Flood Survival Strategies of Overwintering Snakes

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Dedication

This MSc. is dedicated to my good friends and family that I lost along the way, Rob† (1971-2008), Paulette† (1964-2013), Jon† (1940-2015), Mom† (1930-2017), Kevin† (1945-2020), Rose† (1933-2020) and Nancy† (1957-2020).

Abstract

This thesis investigates snake flood survival during hibernation and how anthropogenic habitat alteration and climate variability may affect habitat quality and overwintering survival. Chapter one reviews the current understanding of ecophysiology of hibernation in snakes. In chapter two, I introduce a winter habitat model of a subterranean space that remains flood and frost-free, referred to as the "life zone," where snakes survive winter. I analyzed 11- winters of hibernation habitat data and 18-yrs of population mark-recapture data to assess the effects of the first flood event on an endangered Massasauga population. Following the flood event, snake observations declined despite hundreds of hours of search-effort. At the population level this was evidence of poor winter survival and recruitment post flood. The direct cause of mortality was not determined but poor winter survival in areas with a depleted life zone was statistically supported.

In the third chapter, I measured the metabolic rate ($\dot{M}_{O_2}$) at 5°C for three snake species that inhabit my study area. I varied water level conditions and measured activity and dive behaviours continuously during experiments. I found differences between species in their resting metabolic rate, which I attributed to body size differences. I confirmed, cutaneous respiration occurs at a low rate and was significantly upregulated during a forced dive (flood event). Therefore, there is an intrinsic physiological response to a flood event in neonatal snakes. However, post-flood recovery indicated a greater oxygen demand after the short-forced dive. An oxygen debt was incurred during a short-forced dive under normoxic conditions. My conclusions are, 1) hibernation habitat (i.e., life zone) must include a non-freezing, non-flooding aerobic space throughout winter to maintain snake survival. 2) cutaneous respiration is a short-term flood survival strategy. I found no support for a complete aquatic hibernation strategy 3) the energy

costs of a full-dive is additive to the recovery energetic costs of a flood event. A neonatal snake wintering energy budget is proposed, and winter mortality conservation issues are discussed in chapter 4.

Acknowledgements

Preamble and Statement of Authorship Contribution

As part of my duties as the local, provincial biologist with the Ministry of Natural Resources and Forestry (1984 to 2016 MNRF) until my retirement on Sep 30, 2016, I initiated and directed a long-term mark-recapture study (1998 to 2016) of an isolated, endangered population of Massasauga rattlesnakes. This long-term study included a four-year radio telemetry study (2000 to 2004) of adult rattlesnakes, and a long-term winter habitat quality investigation (2003 to 2016). This information was used within government documents to aid in establishing recovery planning objectives for this population. Mark-recapture, habitat quality, and *in situ* winter survival experiments continue today that were inspired by both the historical information and by laboratory and field experiments conducted during graduate studies at Brock University. Most of the historical data are not published except for neonatal and maternal birth data (Hileman et al., 2017), adult survival estimates during telemetry (Jones et al., 2012), and genetic assessments of isolation and inbreeding depression (Chiucchi and Gibbs, 2010; Gibbs and Chiucchi, 2012). In addition, flood survival estimates are contained within chapter 2 of this thesis which is accepted for publishing in the Journal of Herpetology (Yagi et al., in press). Conclusions from this long-term study suggest there was a significant decline in Massasauga encounters following the first flooding of the central mined peatland which occurred during hibernation in 2006. I found that Massasauga overwinter survival was significantly correlated to a loss in hibernation habitat quality. My co-authors and I concluded that the study site might be acting as a wintering ecological trap on the population (Yagi et al., In Prep; Chapter 2). Snake winter survival may have been affected by flooding, freezing or a change in subterranean air quality induced by factors such as past peat mining and stochastic weather events.

This long-term project involving complex interactions and conservation issues, lured me into returning to University to improve and update my academic skills. I began graduate studies at Brock University in 2013 on a part time basis to measure respiration strategies and dive behaviour at low temperatures in three snake species from a comparative perspective. I am continuing my research today by conducting annual winter survival experiments of neonatal snakes within the study area. A technique I call "forced or assisted hibernation," is now being used to assist the dispersal and survival of neonatal snakes at my study site. There is interest across the range of practising biologists in developing this technique for other populations (i.e., Ojibway Prairie in Windsor, ON) and other species and habitats (i.e., Gray ratsnake Carolinian population and Blue Racer, Pelee Island, ON). The application of knowledge gained during my graduate studies continues today.

This thesis contains a general introduction regarding the species of interest and the ecology and physiology of hibernation written by me. A detailed literature review is contained within Chapter 2. This article is based on the life zone concept and methods I developed by myself, guided by my mentor Dr. R.J. Planck† and the long-term data I collected before starting my MSc, but written and synthesised during my graduate studies. I alone have the right of authorship of these data collected during my career at MNRF and have the support of my past supervisor (Mr. Joad Durst, MNRF). This article was primarily written by me and edited by the co-authors. Chapter 3 is a series of lab experiments comparing respiration and behaviour strategies of three temperate neonatal snake species during simulated wintering conditions and represents experiments conducted as a graduate student. This chapter is followed by a general discussion and conclusions from a conservation biology perspective.

Statement of Ethics

All aspects of this thesis were completed under the authority of provincial legislation, Fish and Wildlife Conservation Act, Ontario Endangered Species Act, Ontario Provincial Parks Act, Ontario Ministry of Natural Resources and Forestry Animal Use Permits (#1998-55 to 2016-55) and Brock University Animal Use Permit (# 13- 06-01) with annual renewals.

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Chapter 1: General Introduction

Global declines in biodiversity relate mainly to anthropogenic factors that cause habitat loss,

fragmentation, pollution, and over harvest (IUCN, 2018). The International Union for

Conservation of Nature (IUCN) includes over 28,000 species listed as threatened with extinction.

Reptiles and amphibians constitute about 12 % of all listed species and 19% of all assessed

reptiles and amphibians are threatened with extinction (IUCN, 2018). However, the IUCN

criteria may not necessarily reflect localized population declines or small geographical range

losses. In this regard, national, provincial, or state designation adds context. In Canada, reptiles

and amphibians' amount to 8% of all endangered species and 57.8% of all reptiles and

amphibians in Canada (n=102) are a species at risk (Environment and Climate Change Canada,

2020).

Most species in the world are ectothermic (Chapman, 2009). The use of the term ectotherm in

this thesis refers to vertebrate ectotherms, primarily reptiles and amphibians. Ectotherms rely on

environmental cues such as temperature and moisture to complete their annual life cycle (Huey,

1982). In this group, ambient temperatures affect biological functions such as metabolism,

feeding, movement, reproduction, and growth (Huey, 1982; Baldwin et al., 2006). There are

upper and lower thermal limits that determine ectotherm survival (Sinervo et al., 2010). The

amount, distribution, and rate of change in temperature or hydroperiod affect activity patterns,

distribution, and abundance of ectotherms worldwide (Huey, 1982; Baldwin et al., 2006;

Karraker and Gibbs, 2009).

Climate variability effects, such as increased environmental stochasticity (i.e., flood,

temperature, drought, polar vortices, snow cover; IPCC, 2018), may play an important role in

ectotherm overwinter survival trends. Especially in habitats that have been altered by human

activities such as mining, drainage, land clearing, and urbanization. In these habitats, natural hydroperiods, vegetation, surface soils and microtopography are impaired (DiMauro and Hunter, 2002; Holden and Burt, 2003; Willard and Berhardt, 2011). Places for ectotherms that provide environmental stability and protection from winter extremes may be the best strategy to successfully spend a long Canadian winter.

The objectives of my thesis are to better understand the effects of environmental stochasticity (i.e. unusual flood events) on snake hibernation habitat, snake survival in an anthropogenically altered ecosystem, and the behavioural, and physiological strategies neonatal snakes may use to survive winter under various water level scenarios. I begin with a literature review of the ecophysiology of ectotherm hibernation. Within this review there are some research assumptions regarding snake aquatic hibernation and flood tolerances. In my thesis statement, I outline specific objectives to address some of these assumptions.

Ecophysiology of Ectotherm Hibernation

Ecology and Behaviour

All temperate ectotherms have a biphasic life history comprised of an active (spring to fall) and inactive (fall to spring) overwinter season. The overwintering season can be longer than the active season depending upon latitude and altitude (average six months; range 4 to 8 months) (Gregory, 1982; 2009; Ultsch, 1989). Overwintering sites are referred to as hibernaculum or dens (Weatherhead and Prior, 1992; Johnson, 1995; Kingsbury and Coppola, 2000).
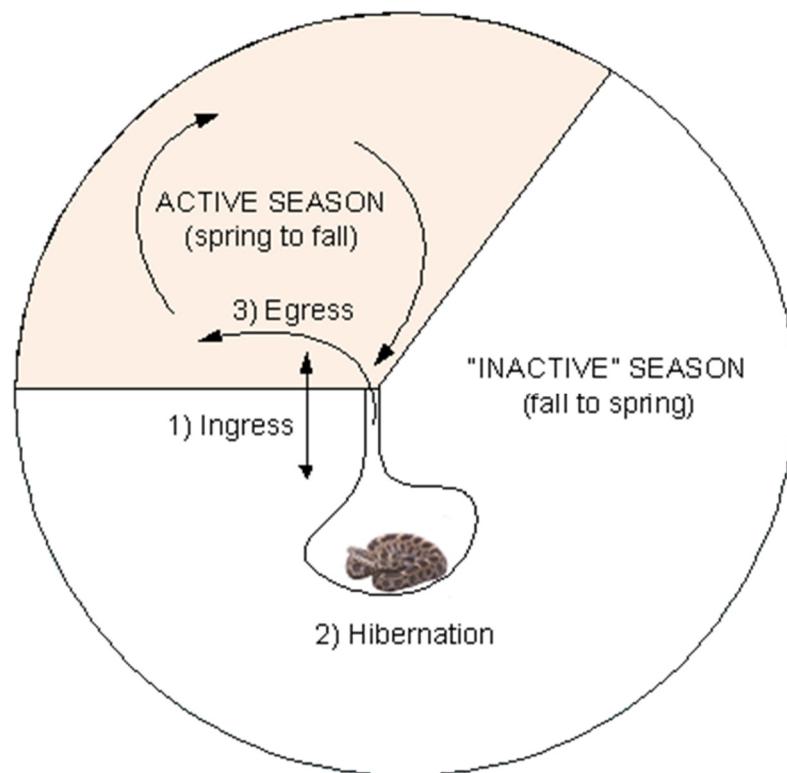
Fig. 1.1 Conceptual diagram of a temperate snake's annual biphasic life history, with active (< 6 months) and inactive seasons (> 6 months) and the three main phases of overwintering, (1) ingress (2) hibernation and (3) egress.

Overwintering refers to a prolonged aphagic period sequestered within a hibernaculum until environmental conditions become favourable for foraging, growth, movement, and reproduction

(Fig. 1.1). The overwintering season includes three main phases; (1) ingress - entering a hibernaculum (i.e., wintering site), which may involve several up and down movements (2) hibernation occurs within the hibernaculum; and (3) egress - emerging from the hibernaculum (Gregory, 1982; Wallace and Diller, 2001; Nordberg, 2013; Shipley et al., 2013) (Fig. 1.1). In northern hemisphere, ingress occurs in the fall season and is characterized by lower environmental temperatures and vertical movements into and out from a subterranean feature and a final decent into the hibernaculum. All temperate ectotherms cease feeding and enter the hibernaculum when ambient temperatures decline below 15°C (Aleksiuk, 1976; Gregory, 1982).

Hibernation is a state of metabolic depression during a seasonal period of low ambient temperatures within the hibernacula which reduces an ectotherm's overall activity, and is synonymous with a period of energy conservation (Gregory, 1982; Boutilier et al., 1997; Costanzo, 1985; 1986; Macartney et al., 1989; Ultsch, 1989). The third phase of overwintering, egress, is characterized by arousal, increases in activity and metabolism, and movement out of the hibernaculum (Nordberg and Cobb, 2016). Ectotherms employ behavioural strategies (i.e., habitat selection, site fidelity, cold hardiness, low activity, thermoregulation, diving) and physiological strategies (low metabolic rate, aerobic and anerobic metabolism, cutaneous and pulmonary respiration) to manage a prolonged aphagic period in the confines of a hibernaculum that may or may not change in temperature, water content or air quality.

Many temperate reptiles, especially the older life stages (adult and juvenile) are philopatric, utilizing homing behaviour to return to previously occupied hibernation areas (Weatherhead and Prior, 1992; Johnson 1995; Sage, 2005; Refsnider et al., 2012; Zappalorti et al., 2014). This behaviour is called 'hibernation site fidelity'. First time hibernators (i.e., neonatal) have no previous experience in hibernation and must rely on external cues to locate a hibernaculum.

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Most snakes do not construct a hibernaculum; they rely instead on the presence of subterranean spaces that have surface access holes. Subterranean spaces that offer some consistency or resiliency to environmental change may likely provide the best chance for survival. Hibernaculum may be singly or communally occupied with many individuals and multiple species (Gienger and Beck, 2011; Harvey and Weatherhead, 2006b; Johnson, 1995; Litzgus et al., 1999; Prior, 1997). Hibernacula are important habitat features for temperate snakes because they are often rare in the landscape, are occupied for the majority of the snakes' annual lifecycle and repeatedly used year after year.

Ectotherms overwinter in a wide range of terrestrial, wetland and aquatic habitats (Gregory, 1982; Costanzo 1986; Kingsbury and Coppola, 2000; Sage, 2005; Harvey and Weatherhead, 2006aNordberg, 2013; Riley et al., 2014, Zappalorti et al., 2014). Hibernacula subsurface habitat characteristics likely protect the individual from potentially lethal effects of freezing, desiccation, hypoxia and predation. However, the subsurface characteristics of the hibernaculum are not well described to make comparisons between the variety of habitats used by ectotherms. Hibernacula may vary in temperature, and the amount of water and oxygen present depending on habitat and location.

Terrestrial and Semi-Terrestrial Habitat

Terrestrial hibernators are challenged by freezing temperatures (i.e., descending frost layer) and potential for desiccation (Ultsch and Anderson, 1988; Ultsch, 2006; Riley et al., 2014). Terrestrial and semi-terrestrial wintering sites may also become anaerobic or hypoxic when atmospheric oxygen exchange is blocked by snow, ice or flooded conditions (Boutilier, 1990). The type of soil and the presence (or not) of a groundwater table may also contribute to the aerobic and thermal quality of the hibernaculum. Organic soils that have an active microbial

community produce biogases, water, and heat as end products of carbon metabolism (Charman et al., 1994; Reddy et al., 2000; Ingett et al., 2005). In contrast to organic soils, sand or rocky soils would have higher porosity and therefore greater air exchange but would also be considerably drier (Inglett et al., 2005). Since air exchange would be greater it is also likely that temperatures within a sandy or rocky hibernaculum would be colder due to deeper frost penetration. Comparative studies of the thermal and moisture content of hibernation sites have not been conducted. Hibernacula that are too dry are associated with post-hibernation mortality in gray ratsnakes (Prior and Shilton, 1996). Hibernation sites that are anthropogenic (i.e. altered natural habitat or man-made) may meet all or some of the needs for overwintering ectotherms. However, altered sites may be attractive during fall site selection but may change during hibernation and not meet all the needs to sustain life during winter. These sites are ecological traps (Battin, 2004) and may be a problem for naïve life stages. Climate change effects of increased temperature and climatic variability may aggravate the ecological trap effect on populations affecting recruitment and long-term sustainability of populations (Sinervo et al., 2010; Battin, 2004).

Aquatic Habitat

Amphibians (i.e., Ranidae) and freshwater turtles are known to overwinter aquatically and under hypoxic conditions (i.e., ice covered waterbodies). Entirely aquatic hibernation for snakes is not usual (Ultsch, 1989), and there are limited evidence of snakes surviving prolonged exposure to hypoxia (Costanzo, 1989a). Aquatic sites would vary in the amount of oxygen present especially during prolonged ice cover (Boutilier et al., 1997). Therefore, obligate aquatic hibernators would need to manage both cold temperatures and low ambient oxygen (i.e., hypoxia) (Ultsch, 1985; Ultsch, 1989; Boutilier et al., 1997; Tattersall and Ultsch, 2008). Cold submerged frogs can behaviourally adjust their position with respect to gradients in temperature

and oxygen (Tattersall and Boutilier, 1997). Radio tracked Spotted turtles (*Clemmys guttata*) dig into the banks of shorelines at the water edge in the fall season and possibly use burrows partly water filled with an air space that does not freeze (Litzgus et al., 1999; Yagi and Litzgus, 2012). Snakes may use hibernaculum where the groundwater table may be within the spaces occupied by snakes (Johnson, 1995). If snakes successfully overwinter entirely aquatically for the duration of winter, they must also have adaptations to obtain oxygen cutaneously and be able to manage hypoxia especially if the aquatic site becomes ice covered.

Consequently, spaces that change or undergo stochastic variation beyond the limits of an animal's ability to maintain homeostasis may result in death (Shine and Mason, 2004; Harvey et al., 2014; Yagi et al., in press). Climate variability or environmental stochasticity (IPCC, 2018) may play an important role in overwinter survival trends especially for habitats that lack resistance to water level or temperature fluctuations or involve first-year hibernators, that may select a poor wintering site (Yagi et al., in press). There is evidence that snakes can die during winter when the hibernaculum suddenly floods or freezes (Harvey et al., 2014; Shine and Mason, 2004). However, the lack of sufficient oxygen may also have contributed to their death. Long-term survival within various water levels across the range may be related to the stability of the thermal, air quality and water level regimes. More research is needed that collects hibernation habitat parameters and survival in a standardized manner across the range of temperate snakes (Chapter 2).

Thermal Ecology and Cold Hardiness

Environmental temperature is an important determinant in defining vertebrate ectotherm habitat use and activity periods (Huey, 1982). Ectotherms use behavioural thermoregulation during the active season (Huey, 1982), and those occupying a finite space such as a pond or

hibernaculum likely continue to use a similar strategy during winter (Tattersall and Boutilier, 1997). Some ectotherms do not overwinter in a hibernaculum, instead they hibernate in the forest floor or leaf litter, and they survive winter in a frozen state. These species are freeze tolerant for example, Wood frog, Chorus frog, Gray tree frog, Spring Peeper (Storey and Storey, 1992; Costanzo and Lee, 1995); hatchling Painted turtles (Storey et al., 1988). Other species, such as most snakes, turtles and frogs behaviourally avoid freezing surface temperatures by sequestering themselves in thermally buffered environments (Gregory, 1982; Ultsch, 1989; Costanzo and Lee, 2013). Gartersnake spp. can supercool their bodies below the freezing point of body fluids and avoid freezing (freeze avoidance) allowing them to extend their time above ground when ambient temperatures fluctuate around 0°C (Costanzo and Lee, 1995; Costanzo and Lee, 2013). Gartersnake spp. are the most northerly snake in North America and the subspecies, Red-sided Gartersnake (*Thamnophis sirtalis parietalis*) has been found under snow near their hibernacula in the spring or fall seasons. The snow layer provides a subnivean environment that is buffered from extreme temperature fluctuations, and this species is known to survive freezing in the short-term (Costanzo and Claussen, 1988; Churchill and Storey, 1992a, b; Storey and Storey, 1996). However, based upon laboratory experiments, Gartersnakes cannot survive prolonged periods in a frozen state. The ability to endure exposure to sub-zero temperatures is considered a short-term ecologically relevant scenario during ingress or egress and not a long-term wintering strategy for this species (Storey and Storey, 1996; Costanzo and Lee, 2013). The freeze tolerance of other temperate snake species is unknown. The general understanding is that temperate snakes avoid surface freezing temperatures and retreat below ground surface and remain there until surface temperatures allow for the return of active season behaviours (i.e., reproduction, feeding, movement, predator defence).

