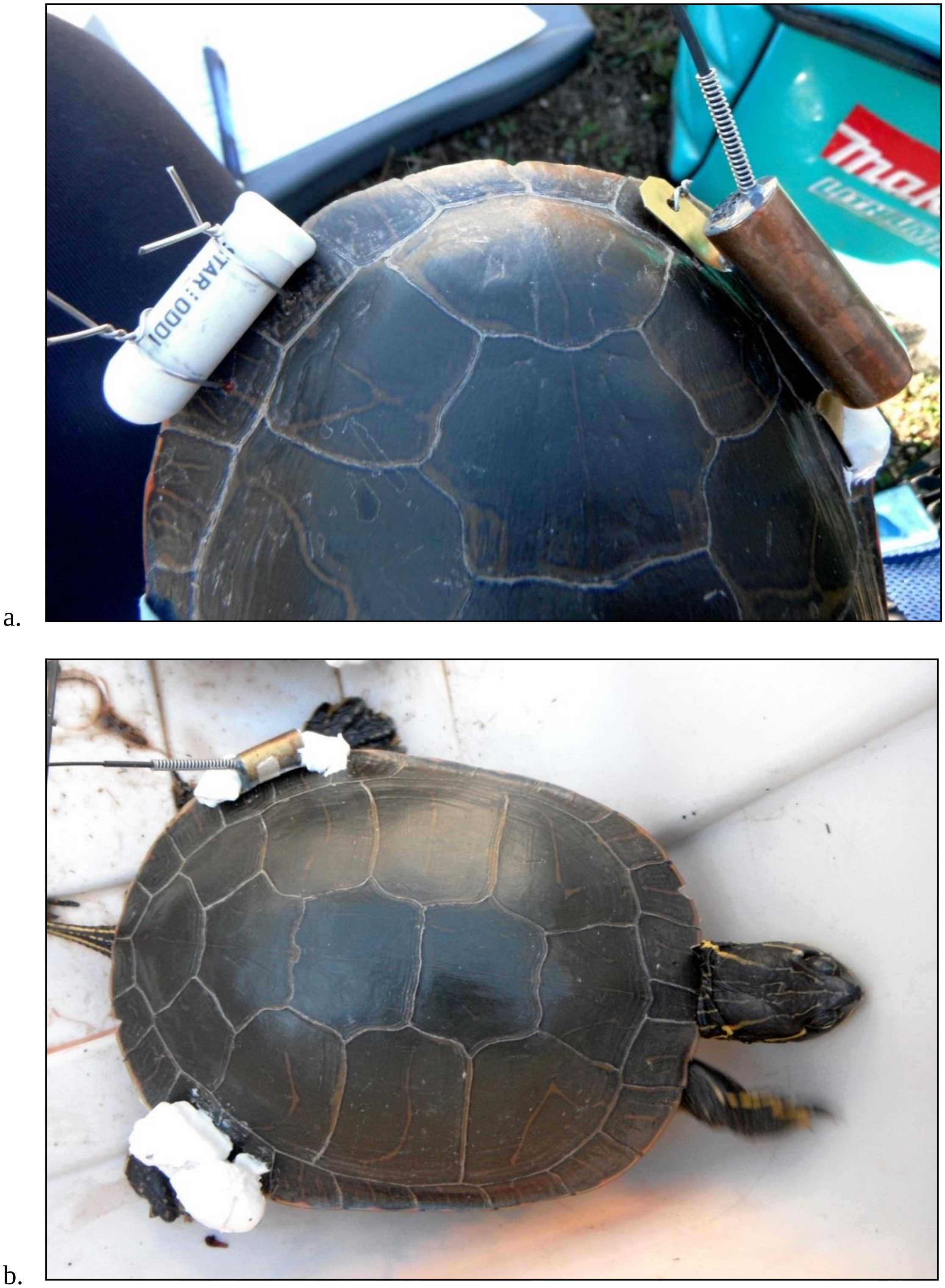


Figure 2-1: a. Radio transmitter (gold) and depth recorder (white) attachment and placement on posterior of Western Painted Turtle (*C.p. bellii*) carapace. Wires on the depth recorder were trimmed similar to those on the radio transmitter prior to marine epoxy placement b. Radio transmitter and depth recorder attachment on posterior of Western Painted Turtle (*C.p. bellii*) with marine epoxy. Photos by author.

Spatial Arrangement of Overwintering Turtles

During the winters of 2012-13 and 2013-14, I collected data on each turtles' location using a wide-band radio receiver (Lotek Biotracker), aYagi three-element antenna and a hand-held GPS instrument (Garmin GPSMAP 64S). When conditions did not allow safe travel over ice, I estimated turtle locations from safe points using triangulation. Once ice cover permitted safe travel, I was able to pinpoint the exact location of each telemetered turtle and collect associated habitat data.

Terminology

A number of terms have been used interchangeably in the literature to describe groups of animals, including communal, congregated and aggregated (among others); herein I have chosen to use the term *congregated* and to define it as an animal's location within 5 m of another animal (see similar boundaries used by Brown and Brooks 1994, Edge *et al.* 2009). Similarly, I refer to animals located more than 5 m from one another as *dispersed*.

Conditions at Overwinter Sites

The ambient temperature at each turtle's location was determined using the pulse-rate of the transmitter. Using an ice auger (Kovacs Enterprises Inc.), a 5-cm hole was made through the ice, enabling me to measure ice depth (cm), water depth (cm), dissolved oxygen (mg/L), water temperature (°C) (using a YSI Model 85 Handheld Oxygen, Conductivity, Salinity and Temperature System). I also obtained data on reservoir elevation (MASL), precipitation (mm) and local air temperature (°C). Air temperature and precipitation data were retrieved from Environment Canada's 'Revelstoke Airport Auto British Columbia' weather station (located at 50.5729 N, 118.1034 W, Environment Canada 2014). Reservoir elevation data (MASL) was recorded at the Fauquier hydrometric station established by BC Hydro (located at 49.5220 N, 118.0448 W, BC Hydro 2013-14).

In the second winter of the study (November 2013 – March 2014), I continued the same procedures described above, and I also used underwater cameras (GoPro Hero 3 (Black Edition) and Jetview Electronics Motorized 360° Video Camera JE-CM27-AS with DVR) in an attempt to gain visual contact with the turtles. I attached game cameras (Bushnell Trophy Cam) to trees along shorelines to track ice cover by taking a photo each day at 11:00 AM. Cameras at reservoir sites were positioned in the vicinity of known overwintering turtle locations, and at the isolated site (TP) the camera was placed along the opposite shore from turtle locations.

Conditions at Reference Sites

The different overwintering tactics used byf turtles between water bodies required different approaches to enable comparisons between turtle locations and reference sites. At the AP site, data from the first winter revealed the turtles were using two main areas to overwinter (see Results). In the second winter, I therefore collected fine-scale data on the habitat used and available to each hibernating animal using identical procedures to those outlined above, sampling ice depth (cm), water depth (cm), temperature (°C) and dissolved oxygen (mg/L) at random directions 2 m and 5 m from each turtle at AP. This methodology only was used at AP since turtles at MS displayed congregated hibernation in the first study winter, and thus I focused there on obtaining comparative habitat data using a broader approach within the entire water body.

Habitat data were collected throughout water bodies using a network of recording stations. Dissolved oxygen (mg/L) and temperature (°C) were monitored by LGL Ltd. throughout the winter months of 2012-13 at all water bodies using 4 PME MiniDot Dissolved Oxygen Loggers (Precision Measurement Engineering, USA) (1 TP, 1 MS, 2 AP) (Figures 2-2 and 2-3). The following winter (Jan – April 2014) I positioned seven dissolved oxygen/temperature stations (3 HOBO U26-01, Onset, USA and 5 PME) near known locations of overwintering turtles (2 TP, 3 MS, 2 AP). Loggers were secured to the top of either a submerged milk crate or laundry basket to keep them above the substrate and within the water column. The data loggers were programmed to record at least every 60 minutes over the course of the winter.

During the second winter of my study, I collected reference temperature profiles of the water column through a network of 30 temperature monitoring stations placed throughout each of my three study sites (11 MS, 9 AP, 7 TP) (Figures 2-2 and 2-3). To do this, I drove 1.5 m length sections of concrete reinforcing bars (‘rebar’) into the lake substrate in an array of locations haphazardly chosen to represent the range of conditions occurring in the sites. The precise locations were chosen based on my own knowledge of the physical structure of the water bodies, as well as the knowledge gained to date on the hibernating sites of turtles. Each stake had a minimum of three iButton® data loggers encased in waterproof cases and affixed

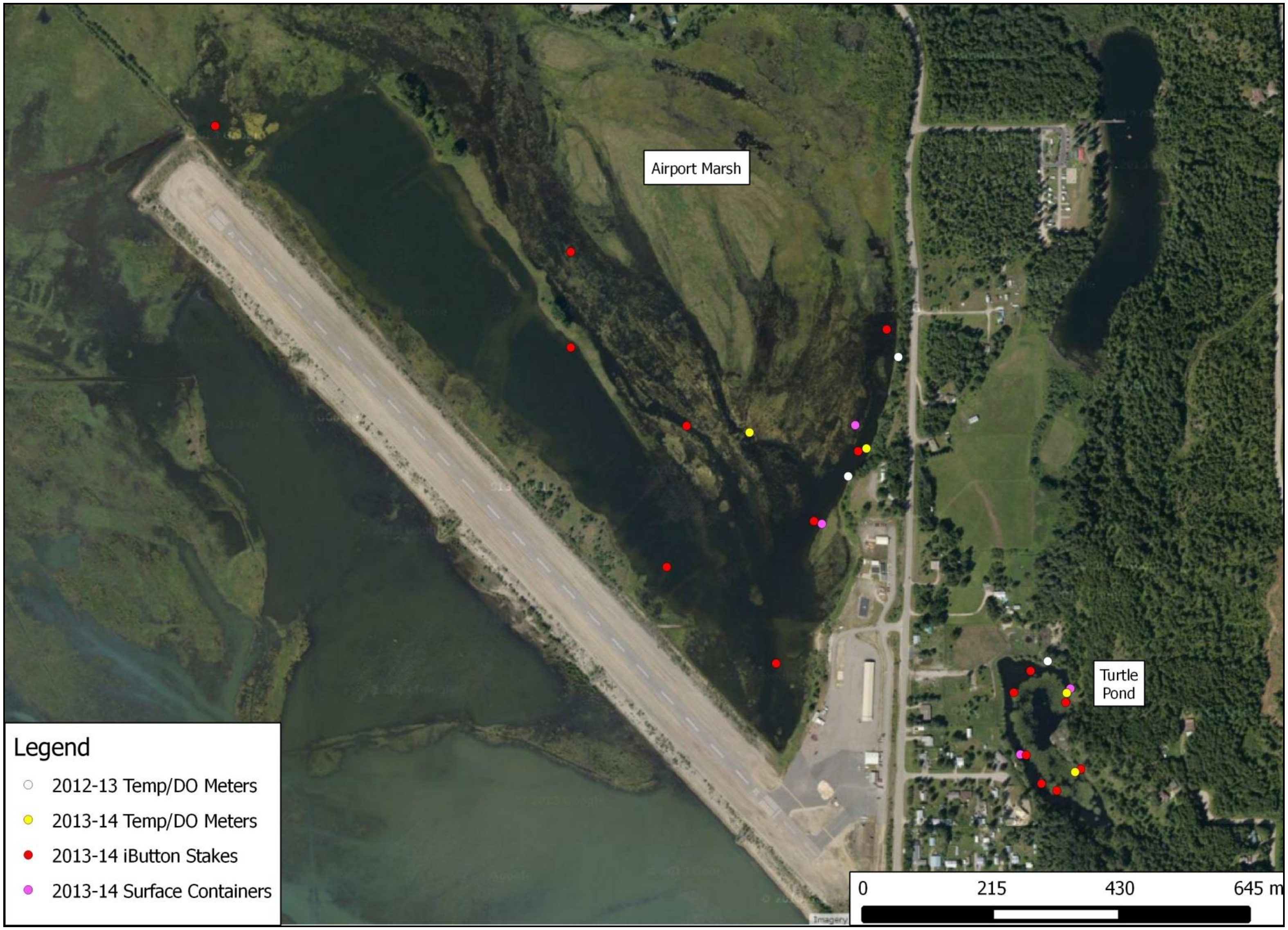


Figure 2-2: Location of automated data-recording stations in Airport Marsh and Turtle Pond in both study winters.

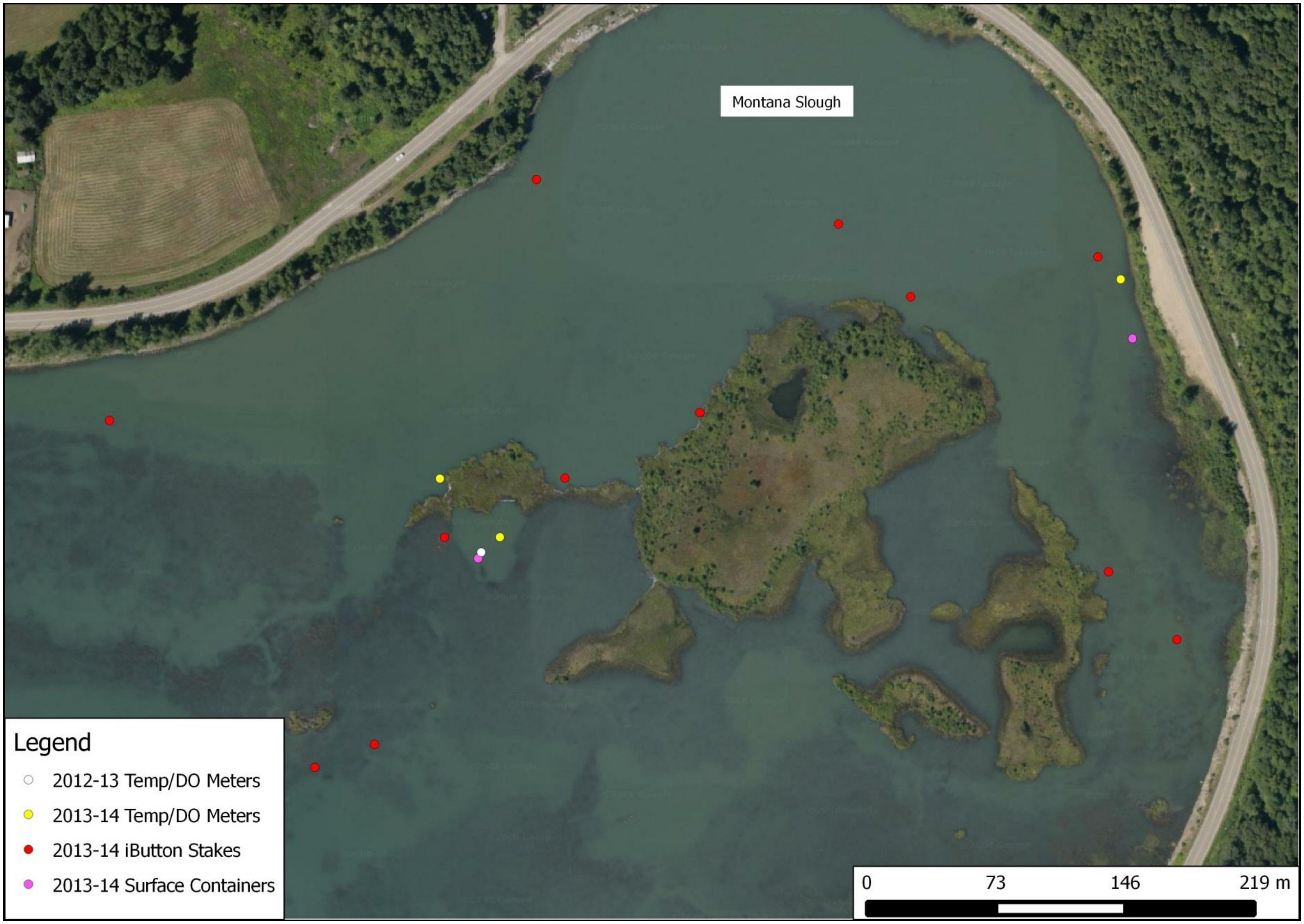


Figure 2-3: Location of automated data-recording stations in Montana Slough in both years.

to the stake with zip ties and duct tape. Though the exact depths of the data loggers changed in accordance with water levels and ice thickness over winter, their data reflected temperatures at the substrate level and the midway and surface points in the water column. The data loggers were programmed to take a reading every 4 h. Additionally, to monitor temperature and ice conditions along the shoreline, I placed two submerged containers at each location, each containing one iButton®, approximately 10.5 m (± 5.1) from the shoreline at depths between 15-40 cm. In total, 98 iButtons® were used to record water temperatures in all locations over the second winter (November 2013 – May 2014).