Voluntary Dive Behaviour

Voluntary diving may be an important strategy to survive within a hibernaculum that has an elevated groundwater table or within a hibernaculum that suddenly floods. Reptiles such as crocodiles, Iguanas, frogs, turtles, sea snakes and sea kraits are well known for their diving abilities (Schreer and Kovacs, 1997; Brishoux et al., 2007b; Tattersall, 2007; Ultsch et al., 2004; Ultsch, 2006; Ultsch, 2013; Udyawer et al., 2016). Comparatively, dive length, breath holding (apnoea) and deep dives are correlated with large body size in endotherms, but these same variables were not strongly correlated with ectotherm dive duration (Shelton and Boutilier, 1982; Brishoux et al., 2007a). Ectotherm dive length is also significantly longer than dive lengths in endotherms (Boutilier, 1984; Brishoux et al., 2007a); environmental temperatures may be important in dive length. Time spent diving at cold temperatures may be longer because of the inherent lower thermally dependent metabolic rate, and the irregular respiration rate (i.e., apnoea) associated with ectotherms (Boutilier 1984, 1988).

Cold hardy frogs are excellent models of study for researching hibernation relevant dive behaviour in ectotherms (Shelton and Boutilier, 1982; Ultsch, 1996; Tattersall and Boutilier, 1997; Tattersall, 2007). Amphibian anatomy includes a pulmonary and cutaneous respiratory and circulatory system to enhance blood flow to the skin (Boutilier et al., 1986; Hicks 2002; Tattersall 2007). Reptiles do not have this anatomical feature, instead rely on a right to left shunt of blood flow within the heart to bypass the lungs during a prolonged dive (Hicks, 2002). Although temperate snake voluntary dive behaviour is not well studied, dive length in ecologically relevant scenarios (i.e., within a partly or flooded hibernaculum at low temperatures) for temperate snakes is challenging to confirm in situ (Sage, 2005). Laboratory experiments using digital camera systems provide an opportunity to quantify dive length,

frequency and continuous monitoring may provide insight into dive limitations, voluntary position, dive intervals and other behaviours with respect to water level. This is a focus of interest in this thesis.

Physiology

Overwintering reptiles and amphibians that are sequestered in their hibernaculum for winter due to surface freezing temperatures, do not feed (i.e. aphagic) and cannot leave to an alternate location (except within the hibernaculum) when the hibernaculum environment changes. An overwintering ectotherm faces stressors of a confined space, limited by cold temperatures, hypoxia and limited energy stores. From an ecological perspective, there is variation in the type of hibernaculum used by ectotherms in terms of amount and duration of water (Fig. 1.2). From a behavioural perspective an ectotherm is limited to responses based upon thermoregulatory abilities, movement, and maintaining a position with respect to the water level or diving (Fig. 1.2). From a biological perspective, an ectotherm faces challenges of cold temperatures, reduced energy stores, hypothermia, low metabolism, reduced kidney and heart rates and an overall reduction in activity (Fig. 1.2). Physiological mechanisms such as homeostatic regulation are central to an animal's ability to control changes within their body, despite changes happening in the surrounding environment (Modell et al., 2015). Metabolism via pulmonary and cutaneous gas exchange at cold temperatures under ecologically relevant water levels are the physiological strategies of interest in this thesis.
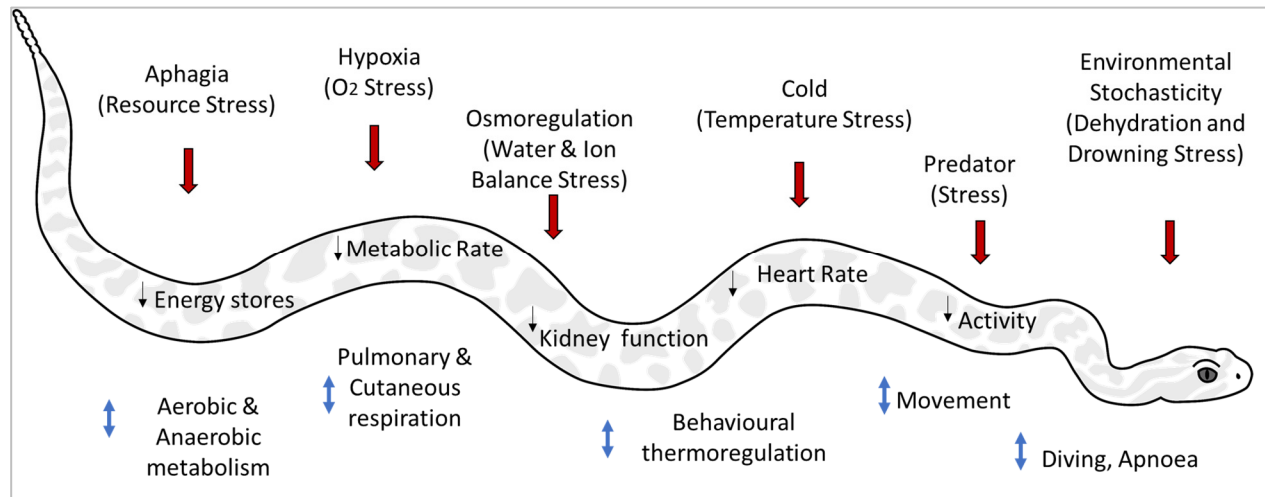
Fig. 1.2. Overwintering snake model indicating the physiological and behavioural responses to hibernation (black arrows). Stressors are indicated by red arrows acting on the organism. Blue arrows indicate behavioral or physiological coping strategies.

Homeostasis

Homeostasis is "the maintenance of a stable internal environment by an organism in the face of a changing external environment by varying internal activity using negative feedback mechanisms to minimize the error signal" (Modell et al., 2015; page 264). The "internal environment is the extracellular fluid where the body's cells live" (Modell et al., 2015; page 264), also termed "internal milieu" (Bernard, 1878). Overwintering survival, therefore, depends upon maintaining physiological homeostasis for the duration of winter. Hibernation sites that are stable with respect to flooding and do not freeze, may enhance winter survival. Alternatively, hibernation sites that are stochastic with respect to water level and freezing may result in poor winter survival.

Temperature Effects

By entering a subterranean space in the fall season, an ectotherm can take advantage of a thermally buffered environment that does not fluctuate as much as in the air.   For ectotherms, a 10 °C reduction in ambient temperature generally causes a 2 to 3-fold reduction in most biological rate functions. This is implied from the Arrhenius equation (Arrhenius = van't Hoff = = Q_{10} effect) but is typically referred to as the Q_{10} effect, which is a physiological response to a change in a biological rate function when temperature changes by a factor of 10°C (Aleksiuk, 1976; Equation 1).

$$Q_{10} = \left(\frac{R_1}{R_2}\right)^{\left(\frac{10}{T_2-T_1}\right)} \qquad \text{Equation (1)}$$

Where.

- Q_{10} is the factor by which the reaction rate changes with a 10°C change in system temperature.

- R_1 is the measured metabolic rate at temperature (T_1).

- R_2 is the measured metabolic rate at temperature (T_2).

Cold temperatures reduce all biological functions including metabolic rate, respiration rate, heart rate, blood flow and kidney function in ectotherms (Ultsch, 1989; Jackson, 2007; Tattersall, 2007). However, metabolic rates measured at declining cold temperatures show a much greater depression than can be explained by Q_{10} alone (Aleksiuk, 1976; Ultsch, 1989; Boutilier et al.,

1997; Tattersall and Boutilier, 1997;). Cold induced metabolic depression, or torpor is likely an important survival consideration for small snakes to conserve energy for a long winter.

Torpor

The physiological state called torpor is a state of controlled metabolic depression (Staples, 2016; Geiser, 2013). Torpor is not well studied in terms of a survival strategy for reptiles under ecologically relevant scenarios (Gregory, 1982; Ultsch, 1989). Costanzo (1986) reports an observation of "suspended animation" in Eastern Gartersnakes hibernating submerged (forced submergence or forced dive) in cold (<5˚C) water. The snakes appear comatose, but "revived when removed from the water" (J. Costanzo personal communication). However, there was no follow up post submergence survival assessment. Whether Eastern Gartersnakes enter this extreme low state of metabolism and survive under natural conditions is not known and studies on other snake species have not been completed (Gregory, 1982).

Aphagia

Ectotherms do not eat during winter and entirely depend upon stored energy reserves (lipid, glycogen and protein) to meet metabolic energy needs during a prolonged aphagic period. Both glycogen and lipids were utilized during hibernation of Red-sided Gartersnakes (*Thamnophis sirtalis parietalis*) at a northern den site (latitude 50°) (Aleksiuk and Stewart, 1971; Derickson, 1976). A study of Eastern Gartersnakes (*Thamnophis sirtalis sirtalis*) from a site in Wisconsin (latitude 44°), concludes they use lipids during hibernation (Derickson, 1976). Another study of Eastern Gartersnakes (latitude 44°) indicates glycogen and protein reserves and not lipids are used as an energy source during a 165-day hibernation period (Costanzo, 1985). The differences in substrate use is attributed to the age class differences of the sampled animals. Juvenile snakes use lipids during winter compared to adults that do not (Derickson, 1976; Costanzo, 1985). Adult

lipid conservation may be a strategy for annual ova development (vitellogenesis; Price, 2017) at southern latitudes. The higher depletion of lipid storage from adults collected at the northern den site may also relate to a lower frequency of annual reproduction at northern latitudes (Derickson, 1976). Differences in substrate use may be a consideration in winter survival of neonates which may have limited time to store fat prior to egress.

Juveniles tend to lose more weight and derive more energy from fat reserves than do adults (Aleksiuk and Stewart 1971; Derickson, 1976; Gregory 1982; Ultsch 1989). However, this is not always true as there is also a latitudinal effect on substrate use by ectotherms during winter (Costanzo, 2019). For a southern population of adult Eastern Gartersnakes, the energy budget for hibernation is19.9 kJ with 48% from liver and muscle glycogen stores and 52% from liver and muscle proteins (Costanzo, 1985). Aleksiuk and Stewart (1976) estimate a 25.3 kJ energy budget for adult Red-sided gartersnakes which comprises 50% energy from lipids (Derickson, 1976). Lipid and protein catabolism require aerobic metabolism whereas carbohydrates can also be metabolized without oxygen via glycolysis (anaerobic metabolism). Energetic data from small neonatal snakes at cold temperatures is not available. However, most neonatal snakes do not reproduce their first active season, therefore it is reasonable to assume they would use all three types of energy stores to meet metabolic needs during winter.

Water and Ion Balance

Environmental changes that increase the groundwater level within a hibernaculum may increase physical activity by initiating movement into or out of the water. Increasing activity relating to water levels may be important for maintaining water and ion balance for some species at cold temperatures. Kidney function and heart rate at low temperatures is affected by Q_{10} effects, thus in the cold, whether kidneys can adequately maintain their role in water and ion

balance is uncertain (Gregory, 1982; Ultsch, 1989). Low temperatures may even shut down kidney function in ectotherms. In captive reptiles, kidney disease is often attributed to cold and dry housing conditions (Lock, 2017). Thus, snakes inhabiting a dry and cold hibernaculum may not be able to maintain kidney function. Kidney function is necessary for filtering nitrogenous waste products such as ammonia from the blood stream (Wright, 1995). Snakes are aphagic during winter yet require energy for metabolic needs and the catabolism of protein is one of three energy sources during hibernation (Alesiuk and Stewart, 1971; Costanzo, 1985). The final nitrogenous waste product for terrestrial reptiles is uric acid and is an energy demanding process of the uric acid cycle (Wright, 1995; Salway, 2018). Although kidney function is not a focus of this thesis it may be a factor in snake survival if kidney function fails to eliminate nitrogenous waste or balance water uptake with losses, and gains from the environment (Gregory, 1982).

Metabolism

Metabolic rate is the amount of energy used by an animal per unit time and is affected by environmental temperatures, seasonal cues, and body size (Aleksiuk and Stewart, 1971; Ultsch, 1973; Gregory, 1982; Seebacher, 2009; Baker et al., 2017). Larger animals generally consume more oxygen than smaller individuals (Hemmingsen, 1960; Dmi'el, 1972; Bennett and Dawson, 1976; Feldman and McMahon, 1983; Andrews and Pough, 1985; Ultsch, 2013). For the aquatic salamander (*Siren lacertina*), which utilizes almost exclusively cutaneous gas exchange, body surface area was highly correlated with $\dot{M}_{O_2}$ (Ultsch, 1973). Overwintering ectotherms in cold hibernacula have a very low metabolic rate, using stored energy reserves to maintain life processes through aerobic and anaerobic metabolic pathways (Gregory, 1982; Ultsch, 1989). For the purposes of this thesis, I measured oxygen consumption metabolic rate ($\dot{M}_{O_2}$) at constant cold temperatures (5 °C), which was measured as the amount of O_2 consumed over time, during

controlled experiments within a confined space called a respirometer, and was a focus in Chapter 3.

Hypoxia tolerance

Although hypoxia tolerance is not a focus of this thesis, some background of this principle is relevant to my study site, which exhibits very low groundwater dissolved oxygen and flood events during winter (Chapter 2). A vertebrate ectotherm may respond behaviourally to a low oxygen environment (i.e., ice-covered pond or burrow, or flooded burrow) by changing activity patterns; or physiologically by changing respiratory and metabolic pathways (Boutilier et al., 1997; Tattersall and Boutilier, 1997; Tattersall and Ultsch, 2008; Nilsson, 2010). The first reaction by an organism, when exposed to hypoxia, is to adopt mechanisms that allow for the maintenance of oxygen uptake from the environment (Nilsson, 2010). These animals regulate oxygen consumption because they can increase the efficiency of oxygen uptake through a respiratory organ (i.e., gills or lungs) or perhaps increase circulation to the skin to facilitate cutaneous respiration (Meyers et al., 1980, Boutilier et al., 1986, Tattersall, 2007).

The lowest level at which an animal can maintain $\dot{M}_{O_2}$ is denoted by the critical oxygen concentration $[O_2]$ crit or the critical oxygen tension (P_{O_2}crit; Fig.3). Most bony fish, for example, are oxygen regulators, as are most mammals and birds (Ultsch et al., 1981; Butler and Jones, 1997; Svendsen et al., 2019). Oxygen regulators maintain their oxygen consumption along an environmental hypoxia gradient until the amount of available oxygen falls below P_{O_2}crit (Svendenson et al., 2019). Below this level, they become oxygen conformers as the amount of oxygen use declines directly with what is available (Svendsen et al., 2019). Survival below P_{O_2}crit requires anaerobic metabolism or mechanisms to allow for substantial metabolic suppression, such as exhibited by the overwintering Ranid frog (Utsch, 2004, Tattersall and

Ultsch, 2008; Fig. 1.3). When P_{O_2} is high as in air or well-oxygenated water (i.e., normoxic),

animals regulate their oxygen consumption according to their needs. When the amount of oxygen

diminishes (hypoxic) in the environment, the P_{O_2}crit is also reduced, such as during prolonged

submergence (Ultsch, 1973; Tattersall and Boutilier, 1997; Nilsson, 2010). Recent commentary

involves a critical look at the usefulness of P_{O_2}crit values for aquatic breathers advocating other

tests to describe species hypoxia tolerances (Wood, 2018). The counter-argument reinforces the

validity of P_{O_2}crit correlations across species and environmental oxygen gradients and is

ecologically relevant (Regan et al., 2019). P_{O_2}crit does not in itself describe species hypoxia
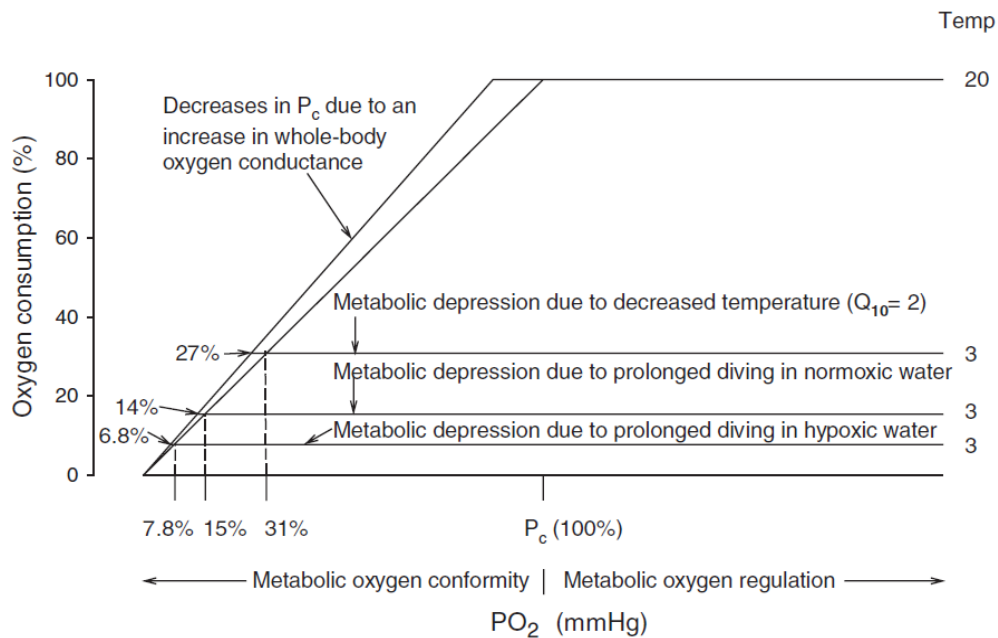
Fig. 1.3 An analysis of factors that contribute to a fall in P_{O_2}crit in overwintering submerged frogs. Starting assumptions are for air breathing frog at 20° C with P_{O_2}crit of 80mmHg = 100% oxygen in air. Metabolic depression decreases P_{O_2}crit, due to low temperatures (3° C). Prolonged submergence and hypoxia further reduce P_{O_2}crit allowing the frog to remain aerobic under hypoxic conditions (adapted from Tattersall and Ultsch, 2008; page 129)

tolerances. Instead, P$_{O_2}$crit describes an organism's oxygen regulatory ability within the context of relevant environmental oxygen concentrations (Regan et al., 2019; Fig. 1.3).

There is considerable variation amongst vertebrates in their ability to extract oxygen from hypoxic environments (Nilsson, 2010). A few vertebrates, such as goldfish (*Carassius auratus*) and some turtles are adapted to living, at least part of their annual life cycle, in oxygen-depleted environments (Bickler and Buck, 2007). For example, aquatic hibernating turtles such as Painted turtles (*Chrysemys* sp.) and Pond Sliders (*Trachemys* sp.) can survive prolonged submergence during winter by relying on anaerobic metabolism (Ultsch, 2006). These turtles achieve this by managing the acidosis produced during prolonged submersion with reduced metabolism and by buffering the toxic lactic acid using shell and body stores of Ca+2 (Ultsch, 1989; Jackson, 2002; Jackson, 2004). Snakes can also initiate anaerobic metabolism under hypoxic conditions (Costanzo, 1989a), but they lack large, endogenous, mobilizable stores of Ca+2 to manage lactic acidosis similarly. The Common frog (*Rana temporia*) overwintering in hypoxic water at 3 °C can reduce blood P_{CO_2} without build-up of lactate via cutaneous gas exchange (Boutilier et al., 1997). Researchers investigating a communal den located in an abandoned farm well in Wisconsin assumed the possibility that snakes remained submerged for 165 days due to the likelihood of subsurface ice cover (Costanzo, 1989a). Observations of Eastern Gartersnakes and Foxsnakes (*Pantherophis vulpinus*) submerged or periodically diving and coming to the surface were made in the spring and fall seasons; and the presence of ice cover with or without an air space in the well was not confirmed (Costanzo, 1986). Lactate levels were not significantly different between lab submerged Eastern Gartersnakes (< 20 hr) and emerged captures (Costanzo, 1989a). Indicating Eastern Gartersnakes were either able to regulate oxygen consumption behaviourally by diving and apnoea, or by conforming to low ambient oxygen

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levels via cutaneous respiration. Studies involving other snake species or under variable water conditions have not been undertaken. Survival using prolonged anaerobic metabolism in snakes seems unlikely.