Data Analysis

Habitat variables were analyzed using R (version 3.0.2). Statistical significance was guided by $\alpha = 0.05$. Stated means are given ± SD, unless otherwise noted. Comparisons between water bodies or movements were made using repeated-measures of Anova (RMANOVA). A linear mixed-effects modeling (packages lmer and lmerTest in R) was used to conduct a resource selection-test using habitat data from turtle and non-turtle locations at AP. Spatial analysis was completed using QGIS 2.0 Dufour, 2.6 Brighton (www.qgis.org) and Google Earth. Graphs were created using SigmaPlot (Version 8.0).

RESULTS

All told, I collected telemetry data from 50 turtles; 19 of these animals carried radio transmitters through the winter of 2012-13, with another 31 turtles similarly monitored over winter 2013-14. One of the tracked turtles was found floating dead in AP in May 2014. This individual overwintered in a location with no standing water from Dec 2013 – Mar 2014 suggesting it died from freezing or desiccation. Six of the 31 turtles in the second winter could not be located prior to or during the winter: three of these six missing animals were subsequently live-trapped in the spring of 2014, and a fourth was located using telemetry. Six turtles successfully carried depth recorders over winter, though one of the recorders became damaged and did not record usable data. Five additional turtles carried iButtons®, only three of which were retrieved. To the best of my knowledge none of the devices attached to the turtles became dislodged or lost.

Warmer temperatures during the first winter resulted in thinner average ice depths than in the following year (Table 1). Camera records (taken in the second winter only) indicated the AP and MS water bodies were ice-covered for the same period of time (130 days), whereas the surface of TP remained frozen for longer (147 days).

Reservoir fluctuations during my study were within the range of those recorded over the past decade, except from December 2012 - early January 2013 when water levels were higher than normal (refer to Chapter 1, Figure 1-5). Between the two study years, a relatively larger and more rapid drop in water levels occurred during the first winter.

Did turtles in different locations show different overwintering tactics?

Patterns of dispersion overwinter

Variation in the spatial arrangement of overwintering turtles was evident between reservoir locations in the first winter of study, when all turtles at MS overwintered congregated, and all turtles at AP overwintered dispersed (Figures 2-4 and 2-5). The congregation in MS was created by all telemetered turtles in this area gathering in Winter Pond, with an average distance of 3.4 m (± 1.8) between individuals. In the second winter, turtles at AP again showed a dispersed spatial arrangement (n = 7), except for two turtles located closer together in February (1.5 m apart). More substantial changes occurred at MS in the second winter: two turtles congregated in Winter Pond during the winter (average 2 m ± 0.9), and two others congregated within the main arm of Montana Slough in February and March (average 3.6 m ± 2 apart). The other three telemetered animals at MS in the second year overwintered dispersed: two within the main arm and one in Winter Pond over 5 m from the other telemetered turtles. Isolated from the reservoir in TP, turtles hibernated in a dispersed pattern in both winters.

Overwintering turtles in all three sites buried themselves in substrate, as suggested by data from the depth recorders and 21 failed attempts to obtain visual contact using the submersible cameras. By comparing manually measured water depths at turtle locations to depths recorded simultaneously by the depth recorders, I estimated the turtles were on average buried 26.3 cm ± 37 into the substrate (Figure 2-6). Additionally, one turtle was observed partially buried at TP on Dec 15, 2013 (Figure 2-7) and a second turtle was confirmed buried in March 2014 at a stream site in AP.

Table 1: Mean monthly air temperature (°C) and ice depth (cm) (measured from Dec – Mar) for the Revelstoke Reach portion of the Arrow Lakes Reservoir during the two winters of study.

Year	Mean Air Temperature (°C)				Ice Depth (cm)
	December	January	February	March	
2012-13	-1.1 ± 2.4	-2.7 ± 2.7	0.7 ± 1.4	2.8 ± 2.5	25 ± 8.4
2013-14	-3.6 ± 5.2	-1.8 ± 2.8	-6.1 ± 5.8	0.9 ± 2.6	36 ± 16.8

Figure 2-4: Overwintering turtle locations in February of both 2013 (yellow) and 2014 (red) at Airport Marsh and Turtle Pond, Revelstoke Reach, Arrow Lakes Reservoir.

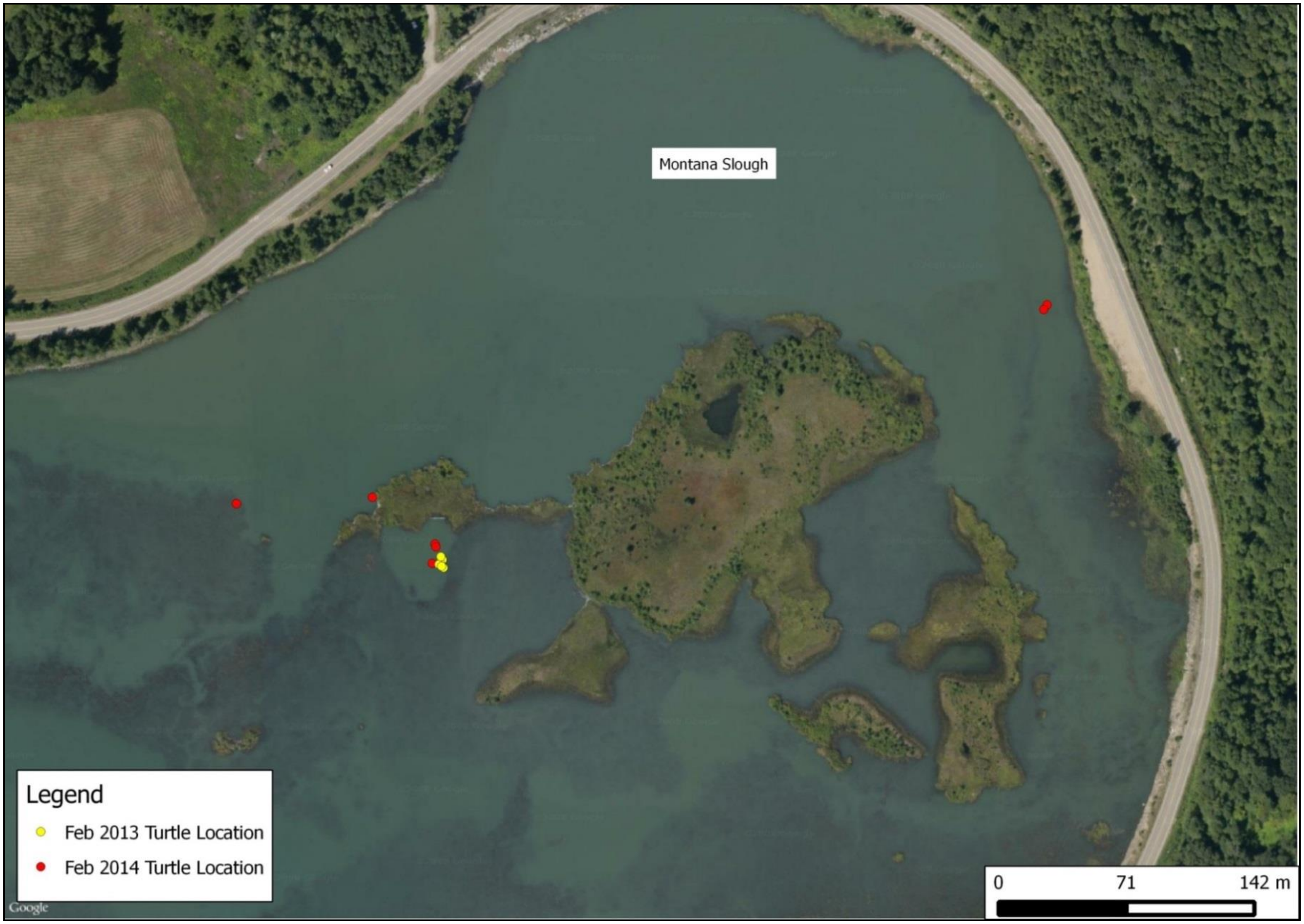


Figure 2-5: Overwinter turtle locations in February of both 2013 (yellow) and 2014 (red) at Montana Slough, Revelstoke Reach, Arrow Lakes Reservoir.

Males and females were apparent in the congregations of turtles in both winters. In the first winter one female was present of the six congregated turtles in Winter Pond at MS; however, in all months of the winter she was positioned near the center of the male group, averaging 5.4 m distance from each of the five males from January to March. In the second winter two male-female pairs hibernated congregated in separate locations of MS.

Site fidelity

Of the nine turtles that were followed over both winters, only one turtle in AP showed any degree of fidelity to a specific hibernating site, having only 9.4 m between its 2013 and 2014 locations. Other turtles (n=6) using the same water body in both years had specific hibernating sites separated by on average 257 m (SD ± 204).

Movements between water bodies

The majority of turtles followed over two study winters (7/9) remained in the same water body. One exception was a turtle that spent 2013 in AP along the southern shore and travelled to MS (~4.1 km south) to overwinter in 2014. Another turtle showed the opposite: overwintering in 2013 within Winter Pond at MS, then travelling to the northern end of AP (~5.4 km) and spending winter 2014 in a flowing ice-free stream.

Did habitat conditions vary between locations?

Habitat conditions in the two reservoir water bodies differed very little in either year. In the first winter, water depth was significantly deeper at turtle locations in MS (congregated hibernation) compared to AP (dispersed hibernation) ($F_{1,2}$ = 32.07, P = 0.03; Figure 2-8). In the second study winter, ice depth was the only metric to vary between turtle locations in each of the water bodies, being thinnest at MS ($F_{2,6}$ = 5.6, P = 0.04) (Figure 2-9). Within sites, the small pond where turtles congregated in MS recorded significantly lower dissolved oxygen levels compared to loggers in the main body of water ($F_{1,2}$ = 22.06, P = 0.04) in the second winter.

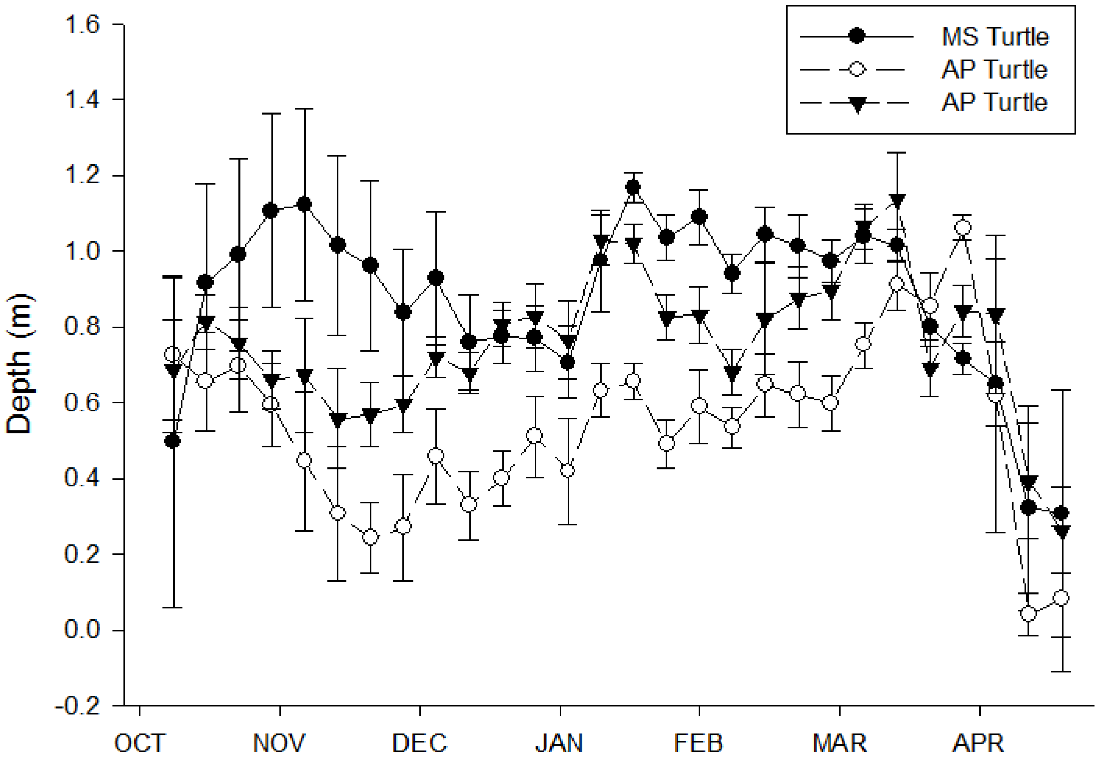


Figure 2-6: Mean weekly depth (±SD) of three turtles carrying depth recorders (n = 2 Airport Marsh and n = 1 Montana Slough) from October 2013 to April 2014.

Figure 2-7: Partially buried Western Painted Turtle beneath ice at Turtle Pond viewed through hole drilled with ice auger, Dec 15 2013. Photo by author.

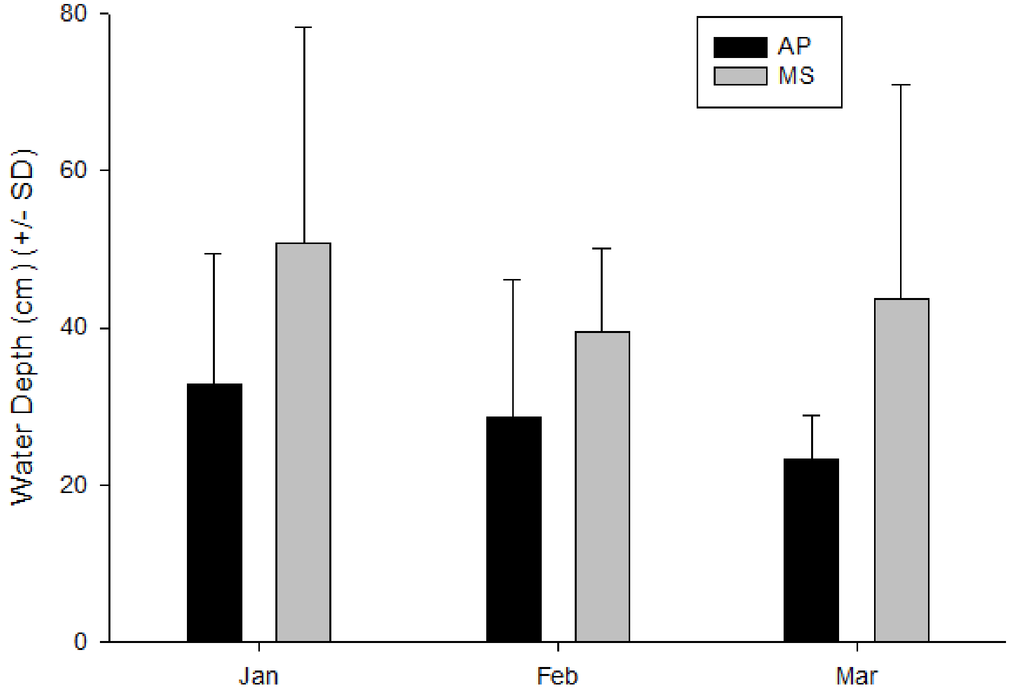


Figure 2-8: Mean water depths (cm) (+SD) at turtle locations in Airport Marsh and Montana Slough in the first study winter (2013).

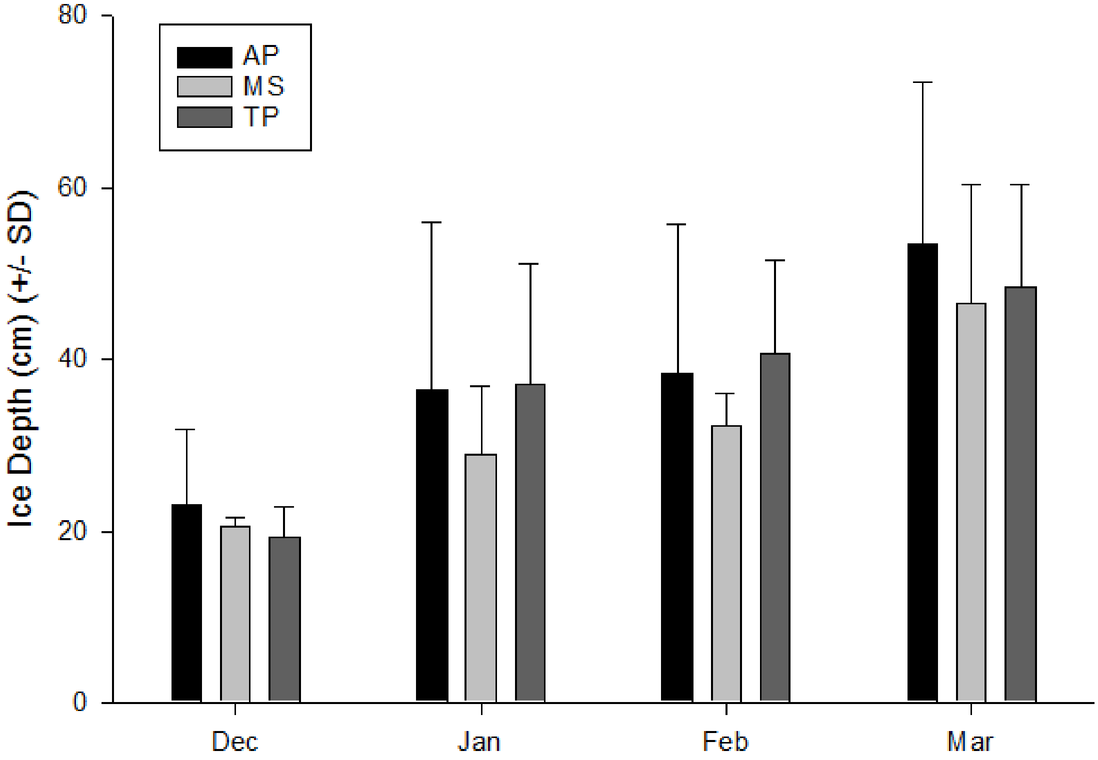


Figure 2-9: Mean ice depths (cm) (+SD) at turtle locations in all water bodies in the second winter (2013-14) (AP = Airport Marsh, MS = Montana Slough, TP = Turtle Pond).

Habitat comparison between turtle and reference locations

During the first winter, turtle locations at AP did not vary significantly in temperature or dissolved oxygen levels compared to measurements recorded at the stationary loggers ($P > 0.05$). In MS both the water temperature recorded at turtle locations within the small pond and temperatures recorded by the radio transmitters attached to the turtles' carapace were colder than the water temperatures recorded at stationary loggers throughout the water body (T-tests, $P \leq 0.01$) (Figure 2-10). Dissolved oxygen at MS turtle locations did not differ from stationary loggers ($P > 0.05$). Turtle carapace temperatures did not vary significantly between water bodies in the first year (F-tests, $P > 0.05$) (Figure 2-11).

In the second winter, dissolved oxygen was not significantly different between turtle locations and stationary loggers in any water bodies, except at MS where turtle locations measured higher dissolved oxygen in comparison (T-test, $P = 0.04$). Conversely, temperatures were significantly lower at turtle locations compared to stationary loggers in both AP (T-test, $P = 0.03$) and TP (T-test, $P < 0.01$) in this winter, but not at MS ($P > 0.05$). Turtles at MS in the second winter recorded significantly higher carapace temperatures than both AP and TP (Dec – Mar: T-tests $P < 0.01$) (Figure 2-11). Despite turtles being buried in substrate, carapace temperatures at all sites in the second winter were most comparable to mid- and high-column temperatures (Figure 2-12).

Turtle Presence Models

At the 2 and 5 m scales, the best model for predicting turtle presence at AP was that containing water temperature alone as the explanatory variable. No other models (both univariate and multivariate) alone or in combination had rankings close to this model (i.e., $\Delta AIC \leq 2$, Table 2). Water temperatures at turtle locations were colder than those measured both 2- and 5-m away. The predictive accuracy of this model using the 2-m data correctly predicts turtle presence 53% of the time, and the 5-m model 56% of the time.

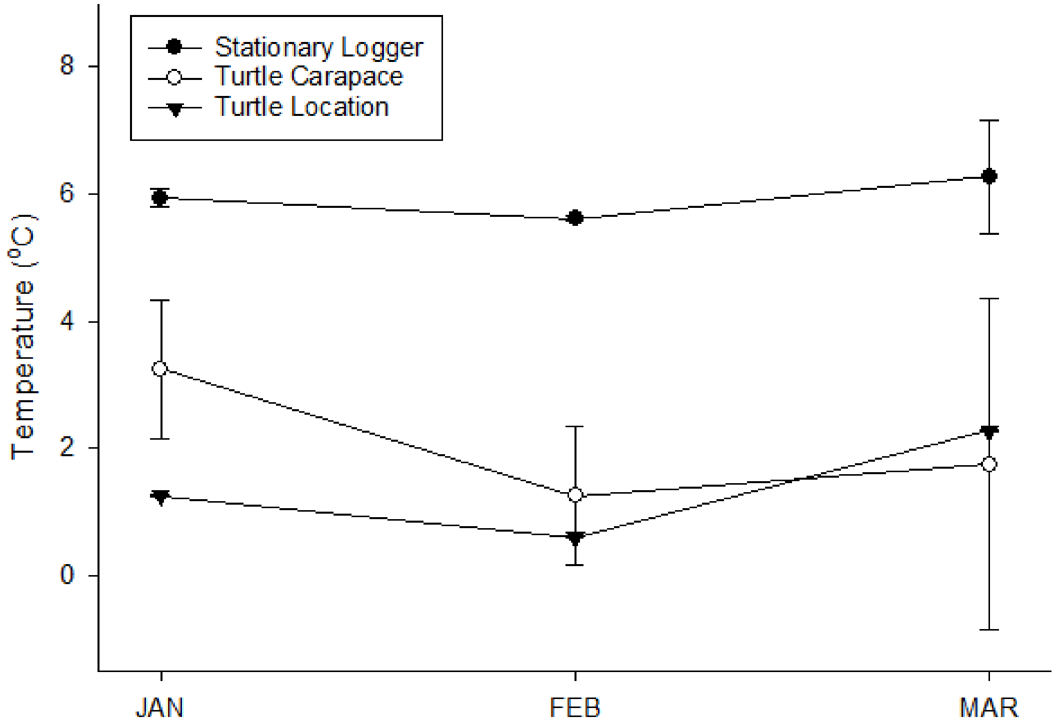


Figure 2-10: Mean monthly logger, turtle carapace and turtle location temperature at Montana Slough, January – March 2013. All means show ±SD.

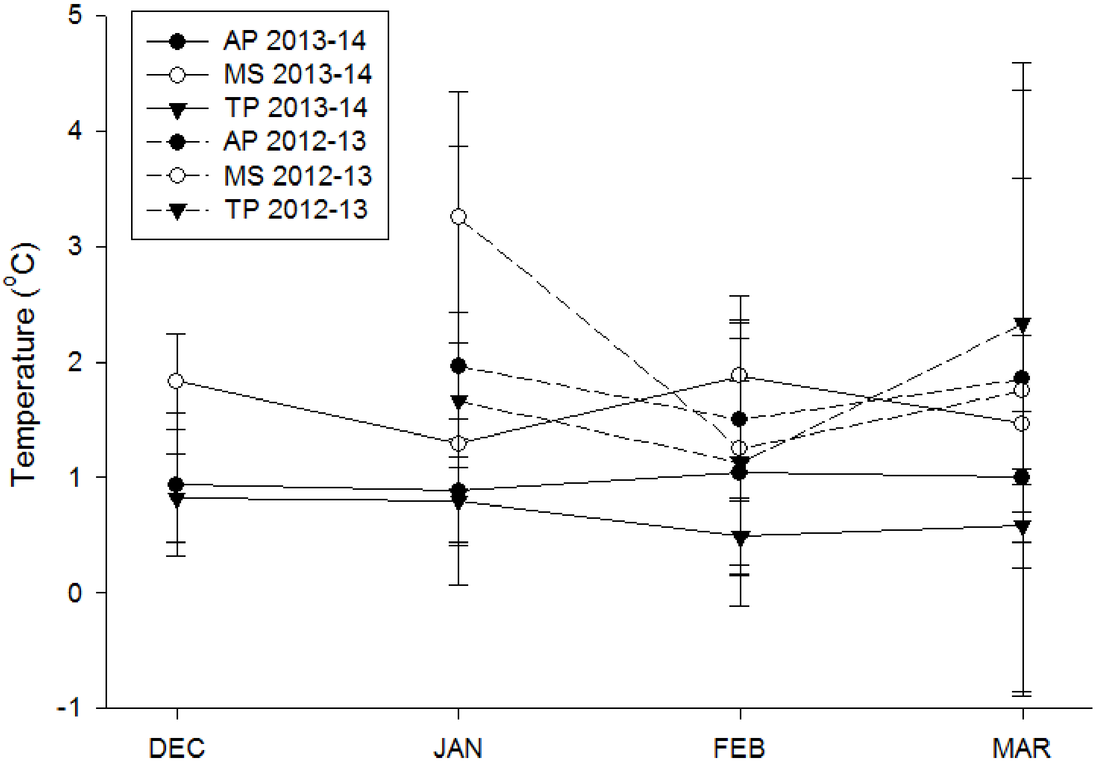


Figure 2-11: Turtle carapace temperatures (°C) over both study winters at all locations (AP = Airport Marsh, MS = Montana Slough, TP = Turtle Pond). All means show ±SD.

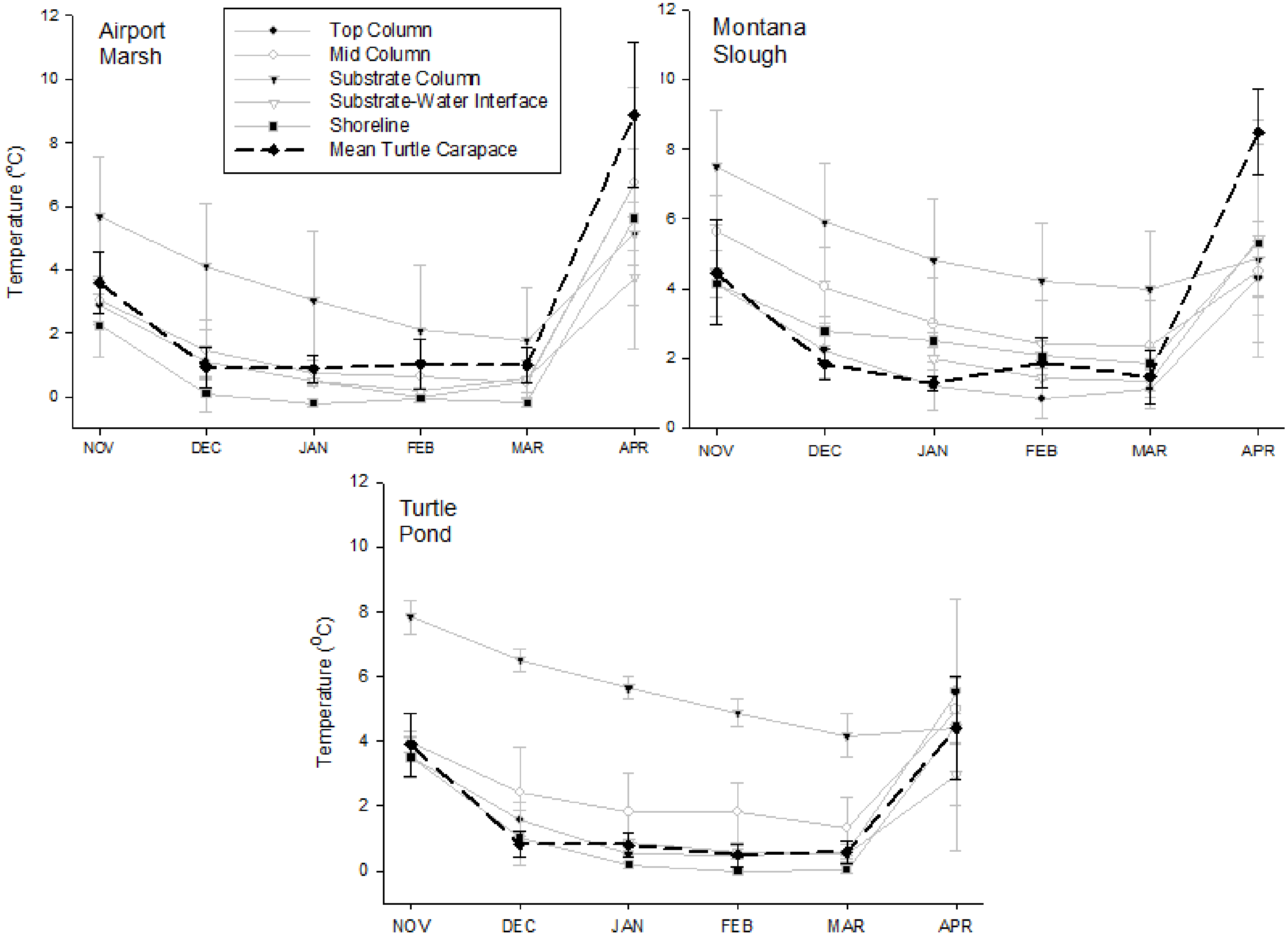


Figure 2-12: Mean water body temperature (±SD) profiles showing high, mid, substrate, surface-water interface and shoreline temperatures in comparison to mean turtle carapace temperature for all water bodies from Nov 2013 to Apr 2014.

Table 2: Summary of AIC and ΔAICc values for the top five candidate models assessing the influence of water temperature (°C), dissolved oxygen (mg/L), ice depth and water depth (cm) at the 2 and 5-m scales on turtle presence at Airport Marsh in the second study winter (2013-14). Repeated measures were accounted for by including the turtle identifier as a blocking variable within months (i.e., Month|Turtle).

Explanatory Variables (2 m)	**AIC**	**ΔAICc**
Temperature ~ ID	*120.43*	
Dissolved Oxygen ~ ID	125.46	5.03
Water Depth ~ ID	127.85	7.42
Ice Depth ~ ID	127.8	7.37
Temperature + Dissolved Oxygen ~ ID	129.94	9.51

Explanatory Variables (5 m)	**AIC**	**ΔAICc**
Temperature ~ ID	*120.45*	
Dissolved Oxygen ~ ID	125.73	5.28
Water Depth ~ ID	125.78	5.33
Ice Depth ~ ID	128.19	7.74
Temperature + Dissolved Oxygen ~ ID	130.18	9.73

Note: *Italics* indicates top model

Do changes in reservoir levels elicit movements in overwintering turtles?