Gas Exchange Strategies

Respiration is the exchange of gases (oxygen and carbon dioxide) between an organism and their environment (Boutilier, 1988). Most ectothermic vertebrates such as the orders, Crocodilia, Testudines, and Squamata, almost exclusively use pulmonary gas exchange throughout most of their life cycle relying on the presence of air derived oxygen to maintain life processes (Ultsch, 1989). The larval stages of most amphibians and neotenic adults retain gills (e.g., Axolotls, Mudpuppy, newts) to respire aquatically (Feder and Burggren, 1985). Cutaneous gas exchange abilities are well studied in amphibians and not as well studied in terrestrial reptiles. Lungless salamanders (family Plethodontidae), two species of salamanders (family Hynobiidae), one caecilian (*Typhlonectes eiselti*) and one frog (*Barbourula kalmantanensis*) exclusively use cutaneous gas exchange (Feder and Burggren, 1985; Hutchison, 2008). Whereas some snakes, turtles, metamorphic salamanders, and most frogs and toads employ a bimodal gas exchange strategy, relying on cutaneous gas exchange during certain activities such as overwinter or during diving (Feder and Burggren, 1985; Ultsch, 1989, Costanzo, 1989x; Udyawer et al., 2016). Lake Titicaca frog (*Conraua goliath*) with extensive skin folds and, Hairy frogs (with skin papillae) can enhance the effective surface area by extending folds of skin into the water to enhance cutaneous respiration (Hutchinson, 2008). Lunged amphibians including Ranid frogs, increase cutaneous respiration by increasing blood flow to the skin when submerged (Burggren and Moalli, 1984; Boutilier et al., 1986; Tattersall, 1997; Tattersall and Ultsch, 2008). Overwintering turtles use cloacal or buccal gas exchange while submerged in cold water (Ultsch, 1989; Jackson

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2002; Ultsch, 2006). Snakes and lizards have scales and therefore a thicker keratinized skin. However, when the skin is stretched there are thinner skin areas between the scales (Fig. 1.4). Theoretically, snakes may also be able to expose these thinner sections to improve cutaneous gas exchange.

The ability to exchange O_2 and CO_2 cutaneously is dependent upon the presence of a pressure gradient to facilitate the passive diffusion of gases across the skin from the surrounding environment (Feder and Burggren, 1985). There are limits to the effectiveness of cutaneous respiration, governed by physical laws (Fick, 1870). According to Fick's gas diffusion law (Fick, 1870), the rate of diffusion through the skin depends upon the skin surface area and thickness or permeability to gases, the diffusion coefficient of the gas in air or water and the partial pressure of gas on either side of the skin (PO2ext > PO2int) (Feder and Burggren, 1985; Equation 2). According to Fick's Law, the diffusion of oxygen through the skin would be reduced by thicker skin, a smaller skin surface area, and a small difference in the partial pressure of oxygen (Fick's Law; Equation 2).

$$D \propto \frac{A \times ß \times (P_{O_2\ ext} - P_{O_2\ int})}{x} \qquad Equation\ 2$$

Where.

- D is the rate of diffusion through the skin

- A is the skin surface area

- $ß$ is skin's diffusion coefficient of the gas in air or water

- x is skin thickness or permeability to gases

- $P_{O_2\ ext}$ is the partial pressure of oxygen on the exterior side of the skin.

- $P_{O_2\,int}$ is the partial pressure of gas on inner side of the skin.

The diffusion rates of respiratory gases are inversely proportional to their molar masses (Ekert et al., 1978). Therefore, O_2 will diffuse faster than CO_2, but skin has a greater capacitance to diffuse CO_2 more rapidly than O_2, resulting in 20–40 times faster CO_2 skin diffusion rate (Feder and Burggren, 1985). Increased skin permeability to respiratory gases may also affect H_2O balance and osmoregulation (Feder and Burggren, 1985). Cutaneous diffusion of other molecules depends upon their respective diffusivities (Dunson, 1978). Diffusivity of respiratory gases are
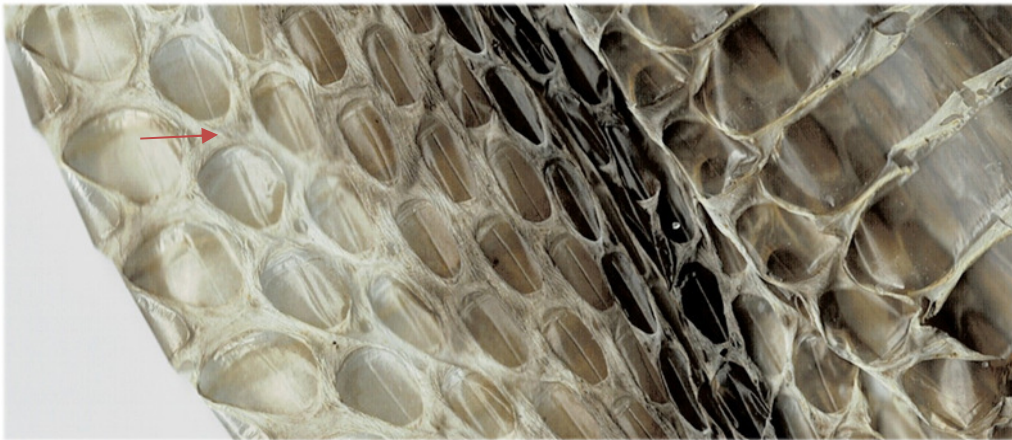
Fig. 1.4 Shed snakeskin of the Eastern Gartersnake (top) and Massasauga rattlesnake (bottom). Red arrows point to the thinner sections of skin between the oval shaped scales.

also affected by mucous or, in the case of oxygen, the presence of a hypoxic boundary water layer next to the skin, that adds to the total diffusion distance and therefore would further impair oxygen diffusion rates (Feder and Booth, 1992). Therefore, gas exchange is limited by the thickness of the diffusion boundary layer, skin thickness and the respiratory medium (Feder and Booth, 1992); and, thus the ability to derive oxygen cutaneously is likely species and size specific.

The solubility of oxygen in water is greatest at high pressure and cold temperatures (Wetzel, 1975). The amount of oxygen dissolved in pure water at high pressure is a small fraction ($\sim 5\%$) of the amount of oxygen present in air (Wetzel, 1975). For example, the capacitance for oxygen (ß_{air}) at 5 °C is 52.95 $\mu mol \cdot L^{-1}O_2 \cdot mmHg^{-1}$ compared to the solubility in water (ß_{water}) which is 2.5262 $\mu mol \cdot L^{-1}O_2 \cdot mmHg^{-1}$. There is a difference in the amount of oxygen available for aquatic respiration depending on the rate of atmospheric oxygen exchange within different environments. For example, high velocity streams with waterfalls have higher oxygen content than slow moving or deeper aquatic environments such as ponds, lakes, oceans, subterranean, groundwater and bog ecosystems (Wetzel, 1975). Most relevant to this thesis is measuring the ability of small snakes to metabolize oxygen from ideal, normoxic water or air media at cold temperatures similar to the expected wintering conditions within a hibernaculum. Furthermore, cold water contains higher dissolved oxygen content, which may be able to prolong dive performance for skin breathing vertebrates (Ultsch, 1985; Ultsch, 1989; Ultsch, 2006; Tattersall and Boutilier, 1997; Tattersall and Ultsch, 2008). However, once a submerged animal returns to air breathing, there may be physiological costs or oxidative stress to pay during the dive recovery period (Hermes-Lima and Zenteno-Savin, 2002).

Partitioned Respirometry

Partitioned respiration experiments provide an opportunity to measure differential oxygen uptake for bimodal breathers such as most amphibians and reptiles (Boutilier, 1984; Feder and Burggren, 1985; Boutilier and Shelton, 1986), and thereby ascertain their capacities for breathing underwater when submerged or diving. Bimodal respiration in temperate snakes is not as well studied as in amphibians (Ultsch, 1973; Moalli et al., 1980; Boutilier et al., 1986; Tattersall and Boutilier, 1997; Tattersall et al., 2013). Amphibians have a separate cutaneous circulatory system of pulmonary origin, while snakes do not (Hicks, 2002). The significance of this is that blood that is already oxygenated circulates to the skin in snakes, whereas deoxygenated blood reaches the amphibian skin. However, snakes can shunt blood flow within the heart away from the lungs toward the body (Hicks, 2002). This shunt may be important during a prolonged dive by lowering oxygen content of the blood to enhance diffusion of oxygen from the surrounding environment through the skin and back into the body (Hicks, 2002). Costanzo (1989a) has shown that Eastern Gartersnakes use both pulmonary and cutaneous respiration during hibernation when both air and water are present. Costanzo (1989a) also shows that Eastern Gartersnakes can survive prolonged forced submergence (approximately 17.5 hr in 20L chamber) in normoxic water at cold temperatures (approximately 5˚C)[1]. During a dive, oxygen sources would come from stored air in the lungs and from the available oxygen dissolved in water via cutaneous respiration (Brishoux et al., 2007; Udyawer et al., 2016). There is a physiological limit to the length of an aerobic dive followed by an oxygen debt (Butler, 2006; Rodgers and Franklin, 2017). The ability to maintain oxidative metabolism in the short term under forced dive conditions is ecologically relevant to a terrestrial hibernaculum that suddenly floods to the

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[1] Personal Communication with Jon Costanzo in 2014 that all Gartersnakes forcibly submerged were alive before sacrificing to measure lactate and other values

surface or when groundwater fills up to the frost or ice layer and then retreats. Dive recovery oxygen uptake following submergence and the amount of time a snake spent voluntarily submerged vs coming to the surface to breathe air was not measured in this study (Costanzo, 1989a). Prolonged hypoxic events, such as prolonged dives, may cause oxidative stress with the potential for increased production of ROS and cellular damage (Hermes-Lima and Zenteno-Savin, 2002), which may affect survival. Comparing a snakes' oxygen uptake in water with and without air present can help assess whether cutaneous respiration pathways can be upregulated during a forced dive (i.e. flood event). Measurements of post dive recovery oxygen uptake will help to understand whether an oxygen debt was incurred and determine whether cutaneous respiration is a short or longer-term flood survival strategy (Shelton and Boutilier, 1982; Boutilier and Shelton, 1986; Tattersall et al., 2013).

Closed System Respirometry is a method for measuring whole body oxygen depletion in either air or water media within a finite space. Factors such as experimental length, size of the respirometer relative to the animal and the circulation of the respiratory media within the respirometer can affect the measurements of P_{O2} (Steffensen, 1989). In a closed system with aquatic respirometry, a hypoxic boundary layer may develop around the animal, impairing gas exchange and thereby lowering $\dot{M}_{O_2}$ (Pinder and Burggren, 1986; Tattersall and Boutilier, 1997; Tattersall and Boutilier, 1999). The activity of the animal, temperature, presence of bacteria, accumulation of CO_2, nitrogenous waste and other metabolites within the respirometer will also influence the measurements of P_{O2} (Steffensen, 1989). Therefore, experimental design should consider temperature, aphagia, presence of a hypoxic boundary layer, the size of the respirometer relative to the size of the animal and complete experiments in a short time as possible while considering the potential for the confounding effects of animal activity (Steffensen, 1989).

Closed system respirometry was used in my thesis because of the ease of use, the ability for

multiple simultaneous measurements, and the ability to scale the respirometer size to the size of

the snake to improve P_{O2} detection. The partitioned approach using closed system respirometry

will aid in our knowledge about small sized snakes and species flood tolerances during

hibernation, which is a focus of this thesis.

Species of Interest

The Eastern Gartersnake (*Thamnophis sirtalis sirtalis*) (Linnaeus, 1758), Red-bellied snake

(*Storeria occipitomaculata* =Northern, *Storeria occipitomaculata occipitomaculata* Storer,

1839) and the Massasauga (*Sistrurus catenatus* =Massasauga, *Sistrurus catenatus catenatus*

Rafinesque, 1818; Crother et al., 2017) are examples of temperate, viviparous species that

occupy a similar geographic range, habitat and phenology and therefore are of interest from a

comparative perspective. All three species use the same terrestrial wintering habitat in my study

site in Port Colborne, ON. The Eastern Gartersnake is the most abundant snake species, followed

by the Red-bellied snake (OMNRF, unpublished data). The Massasauga is endangered in the

Carolinian region of Canada including my study site (Parks Canada, 2015). Due to this status,

additional regulatory constraints were placed on scientific research. However, permission was

granted to use neonates collected from Parry Sound, ON which are not endangered, for

metabolism and dive experiments.

Eastern Gartersnake

The Eastern Gartersnake (*Thamnophis s. sirtalis*) is a common species from the Natricidae

family (Fig. 1.5A). This species is widespread and is generally found in a diverse range of

habitats and further north than the Massasauga (Fig. 1.5B). The subspecies, Red-sided

Gartersnake (*Thamnophis sirtalis parietalis*) is found at the highest latitude of any North

American reptile (Crews and Garstka, 1982). This species is often used as a model organism of study in the areas of behavior, physiology, and ecology (Gregory, 1982; Ultsch, 1989). Eastern Gartersnakes give birth in late summer or early fall season (Seigel and Fitch, 1984). Litter sizes

Fig. 1.5 A) Eastern Gartersnake (*Thamnophis sirtalis sirtalis*) photograph was taken from the study site by Curtis Abney. B) North American Distribution of the Eastern Gartersnake (*Thamnophis s. sirtalis*). Modified from Nature Serve Canada and overlaid onto Google Earth imagery. Black arrow indicates approximate location of the study site located in Port Colborne, Ontario Canada.

are large ranging from 6 to 78, and neonates range from 100 to 229 mm TL and weigh about 2 grams or less at birth.

In some places, Gartersnake spp. are known to communally hibernate (Gregory, 1974; Costanzo, 1986; Shine and Mason, 2004) with the same and other species or hibernate singly. Hibernation sites include natural terrestrial and semiterrestrial habitats (root holes, animal burrows, caves, fractures in bedrock, karsts) and anthropogenic sites such as wells, cisterns, building foundations and spaces around fence posts (Rowell, 2012). Thousands of Red-sided Gartersnakes (*Thamnophis sirtalis parietalis*), a subspecies of the Eastern Gartersnake, communally hibernate within subterranean karst features at the Narcisse dens in Manitoba (Gregory, 1974).

Communal hibernation has also been observed at my study site. An aggregation of Eastern Gartersnakes were found emerging from underneath a deteriorating railbed in early spring. In addition, two or more Eastern Gartersnakes were observed emerging from a hibernaculum occupied by at least two Massasugas (1 telemetered). I have also observed Eastern Gartersnakes emerge first from the same hole about midday followed by two adult Massasugas later the same day. The hibernaculum was in an anthropogenically altered portion of the study area with shallow soil depth. Later that same day a neonate Massasauga emerged from a small hole under a blueberry bush not far from the communal hibernaculum.

Red-bellied Snake

Another widespread, but not a well-studied species, is the Red-bellied snake (= Northern red-bellied snake *Storeria o. occipitomaculata*; Crother et al., 2017), family Natricidae (Fig. 1.6A). The Red-bellied snake is a small, cryptic viviparous snake (Halliday and Blouin-Demers, 2015). They are variable in colour (reddish brown to grey and black) with a light patch or collar at the

Fig. 1.6 A) Red-bellied snake of a grey-black morph from the study site showing the characteristic red underbelly photo by Curtis Abney. B) North American Distribution of Red-Bellied snakes (*Storeria occipitomaculata*), modified from distribution maps available from NatureServe Canada and overlaid onto Google Earth imagery. Black arrow indicates approximate location of my study site located in Port Colborne, Ontario Canada.

base of their head. They are distinguishable from other snakes by their bright reddish to orange coloured underbelly (Fig. 1.6A).

   The Red-bellied snake is found in similar habitats as the Eastern Gartersnake and Massasauga, but its range does not extend as far north as Gartersnake spp. (Fig. 1.6B; Rowell, 2012). Minnesota and Manitoba studies found this species using inactive ant hills and animal burrows as hibernacula (Lang, 1969). This species may hibernate singly or communally in large

Fig. 1.7 Red-Bellied snake neonate captured in early October at my study site. Photo by Cathy Blott

numbers with their own and other species including the Eastern Gartersnake and Smooth Greensnake (*Opheodrys vernalis*) (Lang, 1969). They are one of the first snake species seen in the spring and one of the last ones observed in the fall in my study site. They hide during the day and are active later in the day or at night. The Red-bellied snake gives birth in late summer to early fall (late July to September) and they are often quite small at time of ingress in my study site (Fig. 1.7). Blanchard (1937) found litter size to range from 1 to 13 and newborn total length to be 67 to 98 mm at a study site in Northern Michigan. In New York state newborns ranged from 42 to 75 mm SVL (Brodie and Ducey, 1989). They are fossorial and feed on soil

invertebrates including ant and beetle larva. Growth may be rapid up to the fall season. Harding

(1997) suggests they may double their birth size within the first year.

Massasauga

The Massasauga is a rattlesnake from the family Viperidae. This is a medium sized

rattlesnake species found in eastern North America within the central lowland physiographic

region (Rowell, 2012) (Fig. 1.8A and 1.8B). Although not a common species, general habitat use

across their range includes deciduous, mixed and coniferous forest, prairie, fen, bog, rock

barrens, meadow, islands, shorelines, shrub tickets and swamps (Rowell, 2012).

Fig.1.8 A) Eastern Massasauga (*Sistrurus catenatus*) neonate from Niagara study site. B) North American Distribution of the Eastern Massasauga rattlesnake (*Sistrurus catenatus*), modified from distribution maps available from Nature Serve Canada and overlaid onto Google Earth imagery. Black arrow indicates approximate location of my study site located in Port Colborne, Ontario Canada.

In Ontario, there are four geographically distinct areas with extant rattlesnake populations:

Windsor, Niagara, Bruce Peninsula and Eastern Georgian Bay (Ray et al., 2013; Parks Canada,

2015). Although listed as least concern (LC) by the International Union for Conservation of

Nature and Natural Resources (IUCN) red list, geographically based assessments consider

Sistrurus catenatus at risk across its range and relatively more secure in Michigan and Eastern

Georgian Bay, Ontario (OMNRF 2015; Parks Canada 2016). Mitochondrial DNA analysis was used to define the genetic variation in extant populations (Pearce, 2001; Ray et al., 2013). All populations across the Massasauga rattlesnake range are considered allopatric except for populations within Michigan and central Ontario where there remains the highest genetic diversity and gene flow (Dileo et al., 2013). Genetic diversity between Massasauga populations, therefore, is highest in the northern range limits and less diverse in southern populations (Ray et al., 2013) confirming a lack of contemporary gene flow (Dileo et al., 2013) and possibly genetic drift or adaptation (Gibbs et al., 1997). Further genetic research across the range has found no evidence of inbreeding depression (Gibbs and Chicuchii, 2012). Therefore, if inbreeding is occurring, inbred individuals are not surviving to breed. Genetic evidence also suggests, the effective population size is small relative to other populations across their geographic range. Small population size is thought to be related to an ecological (i.e. habitat quality) problem. Although genetic drift cannot be dismissed, inbreeding depression is not a contributing factor that explains the small population size in the study area (Chiucchi and Gibbs, 2010; Gibbs and Chiucchi, 2012; Baker, 2018).