Turtles commonly displayed movements throughout the winter at both the reservoir and non-reservoir locations, ranging from 0 – 95 m over the winter months. There was not a significant difference in distance moved by turtles between the water bodies in either year or between the two different tactics used (annual comparisons analyzed by month, F tests, all Ps > 0.07, Figure 2-13; by tactic, F-tests, all Ps > 0.20).).

Habitat Conditions between Movements

Turtle movements during the first winter did not result in the animals experiencing notably different conditions from their previous location (F-tests, all P > 0.06). In the second winter at MS, turtles moved to areas of lower temperature and lower dissolved oxygen levels between January and February, and between February and March moved to areas with increases in both these metrics (DO: $F_{2,8} = 5.45$, P = 0.03; Temp: $F_{2,8} = 4.35$, P = 0.05). At TP, turtles moved to areas with increasing dissolved oxygen throughout the winter ($F_{2,7} = 13.02$, P = < 0.01). Water temperature and dissolved oxygen levels did not change significantly between turtle movements at AP (F tests, P > 0.05).

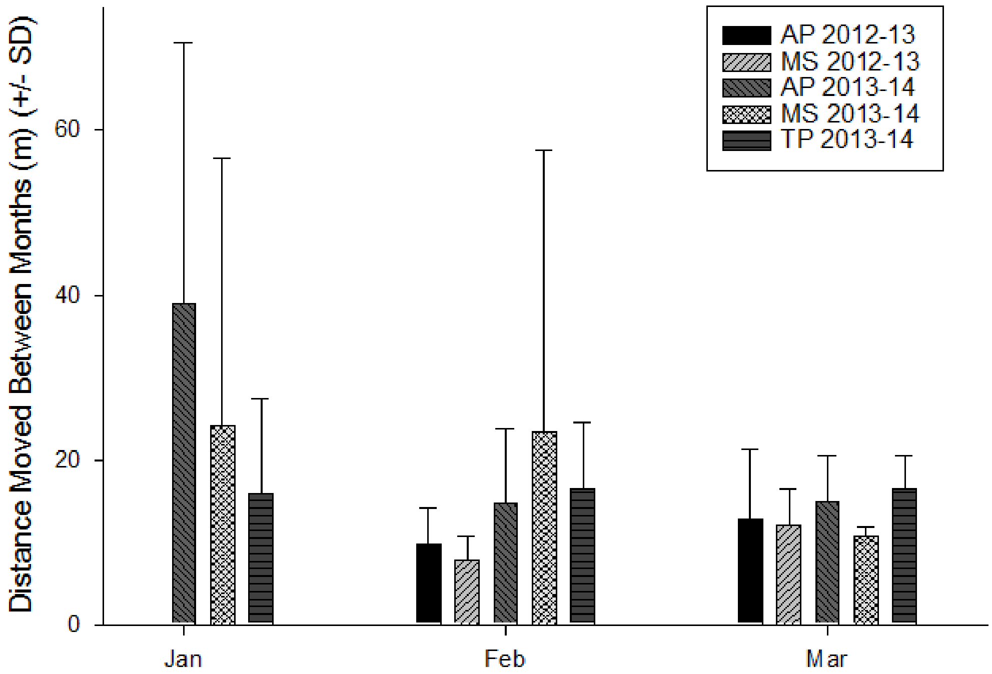


Figure 2-13: Mean distance moved by turtles (+SD) among winter months at each location shown for both years (AP = Airport Marsh n = 17, MS = Montana Slough n = 13, TP = Turtle Pond n = 10).

DISCUSSION

This study is the first to focus on the overwintering ecology and behaviour of a population of Western Painted Turtles (WPT, *Chrysemys picta bellii*) within a fluctuating reservoir environment. The results of my research indicate the variation in tactics used by overwintering turtles is not correlated with water temperature, dissolved oxygen or water depth. Additionally, this population of WPT was not negatively affected by the fluctuating water table as a result of hydroelectric operations during winter. These animals appear able to respond to local conditions by using sites with suitable habitat characteristics for winter, namely water temperature (°C) and subsequent water depth (cm). The extremely low incidence of overwinter mortality (apparently due to freezing) that I detected suggests the animals avoid freezing temperatures successfully. The prevalence of overwinter movements also suggests turtles retain the ability to avoid unfavorable conditions should they result from fluctuating water levels.

In general, this population of Western Painted Turtles are using overwintering sites with shallow water depths (< 1 m), soft substrate into which they bury, normoxic oxygen content (> 0.5 mg/L) and cold but above freezing water temperatures (~ 0.37 – 1.1°C). These overwinter habitat conditions are comparable to those reported in similar studies of this species (Ernst 1972, Peterson 1987, Taylor and Nol 1989). Other studies found overwintering Painted Turtles did not bury into substrate during winter, although they did select locations with similar attributes (shallow depths, close to shore) (St. Clair and Gregory 1990, Crocker *et al.* 2000, Rollinson *et al.* 2008).

Why are turtles showing variation in their overwintering tactics?

This study verified the use of different overwintering tactics (i.e., congregated vs. dispersed), confirming that suggested by Basaraba (2014). In the first winter of study, turtles used the small pond structure in the MS location exclusively, providing deeper water depths for overwintering. Deeper water depths would make the animals less susceptible to changes in water levels brought about by reservoir operations. The tactics used at both AP and TP were consistent over both study winters, but not so at MS. The variation in tactics between years at MS suggests the criteria by which sites are used may not be consistent among individuals over years, especially since this is an environment that is constantly undergoing fluctuation (see Brown and Brooks 1994). Habitat conditions did not vary between tactics in the second winter, although micro-habitat conditions at turtle locations within MS did vary somewhat. Turtles overwintering in the small pond in the second winter experienced near anoxic conditions, compared to the normoxic conditions in the main body of MS.

I observed both congregated and dispersive hibernation tactics within this population of Western Painted Turtles. Congregated hibernation has been documented in similar studies of freshwater turtles at their northern extent (Brown and Brooks 1994, Litzgus *et al*. 1999, Crocker *et al.* 2000, Edge *et al*. 2009, Newton and Herman 2009). Researchers have speculated turtles may overwinter congregated to optimize mating opportunities prior to or following hibernation (Gregory 1982, Ultsch 1989). This is a plausible theory for this population, as both sexes were commonly represented within the congregations of overwintering turtles.

Congregated hibernation also may indicate a lack of suitable overwintering sites (Gregory 1982, Ultsch 1989, Newton and Herman 2009, Edge *et al*. 2009). This can be inferred by high levels of site fidelity (Gregory 1982): if an animal returns to the same spot in consecutive years, it may be out of necessity rather than selection. I observed only one instance of site fidelity, indicating suitable overwintering conditions do not appear to be limited in the three water bodies I studied. Of the nine turtles followed over two winters, two relocated to a different water body altogether, suggesting a range of suitable overwinter habitat is available.

Turtle Behaviour and Habitat Availability

My inability to make visual contact with turtles under the ice, coupled with depth recording from the animals, jointly suggests that the animals buried into the bottom substrate to overwinter. This behaviour has been documented in other freshwater turtle populations at northern latitudes and is thought to provide protection from predators while reducing the threat of freezing into surface or water-column ice (Ultsch *et al*. 1985, Ernst *et al.* 1989, St. Clair and Gregory 1990, Brown and Brooks 1994). Certainly, otter (*Lontra canadensis*) observations and sign were routine at the study site (Duncan, pers. obs) and this animal has been implicated elsewhere in overwinter mortality of turtles (Brooks *et al.* 1991).

Turtle carapace temperatures in all locations were colder compared to the average substrate temperatures measured at the stationary loggers which provided a profile of available conditions. Had I not used depth recorders on turtles and instead relied on the thermal data to infer the turtles' position (see Rollinson *et al.* 2008), I may have concluded the turtles were *not* buried in the substrate given their near-freezing carapace temperatures. Additionally, this suggests these animals may be displaying thermoregulatory behaviours in selecting colder sites to maintain metabolic suppression. Rollinson *et al*. (2008) found Painted Turtles utilizing thermally-stable environments near 0°C to overwinter, and similar studies have found freshwater turtles overwinter in locations colder than available adjacent habitats [Painted Turtles (*Chrysemys picta*) (Taylor and Nol 1989, Crawford 1991), Spotted Turtles (*Clemmys gutta*) (Litzgus *et al.* 1999, Rasmussen and Litzgus 2010), Wood Turtles (*Glyptemys insculpta*) (Greaves and Litzgus 2008), Blanding's Turtles (*Emydiodea blandingii*) (Edge *et al.* 2009), and Snapping Turtles (*Chelydra serpentina*) (Paterson *et al.* 2012)]. Near-freezing temperatures for overwintering may help suppress metabolism and subsequently reduce oxygen demands (Herbert and Jackson 1985a, 1985b, Ultsch and Jackson 1982, Ultsch 1985/89 in Greaves and Litzgus 2008), ultimately conserving energy that can later be diverted into growth and reproduction (Rollinson *et al.* 2008). Similar to this idea, lab studies on frogs (*Rana temporaria*) show that under hypoxic conditions voluntary hypoxia occurs, whereby the temperature preference of the animals changes compared to that in normoxic water (Tattersall and Boutillier 1997). This behavioural response to hypoxia helps preserve metabolic stores and delays the onset of lactic acidosis (Tattersall and Boutillier 1997).

Do reservoir operations impact overwintering turtles?

Movements and relocations

This population of Painted Turtles displayed notably longer movements throughout winter in comparison to similar studies (Sexton 1959, Gibbons 1968, Taylor and Nol 1989, St. Clair and Gregory 1990). Overwinter movements by freshwater turtles have been documented in similar studies, and often cease once the water reaches a certain temperature or level of anoxia (Taylor and Nol 1989, St. Clair and Gregory 1990, Crocker *et al.* 2000). Documented movements tend to be of short distance, up to 3 m; however Crocker *et al.* (2000) reported movement of 100 m by an Eastern Painted Turtle in Rhode Island between late February and early March, comparable to the 95 m travelled by one individual between January and February in my study.

Various reasons have been postulated as to why movements, such as those recorded in this study, occur over winter. I hypothesize the movements made by this population of Painted Turtles may be required to aid in regulation of plasma lactate by exiting from the mud, utilizing dissolved oxygen via extrapulmonary needs, and again burying to avoid potential predators. Extrapulmonary oxygen uptake is thought to be an important adaptation for freshwater turtles overwintering beneath the ice at northern latitudes (Ultsch and Jackson 1982, Ultsch *et al.* 1985, Ultsch *et al.* 1999, Crocker *et al.* 2000, Greaves and Litzgus 2007). Reese *et al.* (2004) confirmed that Western Painted Turtles (*Chrysemys picta bellii*) have the ability to utilize both anaerobiosis/aerobiosis during long-term submergence in oxygenated water. Based on the variability in depths and temperatures from depth recorder data and the mean distance moved between months, it appears movements by this population were made within the water column and not by shuffling through the substrate. In future, this could be investigated by sampling plasma lactate levels throughout winter and looking for trends between movements and the extent of acidosis.

There was no clear association between habitat variables and turtle relocations, and turtles did not relocate to areas of deeper water depth as might be expected with changing water levels. The only consistent trend was apparent at TP where dissolved oxygen concentrations increased between movements throughout the second winter. This did not appear to be a natural trend within the waterbody, based on the stationary logger data, therefore it is possible turtles were intentionally moving to areas of higher dissolved oxygen in this water body. Due to the limited amount of location data (one point per month), my data do not provide a conclusive test of whether turtles in the reservoir move in response to sudden changes in ice or water levels. Nevertheless, these movements do show that the turtles are able to successfully move to different areas within water bodies throughout winter. As few turtles exhibited long-distance movements between water bodies, this may indicate most turtles are able to fulfill all stages of their life cycle within one water body.

CONCLUSIONS

My data suggest the variation in tactics used by turtles hibernating in this study area is not linked to habitat conditions (water temperature, dissolved oxygen and depth). This population does not appear to be negatively affected by the fluctuating water table. Suitable overwintering habitat does not appear to be limiting at this location, and the turtles appear to be resilient to the changing water levels and also have the ability to move beneath the ice if conditions become unfavorable. As well, turtles may use hibernation sites with colder temperatures in an attempt to exploit voluntary hypothermia and reduce metabolic losses. Turtles in all water bodies buried in substrate to overwinter, likely to avoid the active otters and perhaps to provide an additional buffer from fluctuations. I hypothesize turtle movements throughout winter may be an attempt to regulate plasma lactate via extrapulmonary needs and/or to facilitate closer access to potential mates.

LITERATURE CITED

Aleksiuk, M. 1976. Reptilian hibernation: evidence of adaptive strategies in *Thamnophis sirtalis patietalis*. Copeia. 1976(1): 170-178.

Basaraba, N. 2014. The Ecology of Western Painted Turtles (*Chrysemys picta bellii*) in a Northern Canadian Reservoir. MSc Thesis. Thompson Rivers University. 110 p.

BC Hydro. 2007. Columbia River Project Water Use Plan. Revised for Acceptance by the Comptroller of Water Rights. BC Hydro Power Corporation. Burnaby, BC. 65 p.

BC Hydro. 2013 – 2014. Hydrometeorologic Information. Climate, snow and/or surface water station: Arrow Reservoir at Fauquier (FQU). Updated hourly. Available: http://www.bchydro.com/info/res_hydromet/data/fqu.txt.

Bickler, P.E. and Buck, L.T. 2007. Hypoxia tolerance in reptiles, amphibians and fishes: Life with variable oxygen availability. Annual Review of Physiology. 69: 145-170.

Brooks, R. J., Brown, G. P., and Galbraith, D. A. 1991. Effects of a sudden increase in natural mortality of adults on a population of the common snapping turtle (*Chelydra serpentina*). Canadian Journal of Zoology. 69: 1314-1320.

Brown, W.S. and Parker, W.S. 1976. Movement ecology of *Coluber constrictor* near communal hibernacula. Copeia. 1976(2): 225-242.

Brown, G.P. and Brooks, R.J. 1994. Characteristics of and fidelity to hibernacula in a northern population of Snapping Turtles, *Chelydra serpentina*. Copeia. 1994(1): 222-226.

Carpenter, C.C. 1957. Hibernation, hibernacula and associated behavior of the Three-Toed Box Turtle (*Terrapene Carolina triunguis*). Copeia. 1957(4): 278-282.

Canadian Council of Animal Care. *n.d*. CCAC species-specific recommendations on: Amphibians and Reptile. Ottawa, Ontario. [Web accessed; June 2014. http://www.ccac.ca/Documents/Standards/Guidelines/Add_PDFs/Wildlife_Amphibians_Reptiles.pdf].

Christiansen, J.L. and Bickham, J.W. 1989. Possible historic effects of pond drying and winterkill on the behavior of *Kinosternon flavescens* and *Chrysemys picta*. Journal of Herpetology. 23(1): 91-94.

Claussen, D.L., Daniel, P.M., Jiang, S., and Adams, N.A. 1991. Hibernation in the Eastern Box Turtle, *Terrapene c. carolina*. Journal of Herpetology. 25(3): 334-341.

Costanzo, J.P. and Lee, R.E. 2013. Avoidance and tolerance of freezing in ectothermic vertebrates. The Journal of Experimental Biology. 216(11): 1961-1967.

Costanzo, J.P., do Amaral, M.C.F., Rosendale, A.J., and Lee, R.E. 2013. Hibernation physiology, freezing adaptation and extreme freeze tolerance in a northern population of the wood frog. Journal of Experimental Biology. 216(18): 3461-3473.