Adult Massasaugas breed in the summer to early fall season, retain sperm in oviducts until the spring when ovulation and fertilization occur, and give birth in late July to mid-September (Aldridge et al., 2014). Spermatogenesis begins in June and peaks in August-September and generally parallels ova development (Aldridge et al., 2014). In Illinois, vitellogenic follicles are 20mm length by September and overwinter at that size (Aldridge et al., 2014). Generally, females reach sexual maturity at age 3 or 4, with older snakes first age of reproduction at higher latitude. Females may also give birth in alternate years depending upon latitude (Yagi et al., 2018). Adult females' SVL ranges from 566 to 739mm with the largest from more northern

populations (Hileman et al., 2017). Litter size (4 to 13.3) averages 9.3 neonates/litter across the range with larger litters reported from northern latitudes (Hileman et al., 2017). Range wide birth mass varies from 8.3–11.6g.

Massasaugas enter their hibernacula in Sep–Oct and generally hibernate singly, however this is not always the case and occasionally share wintering sites with conspecifics and other species (Smith, 2009; Harvey et al., 2014; A. Yagi pers. obsv). Across the range of the Massasauga, hibernation site locations verified by radio telemetry indicate conditions ranging from abundant surface water to surficial dry conditions at the time of ingress (Weatherhead and Prior 1992; Johnson, 1995; Sage 2005; Harvey and Weatherhead 2006b; Yagi et al., In Press). Massasaugas exhibit hibernation site fidelity and often return to previously used hibernacula or nearby previously used hibernacula (Prior and Weatherhead 2006; Johnson, 1995; Harvey and Weatherhead 2006b; Sage 2005; Yagi et al., In Press).

Life Stage of Interest Neonatal snakes are naïve hibernators. They have no previous experience in hibernation and must rely on cues to locate a hibernaculum. Timber rattlesnakes and Eastern Gartersnakes are known to use conspecific pheromone trails to locate communal hibernacula (Brown and MacLean, 1983; Costanzo, 1989b). Temperature and structural cues play a role in homing behaviour of Northern Pacific rattlesnakes (*Crotalus oreganus*); Gienger and Beck, 2011). Other species such as Gray ratsnake and Blue Racer avoid scent trails made by older and larger conspecifics; instead they join communal dens at an older, larger size (Rosen, 1991: Prior, 1997; LeMaster et al., 2000). Neonates that do not hibernate communally and those avoiding or not detecting scent trails may use temperature and moisture gradients or the presence or absence of predators as cues in selecting their first hibernacula. Mothers that gestate and birth nearby their own hibernation site may improve winter survival rates of their young, generally supporting

Shine's (1995) 'maternal manipulation hypothesis' (Webb et al., 2006). This hypothesis is similar to 'mother knows best' principle that is applied in the context of insect herbivores. Where mothers select oviposition sites to enhance offspring survival (García-Robledo and Horvitz, 2012). This principle is in contrast with 'optimal bad motherhood' principle where mothers choose sites in the best interest of the mother's survival (Mayhew, 2001). Applying this concept to viviparous snakes, an optimal gestation site would be nearby high-quality hibernation sites which may improve the chances of neonatal winter survival (i.e. Shine's hypothesis). Whereas if the mother cannot find suitable thermal quality for gestation near her hibernation site, and she moves to alternate habitats that may be suitable for her own survival (*i.e.,* optimal gestation) but not hibernation, her choice may decrease the likelihood her offspring may survive winter if they choose to remain nearby the birth site over winter. This concept generally supports the 'optimal bad motherhood' principle (Mayhew, 2001; García-Robledo and Horvitz, 2012). Habitats that are anthropogenically altered (farm fields, excavations, roads, partially mined peatlands) may meet summer thermal needs for gestation but not wintering needs for offspring. Habitats that are attractive to some life stages and not others are ecological traps (Battin, 2004).

Thesis Statement

This thesis contains an 11-year field study of hibernation habitat ecology with implications for winter survival of Massasaugas inhabitating an anthropogenically altered wetland that has undergone habitat quality changes and recent environmental stochasticity. I postulated that Massasauga survival was reduced following the first flood event of the centrally mined peatland, because observations of Massasaugas, especially gravid females, and neonatal snakes, also declined. I concluded that in order to survive a flood event during hibernation, snakes would need to either endure the flood by breath holding, or facilitate oxygen uptake using cutaneous

respiration in an anoxic or hypoxic aquatic hibernaculum for the duration of the flood event. The less likely alternative is to leave the flooded burrow, endure surface freezing temperatures, avoid predators, and seek out an alternative wintering site. To provide insight into strategies snakes may use to survive variable water conditions, I simulated two winters in laboratory conditions measuring respiration strategies, voluntary dive behaviours, and general activity of three snake species that inhabit my study area. They are the Eastern Gartersnake, Massasauga, and the Red-Bellied snake.

The main objectives of this study were to (1) analyze environmental and hibernation habitat factors that might affect overwintering survival of neonatal snakes at my study site (2) assess species differences in voluntarily dive response and activity at low temperatures (3) assess species differences in respiration strategies (pulmonary and cutaneous) which might suggest differences in overwintering survival strategies and energy budget.

Neonatal snakes represent a life history stage of interest because of several factors: (1) they are relatively more abundant in the pre-hibernation season than other life stages; (2) from a behavioural perspective, they are naïve hibernators and therefore have no previous experience managing environmental change during winter; (3) they represent a life stage that has a higher natural mortality than adult snakes, which is of interest from a species recovery perspective; (4) they are not part of the reproductive portion of the population, therefore, their use in the lab is less taxing on a population than removing adults for an overwintering study; (5) overwintering behaviour and metabolism studies have not been completed on newborn and few have been completed using small snakes and therefore their study is novel and of scientific interest; (6) neonatal survival in flooded environments places a great interest in understanding cutaneous respiration abilities of small snakes; (7) comparative studies involving three snake species during

winter are important because different life histories may mean different overwintering strategies; and (8) finally, if at least two species have similar behaviour and physiology when facing the same environmental challenges, then flood survival inferences can be made to justify the use of the more common species as an overwintering model for testing hibernation habitat suitability prior to the release of a species of conservation concern, which is a focus of my research today.

Chapter 2: A Long-term Study on Massasaugas (*Sistrurus catenatus*) Inhabiting a Partially-mined Peatland: Presenting a Standardized Method to Characterize Snake Overwintering Habitat

A version of this chapter is published by the Journal of Herpetology under the authorship A.R. Yagi, R.J. Planck†, K.T. Yagi, and G.J. Tattersall

Abstract.— Temperate snakes occupy overwintering sites for most of their annual life cycle. Microhabitat characteristics of the hibernaculum are largely undescribed yet are paramount in ensuring snake overwintering survival. We hypothesized that snakes survive hibernation within a vertical subterranean space that we termed a "life zone" (LZ), that is aerobic, flood, and frost-free throughout winter. We studied an isolated, endangered population of Massasaugas (*Sistrurus catenatus*) inhabiting an anthropogenically-altered peatland and monitored the subterranean habitat during a period of environmental stochasticity. Initial radio telemetry confirmed that snakes moved between altered and natural habitats during the active season and showed hibernation site fidelity to either habitat. We used a grid of groundwater wells, and frost tubes installed in each hibernation area to measure LZ characteristics over 11 consecutive winters. The LZ within the impacted area was periodically reduced to zero during a flood-freeze cycle, while the LZ in the natural area was maintained. Model selection analysis revealed that soil-depth and flood status best predicted LZ size. Thermal buffering and groundwater dissolved oxygen increased with LZ size, and annual Massasauga encounters were significantly correlated with LZ size. This analysis suggests that a population decline occurred when LZ size was reduced by flooding. Our data give support to the importance and maintenance of an LZ for successful snake hibernation. Our methods apply to subterranean hibernation habitats that are at risk of environmental stochasticity, causing flooding, freezing, or hypoxia.

Introduction. — Snakes inhabit a range of climates and those living in temperate ecosystems must adapt behaviorally and physiologically to the seasonal changes in their environment (Huey, 1982; Gregory, 1982; Ultsch, 1989). Temperate snakes have adapted to their climate by employing a biphasic life-history strategy (Huey, 1982). During the above-ground or the active season, surface temperatures are conducive for movement, foraging, growth, and reproduction (Harvey and Weatherhead, 2006a). When surface temperatures are suboptimal, snakes retreat below ground during the overwintering or hibernation phase (Cowles, 1941; Gregory, 1982; Ultsch, 1989; Costanzo and Lee, 2013). In some latitudes or altitudes, the overwintering phase may account for more than half of the animal's annual life cycle (Gregory, 1982). A typical snake overwintering site consists of a subterranean space with access to the surface that could include crayfish and mammal burrows, rotting tree roots, fractures in bedrock, caves, or holes weathered by wind, or water (Weatherhead and Prior, 1992; Johnson, 1995; Kingsbury and Coppola, 2000; Harvey and Weatherhead, 2006b). The depth, width, or ecological characteristics of the subsurface wintering site are not well understood (Prior, 1997; Sage, 2005; Smith, 2009), however likely vary with soil type, geology, hydrology, vegetation, climate, and topography.

Radiotelemetry has provided insights into hibernation thermal ecology, the locations of hibernacula, site fidelity (Macartney et al., 1989; Johnson,1995; Sage, 2005; Harvey and Weatherhead 2006b), and the timing of ingress and egress (Nordberg and Cobb, 2016). Surgically implanted temperature dataloggers or hygrochron dataloggers fused to rattles, and doubly-labeled water have also been used to study the ecophysiology of hibernation (Agugliaro, 2011; Nordberg and Cobb, 2016). Since small numbers of relatively old and large snakes are monitored during radiotelemetry studies, (Johnson, 1995; Harvey and Weatherhead 2006b;

Refsnider et al., 2012; Zappalorti et al., 2014), survival rates are difficult to determine (Jones et al. 2012). Radiotelemetry studies cannot locate all hibernation sites used by solitary hibernators. Therefore, estimating population level impacts using radiotelemetry alone may be inaccurate (Jones et al., 2012). Furthermore, the invasive surgical procedures associated with radiotelemetry itself may contribute to snake mortality (Lentini et al., 2011). Locating hibernacula using more invasive methods such as excavation (Cowles, 1941; Burger et al., 2012) or probing burrows with fibre optic scopes (Sage, 2005; Smith, 2009) may directly change the behaviors of hibernating snakes or alter the physical conditions of the site.

During hibernation, snakes survive by freeze-avoidance or supercooling, and exhibit variable tolerances to water inundation and hypoxia. Snakes generally avoid freezing by retreating into a thermally buffered subterranean space in the autumn and remain there until spring (Costanzo and Claussen, 1988; Storey and Storey, 1992; Gienger and Beck, 2011). Snakes are freeze-intolerant (Costanzo and Claussen,1988; Storey and Storey, 1996; Costanzo and Lee, 2013), even species with very northerly distributions (Churchill and Storey, 1992; Lee and Constanzo, 1998; Andersson and Johansson, 2001; Costanzo and Lee, 2013). Supercooling (Storey and Storey, 1996) is only a short-term strategy for snakes at high latitude (Storey and Storey, 1992; Andersson and Johansson, 2001). There is some indirect evidence that hibernating snakes may drown if the hibernaculum floods (Viitanen, 1967; Prestt, 1971; Shine and Mason, 2004; Harvey et al., 2014) although access to water during overwintering can be beneficial to overall survival (Costanzo, 1989a,b; Prior, 1997; Costanzo et al., 2001). Snakes may survive flooding events in the field (May et al., 1996; Seigel et al., 1998; Kingsbury and Coppola, 2000) and in the laboratory (Constanzo, 1989a; Todd et al., 2009). However, the ability to remain completely submerged for the entire duration of hibernation seems unlikely and may vary among species and

within species depending on hibernacula site quality (Gregory, 1982; Ultsch, 1989; Rollinson et al., 2008).

The survival of overwintering ectothermic vertebrates depends on the reduction in metabolic rates when exposed to cold environmental temperatures (reduced O_2 demand) and the ability to obtain oxygen via cutaneous respiration (Costanzo, 1989a; Tattersall and Boutilier, 1997; Schulte, 2015). Snakes in hibernacula may experience poor air quality when flooding, snow, or ice block atmospheric oxygen exchange (Boutilier, 1990), particularly when the surrounding organic soils produce toxic biogases (Moore and Knowles, 1989; Charman et al., 1994). Measurement of hibernaculum air quality is challenging, although assessment of groundwater dissolved oxygen (GWDO) could provide an indirect measure of aerobic quality changes within a hibernaculum. We studied Massasauga (*Sistrurus catenatus*) hibernation habitat using a subterranean grid of wells and frost tubes at two sample areas (Mined and Not-Mined), a system that allowed us to avoid the disturbance of neither the ecological functions of the hibernation site nor the hibernating snakes themselves. A 4-yr radiotelemetry study (i.e., 2000-2004) averaging 5.2 Massasaugas per yr with five individuals followed over multiple years (Yagi and Tervo, 2005) demonstrated that 1) individuals moved between "Mined" and "Not-Mined" habitats during the active season, 2) individuals exhibited site fidelity to either Mined or Not-Mined hibernation sites across years, and 3) not all hibernacula could be identified. To survive, we assumed that snakes would move within a vertical subterranean space that is aerobic, flood, and frost-free (Fig. 2.1), and we named this space the "life zone" (LZ). We measured groundwater level (GWL) , frost depth (FD), groundwater temperature (TGW) and dissolved oxygen (GWDO) at each site and hypothesized that 1) if an LZ requires a frost- and flood-free subterranean space, then I expect to find the LZ size to be affected by environmental variables,

Hypothetical Terrestrial Life Zone Model
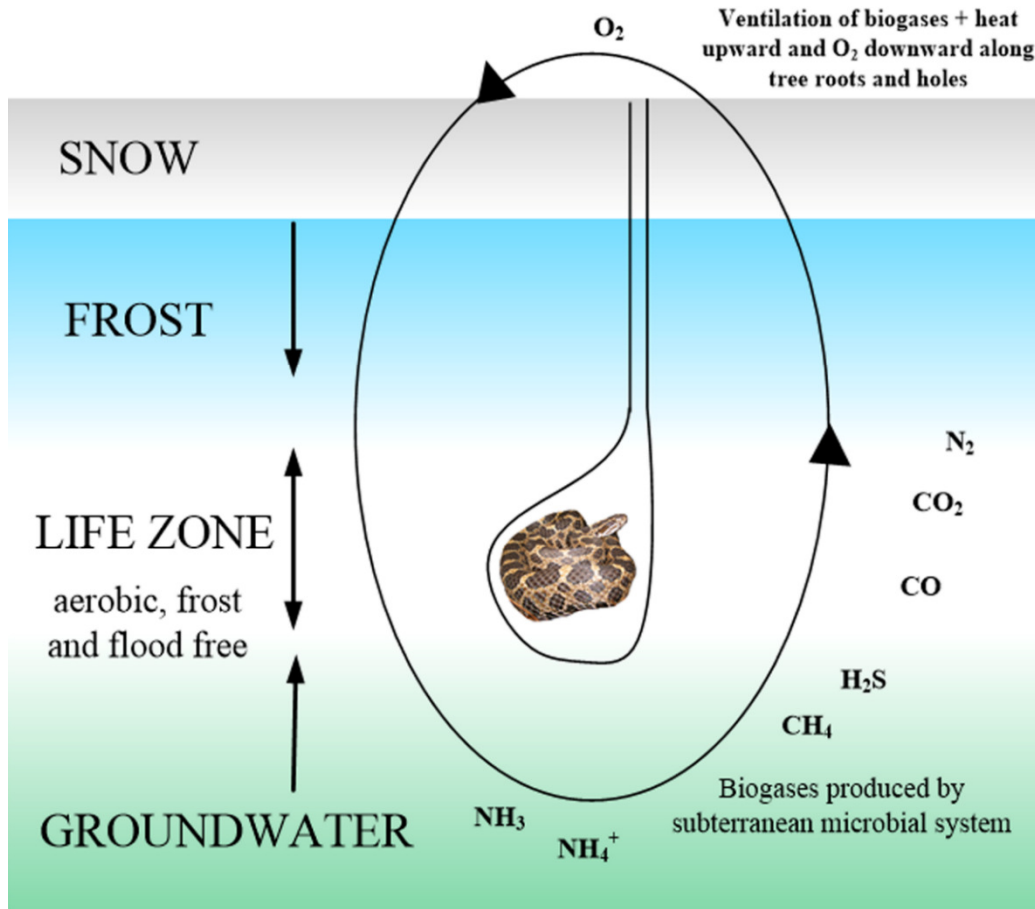
Fig. 2.1 Life Zone (LZ) model hypothesis for terrestrial and semiterrestrial hibernating Massasaugas. Life Zone is the vertical subterranean space that remains aerobic, non-freezing, and flood free throughout hibernation.

such as precipitation and temperature that cause flooding and freezing; 2) if the thermal and

aerobic quality of the subterranean space is affected by the size of the LZ, then I predict an

increasing quality with LZ size; and 3) if successful snake hibernation requires the presence of

an LZ, then we expect to find increased snake overwintering survival with larger LZ size and

higher quality. Additionally, we propose a standardized method for measuring, delineating, and

communicating the characteristics of the LZ within terrestrial and semi-terrestrial habitats using a long-term case study of an endangered Massasauga (*Sistrurus catenatus*) population.

Materials and Methods

Study Site.—The study site is a partially drained and strip-mined peatland, wetland ecosystem within the Great Lakes lowland region, located near Port Colborne ON, Canada, (42° 52' 50" N, 79° 15' 00" W). The wetland is approximately 1700 ha, containing naturalized, and remnant swamp, marsh, and bog vegetation communities, with organic soils underlain by thick clay soil (Browning, 2015). Drainage of the study area began in the 1820s with the construction of the first Welland canal and increased drainage occurred in the 1930s with the construction of channelized water courses within the interior (Yagi and Frohlich, 1998). Constructed watercourses (i.e., drains) intersect both the organic, and underlying clay layer thus shortening the hydroperiod which enabled the harvest of peat soil from the central domed portion of the bog (Browning, 2015; Fig. 2.2). The effects of human disturbance are reflected in the soil core pollens dating to about 180 years ago, with the driest conditions occurring in the 1960s (Nagy and Warner, 1999). In the late 1990's the Canadian and Ontario governments purchased most of the area for restoration, investigation, monitoring, and protection (Frohlich, 1997). Once peat mining ceased in the 1990s, increases in internal drain water-levels occurred due to intrinsic and extrinsic factors such as, growth of vegetation, sediment accumulations, woody debris, constructed peat dams, the natural re-colonization of Beaver (*Castor canadensis*) (Yagi and Litzgus 2012; Browning, 2015), and stochastic wet weather events (Environment and Climate Change Canada, 2016). This wetland is habitat to eight snake species: Eastern Gartersnake (*Thamnophis sirtalis*), Milksnake (*Lampropeltis triangulum*), Smooth Greensnake (*Opheodrys vernalis*), Red-bellied Snake (*Storeria occipitomaculata*), Dekay's Brown Snake (*Storeria*

dekayi), Northern Watersnake (*Nerodia sipedon*), Eastern Ribbonsnake (*Thamnophis sauritus sauritus*), and an endangered population of Massasaugas (Parks Canada, 2015). The wetland is isolated by surrounding agricultural, industrial, and rural land use, and is more than 200 km from other Massasauga populations.