Crocker, C.E., Feldman, R.A., Ultsch, G.R. and Jackson, D.C. 2000. Overwintering behaviour and physiology of eastern painted turtles (*Chrysemys picta picta*) in Rhode Island. Canadian Journal of Zoology. 78(6): 936-942.

Crawford, K.M. 1991. The winter environment of painted turtles, *Chrysemys picta*: temperature, dissolved oxygen, and potential cues for emergence. Canadian Journal of Zoology. 69: 2493-2498.

Edge, C.B., Steinberg, B.D., Brooks, R.J., and Litzgus, J.D. 2009. Temperature and site selection by Blanding's turtles (*Emydoidea blandingii*) during hibernation near the species' northern range limit. Canadian Journal of Zoology. 87: 825-834.

Environment Canada. 2014. Canadian Climate Normals Station Data 1971-2000. [Web accessed June 2014. http://climate.weather.gc.ca/climate_normals/results_e.html?stnID=1372&prov=&lang=e&dCode=4&dispBack=1&StationName=revelstoke&SearchType=Contains&province=ALL&provBut=&month1=0&month2=12].

Ernst, C.H. 1972. Temperature-activity relationship in the Painted Turtle, *Chrysemys picta*. Copeia. 1972(2): 217-222.

Gibbons, J.W. 1968. Reproductive potential, activity and cycles in the Painted Turtle, *Chrysemys picta*. Ecology. 49(3): 399-409.

Gienger, C.M. and Beck, D.D. 2011. Northern Pacific Rattlesnakes (*Crotalus oreganus*) use thermal and structural cues to choose overwintering hibernacula. Canadian Journal of Zoology. 89(11): 1084-1090.

Grayson, K.L., and Dorcas, M.E. 2004. Seasonal temperature variation in the painted turtle (*Chrysemys picta*). Herpetologica. 60(3): 325-336.

Greaves, W.F. and Litzgus, J.D. 2007. Overwintering ecology of wood turtles (*Glyptemys insculpta*) at the species' northern range limit. Journal of Herpetology. 41(1): 32-40.

Gregory, P. T. 1982. Reptilian hibernation. Pages 53–154 in C.Gans & F. H. Pough (eds). Biology of the Reptilia. Volume 13. Academic Press, London.

Harless, M., and Morlock, H. 1979. Turtles: Perspectives and Research. John Wiley & Sons, Inc. New York, USA.

Harvey, D.S., and Weatherhead, P.J. 2006. Hibernation site selection by eastern Massasauga Rattlesnakes (*Sistrurus catenatus catenatus*) near their northern range limit. Journal of Herpetology. 40(1): 66-73.

Herbert, C.V. and Jackson, D.J. 1985a. Temperature effects on the responses to prolonged submergence in the turtle *Chrysemys picta bellii*. I: Blood-acid base and ionic changes during and following anoxic submergence. Physiological Zoology. 58(6): 655-669.

Herbert, C.V. and Jackson. D.J. 1985b. Temperature effects on the responses to prolonged submergence in the turtle *Chrysemys picta bellii*. II: Metabolic rate, blood-acid base and ionic changes and cardiovascular function in aerated and anoxic water. Physiological Zoology. 58(6): 670-681.

Jackson, D.C. 1968. Metabolic depression and oxygen depletion in the diving turtle. Journal of Applied Physiology. 24: 503-509.

Jackson, D.C. 1997. Lactate accumulation in the shell of the turtle *Chrysemys picta bellii* during anoxia at 3°C and 10°C. The Journal of Experimental Biology. 200: 2295-2300.

Jackson, D.C. 2000a. Living without oxygen: lessons from a freshwater turtle. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology. 125(3): 299-315.

Jackson, D.C. 2000b. How a turtle's shell helps it survive prolonged anoxic acidosis. News in Physiological Sciences. 15: 181-185.

Jackson, D.C., Ramsey, A.L., Paulson, J.M., Crocker, C.E., and Ultsch, G.R. 2000. Lactic Acid Buffering by Bone and Shell in Anoxic Softshell and Painted Turtles. Physiological and Biochemical Zoology. 73(3): 290-297.

Jackson, D.C. 2002. Hibernating without oxygen: physiological adaptations of the painted turtle. Journal of Physiology. 543(3): 731-737.

Jackson, D.C., Rauer, E.M., Feldman, R.A. and Reese, S.A. 2004. Avenues of extrapulmonary oxygen uptake in Western Painted Turtles (*Chrysemys picta bellii*) at 10°C. Comparative Biochemistry and Physiology A. 139: 221-227.

Litzgus, J.E., Costanzo, J.P., Brooks, R.J., Lee, R.E. 1999. Phenology and ecology of hibernation in spotted turtles (*Clemmys guttata*) near the northern limit of their range. Journal of Zoology. 77(9): 1348-1357.

Mathies, T., and Martin, D.J. 2008. Overwintering site selection by Short-Horned Lizards (*Phrynosoma hernandesi*) in northeastern Colorado. Journal of Herpetology. 42(1): 163-171.

Meeks, R.L. and Ultsch, G.R. 1990. Overwintering behavior of Snapping Turtles. Copeia. 1990(3): 880-884.

Muir, A.P., Biek R., and Mable, B.K. 2014. Behavioural and physiological adaptations to low-temperature environments in the common frog, *Rana temporaria*. BMC Evolutionary Biology. 14: 110.

Newton, E.J. and Herman, T.B. 2009. Habitat, movements and behaviour of overwintering Blanding's turtles (*Emydiodea blandingii*) in Nova Scotia. Canadian Journal of Zoology. 87: 299-309.

Packard, G.C. and Packard, M.J. 2003. Cold acclimation enhances cutaneous resistance to inoculative freezing in hatchling painted turtles, *Chrysemys picta*. Functional Ecology. 17(1): 94-100.

Paterson, J.E., Steinberg, B.D. and Litzgus, J.D. 2012. Generally specialized or especially general? Habitat selection by Snapping Turtles (*Chelydra serpentina*) in central Ontario. Canadian Journal of Zoology. 90: 139-149.

Peterson, C.C. 1987. Thermal relations of hibernating Painted Turtles, *Chrysemys picta*. Journal of Herpetology. 21(1): 16-20.

Rasmussen, M.L., and Litzgus, J.D. 2010. Habitat selection and movement patterns of Spotted Turtles (*Clemmys guttata*): Effects of spatial and temporal scales of analyses. Copeia. 2010(1): 86-96.

Reese, S.A., Crocker, C.E., Jackson, D.C. and Ultsch, G.R. 2000. The physiology of hibernation among painted turtles: the midland painted turtle (*Chrysemys picta marginata*). Respiration Physiology. 124(2000): 43-50.

Reese, S.A., Stewart, E.R., Crocker, C.E., Jackson, D.C. and Ultsch, G.R. 2004. Geographic variation of the physiological response to overwintering in the painted turtle (*Chrysemys picta*). Physiological and Biological Zoology. 77(4): 619-630.

Rollinson, N., Tattersall, G.J., and Brooks, R.J. 2008. Overwintering habits of a northern population of painted turtles (*Chrysemys picta*): winter temperature selection and dissolved oxygen concentrations. Journal of Herpetology. 42(2): 312-321.

Sexton, O.J. 1959. Spatial and temporal movements of a population of the Painted Turtle, *Chrysemys picta* (Agassiz). Ecological Monographs. 29(2): 113-140.

St. Clair, R.C., and Gregory, P.T. 1990. Factors affecting the northern range limit of Painted Turtles (*Chrysemys picta*): Winter acidosis or freezing? Copeia 1990(4): 1083-1089.

Storey, K.B. 2006. Reptile freeze tolerance: Metabolism and gene expression. Cryobiology. 52(1): 1-16.

Storey, K.B. 2007. Anoxia tolerance in turtles: Metabolic regulation and gene expression. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology. 147(2): 263-276.

Strain, G.F., Anderson, J.T., Michael, E.D., and Turk, P.J. 2012. Hibernacula use and hibernation phenology in the Common Snapping Turtle (*Chelydra serpentina*) in Canaan Valley, West Virginia. Journal of Herpetology. 46(2): 269-274.

Tattersall, G.J. 2008. Physiological ecology of aquatic overwintering in ranid frogs. Biological Reviews of the Cambridge Philosophical Society. 83(2): 119-140.

Tattersall, G.J. and Boutilier, R.G. 1997. Balancing hypoxia and hypothermia in cold-submerged frogs. Journal of Experimental Biology. 200(6): 1031-1038.

Taylor, G.M., and Nol, E. 1989. Movements and hibernation sites of overwintering painted turtles in southern Ontario. Canadian Journal of Zoology. 67: 1877-1881.

Ultsch, G.R. and Jackson, D.C. 1982. Long-term submergence at 3°C of the turtle, *Chrysemys picta belli*, in normoxic and severely hypoxic water. Journal of Experimental Biology. 96: 11-28.

Ultsch, G. R., Hanley, R. W., and Bauman, T. R. 1985. Responses to anoxia during simulated hibernation in northern and southern painted turtles. Ecology. 66(2): 388-395.

Ultsch, G.R. 1989. Ecology and physiology of hibernation and overwintering among freshwater fishes, turtles and snakes. Biological Reviews. 64: 435-516.

Ultsch, G.R., Carwile, M.E., Crocker, C.E. and Jackson, D.C. 1999. The physiology of hibernation among painted turtles: the eastern painted turtle *Chrysemys picta picta*. Physiological and Biochemical Zoology. 72(4): 493-501.

Voituran, Y., Jean-Pierre, H., and Grenot, C. 2000. Metabolic adaptations of overwintering European Common Lizards (*Lacerta vivipara*). Physiological and Biochemical Zoology. 73(3): 264-271.

Chapter Three

TURTLE POPULATIONS IN NEIGHBOURING WATER BODIES: COMPARING ANTHROPOGENIC VS. NATURAL HABITATS

INTRODUCTION

The display of different behaviours within animal populations generally is considered to reflect adaptive plasticity, or the use of contrasting tactics that function best under different conditions (Welcomme 1979, Stearns 1989, Sih *et al*. 2011, Tuomainen and Candolin 2011, Sih 2013, Snell-Rood 2013). In some cases, this plasticity may result in contrasting behaviours being detected between conspecifics even in the same population, providing a unique backdrop to investigate how animals respond to altered environments, and what consequences may result. Potential ramifications of behavioural change may include impacts to body condition, access to mates or reduced survival, and (ultimately) evolutionary implications (example in Noble *et al*. 2013). The potential for the evolution of alternative behavioural tactics may be increased in long-lived species (Baird *et al.* 2012), but the effects of utilizing these tactics in populations or species may not always be evident within short term studies. Further, effects (positive or negative) stemming from local environments may be more subtle or indirect (Lomas *et al*., in press), suggesting they may be more readily detected by examining population structures and demographics.

Of particular interest are situations where varying tactics are displayed within populations. If at some scale this variation shows a spatial pattern (i.e., animals in certain areas show different tactics), it suggests a plastic response by the animals to localized conditions. For example, African killifish (*Nothobranchius furzeri*) occupy both permanent and temporary ponds; in the latter habitat, embryonic development and post-hatching growth is more rapid coinciding with smaller adult body size and a shorter life span (Polačik *et al*. 2014). Despite this behavioural variation due to localized conditions, fecundity, physiological aging and propensity to mate do not vary (Polačik *et al*. 2014). Similarly, Berven (1988) showed a variation in the reproductive traits of a single population of *Rana sylvatica* relative to age and environmental conditions between years. The youngest reproductive females [1 year] produced a large number of small eggs, whereas older females [3 years] produced a small number of large eggs (Berven 1988). Clutch size also varied with body size, as a factor of annual variation in precipitation affecting food levels and activity patterns (Berven 1982).

Compared to behaviours associated with reproduction, variation in hibernation tactics for ectotherms, particularly reptiles, appear to occur more across the range of particular species (i.e., over changes in latitude) rather than within populations. For example, northern Painted Turtles (*Chrysemys picta*) are better able to tolerate anoxia at low water temperatures compared to their southern counterparts (Reese *et al.* 2004), and the choice of hibernacula by conspecifics in different populations may vary according to latitudes (e.g., Great Basin Gopher Snake – Williams *et al.* 2015). However, environmental conditions at any specific location may presumably favour a single overwintering tactic, thus making it less likely that different behaviours will be detected in the same population. Indeed, examples of this type of variation in overwintering behaviours are scant, although inter-individual variation in reptile behaviours connected with temperature have been documented, including maximal spring speed and preferred body temperature in lizards (Artacho *et al*. 2013), as well as in defensive behaviours of snakes (Citadini and Navas 2013).

Hibernation strategies in turtles have been well studied, for both terrestrial and aquatic environments. Species that hibernate on land may in some cases develop resistance to subzero temperatures (Ultsch 1989, review by Storey 2006, Currylow *et al.* 2013) whereas aquatic hibernators in general may be able to avoid freezing conditions, but instead may need physiological mechanisms for dealing with prolonged periods of submersion, sometimes in anoxic conditions. The overwintering adaptations of Painted Turtles (*Chrysemys picta*) are particularly well studied, in part because variation in hibernating strategies/tactics may occur between age classes within the same population: hatchling Painted Turtles may remain on land (in the nest) throughout winter, versus adults that consistently hibernate aquatically (Storey *et al.* 1988, Constanzo *et al*. 1995, Lee and Constanzo 1998, Packard *et al*. 1997, Packard *et al*. 2001, Packard and Packard 2001, Constanzo *et al.* 2008, Riley *et al.* 2014). Further, the ability of submerged adult Painted Turtles to tolerate hypoxic conditions for extended periods has been extensively examined (see Jackson 1968, Ultsch and Jackson 1982, Ultsch *et al.* 1985, Herbert and Jackson 1985a, 1985b, Ultsch *et al.* 1999, Reese *et al.*

2000, Crocker *et al.* 2000, Reese *et al.* 2004, review by Storey 2007). This variation in adult Western Painted Turtle behavioural overwintering strategy, however, has not been reported on.

In Chapter 2, I described variation in aquatic hibernation tactics within a population of Western Painted Turtles (*C. p. belli*) near the northern limit of the species. This variation, first detected by Basaraba (2014), shows a spatial pattern: turtles in one section of a reservoir tend to form aggregations over winter, whereas in a neighbouring portion of the same reservoir the hibernating animals tend to be dispersed. Chapter 2 of this thesis presents a more detailed examination of this phenomenon indicating that this variation in tactics was not linked to spatial or temporal patterns of presumed key habitat variables (water depth, dissolved oxygen, water temperature). I also showed that the fluctuating water levels of the reservoir (and possible shifts in ice cover) did not impact the overwinter survival of the animals, nor precipitate movements under ice. An important complement to this baseline work is a more direct comparison of the subpopulations. Information on survival, density, and the sizes of individuals therein is important for understanding the costs and benefits of using different hibernation tactics.

Herein I present a comparison of demographics and other descriptors of the two subpopulations of reservoir turtles. To add to this comparison, I include data from a neighbouring group of turtles that inhabit a smaller body of water not influenced by reservoir operations. Thus, this comparison of hibernating tactics provides a more general and broader study of how habitat quality for these animals varies between anthropogenic (i.e., reservoir) and natural water bodies. My specific objectives were to: (i) compare population sizes and densities of the animals in the three bodies of water, (ii) conduct a body size comparison between all locations, and (iii) evaluate the overwinter impacts on turtle body condition between the non-reservoir and reservoir locations.

METHODS

Study Area

This research took place from January 2013 – May 2014 in a section of the Arrow Lakes Reservoir, lying immediately south of the town of Revelstoke, British Columbia, Canada

(50.9981° N, 118.1956° W). This section of the Columbia River has existed as a reservoir since the implementation of the downstream Hugh Keenleyside Dam in 1968, with further additional effects coming from the upstream Revelstoke Dam (1984) (BC Hydro 2007).

Western Painted Turtles occur further north than any other species of turtle in North America, extending into south-central areas in Canada. Being so far north, winters here are longer with ice cover over the water bodies occurring from late November to early/mid-April (Chapter 2). Following Basaraba (2014), I continued the collection of basic demographic and morphological data on the turtles occupying the two portions of the reservoir where the overwhelming majority of turtles live. Movements by turtles between these two water bodies (approx. 2.5 km apart) occur rarely (Chapter 2). Of these sites, Airport Marsh (AP) is a large marshy bay bordered by an airport runway where turtles overwinter in a dispersed fashion (using the criteria of >5 m between nearest hibernating neighbours – Brown and Brooks 1994, Edge *et al.* 2009). Montana Slough (MS) is a smaller wetland characterized by a floating vegetated island and turtles there tend to hibernate in a more clumped fashion, often gathering within a smaller body of water encircled by vegetation. Turtle Pond is approximately 1 km from the shoreline of the reservoir; it is a small body of water (6.9 ha) that has no direct connection to the reservoir, although at least one incidence of a turtle moving into this pond from the reservoir has been recorded. Because movements between the three sites are rare but present (Chapter 2, Basaraba 2014), I hereafter use the term 'subpopulation' to refer to the groups of turtles inhabiting the different water bodies. For more details on these specific study sites see Chapter 2 and Basaraba (2014).

Turtle Capture and Morphology

Basaraba (2014) reported female-biased capture using basking traps, so I used hoop traps exclusively during my trapping periods. In spring (May) and late summer (August-September/October) of both 2013 and 2014, I captured turtles using hoop traps baited with sardines and/or cat food. Traps were checked at a minimum every 12 hours. Traps were set at all study locations: Airport Marsh (AP), Montana Slough (MS) and Turtle Pond (TP). I also captured turtles opportunistically by dip net or hand capture. Adult turtles were notched with an individual code using a triangular file (Cagle 1939). Juvenile turtles were not marked to preclude damage to the shell before full ossification (Cagle 1939). However, measurements and photos for identification were collected. Morphological measurements recorded for all turtles were carapace length (cm) and weight (g). Turtles were sexed based on secondary sexual characteristics and the relative positioning of the cloaca (Macartney and Gregory 1985). Samples of adult turtles were equipped with radio transmitters as part of the Chapter 2 study on overwinter behaviour. These animals furnished precise measurements of habitat use, movements and survival.

Data Analysis

I combined data collected by Basaraba (2014) over 2011-12 with my dataset from 2013-14 to estimate the number of turtles in each of the subpopulations using K-sample mark/recapture methods (Krebs 1999) available through MARK software. In the absence of genetic data, these subpopulations were considered to be closed due to the low frequency of detections for turtles moving between them (Basaraba 2014, Chapter 2). Turtles were grouped according to the year they were captured in (2011, 2013 or 2014). Because the reservoir undergoes fluctuation in water levels on an annual cycle, available turtle habitat also fluctuates, therefore I used telemetry data to represent the areas used by these animals in the two locations within the reservoir and constructed minimum convex polygons (QGIS Version 2.6 Brighton) to convert population estimates to densities. The population density of TP was estimated using the surface area of the water body, since turtles were located throughout the enclosed polygon area over the course of each study year.

Turtle body size comparisons involved data collected by both Basaraba (2014 unpublished) and myself. In 2011, turtles were captured from April – September, in 2013 in May, August – October and in 2014 in May. Differences between means for turtle size [carapace length, weight] were determined using a Kruskal-Wallace test [H] with a post-hoc Dunn's Test or ANOVA [F] with post-hoc Tukey Test (depending on homoscedasticity) using the software package R (version 3.02). Homogeneity of variances was tested using Fligner's Test due to departures from normality (Conover *et al*. 1981). Body condition was estimated using the residuals of a curvilinear regression of weight (g) against carapace length (cm) for Apr-June capture data, and comparing differences between the subpopulations using ANOVA (Schulte-Hostedde *et al.* 2005, Litzgus *et al*. 2008, Polo-Cavia *et al*. 2010). Only males were used in the body condition analysis because adult females would be carrying clutches following winter, introducing a bias in their weight-carapace length ratio. My interpretation of statistical significance was guided by using $\alpha = 0.05$.

RESULTS

Captures and Population Estimates

The total number of individual turtles captured using hoop traps at AP inclusive of three trapping sessions from 2011 and 2013-14 was 38 turtles. Twenty-seven turtles were captured at MS, and 29 turtles were captured at TP. Population estimates determined from these captures were 75 ± 8.6, 54 ± 15.9 and 52 ± 6 for the AP, MS and TP water bodies, respectively. These in turn produced density estimates of 0.6 turtle/ha in AP and 1.3 turtles/ha in MS. Using the surface area of TP produced a density estimate of 8 turtles/ha.

Body Size and Condition between Locations

A strong relationship was shown between turtle weight (g) and carapace length (cm) for both adult and juvenile turtles across all locations (Figure 3-1). Turtle weights and lengths of female turtles captured at TP were higher than females captured at both MS and AP (Dunn's Post-Hoc "Bonferroni" (weight) $P < 0.002$, (length) $P < 0.02$). Male turtles at TP were also larger in both weight and length than male turtles at MS (Tukey Post-Hoc (weight) $P = 0.02$, Dunn's Post-Hoc "Bonferroni" (length) $P < 0.003$) however were comparable to those at AP. Interestingly, juvenile turtles at MS were larger than those captured at AP (both weight and length, $F_{1,18} = 9.78$ (weight) and 11.55 (length), $Ps < 0.05$). However, there was not a significant difference in male turtle body condition between locations following winter ($F_{2,40} = 0.03$, $P = 0.97$) (Figure 3-2).

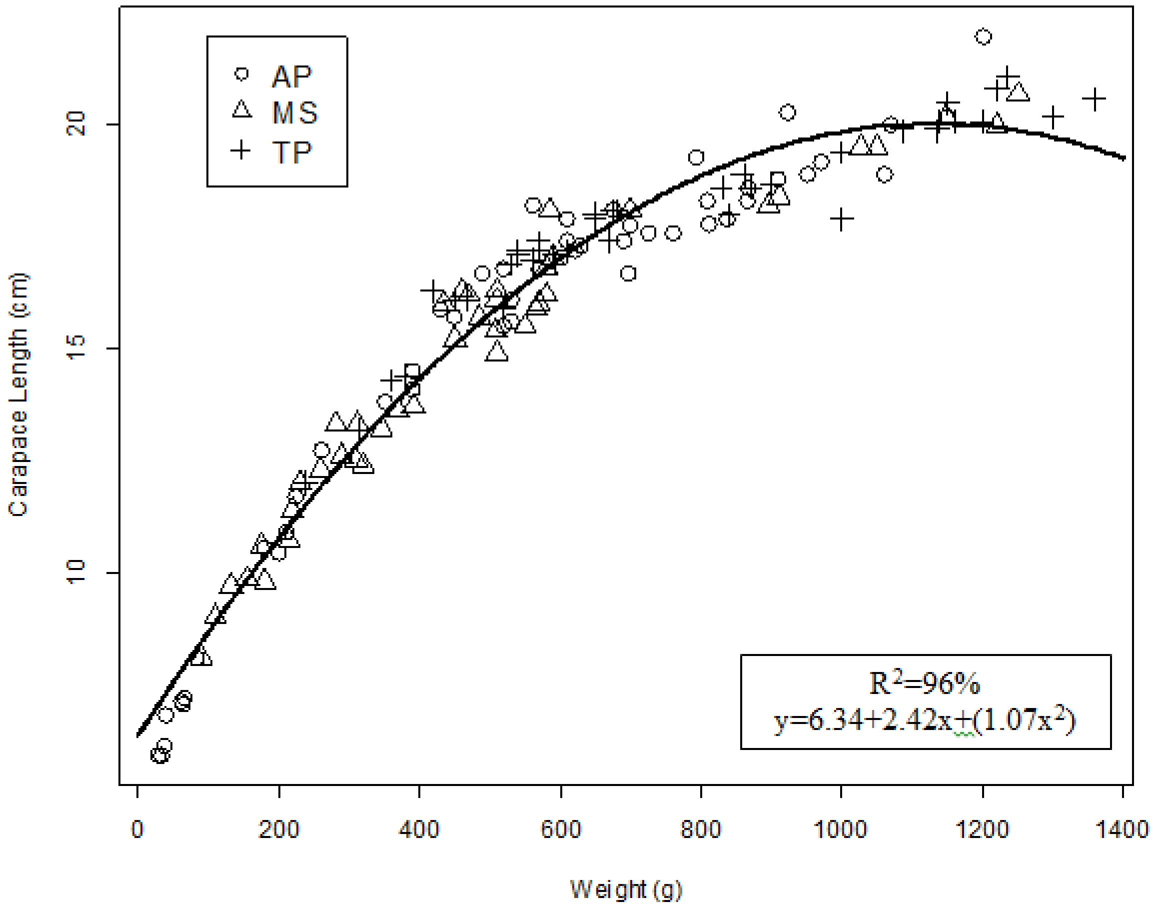


Figure 3-1: The relationship between carapace length (cm) and weight (g) for Western Painted Turtles captured in 2011 (Apr-Sept), 2013 (May, Aug-Oct) and 2014 (May) in all three study sites (AP = Airport Marsh, MS = Montana Slough, TP = Turtle Pond) at Revelstoke BC, Canada.

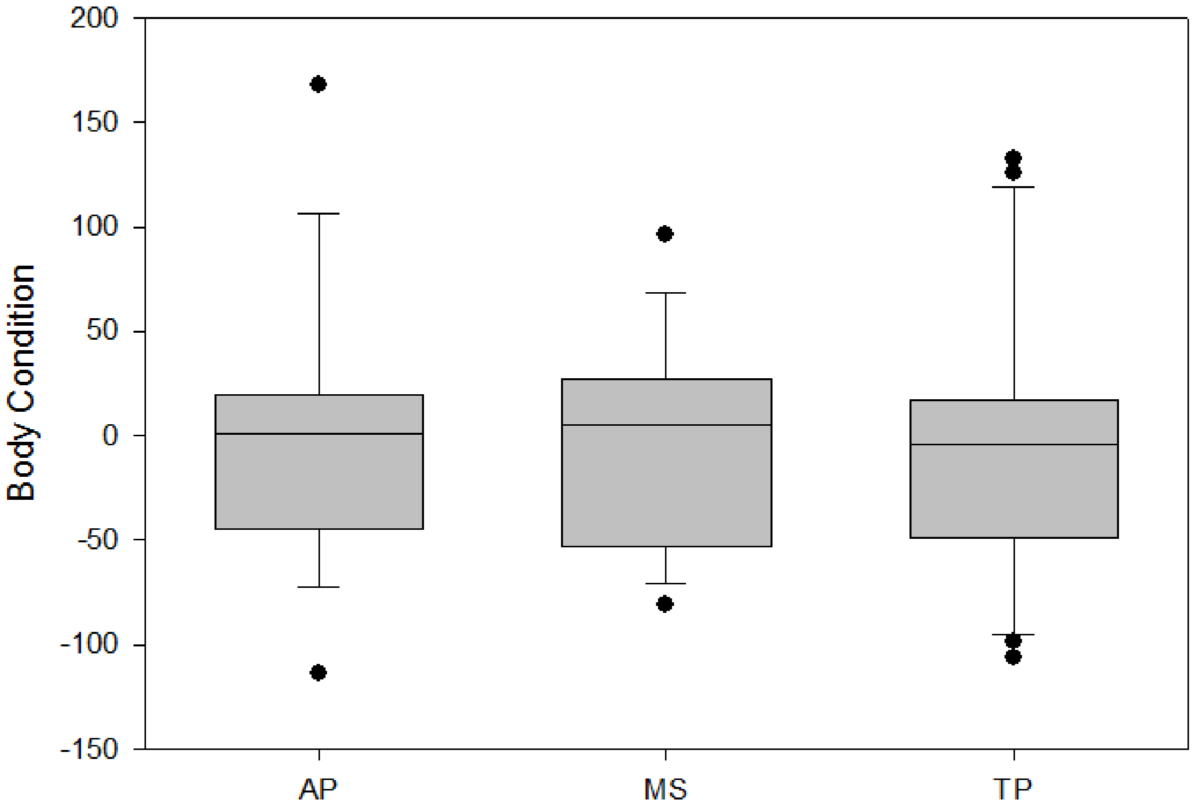


Figure 3-2: Comparison of mean body condition scores (residuals from the regression of weight (g) on carapace length (cm)) ± SE of **male** Western Painted Turtles captured in each study location in 2011, 2013 and 2014 (Apr-June). (AP N = 14; MS N = 13; TP N = 16).

DISCUSSION AND CONCLUSIONS

Perhaps the most striking result from my analysis is that the density of turtles in the smaller Turtle Pond was much higher than that in the two reservoir subpopulations, yet the animals at this location were not smaller, and (at least the males) did not differ in body condition. Presumably, the habitat at TP provides better conditions (foraging, basking, rearing etc.) and therefore is able to provide a higher carrying capacity in comparison to the other subpopulations. I hypothesize that the large amount of algae within the pond may provide ample food for the turtles, which may help them reach larger sizes. I also hypothesize that the prevalence of basking substrates available within Turtle Pond may draw turtles to this location, as there are few logs and rocks available within the reservoir for turtles to use. Nevertheless, density estimation of the reservoir populations are comparable to other population estimates at the northern extent of the species range (0.9 – 7.2 turtles/ha Macartney and Gregory 1985). It should be noted that my estimates of the reservoir locations are lower than the previous estimate for this population (2.0-2.2 turtles/ha in AP and MS combined, depending on water level, Basaraba 2014). This may be indicative of a shift in population sizes, or may also be a result of different methods used to calculate surface area.

Despite the differences in densities between the three sites, there were no detectable differences in body condition between male turtles early in the spring. Intuitively, one would consider body condition to be influenced by density through intraspecific competition. Body condition indices have been negatively correlated with population densities in the marine green turtle (*Chelonia mydas*) (Bjorndal *et al*. 2000), however, studies on freshwater turtles are lacking (but see Kennett and Georges 1990 relating body condition to different habitat types). Additionally, my analysis included metrics taken in *spring* as indicators of body condition (weight, carapace length) to represent physical stress as a result of overwintering between the subpopulations. I recommend comparing measurements prior to overwintering (once foraging ceases) to provide more information on the physical effects of density between subpopulations; however, I recognize these data would be difficult to collect given the challenge of capturing turtles when they are not feeding.

All told, the results herein and that presented in Chapter 2 do not suggest any ramifications (positive or negative) for the different hibernating tactics detected in this population of Western Painted Turtles. As well, I did not detect any noticeable differences in the body condition indices of the different subpopulations living in or adjacent to the reservoir regardless of physical and temporal differences between locations. These turtles do not appear to be negatively affected despite some obvious differences in the conditions facing turtles in the Turtle Pond versus the larger reservoir water bodies.

However, differences detected in the subpopulation of turtles inhabiting Turtle Pond compared to the reservoir sections (Airport March and Montana Slough) strongly suggests some factor(s) other than those I examined are influencing local densities and body size distributions of this animal. Bennett *et al.* (2009) found a similar result comparing Northern Map Turtle populations between fragmented and intact sites; turtles in the control (intact) sites were significantly larger than those in the fragmented (dammed) sites. It is possible that TP provides superior habitat or resources such as food availability, basking structures, overwintering conditions and/or access to mates. Another reason may be that TP is in fact an *open* population having constant immigration occurring from elsewhere, which I was unable to measure in my short study period. Further, density estimates for aquatic turtles will be influenced by the precise method(s) used to calculate habitat area. Certainly, defining area seems more intuitive when dealing with a small, landlocked body of water such as TP compared to the much broader reservoir basin that encompasses the AP and MS subpopulations. Nevertheless, the magnitude of difference in my density estimates across subpopulations seems to suggest that a considerably higher density of turtles occurs in TP, no matter what algorithm is used in the calculations.