  Measurement of the LZ .—The present LZ study began near the end of the 4-yr radiotelemetry study (i.e., winter of 2003–04 to winter of 2013–14). We chose Mined and Not-Mined LZ study areas based upon Massasauga hibernation site fidelity data from the initial radiotelemetry study. Although snakes returned annually to their hibernation area, they often selected different but nearby hibernacula in subsequent years. Also, original capture locations did not always match hibernation areas, especially when captured in the summer, indicating a broader habitat use by this population during the active season (Yagi and Tervo, 2005). Encounter data showed that not all snakes overwintered within the two known hibernation areas (Yagi and Tervo, 2005). Therefore, hibernation site-specific survival trends are not possible to differentiate from an overall population trend.

  Groundwater level (cm), FD (cm), groundwater temperature (T_{GW} °C), GWDO (mg/L), and snow-height (cm) were measured manually in a well-field grid within the Mined and Not-Mined hibernation study areas for 11 winters. Winters were defined annually from 1 November to 30 April. The LZ (cm) calculation equals the difference between the top-of-well-pipe water-level (T) and the height of well above-ground (H) plus FD (LZ = T – (H + FD); or simply LZ = GWL – FD (Fig.2.3). Observations of surface flooding or ice formation in the two hibernation areas constituted a zero LZ. We calculated the LZ groundwater thermal buffering function (T_{BUFF} °C) for each study area as the difference between T_{GW} and daily mean air temperature (T_{AIR}), or $T_{BUFF} = T_{GW} - T_{AIR}$ (Environment and Climate Change Canada, 2016).
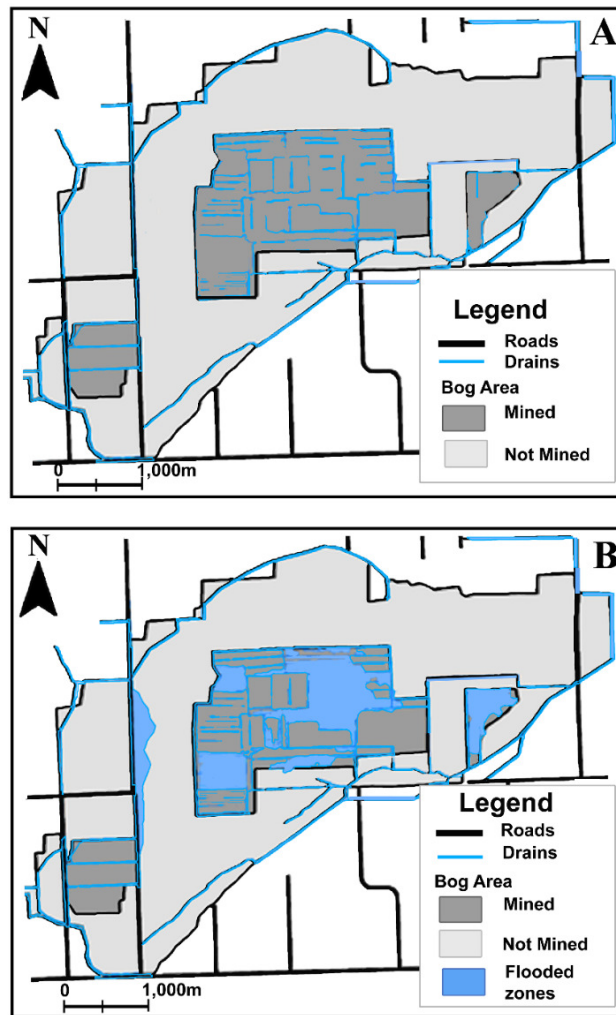
Fig. 2.2 The Massasauga population study area is a partially strip-mined peatland located in southern Ontario, Canada. Figure A shows the amount of surface water present during dry weather cycles (1998–2005; 2011–2014). Figure B shows the amount of surface water present during a wet weather cycle (2006–2011). The first known flood event occurred from 10 October 2006 to 13 October 2006. Hibernation study areas are data sensitive and so are not shown.

More than 1 km of forested peatland separates the LZ study areas, and study areas are also within different hydrologic sub-catchments (Browning, 2015), but otherwise have a similar vegetation community (*Betula pendula*, *Aronia melanocarpa*, *Vaccinium* sp., *Rubu*s sp.) with holes at the surface. The Mined site is low-lying with exposed organic soil surface flattened and compacted from past peat mining activities. The Not-Mined site is higher in elevation with more microtopography.

An important hydrological event occurred three years into the LZ study when the Mined study area (0.8 ha) and approximately 317 ha within the central peat barrens flooded for the first time (Fig. 2.2; Yagi and Litzgus, 2012). This first flood event occurred during a stochastic storm from 10 October 2006 to 13 October 2006 that included mixtures of snow, rain, and freezing temperatures (Environment and Climate Change Canada, 2016). The flooded area accounted for over 30% of the central peat barrens, and the Mined study area continued to experience flood events over the next five winters (i.e., winter of 2006–07 to winter of 2010–11). The flood events were followed by a dry weather cycle with a severe drought with central peatland wild-fires in 2012 (Fig. 2.2).

Detailed Life Zone (LZ) Methods.—Groundwater wells paired with frost tubes were installed, forming a grid pattern, across both Mined and Not-Mined hibernation study areas. Thirty-meter and 50-m spacings were used for Mined and Not-Mined well fields, respectively. Weekly measurements averaged 12 wells for Mined (0.8 ha) and 24 wells in the Not-Mined (3.75 ha) areas. Wells and frost tubes were placed approximately 1m away from any surface hole, to avoid impacting existing snake burrows. Groundwater wells were made from 1.83 m length of black 5 cm diameter ABS pipe and drilled several 0.5 cm diameter holes perpendicular through the sides of the bottom one-third portion, and then placed a cap on top (Fig. 2.3). To install a well, a

vertical hole was bored into the ground surface up to 1.2 m depth using a hand-operated 5 cm diameter soil auger. The well was placed vertically into the hole and tamped down the soil around the pipe at the ground surface interface. The depth to clay (i.e., organic soil-depth cm) was measured at installation. This measurement was limited to the maximum length of the auger ~1.2 m. Fibric soil and subterranean spaces were also noted when they occurred.

A frost tube was constructed in two parts, following a design developed by the US Forestry Service (Patric and Fridley, 1969). For the first part, we made an outer casing from 1.3 cm diameter PVC conduit, and cut it into 60 to 100 cm lengths depending on soil-depth. One end of the casing was covered with 3M ™ Duct tape and pushed vertically into the ground, flush to the surface. The casing was located approximately 1 m from the well. For tighter soils, I recommend the use of metal rebar to make the hole. The inner second part was constructed from 0.95 cm OD clear, flexible tubing sealed at the bottom with a cured waterproof sealant and a 2 cm piece of wooden doweling to form a plug. The flexible tubing fits tightly inside the outer casing. We delineated the corresponding ground surface by marking the clear tube with an indelible felt-tipped pen. We filled the clear tube with red food-dye colored-water up to this mark. I used waterproof tape to secure the top of the frost-tube to prevent water leakage and attached a guidewire through the tape to a nearby post or shrub to help locate the frost-tube under the snow. Frost depth (FD, cm) was measured as ice formation within the red water-filled tube which changes color from red to clear, giving a distinct visual demarcation of ice. Groundwater level (GWL, cm) was measured in the well from the top of the pipe using an electronic measuring tape (Heron Instruments Little Dipper™ 22 m), which beeps when it encounters water. LZ size (cm) equals the space between FD and GWL (Fig. 2.3). Manual data collection occurred weekly between 01 November and 31 April, from 2003 to 2014.
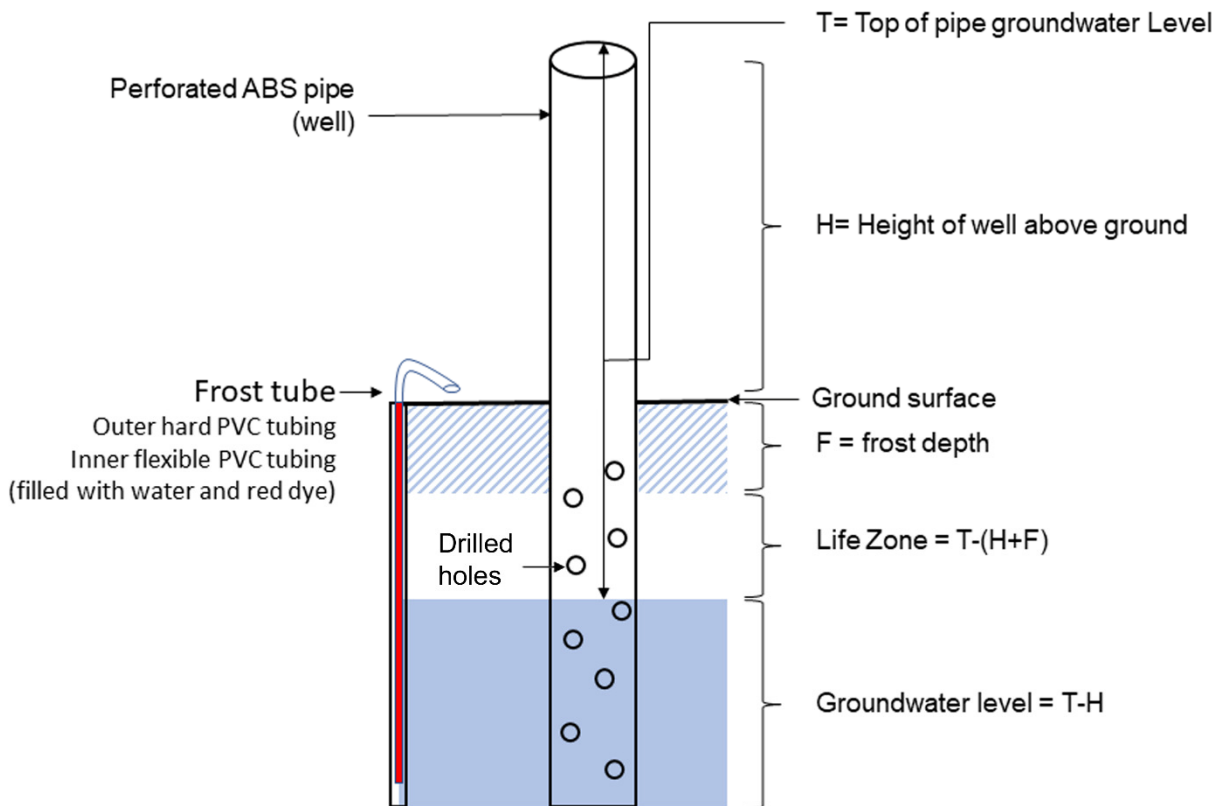
Fig. 2.3 Hibernation habitat study methods developed for a partially mined peatland ecosystem inhabited by a Massasuga population located in southern Ontario, Canada. Life Zone methods include a grid of groundwater wells paired with a frost tube in each study area (Mined and Not-Mined). Life Zone (LZ) equals the groundwater level (GWL) minus frost depth (FD). All measurements are relative to the ground surface.

Groundwater temperature (TGW °C), and dissolved oxygen (GWDO mg/L) were measured within the top 10 cm groundwater layer inside the well, using a DO meter (YSI 55 or 550 models) calibrated to zero salinity, and surface elevation (183 m asl). We calculated the LZ groundwater thermal buffering function (TBUFF °C) for each study area as the difference between TGW and daily mean air temperature (TAIR) (Environment and Climate Change Canada, 2016). Snow-height (cm) including any ice accumulations were measured using a measuring tape. The flooded zone was mapped from field observations using a handheld Garmin

GPS (model eTrex 20x) and digitized into orthogonal corrected aerial imagery using ©ArcGIS mapping software. Surface flooding and ice formation were recorded at a well site, and where this occurred, we scored LZ equal to zero cm.

Over the 11-yr LZ study period, a strict weekly schedule was not always feasible, or field equipment was not functioning, resulting in data gaps. For missing GWL, we used the average between subsequent measurements and assumed the levels did not change drastically between visits. We used the average daily FD value for the study area to fill weekly gaps to complete the LZ calculation. Although this had a smoothing effect on the data, we were able to retain the spatial context. The data correction occurred 146 times out of 3170 (4.6 %). We omitted blank GWDO and T_{GW} data from further analysis.

Massasauga Population.— All methods were completed in accordance with provincial legislation and Ministry of Natural Resources and Forestry Animal Care annual approvals (#98-55 to #15-55). I used annual (April to November) mark-recapture (M-R) data from 1998–2016 to monitor population trends (OMNRF, unpublished). All captured adults were tagged subcutaneously with a Passive Integrated Transponder (PIT) (Parent, 1997). Neonatal snakes were identified by their head and mottle pattern. I estimated age from several biological parameters such as snout-vent length (SVL), weight, reproductive maturity, and the number of rattles within a complete rattle sequence. I assumed that two rattles form per year for this population, which is consistent with local data and other rattlesnake populations at similar latitudes (Fitch, 1985; Macartney et al., 1990; Aldridge and Brown, 1995). All encountered snakes were aged in this manner to confirm the presence in the population and allow us to discern a pre-flood group from post-flood recruits. The number of snakes considered to be adults (N_{adults}) were aged three years and older.

We did not use traditional calculations for population estimates due to the cryptic nature of this species, our low annual recapture rates, and uncertainty in annual survival because of the presence of impaired habitat and environmental stochasticity. Instead we built a time-series of known individuals (N_{indiv}) for the overall sampling area and LZ study period. The calculation for N_{indiv} included all encountered snakes per year plus those presumed present due to their age estimates from future encounters (N_{indiv} = new captures (C) + recaptures (R) + known undetected (U)). We calculated the yearly rate of detection (D) as (C + R) / N_{indiv}. Snakes not re-captured up to 10 years after the last encounter were hence removed from the sample population at the last encounter event. We calculated the proportion of adults in the population (P_{adults}) as N_{adults}/N_{indiv}.

Statistical Analyses.—We used the program R to complete all statistical analyses and graphing (version 3.5.0; R Core Team, 2018). We tested parameters and model residuals for normality using a Shapiro-Wilk test and visually using qq-plots. We used nonparametric tests (i.e., Kruskal-Wallis and Dunn's test) when transformations failed to correct for violations of parametric statistical assumptions.

Weather Data Analysis.—We downloaded 16 winters (1998–2014) of historical weather data from the nearest weather station with vetted data (Port Colborne or Hamilton, Ontario). This period encompassed the broader 16-yr Massasauga population study, including the 11-yr LZ study. Thus, allowing us to contextualize the LZ study within recent winter climate trends concerning precipitation and temperature. We partitioned the monthly weather data to match the LZ study period and extracted four weather variables for analysis; precipitation (snow, rain, total), and the number of freezing days (i.e., mean daily temperature < 0 ˚C; Environment and Climate Change Canada, 2016). A principal component analysis (PCA) was used to reduce the winter weather records into useful categories for analysis. A K-means cluster analysis with the

K-means function was used to assess the sums of squares based on the different number of

clusters. By plotting the total within-groups sums of squares against the number of clusters, the

sudden change in slope depicted an optimal value of three winter-types (Everitt et al., 2011). We

partitioned the three winter-types into groups defined by temperature and precipitation trends:

cold-dry (winter-type 1), mild-wet (winter-type 2), and cold-wet (winter-type 3). A data

reduction of the same weather data was achieved using PCA with the FactoMineR package (Lê

et al., 2016). All variables were centered and scaled to unit variance before the PCA. The

predominant axis (PC1) described a measure of rainfall (from dry to wet), while the second axis

(PC2) combined the number of days below 0 °C (from mild to cold) and total precipitation as

snow.

   Life Zone Analysis.—The LZ is the space between the FD and GWL. Therefore, according to

my hypothesis, when GWL exceeds the ground surface or frost reaches the GWL, LZ size equals

zero. However, we used negative LZ values to avoid zero truncation issues during the model

analysis (Zuur et al., 2009). Fixed and random effects (Table 2.1) were analyzed with respect to

the response variables LZ size and natural log-transformed GWDO and T_{BUFF} using linear mixed

effect models on the full dataset with the lmer function from the lmerTest package. Adjusted

degrees of freedom were estimated using the Satterthwaite method. A model selection analysis

using the AICc function from the AICcmodavg package was used to assess the fixed effects that

best predict LZ size, ln GWDO, and T_{BUFF} (Akaike, 1987). A Type III ANOVA was used to

analyze the significance between fixed effects within the Akaike top model.

   Massasauga Survival Analysis.—We used a linear model to assess differences in detection

by flood groupings. An annual flood survival estimate was calculated for the pre-flood group

accounting for known deaths. I used a generalized linear model with a Poisson distribution to

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compare the previous winter's fixed effects (LZ size, T_{BUFF}, GWDO, T_{GW}, snow-height) against the following active season N_{indiv} (Table 2.1). Akaike model selection was used to determine the top model. A Type II Analysis of Deviance was used to assess the significance of the fixed-effects within the Akaike top model, using the ANOVA function from the R car package.

Results

Winter Weather Trends.— The PCA combined local winter environmental conditions into PC1 (rainfall) and PC2 (snow and number of freezing days), which clustered into three winter-types (Fig. 2.4; Table 2.2). Winter-type 1 (cold-dry) occurred four times but only once during the LZ study. Winter-type 2 (mild-wet) occurred four times during the LZ study. Winter-type 3 (cold-wet) occurred six times (Fig. 2.4).

LZ Size Dynamics.—The Not-Mined area had significantly deeper organic soils (mean = 167.9 cm, SD = 53.3) vs Mined (mean = 71.3 cm, SD =20.7; $t_{(30)}$ = 8.49, $P < 0.001$). Maximum recorded frost depth (29.8 cm) was in Not-Mined habitat during winter 2007, after the first flood event. The Mined LZ size was significantly larger before, than after flooding ($P < 0.001$; Table 2.3). Mined LZ size was reduced to zero periodically (312/658 measurements = 47% zeros) during flood events (Fig. 2.5). Not-Mined LZ size was not significantly different throughout the 11-yr study ($P = 0.49$; Table 2.3; Fig. 2.5). The model that best explained the variance in LZ size included the fixed effects of site-soil-depth (cm) and flood-condition (Table 2.4). Type III ANOVA indicated that site-soil-depth and flood-condition were significant effects on LZ size (Table 2.5).

Groundwater Temperature.— Mined T_{GW} declined significantly following flood events ($P<0.001$), whereas Not-Mined T_{GW} did not ($P=0.08$,Table 2.6). T_{GW} was not measurable when a well was flooded or frozen (361 out of 3170 sampling events, 11.4%), causing data gaps and

imbalances in the Mined area dataset during the flood maxima, winter 2008. There were also 75

out of 3170 (2.3%) sampling events that were too dry to measure groundwater attributes. Mined

T_{BUFF} was not significantly different due to flooding ($P = 0.11$; Table 2.6); and Not-Mined T_{BUFF}

was significantly greater after flooding ($P < 0.001$; Table 2.6). The top model for T_{BUFF} included

the significant fixed effects of LZ size, snow-height, site-soil-depth, flood-condition, and PC1

(Table 2.4, 2.5).