A number of direct and indirect indicators have been used as measures of habitat quality, including but not limited to survival, reproduction, body condition, spatial and temporal patterns, and environmental constraints (Johnson 2007). Whether density is an accurate predictor of habitat quality has been subject to considerable debate and study (Van Horne 1983, Johnson 2007). Assuming the reservoir water bodies have suboptimal habitat because of lower turtle densities may not be correct; one study found freshwater turtle densities (including *Chrysemys picta* and other species) were greater in rural ponds of a smaller size compared to larger urban/man-made ponds (Witczak *et al.n.d.*). Population densities in long-lived species may not respond immediately to habitat alterations, therefore long-term population monitoring efforts may be required to determine if the habitat quality is suboptimal in the reservoir (Eskew *et al.* 2010).

The overarching question behind my research on these turtles is why do turtles within the two sections of the reservoir demonstrate different hibernating tactics? These animals have shown plasticity in their overwintering behaviour by altering tactics used between subpopulations (Chapter 2). However, the behaviours shown do not appear to be related to the key habitat variables that I measured [temperature, dissolved oxygen, water depth], nor do they appear to impact the survival and body condition of turtles utilizing the different behaviours. Researchers have suggested animals may overwinter congregated to facilitate easier access to mates (Gregory 1982, Ultsch 1989) or out of necessity due to a lack of suitable overwintering habitat (Gregory 1982, Ultsch 1989, Newton and Herman 2009, Edge *et al*. 2009). Ample overwintering habitat does appear to be available to turtles at all locations; however the role of mate proximity in affecting hibernation tactics is plausible for this population as both sexes were present in the congregated hibernators. It may also be that the factors influencing site selection by hibernating turtles varies between years (see Brown and Brooks 1994), something which I failed to detect with my environmental measurements (Chapter 2). Such changes (if they occur) may be more readily detected over a longer study of overwintering by these animals. Nevertheless, this population may be more likely to persist as individuals appear capable of plastic responses to changing environments (both spatially and temporally) (Ghalambor *et al.* 2007).

LITERATURE CITED

Artacho, P., Jouanneau, I. and Le Gaillard, J-F. 2013. Inter-individual variation in thermal sensitivity of maximal spring speed, thermal behavior and resting metabolic rate in a lizard. Physiological and Biochemical Zoology. 86(4): 458-469.

Baird, T.A., Baird, T. D. and Shine, R. 2012. Aggressive transition between alternative male social tactics in a long-lived Australian Dragon (*Physignathus lesueurii*) living at high density. PLoS ONE. 7(8): 1-8.

Basaraba, N. 2014. The Ecology of Western Painted Turtles (*Chrysemys picta bellii*) in a Northern Canadian Reservoir. MSc Thesis. Thompson Rivers University. 110 p.

BC Hydro. 2007. Columbia River Project Water Use Plan. Revised for Acceptance by the Comptroller of Water Rights. BC Hydro Power Corporation. Burnaby, BC. 65 p.

Bennett, A.M., Keevil, M., and Litzgus, J.D. 2009. Demographic differences among populations of Northern Map Turtles (*Graptemys geographica*) in intact and fragmented sites. Canadian Journal of Zoology. 87: 1147-1157.

Berven, K.A. 1982. The genetic basis of altitudinal variation in the wood frog *Rana sylvatica*. I. An experimental analysis of life history traits. Evolution. 36(5): 962-983.

Berven, K.A. 1988. Factors affecting variation in reproductive traits within a population of wood frogs (*Rana sylvatica*). Copeia. 1988(3): 605-615.

Bjorndal, K.A., Bolten, A.B., and Chaloupka, M.Y. 2000. Green turtle somatic growth model: evidence for density dependence. Ecological Adaptations. 10:269-282.

Brown, W.S. and Parker, W.S. 1976. Movement ecology of *Coluber constrictor* near communal hibernacula. Copeia. 1976(2): 225-242.

Brown, G.P. and Brooks, R.J. 1994. Characteristics of and fidelity to hibernacula in a northern population of Snapping Turtles, *Chelydra serpentina*. Copeia. 1994(1): 222-226.

Carpenter, C.C. 1957. Hibernation, hibernacula and associated behavior of the Three-Toed Box Turtle (*Terrapene Carolina triunguis*). Copeia. 1957(4): 278-282.

Cagle, F.R. 1939. A system of marking turtles for future identification. Copeia. 1939(3): 170-173.

Citadini, J.M., and Navas, C.A. 2013. Inter-individual variation and temperature-dependent antipredator behaviour in the snake *Tomodon dorsatus* (Dipsadidae). Behavioural Processes. 97: 11-17.

Conover, W.J., Johnson, M.E., and Johnson, M.M. 1981. A comparative study of tests for homogeneity of variances, with applications to the outer continental shelf bidding data. Technometrics. 23(4): 351-361.

Constanzo, J.P., Iverson, J.B., Wright, M.F. and Lee Jr., R.E. 1995. Cold hardiness and overwintering strategies of hatchlings in an assemblage of northern turtles. Ecology. 76(6): 1772-1785.

Constanzo, J.P., Lee Jr., R.E. and Ultsch, G.R. 2008. Physiological ecology of overwintering in hatchling turtles. Journal of Experimental Zoology. 309A: 297-379.

Crocker, C.E., Feldman, R.A., Ultsch, G.R. and Jackson, D.C. 2000. Overwintering behaviour and physiology of eastern painted turtles (Chrysemys picta picta) in Rhode Island. Canadian Journal of Zoology. 78(6): 936-942.

Currylow, A.F., Macgowan, B.J., and Williams, R.N. 2013. Hibernal thermal ecology of eastern box turtles within a managed forest landscape. Journal of Wildlife Management. 77(2): 326-335.

Edge, C.B., Steinberg, B.D., Brooks, R.J., and Litzgus, J.D. 2009. Temperature and site selection by Blanding's turtles (*Emydoidea blandingii*) during hibernation near the species' northern range limit. Canadian Journal of Zoology. 87: 825-834.

Eskew, E.A., Price, S.J., and Dorcas, M.E. 2010. Survivorship and population densities of Painted Turtles (*Chrysemys picta*) in recently modified suburban landscapes. Chelonian Conservation and Biology. 9(2): 244-249.

Gienger, C.M., and Beck, D.D. 2011. Northern Pacific Rattlesnakes (*Crotalus oreganus*) use thermal and structural cues to choose overwintering hibernacula. Canadian Journal of Zoology. 89: 1084-1090.

Ghalambor, C.K., McKay J. K., Carroll S. P. and D. N. Reznick. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in New Environments. Functional Ecology. 21(3): 394-407.

Gregory, P. T. 1982. Reptilian hibernation. Pages 53–154 in C.Gans & F. H. Pough (eds). Biology of the Reptilia. Volume 13. Academic Press, London.

Herbert, C.V. and Jackson, D.J. 1985a. Temperature effects on the responses to prolonged submergence in the turtle *Chrysemys picta bellii*. I: Blood-acid base and ionic changes during and following anoxic submergence. Physiological Zoology. 58(6): 655-669.

Herbert, C.V. and Jackson. D.J. 1985b. Temperature effects on the responses to prolonged submergence in the turtle *Chrysemys picta bellii*. II: Metabolic rate, blood-acid base and ionic changes and cardiovascular function in aerated and anoxic water. Physiological Zoology. 58(6): 670-681. Jackson, D.C. 1968. Metabolic depression and oxygen depletion in the diving turtle. Journal of Applied Physiology. 24: 503-509.

Jackson, D.C. 1968. Metabolic depression and oxygen depletion in the diving turtle. Journal of Applied Physiology. 24: 503-509.

Johnson, M.D. 2007. Measuring habitat quality: a review. The Condor. 109: 489-504.

Kennett, R.M. and Georges, A. 1990. Habitat utilization and its relationship to growth and reproduction of the eastern long-necked turtle, *Chelodina longicollis* (Testudinata: Chelidae), from Australia. Herpetologica. 46(1): 22-33.

Krebs, C.J. 1999. Ecological Methodology, 2nd ed. Addison-Welsey Educational Publishers, Inc. USA. 620 p.

Lee, R.E. and Constanzo, J.P. 1998. Biological ice nucleation and ice distribution in cold-hardy ectothermic animals. Annual Review of Physiology. 60(1): 55-72.

Litzgus, J.D., Costanzo, J.P., Brooks, R.J., Lee, R.E. 1999. Phenology and ecology of hibernation in spotted turtles (*Clemmys guttata*) near the northern limit of their range. Journal of Zoology. 77(9): 1348-1357.

Litzgus, J.D., Bolton, F., and Schulte-Hostedde, A.I. 2008. Reproductive output depends on body condition in Spotted Turtles (*Clemmys guttata*). Copeia. 2008(1): 86-92.

Lomas, E., Larsen, K.W., and Bishop, C.A. in press. Persistence of Northern Pacific Rattlesnakes masks the impact of human disturbance on weight and body condition. Animal Conservation.

Macartney M., and Gregory, P.T. 1985. The western painted turtle in Kikomun Creek Provincial Park. Unpublished report, submitted to Parks Branch, British Columbia. 95p.

Meeks, R.W. and Ultsch, R.G. 1990. Overwintering behavior of Snapping Turtles. Copeia. 1990(3): 880-884.

Newton, E.J. and Herman, T.B. 2009. Habitat, movements and behaviour of overwintering Blanding's turtles (*Emydiodea blandingii*) in Nova Scotia. Canadian Journal of Zoology. 87: 299-309.

Noble, D.W.A., Wechmann, K., Keough, J.S., and Whiting, M.J. 2013. Behavioral and morphological traits interact to promote the evolution of alternative reproductive tactics in a lizard. The American Naturalist. 182(6): 726-742.

Packard, G.C., Lang, J.W., Lohmiller, L.D., Packard, M.J. 1997. Cold tolerance in hatchling painted turtles (Chrysemys picta): supercooling or tolerance for freezing? Physiological Zoology. 70(6): 670-678.

Packard, G.C., Packard, M.J. and McDaniel, L.L. 2001. Seasonal change in the capacity for supercooling by neonatal painted turtles. The Journal of Experimental Biology. 204: 1667-1672.

Packard, G.C. and Packard, M.J. 2001. The overwintering strategy of hatchling painted turtles, or how to survive in the cold without freezing. BioScience. 51(2): 199-207.

Polačik, M., Blažek, Řežucha, R., VrtiĺIek, M., Terzibasi Tozzaini, E., Reichard, M., 2014. Alternative intrapopulation life-history strategies and their trade-offs in an African annual fish. Journal of Evolution Biology. 27(2014): 854-865.

Polo-Cavia, N., Engstrom, T., López, P., and Martin, J. 2010. Body condition does not predict immunocompetence of western pond turtles in altered versus natural habitats. Animal Conservation. 13(2010): 256-264.

Reese, S.A., Crocker, C.E., Jackson, D.C. and Ultsch, G.R. 2000. The physiology of hibernation among painted turtles: the midland painted turtle (*Chrysemys picta marginata*). Respiration Physiology. 124(2000): 43-50

Reese, S.A., Stewart, E.R., Crocker, C.E., Jackson, D.C. and Ultsch, G.R. 2004. Geographic variation of the physiological response to overwintering in the painted turtle (*Chrysemys picta*). Physiological and Biological Zoology. 77(4): 619-630.

Riley, J.L., Tattersall, G.J. and Litzgus, J.D. 2014. Potential sources of intra-population variation in the overwintering strategy of painted turtle (*Chrysemys picta*) hatchlings. The Journal of Experimental Biology. 217: 4174-4183.

Rosen, P.C. 1991. Comparative ecology and life history of the Racer (*Coluber constrictor*) in Michigan. Copeia. 4: 897-909.

Schulte-Hostedde, A.I., Zinner, B., Millar, J.,S., and Hickling, G.J. 2005. Restitution of mass-size residuals: validating body condition indices. Ecology. 86: 155-163.

Sih, A. Maud, C.O.F, and Harris, D.J. 2011. Evolution and behavioural responses to human-induced rapid environmental change. Evolutionary Applications. 4(2): 367-387.

Sih, A. 2013. Understanding variation in behavioural responses to human-induced rapid environmental change: a conceptual review. Animal Behaviour. 85(5): 1077-1088.

Snell-Rood, E.C. 2013. An overview of the evolutionary causes and consequences of behavioural plasticity. Animal Behaviour. 85(2013): 1004-1011.

Stearns, S.C. 1989. The evolutionary significance of phenotypic plasticity. Bioscience. 39(7): 436-445.

Storey, K.B., Storey, J.M., Brooks, S.P.J., Churchill, T.A., and Brooks, R.J. 1988. Hatchling turtles survive freezing during winter hibernation. Proceedings of the National Academy of Sciences of the United States of America. 85(21): 8350-8354.

Storey, K.B. 2006. Reptile freeze tolerance: Metabolism and gene expression. Cryobiology. 52: 1-16.

Storey, K.B. 2007. Anoxia tolerance in turtles: Metabolic regulation and gene expression. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology. 147(2): 263-276.

Swanson, D.L. and Burdick, S.L. 2010. Overwintering physiology and hibernacula microclimates of Blanchard's Cricket Frogs at their northwestern range boundary. Copeia. 2: 247-253.

Taylor, G.M., and Nol, E. 1988. Movements and hibernation sites of overwintering painted turtles in southern Ontario. Canadian Journal of Zoology. 67: 1877-1881.

Tuomainen, U., and Candolin, U., 2011. Behavioral responses to human-induced environmental change. Biological Reviews. 86(3): 640-657.

Ultsch, G.R. and Jackson, D.C. 1982. Long-term submergence at 3°C of the turtle, *Chrysemys picta belli*, in normoxic and severely hypoxic water. Journal of Experimental Biology. 96: 11-28.

Ultsch, G. R., Hanley, R. W., and Bauman, T. R. 1985. Responses to anoxia during simulated hibernation in northern and southern painted turtles. Ecology. 66(2): 388-395.

Ultsch, G.R. 1989. Ecology and physiology of hibernation and overwintering among freshwater fishes, turtles and snakes. Biological Reviews. 64: 435-516.

Ultsch, G.R., Carwile, M.E., Crocker, C.E. and Jackson, D.C. 1999. The physiology of hibernation among painted turtles: the eastern painted turtle *Chrysemys picta picta*. Physiological and Biochemical Zoology. 72(4): 493-501.

Van Horne, B. 1983. Density as a misleading indicator of habitat quality. The Journal of Wildlife Management. 47(4): 893-901.

Welcomme, R.L. 1979. Fisheries ecology of floodplain rivers. Longman Inc., New York. 317 p.

Williams, K.E., Hodges, K.E. and Bishop, C.A. 2015. Hibernation and oviposition sites of Great Basin Gophersnakes (*Pituophis catenifer deserticola*) near their northern range limit. Journal of Herpetology. 49(2): 207-216.

Witczak, L.R., Williams, C.J., Price, S.J. and Dorcas, M.E. n.d. Population densities of semi-aquatic turtles in rural, urban and golf course ponds. Davidson College of Biology. Accessed Nov 7 2015. Research presentation available at: http://bio.davidson.edu/people/midorcas/research/herpstaff/StevePrice/Witczak%20and%20Williams%20ppt%20presentation.pdf.

Chapter Four

MANAGEMENT IMPLICATIONS AND FUTURE RESEARCH

Plasticity of behaviour (e.g., the ability to shift tactics) presumably reflects selection for individuals that can respond to fine-scale variations in the environment. As suggested by Darwin (1859), if variation in behaviour occurs across age classes, and is linked to fitness, it may very well be an evolutionary response. In my study population, this variation could be a result of anthropogenic habitat alteration. Identifying the mechanisms by which such plasticity develops is clearly beyond the scope of a short-term study, but my work still contributes to our general knowledge of behavioural ecology and plasticity in animals. The fact that my study animals persist in this fluctuating and somewhat artificial environment is likely due, to some degree, to their ability to show plasticity in response to their surroundings.