Groundwater Dissolved Oxygen.—Mined GWDO (mg/L) was significantly reduced when

flooded ($P<0.001$) and Not-Mined GWDO significantly increased ($P<0.001$, Table 2.6). Mined

Ln GWDO was significantly and positively correlated with mean LZ size before flooding ($F_{1, 34}$

$= 41.25$, $R^2 = 0.55$, $P < 0.001$) but not after ($F_{1,41} = 0.48$, $R^2 = 0.01$, $P = 0.49$; Fig. 2.6). Although

the Not-Mined area did not flood, it did undergo the same wet and dry weather cycles. The

relationship between Not-Mined mean GWDO and mean LZ size was significant before ($F_{1, 32} =$

2.6, $R^2 = 0.14$, $P = 0.03$) but not after ($F_{1,51} < 0.001$, $R^2 < 0.001$, $P = 0.99$) the first flood event

(Fig. 2.6). The top model that best explained the variance in ln GWDO included the fixed effects

LZ size, PC1, flood-condition, and snow-height (Table 2.4). Type III ANOVA for the top model

showed that LZ and PC1 were significant factors while snow-height and flood-condition were

not (Table 2.5).

Massasauga Population Dynamics.—Mean snake detection (D) increased in the study area

from before (Mean = 0.30, SD = 0.11) to during (Mean = 0.52, SD = 0.37) and decreased after

(Mean = 0.38, SD = 0.19) the flood events (Table 2.7). There was no significant difference in D

among these flood groupings ($F_{2,8} = 0.68$, $P = 0.53$). Therefore, we used before and after the first

flood event groups for subsequent analyses. Total search effort during the 11-yr study averaged

735 person·h per year, SD = 354 (Table 2.7). The lowest N_{indiv} occurred in 2008, and the pre-

flood group low occurred in 2010 (Fig. 2.7). The pre-flood group encounters declined by 33% after the first flooded winter, followed by 76% after the second, and 90% by the third winter (Fig. 2.7). The mean age of N_{indiv} in 2004 was 2.5 yr ± 0.50 S.E. (P_{adults} = 0.45), increased to 4.1 yr ± 0.46 S.E. in 2007 (P_{adults} = 0.79), and declined to 1 yr ± 0.6 S.E. by 2010 (P_{adults} = 0.11; Table 2.7). The Akaike top model that predicted N_{indiv} included LZ size over the fixed effects T_{BUFF}, snow-height, T_{GW}, and GWDO (Table 2.8). The N_{indiv} and previous winter's mean LZ was a significant positive relationship with an incidence response ratio of 0.06 (χ^2 = 28.8, df =1, r^2= 0.34, P < 0.001; Fig. 2.8).

Discussion

In our study, anthropogenic habitats and natural habitats show important differences in life zone functions during times of environmental stochasticity. Important factors affecting LZ size in our model analysis were site, soil-depth, snow-height, flood-condition, and winter-type. The significant relationship between LZ size and flood-condition supported our first hypothesis that environmental factors, such as precipitation and temperature, affect the size of the LZ. Thermal buffering and GWDO quality attributes significantly improved in natural areas that maintained an LZ compared to peat-mined habitats that did not. The significant relationship between LZ size, T_{BUFF} and GWDO supports our second hypothesis that LZ size affects thermal and aerobic quality. Finally, the significant relationship between LZ size and Massasauga encounters indirectly supports our third hypothesis that Massasauga survival increases with LZ size. Since natural habitats only maintained an LZ, they likely provided areas of refugia for the Massasauga population.

In our LZ model, we considered that snakes might die from either drowning, freezing, or asphyxiation. However, only the top model showed a positive correlation between N_{indiv} and LZ

size. In altered ecosystems, a large LZ may provide the capacity needed to maintain survival during times of environmental stochasticity. In natural bog ecosystems, a shallow LZ may also provide thermal stability because there is an elevated and stable groundwater table (Smolarz et al., 2018). At our site, we have a degraded ombrotrophic bog ecosystem without stable hydrology or stable Massasauga population. Ombrotrophic means the amount of water within the wetland is derived entirely from precipitation. Therefore, relationships between population trends and LZ functions may be confounded by these complexities. In the case of GWDO, we did not directly measure the air oxygen content in occupied hibernacula. Yet over half the measurements of GWDO were hypoxic, which indicates that cutaneous respiration, as a flood survival strategy, may not be possible here (Costanzo and Lee, 1995; Tattersall and Boutilier, 1997: Jackson, 2007). Therefore, maintenance of an aerobic space within the hibernation site for oxidative metabolism is an important consideration in overwintering survival. Since subterranean air, spaces naturally have low atmospheric oxygen exchange, winter-types vary, and flooding reduces soil oxygen content, additional monitoring is needed to establish the relationship between LZ size, survival, GWDO, snow-height, and air space O_2 (Boutilier, 1990; Cavallaro and Hoback, 2014). Survival experiments that control snake overwintering locations, coupled with LZ measures, would provide direct support of our hypothesis that the presence of an LZ supports overwinter survival.

Our population trends are based upon Massasauga encounters that are backcast using age and refined annually with new M-R and age estimate data. Therefore, N_{indiv} is more reliable in the past and underestimated in the most recent years. Our M-R data are affected by the challenges of finding a cryptic species. Yet our snake detection rate doubled during the first three flood years, then dropped to zero in the fourth flood year (i.e., 2010). Zero captures in 2010 are an important

finding considering there was a spatial search effort bias toward the central peat barrens as we were trying to recapture flood survivors. After 2010 we evened out the search effort and captured Massasaugas in the Not-Mined habitats that were previously undetected. Declining encounters and the significant correlation with LZ size provide indirect evidence of reduced survival in the wetland during this period of environmental stochasticity. We also presented evidence that the mean age of N_{indiv} increased by a factor of 1.6 from 2004 to 2007, then declined by a factor of 4.1 by 2010. The increase in the mean age estimate suggests 1) low recruitment or reproductive output, 2) that adults had a higher initial survival rate than their offspring, or 3) a combination of factors. The subsequent decline in mean age by 2010 is likely related to successful overwintering in the younger age classes. Since post-flood encounters were almost exclusively in Not-Mined habitats that maintained an LZ, females giving birth in higher elevations may have aided neonatal dispersal and survival. Adults likely moved to higher elevation areas and overwintered successfully, emigrated beyond the survey areas, or did not survive hibernation in low lying areas due to site-fidelity. This study presents indirect evidence of the latter. With recent flooding events and the challenges in monitoring a recovering cryptic species, the relationship between LZ functions and survival requires further investigation. We should avoid over-interpreting these results because our summarized data may mask the true relationship between encounters and hibernation habitat quality.

Across the Massasauga range, hibernation sites are often, but not always, found in habitats with an elevated groundwater table (Johnson, 1995; Parent, 1997; Harvey and Weatherhead, 2006b). Habitats that maintain an LZ may offer increased thermal stability and population viability (Pomara et al., 2014; Smolarz et al., 2018). The presence of a consistent snow layer may also provide important thermal buffering, especially when considering the presence of a

thermally stable groundwater table and the thickness of the LZ. In addition to the increased frequency of stochastic events that reduce LZ size, the presence of a snow layer at our study site in southern Ontario is inconsistent between years. Increasingly episodic polar vortices (Charlton and Polvani, 2007) may also increase freeze risk in areas, especially when there is a lack of snow cover and a small LZ. Modeling LZ size and quality functions within various climate-change scenarios under ecologically relevant conditions are important next steps in this research (Pomara et al., 2014). Expanding LZ monitoring into other habitats and soil types are warranted where there is a flood or freeze concern.

Furthermore, demonstrating causality by multiple potential factors is challenging, especially when subsurface mortality and unknown hibernacula locations prevent the confirmation of survival. The continual presence of potentially poor habitat that remains periodically used by Massasaugas during dry weather cycles may indicate the presence of an ecological trap (Battin, 2004). From a conservation perspective, mortality events caused by an ecological trap may increase the extinction risk of a population that may already be compromised by small size, lack of rescue by isolation, genetic drift, and uncertainty surrounding adaptation to environmental stochasticity (Battin, 2004; Bradke et al., 2018). Future studies on this topic should provide opportunities to study the effects of climate variability, habitat quality, winter severity, and the relevance to reptile hibernation success in northern climates.

Tables

Table 2.1. Summary of parameters used in the model selection analysis for each habitat and encounter response variable from an 11-yr hibernation habitat study on a Massasauga population located in southern Ontario, Canada. Linear mixed-effects models are 1) Life zone size (LZ cm), 2) LZ thermal buffering function (T_{BUFF}), 3) Groundwater dissolved oxygen (GWDO mg/L), 4) A generalized linear model compares the previous winter's fixed effects against the following active season Massasauga encounters (N_{indiv}).

Response Variable	Fixed Effects	Random Effects	Data
LZ (cm)	weather cycle (dry or wet) flood (before and after) site (Mined and Not-Mined) site soil-depth (cm) PC1, PC2, Winter-type (1–3)	Year Well-ID Date	Full dataset $n = 3170$
T_{BUFF} ($\Delta°C$) and ln (GWDO) (mg/L)	LZ (cm) snow-height (cm) flood (before and after) site (Mined and Not-Mined) site soil-depth (cm) PC1, PC2, Winter-type (1–3)	Year Well-ID Date	Full dataset $n = 2281$
Massasauga Encounters (N_{indiv})	mean LZ (cm) mean snow-height (cm) mean GWDO (mg/L) mean T_{GW} (°C) mean T_{BUFF} ($\Delta°C$)	Generalized linear models	Annual Means n=11

Table 2.2. Summary of PCA eigenvalues for winter environmental data from a Massasauga population in southern ON, Canada. We downloaded historical weather data for the study area from Environment and Climate Change Canada's weather data website (1998 to 2014). Variables of interest include total rain, total snow, total precipitation, and the number of days when the mean temperature was below zero.

Importance of Components	PC1	PC2	PC3
Variance	1.93	1.6	0.47
% of variance	48.26	39.95	11.79
Cumulative % of variance	48.26	88.21	100

Table 2.3. Life zone summary measurements from Mined and Not-Mined study areas taken during the 11-yr Massasauga hibernation study of a partially mined peatland located in southern ON Canada. We provide maximum frost depths (FD) and minimum-maximum groundwater levels (GWL) relative to the ground surface. LZ size is the difference between GWL and FD. The first flood event occurred on 10 October 2006 and flooding continued over the five consecutive winters. The flood peak occurred in 2008 and was approximately 30cm above the ground surface indicated by parentheses. (* denotes significant p value).

Site	Flood Event	n	Max. Frost Depth (cm)	Min. and Max GWL (cm)	LZ (cm) Mean	SD	Range
Mined	Before	834	23.6	10.8–86.0	40.4*	± 12.6	10.8– 86.0
	After	658	22.0	0–64.2 (30.0)	9.2	± 12.5	0–60.5
Not-Mined	Before	771	26.7	7.5–134.1	51.3	± 15.9	6.9– 134.1
	After	907	29.8	12–103.3	50.4	± 16.7	1.1– 103.3

Table 2.4. Akaike top five linear mixed effect models and the null model results for an 11-yr Massasauga hibernation habitat study located in southern Ontario, Canada. The models are A: Life zone size (LZ cm), B: Thermal buffering function (T_{BUFF}), and C: Groundwater dissolved oxygen (ln GWDO). Fixed effects include winter-type (1,2,3) or PC1, or PC2, site (Mined or Not-Mined) or site-soil-depth (cm), flood (before and after) or weather cycles (dry and wet), and LZ size (cm). For all analyses, random effects were Well-ID, date, and year (Null model). The top models are marked by an asterisk.

	y	Model	AICc	Δ AICc	*df*	ω	
A	LZ	site-soil-depth + flood	22785.1	0	7	0.72	*
		site-soil-depth +flood+ PC1	22787.0	1.9	8	0.28	
		winter-type + site-soil-depth	22805.8	20.7	8	<0.001	
		flood	22812.3	27.2	6	<0.001	
		weather cycle	22825.4	40.4	6	<0.001	
		Null	22830.6	45.5	5	<0.001	
B	T_{BUFF}	LZ + snow-height + flood + site-soil-depth +PC1	6267.5	0	10	0.8395	*
		LZ + snow-height + flood + PC1	6271.5	4.1	9	0.1101	
		LZ + snow-height	6275.2	7.7	7	0.0179	
		LZ + snow-height + flood	6276.1	8.7	8	0.011	
		LZ + snow-height + flood + PC2	6277.1	9.7	9	0.0067	
		Null	6323.1	55.7	5	<0.001	
C	ln GWDO	LZ + snow-height + PC1+ flood + site-soil-depth	4994.5	0	9	0.7874	*
		LZ + snow-height + site-soil-depth	4997.3	2.8	8	0.1948	
		site-soil-depth + snow-height	5004.2	9.7	7	0.0062	
		PC1 + PC2	5004.4	9.9	7	0.0056	
		PC1	5005.2	10.7	6	0.0038	
		Null	5006.9	12.4	5	0.0016	

Table 2.5. The top-ranked linear mixed-effects model ANOVA results for the 11-yr Massasauga hibernation habitat study located in southern Ontario, Canada. The hibernation habitat study examined the following environmental effects; (A) Life zone size (LZ cm) (B) LZ thermal buffering function (T_{BUFF}), and (C) LZ aerobic quality function (GWDO). Random effects were well-ID, date, and year. (* denotes significant P value)

Fixed Effects	SS	MS	Num df	Den df	F value	P value
(A) LZ size ~ site-soil-depth + flood-condition						
site-soil-depth	2373.9	2373.9	1	46.311	40.879	<0.001*
flood	4078.3	4078.3	1	8.611	70.228	<0.001*
(B) T_{BUFF} ~ LZ size + snow-height + flood-condition + PC1 + site-soil-depth						
LZ cm	19.31	19.31	1	918.7	35.34	<0.001*
snow-height	5.49	5.49	1	2186.4	10.05	<0.01*
flood	4.20	4.20	1	171.2	7.69	<0.01*
PC1	6.04	6.04	1	170.5	11.05	<0.01*
site-soil-depth	3.47	3.47	1	50.0	6.35	0.01*
(C) ln GWDO ~ LZ size + PC1 + flood-condition + snow-height						
LZ cm	6.22	6.221	1	396.56	14.56	<0.001*
PC1	6.66	6.6563	1	3.51	15.58	0.02*
flood	2.36	2.3623	1	3.30	5.53	0.09
snow-height	0.10	0.0968	1	375.79	0.23	0.63

Table 2.6. Life zone summary characteristics Mean ± SD from Mined and Not-Mined study areas before and after first flood event which initiated a five-year stochastic flooding period followed by receding floodwaters (* denotes significant p value).

	Mined			Not-Mined		
	Before	After	Test Statistic	Before	After	Test Statistic
LZ Size (cm)	40.4 ± 12.6 n = 834	9.2 ± 12.5 n = 658	$\chi^2 = 898.6$, df 1* $P < 0.001$	51.3 ± 15.9 n = 771	50.4 ± 16.7 n = 907	$\chi^2 = 0.48$, df 1 $P = 0.49$
T_{GW} (°C)	5.62 ± 1.66 n = 579	4.23 ± 2.03 n = 290	$\chi^2 = 113.6$, df 1* $P < 0.001$	5.95 ± 1.48 n = 641	6.10 ± 1.54 n = 718	$\chi^2 = 3.1$, df 1 $P = 0.08$
$T_{BUFF} = T_{GW} - T_{AIR}$	6.17 ± 3.83 n = 579	6.03 ± 4.56 n = 290	$\chi^2 = 2.55$, df 1 $P = 0.11$	6.96 ± 4.89 n = 641	8.44 ± 4.76 n = 718	$\chi^2 = 35.7$, df 1* $P < 0.001$
GWDO (mg/L)	2.42 ± 1.82 n = 579	1.45 ± 1.50 n = 290	$\chi^2 = 64.0$, df 1* $P < 0.001$	1.8 ± 1.40 n = 641	2.71 ± 2.10 n = 718	$\chi^2 = 61.42$, df 1* $P < 0.001$

Table 2.7. A summary of Massasauga population data collected during 11 active seasons following each winter's hibernation study (2004–2014). Encounters (N_{indiv}), mean age (with standard error), search effort (person·h / yr), detection rate (Known-undetected / N_{indiv}), and flood state from 2004–2014 and included. N_{indiv} is the number of new captures, recaptures, and known-undetected snakes, calculated by back-casting age in the population time-series. Flooding events started in winter of 2006–07 and continued through to winter of 2010–11.

Year	N_{indiv}	Proportion Adults	Mean Age	Age SE	Search Effort person·h / yr	Detection Rate	Description
2004	29	0.45	2.5	0.4	1377.0	0.36	Pre-Flood
2005	23	0.48	3.1	0.5	1085.0	0.17	Pre-Flood
2006	21	0.76	3.9	0.6	813.0	0.38	Pre-Flood
2007	14	0.79	4.1	0.5	840.0	0.64	Flood
2008	7	0.57	3.4	1.2	606.5	0.57	Flood
2009	8	0.25	1.5	0.8	851.5	0.63	Flood
2010	9	0.11	1.0	0.5	828.5	0.00	Flood
2011	10	0.10	1.8	0.5	835.0	0.30	Receding flood with flood events
2012	10	0.40	2.3	0.6	150.5	0.10	Post-Flood with Wild-fires
2013	10	0.60	2.9	0.7	275.9	0.40	Post-Flood
2014	8	0.62	3.6	1.0	417.4	0.50	Post-Flood

Table 2.8. Akaike top five generalized linear mixed effect models and the null model results for an 11-yr Massasauga hibernation habitat and population study located in southern Ontario, Canada. Massasauga encounters (N_{indiv}) were set as a function of the fixed effects mean; life zone size (LZ cm), snow-height (cm), LZ temperature buffering function (T_{BUFF}), and LZ aerobic quality (GWDO). N_{indiv} includes all encountered snakes per year plus those presumed present due to their age estimates from future encounters.