The overarching goal of my research was to improve our understanding of the factors and consequences of different overwintering tactics being demonstrated in a population of Western Painted Turtles (*Chrysemys picta bellii*). What made this particularly interesting, although somewhat more complicated, is that the animals were living within a fluctuating, anthropogenic environment (a reservoir). I added to this investigation by also comparing the basic demographics of the animals in the reservoir to those in an isolated pond not influenced by reservoir operations. From a practical standpoint, it also was my intention to provide information that would aid in the crafting of effective management strategies for this particular reservoir. Herewith I provide a specific list of the results of my thesis that have management implications:

- Turtles within one reservoir location are plastic in their overwintering behaviour, and all turtles appear to be resilient to gradual changes in water level in winter;
- Water temperature, dissolved oxygen and water depth do not appear to influence the tactics used by overwintering turtles;
- Turtles are able to move beneath the ice in winter;
- The fluctuation in water levels does not appear to limit suitable overwintering habitat for Western Painted Turtles in this location;

- There were no mortalities detected in my study that could be directly attributed to reservoir operations;
- Differences in the physical environment (between water bodies) do not appear to impact the body condition of turtles (males) throughout hibernation;
- There may be an advantage to those turtles utilizing habitat such as the neighbouring Turtle Pond (e.g., deeper water, prevalence of basking logs, smaller water body, food availability etc.). I base this suggestion on (i) the high density of turtles and (ii) larger size turtles at this location. Certainly, seasonal turtle use and physical habitat parameters in Turtle Pond should be further investigated to determine the attraction to this water body.

All told, this study has implications for the management and conservation of the Western Painted Turtle. The primary overwintering threats to this population of freshwater turtles include: freezing into the ice/desiccation from stranding and predation. . Given this, recommendations for management in anthropogenic environments should involve maintaining habitats that provide water depths sufficient for the animals to avoid freezing or desiccation overwinter, especially as turtles in this study and others (St. Clair and Gregory 1990, Crocker *et al.* 2000) utilize locations with shallow water in winter. Though fluctuations in water levels during my study did not appear to negatively affect overwintering turtles, I still recommend maintaining water levels that provide at least 1 m of water depth throughout winter to avoid the threat of winter mortality.

Overall, some factor(s) must be responsible for the marked difference in population densities I determined between the reservoir subpopulation and the small isolated pond (Turtle Pond). The adult turtles in Turtle Pond were larger overall than those in the reservoir, and also were living in greater density, however few juvenile turtles were captured at this location. Turtle Pond has a number of logs available for basking that have been installed by local landowners. It is possible that this habitat enhancement provides a benefit to the turtles at Turtle Pond, which may contribute to the higher density and/or the larger body sizes found here. I recommend investigating if installing basking logs in the reservoir locations may contribute to increasing turtle densities or body sizes. In addition, further research into the nesting ecology (namely nest survival, number of females nesting and availability of nesting habitat), abundance and growth rates of hatchlings and basking/foraging tendencies of the turtles in each of the locations may provide further insight to this discrepancy in densities.

Even though turtle movements in my study were not directly correlated to changes in water levels, the fact that the turtles displayed winter movements greater than those reported previously for northern Painted Turtle populations is notable. I hypothesize the movements may be an attempt to acquire oxygen from the water column via extrapulmonary means to mitigate plasma acidosis. I also hypothesize that turtles are overwintering in locations with cold water temperatures to initiate voluntary hypothermia and thus reduce metabolic losses throughout winter. Future work analyzing plasma lactate levels collected during natural hibernation in these locations would provide better insight to these hypotheses.

CONCLUSION

Winter is a critical life history stage for many northern species, and animals also inhabiting atypical environments such as reservoirs or existing at the periphery of the species' range may demonstrate responses tailored to the conditions they experience (Lesica and Allendorf 1995, Tuomainen and Candolin 2011, Sih 2013). As ectotherms, herpetofauna will be sensitive to temperature extremes, and in winter most of these species (including species of freshwater turtles) hibernate to avoid unfavourable conditions. Hibernation can occur terrestrially or aquatically, requiring the animal to develop behavioural, morphological or physiological adaptations in order to survive. Behavioural adaptations can include plasticity of behaviours, which may be evident between conspecifics exposed to different habitat conditions. The Western Painted Turtle (*Chrysemys picta bellii*) is a species particularly well-known for its physiological adaptations to overwintering (see Jackson 1968, Ultsch and Jackson 1982, Ultsch *et al.* 1985, Herbert and Jackson 1985a, 1985b, Ultsch *et al.* 1999, Reese *et al.* 2000, Crocker *et al.* 2000, Reese *et al.* 2004, review by Storey 2007), however, less-studied are the behavioural responses of these animals to spatial and temporal changes. Behavioural plasticity, including the ability to shift between overwintering tactics, may be an important compliment to the physiological adaptations that these animals possess. Such characteristics may be particularly important in allowing populations to persist in manipulated environments.

My work has clearly shown that animals in my study population exhibit plasticity in their overwintering tactics. The animals overwinter either congregated or dispersed, neither having a detectable difference on the body condition or survival of the animal. The specific tactic displayed at any point in time did not depend on the habitat conditions that I measured (water temperature, dissolved oxygen, water depth), and overall, suitable overwintering habitat does not appear to be limiting at this location. However, my work also identified variation in both the population density and turtle size between the reservoir and isolated subpopulation. This may be indicative of a higher prevalence of resources (food, habitat etc.), or differential growth patterns occurring in this non-fluctuating environment. Nevertheless, the overwintering portion of these animals' life cycle does not appear to be impacting the ability of the population to persist, and my research contributes to our understanding of how Painted Turtles are able to tolerate both anthropogenic and highly-seasonal environments. Nevertheless, pressures outside of overwintering have been identified for this population of Painted Turtles, including road mortality, loss of critical nesting habitat and an abundance of predators (Basaraba 2014, Hawkes pers. comm.). These factors may be interacting to control or limit the population sizes within the reservoir locations.

My study focused solely on the overwintering ecology of *adult* Western Painted Turtles. Hatchling Painted Turtles spend their first winter in the nest, emerging the following spring and overwintering thereafter in an aquatic environment: this behaviour has been studied extensively, (Storey *et al*. 1988, Costanzo *et al.* 2001, Packard *et al.* 1999, Packard and Packard 2001, Costanzo *et al.* 2004, Dinkelacker *et al.* 2005, among others), yet information on the overwintering ecology and survival of *juvenile* (< 1 year) freshwater turtles remains absent. Determining overwinter habitat use, behavioural ecology, survivorship and changes in growth and weight are needed to determine how recruitment into populations is affected, which may in turn provide insights into population densities. As well, further work investigating the general habitat use, diet, threats and demographics of juvenile Western Painted Turtles would be valuable for identifying conservation priorities for this population.

LITERATURE CITED

Costanzo, J.P., Litzgus, J.D., Iverson, J.B., and Lee Jr., R.E. 2001. Cold-hardiness and evaporative water loss in hatchling turtles. Physiological and Biochemical Zoology. 74(4): 510-519.

Costanzo, J.P., Dinkelacker, S.A., Iverson, J.B and Lee Jr, R.E. 2004. Physiological ecology of overwintering in the hatchling Painted Turtle: multiple-scale variation in response to environmental stress. Physiological and Biochemical Zoology. 77(1): 74-99.

Crocker, C.E., Feldman, R.A., Ultsch, G.R. and Jackson, D.C. 2000. Overwintering behaviour and physiology of eastern painted turtles (*Chrysemys picta picta*) in Rhode Island. Canadian Journal of Zoology. 78(6): 936-942.

Darwin, C. 1859. The Origin of Species By Means of Natural Selection or the Preservation of Favored Races in the Struggle for Life. 1998 Modern Library Paperback Edition. New York. 689 p.

Dinkelacker, S.A., Costanzo, J.P. and Lee Jr., R.E. 2005. Anoxia tolerance and freeze tolerance in hatchling turtles. Journal of Comparative Physiology and Biochemistry. 175: 209-217.

Herbert, C.V. and Jackson, D.J. 1985a. Temperature effects on the responses to prolonged submergence in the turtle *Chrysemys picta bellii*. I: Blood-acid base and ionic changes during and following anoxic submergence. Physiological Zoology. 58(6): 655-669.

Herbert, C.V. and Jackson. D.J. 1985b. Temperature effects on the responses to prolonged submergence in the turtle *Chrysemys picta bellii*. II: Metabolic rate, blood-acid base and ionic changes and cardiovascular function in aerated and anoxic water. Physiological Zoology. 58(6): 670-681. Jackson, D.C. 1968. Metabolic depression and oxygen depletion in the diving turtle. Journal of Applied Physiology. 24: 503-509.

Jackson, D.C. 1968. Metabolic depression and oxygen depletion in the diving turtle. Journal of Applied Physiology. 24: 503-509.

Lesica, P., and Allendorf, F.W. 1995. When are peripheral populations viable for conservation? Conservation Biology. 9(4): 753-760.

Sih, A. 2013. Understanding variation in behavioural responses to human-induced rapid environmental change: a conceptual review. Animal Behaviour. 85(5): 1077-1088.

St. Clair, R.C., and Gregory, P.T. 1990. Factors affecting the northern range limit of Painted Turtles (*Chrysemys picta*): Winter acidosis or freezing? Copeia 1990(4): 1083-1089.

Storey, K.B., Storey, J.M., Brooks, S.P.J., Churchill, T.A. and Brooks, R.J. 1988. Hatchling turtles survive freezing during winter hibernation. Proceedings of the National Academy of Sciences. 85: 8350-8354.

Storey, K.B. 2007. Anoxia tolerance in turtles: Metabolic regulation and gene expression. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology. 147(2): 263-276.

Tuomainen, U., and Candolin, U., 2011. Behavioral responses to human-induced environmental change. Biological Reviews. 86(3): 640-657.

Packard, G.C., Lang, J.W., Lohmiller, L.D. and Packard, M.J. 1999. Resistance to freezing in hatchling painted turtles (*Chrysemys picta*). Canadian Journal of Zoology. 77: 795-801.

Packard, G.C. and Packard, M.J. 2001. The overwintering strategy of hatchling Painted Turtles, or how to survive in the cold without freezing. BioScience. 51(3): 199-207.

Reese, S.A., Crocker, C.E., Jackson, D.C. and Ultsch, G.R. 2000. The physiology of hibernation among painted turtles: the midland painted turtle (*Chrysemys picta marginata*). Respiration Physiology. 124(2000): 43-50

Reese, S.A., Stewart, E.R., Crocker, C.E., Jackson, D.C. and Ultsch, G.R. 2004. Geographic variation of the physiological response to overwintering in the painted turtle (*Chrysemys picta*). Physiological and Biological Zoology. 77(4): 619-630.

Ultsch, G.R. and Jackson, D.C. 1982. Long-term submergence at 3°C of the turtle, *Chrysemys picta belli*, in normoxic and severely hypoxic water. Journal of Experimental Biology. 96: 11-28.

Ultsch, G. R., Hanley, R. W., and Bauman, T. R. 1985. Responses to anoxia during simulated hibernation in northern and southern painted turtles. Ecology. 66(2): 388-395.

Ultsch, G.R., Carwile, M.E., Crocker, C.E. and Jackson, D.C. 1999. The physiology of hibernation among painted turtles: the eastern painted turtle *Chrysemys picta picta*. Physiological and Biochemical Zoology. 72(4): 493-501.

APPENDIX A: Overwintering Turtle Under-Ice Movement Maps

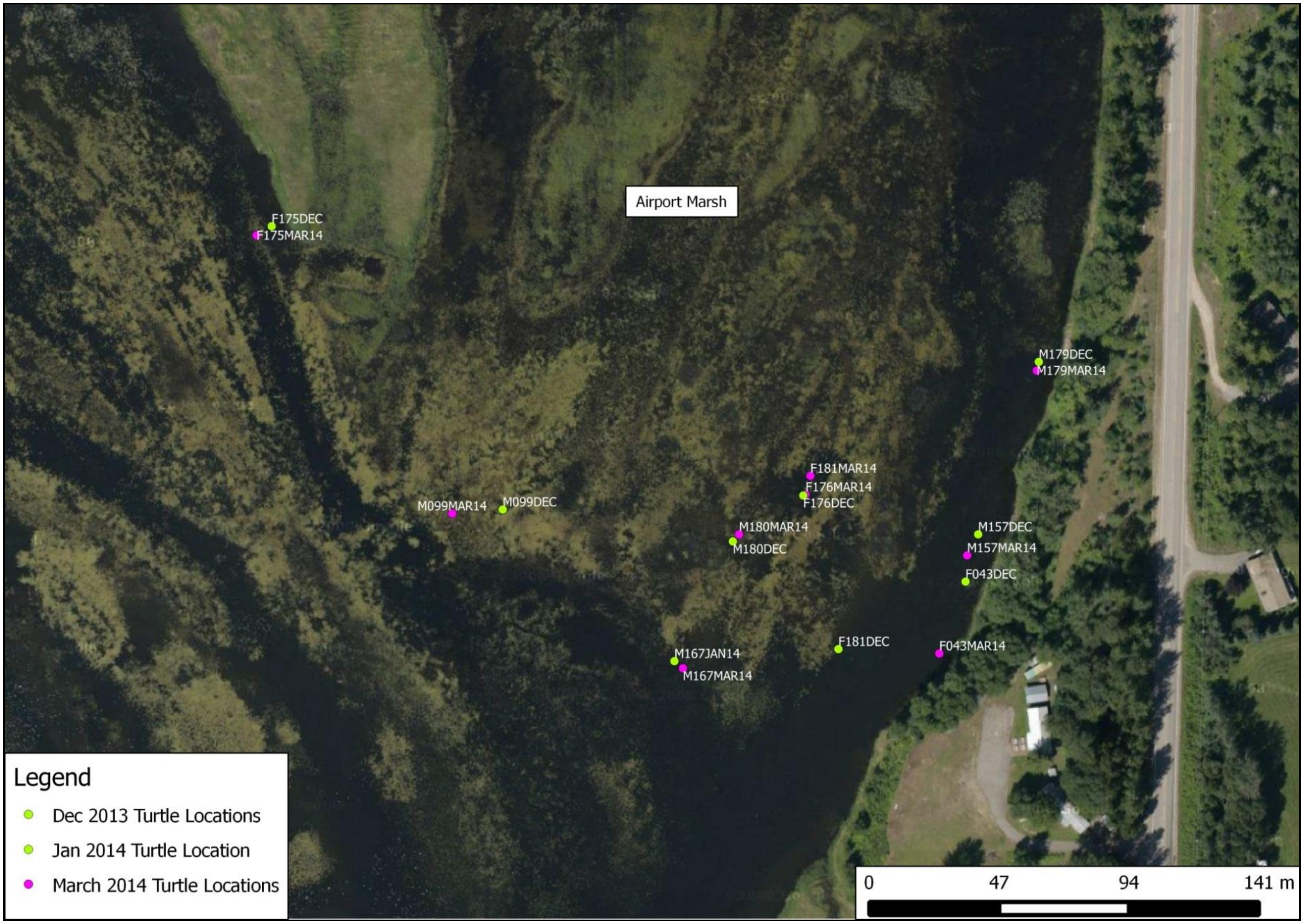


Figure A-1: Overwintering turtle locations at Airport Marsh south in December 2013/January 2014 and March 2014.

Figure A-2: Overwintering turtle locations at Montana Slough in December 2013 and March 2014.

Figure A-3: Overwintering turtle locations at Turtle Pond in December 2013 and March 2014.