Model	AICc	Δ AICc	df	ω
LZ	60.7	0.0	2	0.80
LZ + T_{BUFF}	64.6	3.9	3	0.11
LZ × Snow height	66.6	5.9	4	0.04
LZ × T_{BUFF}	67.9	7.2	4	0.02
LZ + T_{BUFF} + T_{GW}	68.8	8.2	4	0.01
Null	86.4	25.8	1	<0.001

Figures
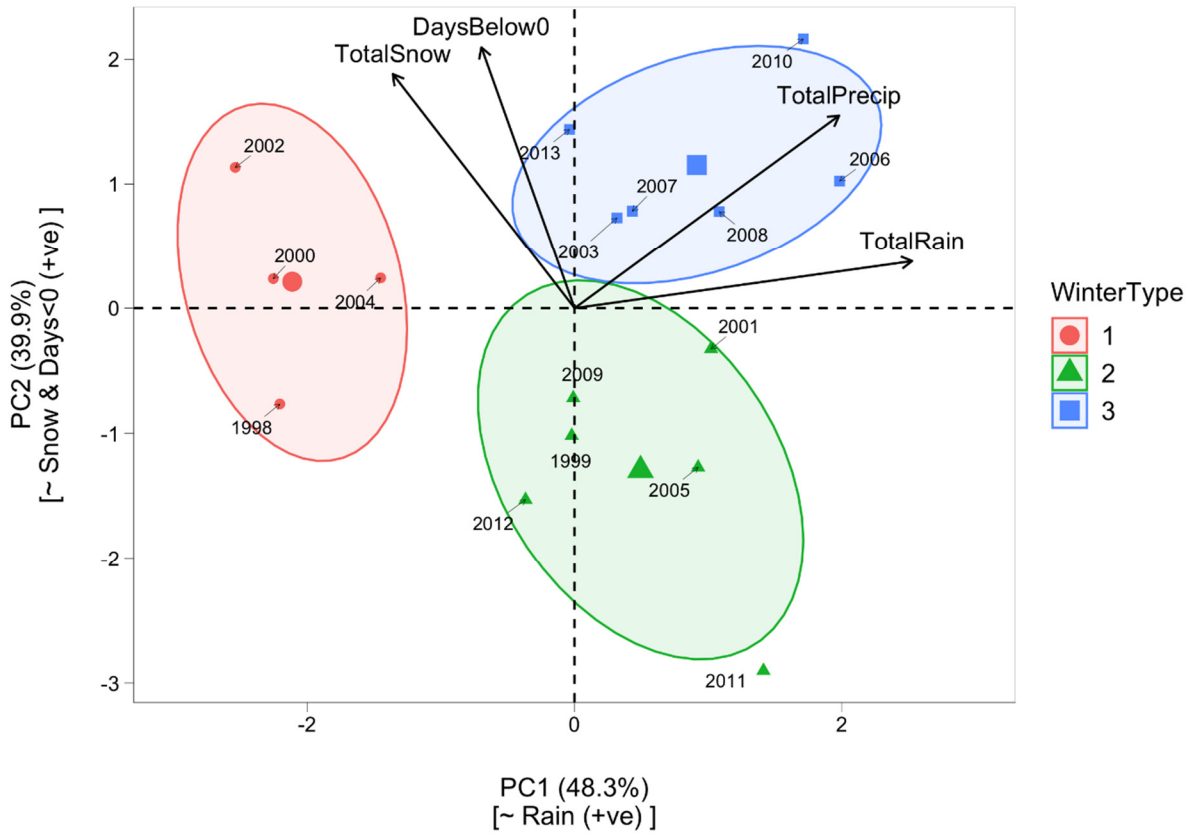
Fig. 2.4 PCA plot depicting the winter-type categories developed for winter environmental data for a Massasauga population located in southern Ontario, Canada. We analyzed local annual winter (November to April) environmental data: total precipitation, rainfall, snow, and the number of days mean temperature is below zero (Environment and Climate Change Canada, 2016).
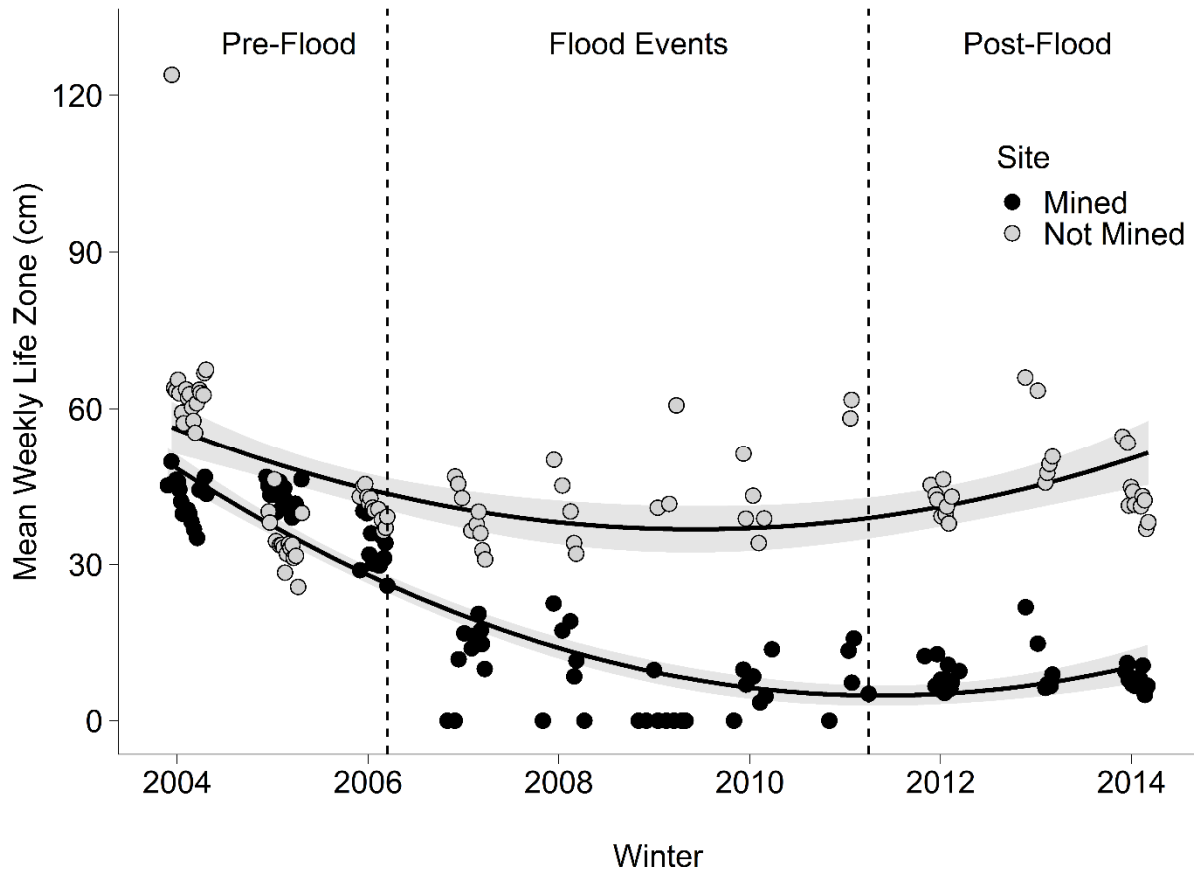
Fig. 2.5 Massasauga hibernation habitat weekly life zone size (LZ cm) by study site (Mined and Not-Mined), over time (November 2003 to April 2014). The study site is a partially strip-mined peatland located in southern Ontario, Canada. Life Zone size was zero for at least one week during winters (2006–2011) because of surface flooding and freezing during a wet weather cycle. Error shown shaded in gray is ± 95% CI.
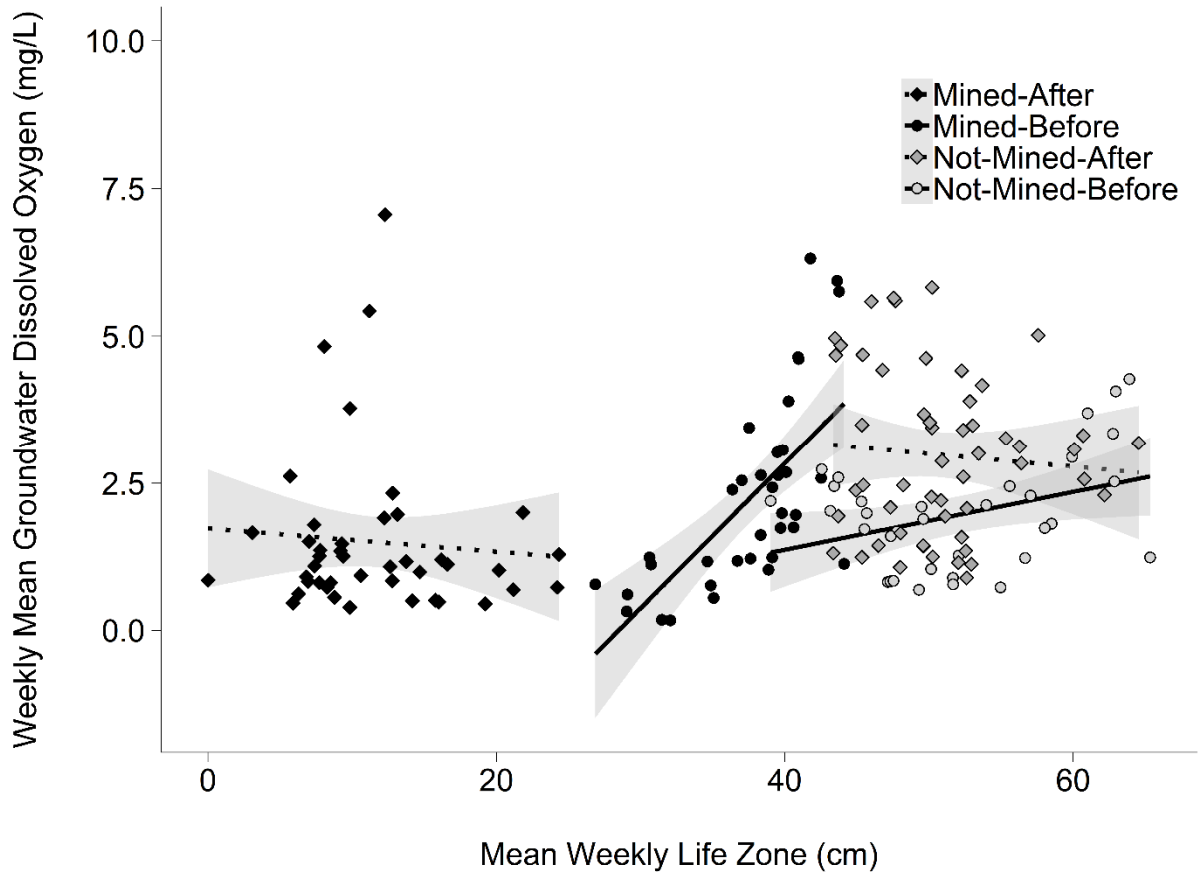
Fig. 2.6 Massasauga hibernation habitat weekly groundwater dissolved oxygen (mean GWDO mg/L) by weekly life zone size (mean LZ cm). The study area is a partially mined peatland located in southern Ontario, Canada. Mined and Not-Mined areas are grouped by before (2003–2005) and after (2006–2014) first flood event. Error shown shaded in gray is ± 95% CI.
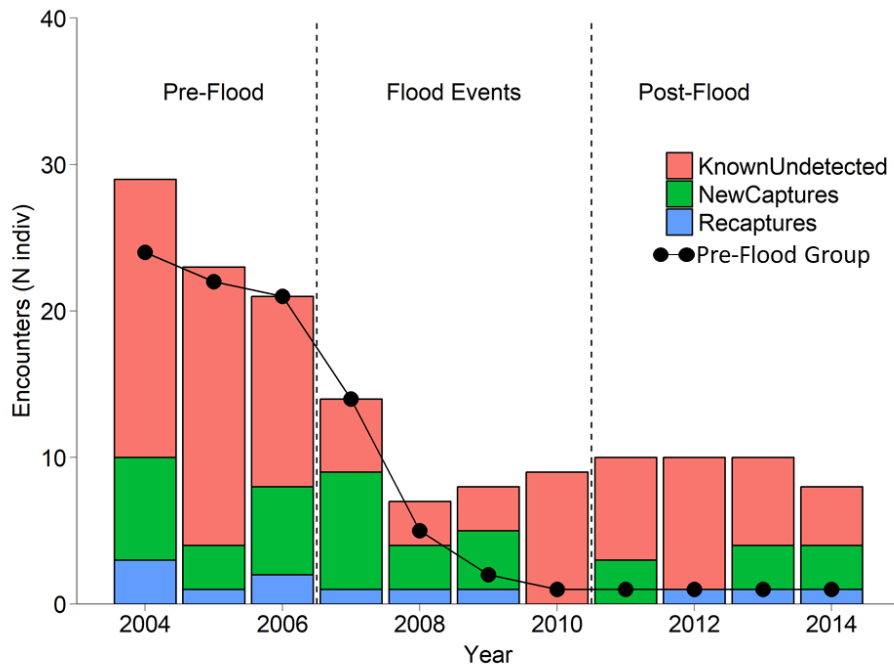
Fig. 2.7 Massasauga encounters (N_{indiv}) from 2004–2014 are calculated from the long-term (1998–2016) encounter dataset for an isolated population located in southern Ontario, Canada. N_{indiv} includes all encountered adult and juvenile Massasaugas plus those presumed present due to their age estimates from future encounters. The pre-flood group encounter trend is indicated to discern post-flood recruitment.
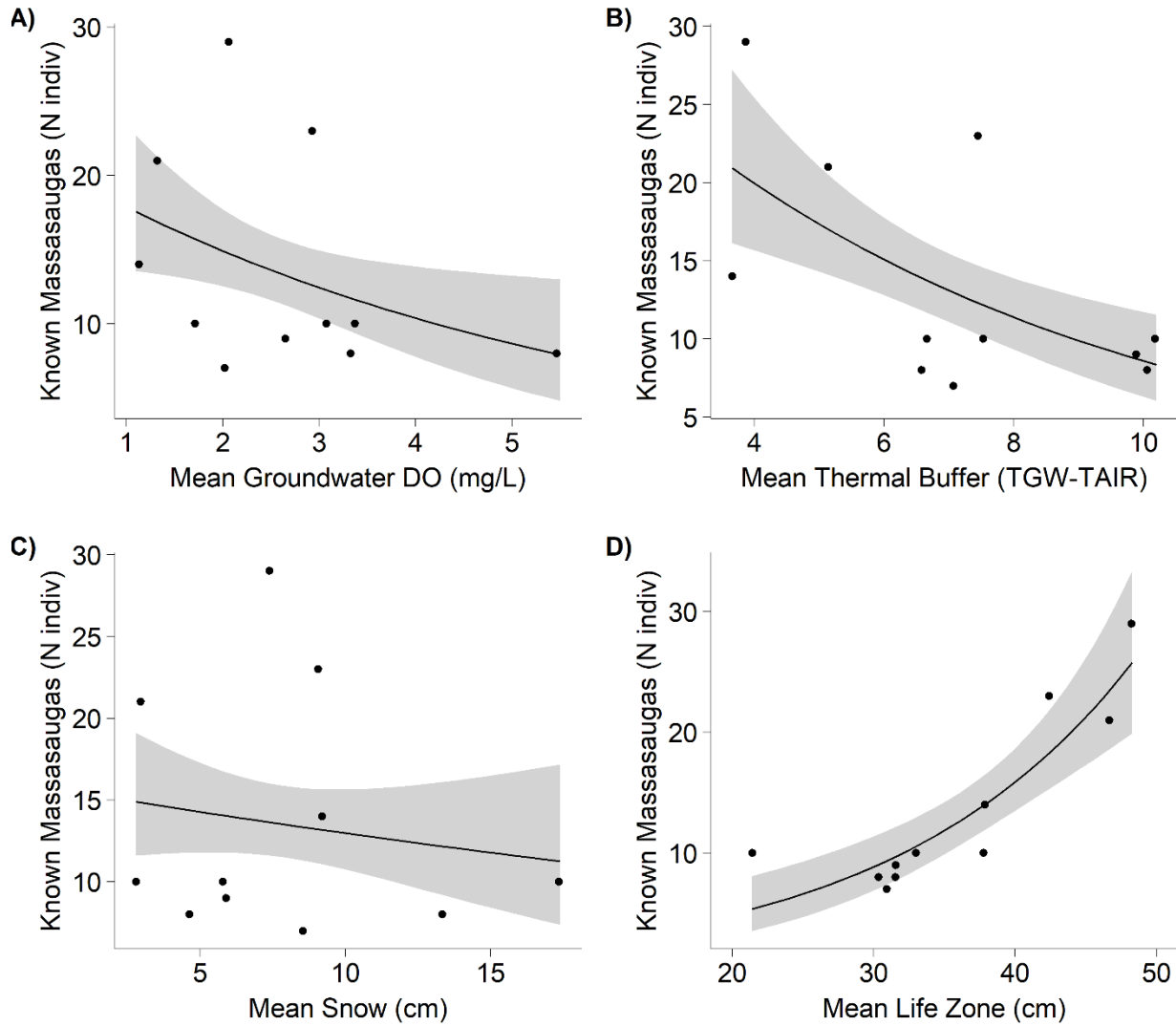
Fig. 2.8 Generalized linear regression models are provided for Massasauga encounters (N_{indiv}) with respect to the independent measures of the previous winter's mean annual, A) groundwater thermal buffer index ($T_{BUFF} = (T_{GW} -$ Mean daily T_{Air}), B) groundwater dissolved oxygen (GWDO mg/L), C) snow-height (cm), and D) the Akaike top model is life zone size (LZ cm). Error shown shaded grey is ± SE. Encounter data are from (1998–2016), for an isolated Massasauga population located in southern Ontario, Canada.

Rationale for Chapter Three

The eleven-year LZ study provided statistically significant and indirect evidence of differential Massasauga survival in my study site after the first flood event. The significance of the flood event was that it occurred during hibernation and included surface freezing temperatures, snow accumulations followed by snow melt and heavy rainfall. In addition, the flood waters covered about 30% of the central peat mined area including a Massasauga hibernation area. Furthermore, flood waters remained elevated in the previously peat mined area for most of the next 5 winters due to a wet climate cycle and increased beaver activity. However, not all hibernation areas were flooded and Massasaugas were found annually but only in higher elevation areas. It remains unclear whether the snakes died from drowning, asphyxiation, freezing or other factors. Since I attributed the decline in Massasaugas to an initial low recruitment followed by an increase in younger snakes and therefore their successful overwintering, I chose neonatal snakes as my subject age class for this study. Chapter 3 contains experiments conducted on respiration strategies and dive behaviour at low temperatures which provide additional insights into overwintering flood survival strategies.

Chapter 3: Comparative Respiration and Behaviour of Temperate Neonatal snakes (Massasauga, Eastern Gartersnake) and Red-bellied snakes during simulated winter conditions

Introduction

Snakes begin hibernation with finite energy stores and remain sequestered within the hibernacula they selected during the fall season and remain there until spring. Philopatric and communal hibernation behaviours by some snake species and in some habitats suggest that good hibernacula are important for annual survival, and good hibernacula are not common (Gregory, 1982; Macartney et al., 1989; Johnson 2000, Gienger and Beck, 2011; Harvey and Weatherhead, 2006b). Snakes are born late in the summer in northern latitudes and may or may not have enough time to feed and increase energy stores before hibernation. Energy conservation may be a determining factor in neonatal snake survival. Therefore, naïve neonatal snakes must find good hibernacula, endure intrinsic changes within their body and any extrinsic changes in the hibernacula to survive a long aphagic period. The presence of water in the hibernacula may be constant (Johnson, 2000, Sage 2005), or change during hibernation (Kingsbury and Coppola, 2000; Shine and Mason, 2004; Harvey et al., 2014) and snakes within a dynamic habitat with respect to water must endure these changes to survive. The constant presence of water may aid in energy and water conservation (Costanzo, 1989a; Costanzo,1989b) or create osmotic and water balance challenges (Gregory, 1982; Ultsch, 1989). Winter survival, therefore, depends on the selection of a good hibernation site and the snake's ability to cope with environmental changes through physiological and or behavioural strategies.

In ectotherms, the catabolism of stored energy (carbohydrate, protein and lipids) is reduced at cold ambient temperatures, as are all biological rate processes (metabolic rate, heart rate,

ventilation rate, and kidney filtering) due to the Q10 effect (Gregory, 1982; Ultsch, 1989). Cold induced metabolic depression or torpor further reduces energy use (Staples, 2016; Geiser, 2013), although not well studied in ecologically relevant scenarios (Gregory, 1982; Ultsch, 1989). Therefore, snakes sequestered in a cold and thermally stable hibernation site would use less oxygen and less energy stores for winter. Ectotherms also use behavioural thermoregulation to move about their habitat to meet their biological needs during the active season (Huey, 1982; Johnson 1995; Sage 2005;Yagi and Litzgus, 2013), and likely continue this behaviour within the hibernaculum unless torpid (Rollinson et al., 2008; Tattersall and Boutilier, 1997). Snakes do not endure winter in a frozen state (Storey and Storey, 1992; Costanzo and Lee, 2013), and therefore would likely avoid a descending frost line within the spatial limits of their hibernacula or freeze and die. A fluctuating frost line would necessitate several movements unless movement is otherwise limited during hibernation. Elevated activity rates in a hibernaculum would deplete energy reserves and may even lower winter survival. Furthermore, unlike turtles, snakes do not have a long-term capacity to manage 'lactic acidosis' incurred during prolonged anaerobic metabolism (Gregory, 1982; Ultsch, 1989; Bickler and Buck, 2007). Therefore, hibernating entirely aquatically in the long-term (i.e. without access to air) within a hypoxic or anoxic environment seems unlikely. Yet it is plausible that the water levels within a hibernaculum may fluctuate and even fill to the frost line temporarily, or flood completely during winter. In an environment with fluctuating water levels or flooded conditions, snakes may increase activity, employ breath holding (apnoea), voluntary diving, and cutaneous respiration strategies to survive winter. The ability of different snake species to survive a sudden flood event is, therefore, of interest.

Hibernation sites for many snake species are often in wetlands or areas with a seasonally elevated water table and therefore, diving is an ecologically relevant behaviour for temperate snakes at all ages (Sage, 2005). Although snake dive research during hibernation is under studied, we can infer behavioural and physiological strategies from species' natural history (Rowell, 2012) and from similar research (Andrews and Pough, 1985; Feder and Burggren, 1985; Ultsch, 1985; Boutilier et al., 1997; Tattersall and Boutilier, 1997; Boutilier and Shelton, 1986; Tattersall and Ultsch, 2008; Udawer et al., 2016). Temperature plays an important role in dive length for ectotherms, with longer dives performed at colder temperatures because of the lowered oxygen requirement (Tattersall and Ultsch, 2008; Udyawer et al., 2016). Higher oxygen content in the water can also result in longer dives (Feder and Burggren, 1985; Boutilier and Shelton, 1986). Ranid frogs remain for months submerged in cold water in ice covered ponds using cutaneous gas exchange (Boutilier et al., 1997; Tattersall and Boutilier, 1997; Tattersall and Ultsch, 2008). "Winter-kill" following a prolonged winter is a global conservation issue for amphibians and suggests there are physiological limits to the duration that frogs can endure winter without access to air (Bradford, 1983; Tattersall, 1997; Tattersall and Ultsch, 2008). Winter-kill of terrestrial or semi-terrestrial hibernating snakes is not obvious, but may be significant (Yagi et al., in press).

Ectotherms perform longer dives than endotherms, and are likely suited to diving because they are naturally intermittent breathers (Boutilier, 1984; Boutilier, 1988), have an irregular heart rate (Costanzo, 1989a), and lower oxygen demand (Brischoux et. al., 2007a,b). In combination with cold temperatures, cutaneous gas exchange, may be enough to sustain metabolic needs in many ectotherms (Feder and Burggren, 1985; Boutilier et al., 1986; Costanzo, 1989a). Reptile cutaneous respiration abilities may be limited, however, by a reduced skin diffusivity due to the

relatively thick keratinised scales (Tu et al., 2002; Lillywhite, 2006; Lillywhite et al., 2009; Fig. 1.4). Therefore, depending on the length of the dive (i.e. flood duration) and ambient oxygen conditions ectotherms may eventually incur an oxygen debt similar to diving mammals and birds (Bradford, 1983; Butler and Jones, 1997; Rogers and Franklin, 2017).

The use of stored pulmonary air may likely occur during the first part of the dive (Boutilier and Shelton, 1986; Boutilier, 1988; Tattersall and Burggren, 2017) and may happen slowly at colder temperatures (Tattersall and Boutilier, 1997). A gradual lung collapse over time was noted in aquatic reptiles and amphibians during prolonged dives (Ultsch et al., 2004). In addition to lung oxygen stores, the respiratory surface for diffusion of gases while submerged would include the outer skin layer and other vascularized body parts including, skin flaps, buccal cavity or cloaca (Ultsch and Jackson, 1982; Hutchinson, 2008). If a diving ectotherm relies on cutaneous gas exchange for respiration, then body size may also be a factor in dive length, with smaller body sizes, or those with, thinner skin (Fick's Law; Feder and Burggren, 1985) or a larger skin surface area being able to dive longer (Ultsch, 1973).

The purpose of this chapter was to compare cutaneous respiration, activity, and dive abilities of three neonatal snake species when exposed to different submergence and emergence conditions at constant cold temperatures. My objectives were to assess whether there were species differences in diving physiology and behaviour of overwintering snakes. I examined species differences in oxygen uptake and behaviour in water and air at constant 5°C temperatures, in a series of closed system respirometry experiments that mimicked ecologically relevant habitats (no water, semi-flooded and flooded). From metabolism data, I estimated the energy costs of hibernation within each type of habitat condition for neonatal snakes. The eventual application is to construct a hibernation habitat energetics model for small snakes. This

model would allow me to estimate energy costs and body mass depletion for small snakes by varying environmental factors, water-level dynamics, winter-type, hibernation length and other life zone characteristics (Yagi et al., in press). This model may also relate well to my ongoing winter survival research. My intention is to have a 3D approach (i.e., subterranean considerations) added to ectotherm climate-change models.

Hypotheses and Predictions

My main hypotheses were that cutaneous respiration would potentially be a significant means of oxygen uptake in an overwintering snake, and both dive behaviour and cutaneous gas exchange would be important flood survival strategies for snakes. Since cutaneous respiration is based upon diffusivity of oxygen through the skin surface, and oxygen content in water is much less than air then cutaneous respiration may not be the main respiration pathway when both water and air are present in the hibernacula. Since the presence of water allows for diving, I expected to see an increase in activity (i.e., voluntary diving) when both water and air are present and therefore total metabolic rate would be elevated when compared to when only air is present. If total metabolic rate remains elevated when both air and water are present and water derived oxygen uptake remains low, then voluntary diving is sustained by lung storage rather than from cutaneous gas exchange. However, when only water is present, such as during a flood event, then cutaneous oxygen uptake would be the only pathway to ensure aerobic metabolism. If cutaneous gas exchange is a flood survival strategy for temperate neonatal snakes, then I expect an increase in cutaneous oxygen uptake from water during a forced dive, reflective of an ability to raise the effectiveness of cutaneous gas exchange. However, if there is an oxygen debt following a forced dive, or if the magnitude of the oxygen debt is large, then cutaneous gas exchange is only a short-term flood survival strategy, reflective of the species tendency to incur oxygen debt to fuel

dive anaerobic metabolism. Further, I predicted differences in the rate of total oxygen consumption between species to be based on body size, activity level and natural history characteristics when placed under various water level conditions.

Methods

Study Animals

All methods were completed in accordance with Brock University animal care (AUP # 13-06-01) and provincial regulations (WSC # 1075269). Eastern Gartersnakes and Red-bellied snakes were collected in late September to early October in 2013 and 2014 from a treed-bog wetland ecosystem located near Port Colborne, ON Canada (42° 52' 50" N, 79° 15' 00" W). Massasauga neonates were collected only in 2014, from gestation sites near Parry Sound, ON Canada (45° 20' 43", 80° 02' 07"). Precise coordinates of capture locations are withheld due to data sensitivity. Collections were performed according to the regulatory requirements of the Ministry of Natural Resources and Forestry (MNRF).

All snakes were brought into the laboratory facilities at Brock University and housed overwinter in a temperature and humidity controlled environmental chamber (© CAN-TROL Environmental systems). Snakes were initially held at temperatures ranging from 15 to 20°C, provided a heated pad under the housing unit, offered food (commercial supplied earthworms, mealworms and pinky mice) and fresh water. Any uneaten food was removed from the housing units and withheld for a minimum of 2 weeks in order to allow time for snakes to purge their stomach contents prior to reducing ambient temperatures. When not in an experiment, all study animals were housed at lab ambient temperatures without heating pads, in individual vented plexiglass enclosed aquaria (~15-20 cm^2), and provided hides and bowls containing clean fresh water topped up weekly throughout the winter.

Snake Age Determination

Neonatal snakes vary from year to year on when they are born (July to September) which may affect their size at time of collection in the fall season. For Eastern Gartersnakes, there were no obvious physical characteristics other than small size to discern neonatal snakes from small juveniles. Neonatal Red-bellied snakes have a pronounced white neck ring that fades as they age, and they are very narrow and short at birth (Fig. 1.7). In 2013, Eastern Gartersnakes were aged based upon their small size (< 5g mass) in the fall season relative to all other observed Gartersnakes. Since neonatal Red-bellied snakes were too small to collect in 2013 (Fig. 1.7), I captured individuals that were similar in size (length and weight) as the Eastern Gartersnakes. In 2014, snakes were aged quantitatively by measuring the total length of all hand captured Eastern Gartersnakes and Red-bellied snakes during weekly searches within the study area throughout the active season. Snakes from the study area were placed in a clear, vented plastic box, digitally photographed over a 0.5 cm^2 laminated grid, and then released. The 0.5 cm^2 grid formed the bottom surface of the box providing a reference scale for each photograph. Total length (TL) (snout to tail tip) of all snakes were later measured using Image J software ™. To distinguish neonates from snakes >1 year old, I divided them into two groups (summer and fall) based upon the first appearance of neonatal snakes. TL frequency histograms were plotted for these two groups followed by a cluster analysis to identify neonatal size classes. Massasauga neonatal age was confirmed by the presence of a single button rattle segment.

Simulated Winter Conditions

To coordinate the release of snakes back to their capture location, the length of hibernation generally followed frost duration and above ground snake observations gathered from my concurrent hibernation study area (Yagi et al., in press). In 2013, ambient temperatures were

gradually reduced over an eight-week period to 5°C and held constant until mid-April, followed by a gradual increase to mean outside temperatures (10°C) two weeks prior to release. In 2013, relative humidity (RH) settings were set at 50% and increased in 2014 to 80% (maximum limit for the lab) to enhance survival, following advice from Dr. Jon Costanzo (Miami University). Photoperiod was reduced gradually from 14 hours light in September to 24 hours darkness in December. Darkness was maintained until the two-week warming period in spring when it was once again increased to 14 hours light and 10 hours dark. Due to additional regulatory restrictions surrounding the use of Massasaugas in 2014 only, lab temperatures and time to 100% darkness were reduced within a 2-week period to accommodate a shortened experimental period ending the second week in December. All snakes were weighed at the beginning and end of each experiment and prior to release using Mettler PE 400 pan scale accurate to 0.01 grams. Snakes that were healthy in appearance following the laboratory hibernation period were released back to their capture location the following spring except for Massasaugas which were donated to the Toronto Zoo as per provincial permit conditions.

Closed system respirometry

I used closed system respirometry to measure the depletion in the partial pressure of oxygen (P_{O_2}) over time in either air or water media within a confined space (i.e. respirometer; Tattersall, 2013). The snake was weighed then placed inside a glass jar of known volume, and capped with a rubber stopper for the duration of the experiment. After a set amount of time a sample of air and/or water was removed from the respirometer with replacement (Fig. 3.1). The partial pressure of oxygen (P_{O_2}) was measured using an oxygen analyser calibrated to ambient air pressure and temperature (Fig. 3.2). The change in P_{O_2} was converted to metabolic rate for

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further analysis. The mole rate of oxygen consumed $\dot{M}_{O_2}$ (μmol$\cdot O_2 \cdot h^{-1}$) in a finite volume (V litres) was calculated from equation 3 (Tattersall et al., 2013).

$$\dot{M}_{O_2} \ (\mu mole\ O_2 \cdot h^{-1}) = \frac{(\Delta P_{O_2}) \times (\beta_{O_2}) \times V}{\Delta t\ (h)} \qquad \text{(Equation 3)}$$

Where

- ΔP_{O_2} is the partial pressure of oxygen measured in the initial sample (air or water) minus the second sample calibrated to water saturated air at known pressure mmHg and temperature 5°C at the time the sample was measured (O_2 at mmHg).

- β_{O_2} is the solubility or capacitance coefficient for oxygen in either air (52.95) or water (2.5262) at 5°C temperature (μmol O_2 / L mmHg).

- V is the net volume of media (air or water) of the jar in litres. This is the volume of the respirometer minus the mass of the organism. For partitioned experiments a known volume of water was added to the jar and subtracted from the net total volume to determine amount of air volume.

- Δ t is the elapsed time between the two samples in hours.
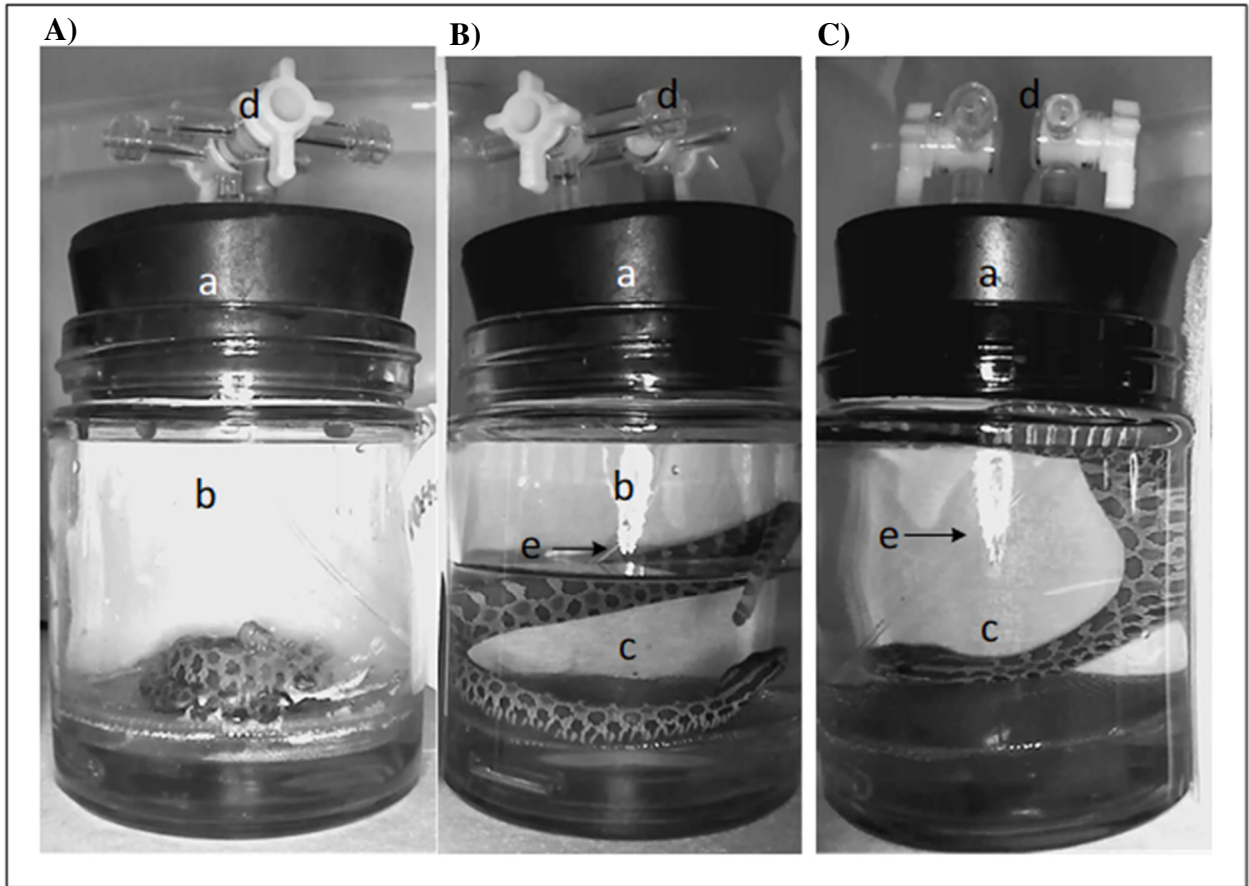
Fig. 3.1 Closed System Respirometry set up for measuring whole body and cutaneous gas exchange in small snakes in air or water media during simulated wintering conditions, darkness, and cold lab temperatures (5°C). Shown here is a Massasauga neonate (A) air filled respirometer, (B) partitioned respirometry air + water media where snakes can voluntarily dive, and (C) Forced dive or full submergence experiment in water only media. The respirometer is constructed from a glass jar (330 ml) (a) rubber stopper (b) air (c) water (d) 3-way stop cock mechanism for extracting sample of air or (e) water through tubing.

Calibration and Electrode Maintenance

A two-point calibration was completed weekly on the Sable System Read-Ox-4H polarographic

oxygen analyzer and micro-electrodes following a change in electrode electrolyte solution. The

two points of calibration were Po_2 of water saturated air at known air pressure at 5° C (upper

limit), and zero calibration (lower limit) using nitrogen gas (for air samples) or sodium bisulfite

($NaSO_3$) crystals dissolved in water (for water samples). In addition to weekly two-point

calibrations, and to accommodate changing air pressure during long experiments, an upper limit

calibration was conducted prior to each sample. The electrode's temperature was maintained at

5°C by housing the electrode within a water-jacket that is continuously supplied by water

pumped from a temperature-controlled water bath (Thermo Scientific Model SC 100).

Sampling Procedure

Two 0.5 ml samples of air or water media were manually removed from the respirometer via a

syringe and replaced with fresh air or water (Fig. 3.2). One ml fresh air or aerated water was

used to clear any previous injected sample and the analyzer calibration was re-checked. Half the
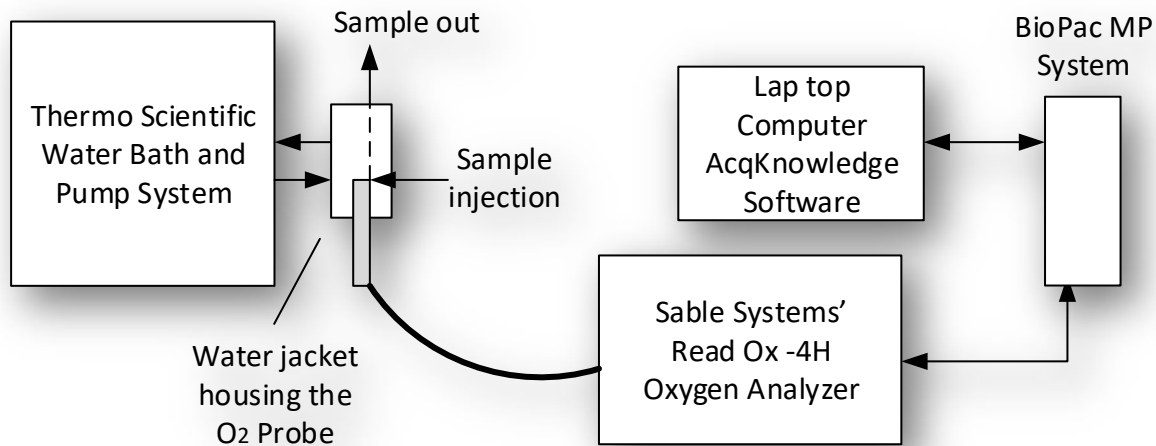
Fig. 3.2 Equipment set up for measuring partial pressure of oxygen in air or water media at constant cold temperatures.

sample was injected into the oxygen analyzer, followed by the second half. Because of the slow

response time and exponential wash-out from the pressure artifact of injection, a stopwatch was

used to maintain the same measurement equilibration period (~ 2 minutes) and the time taken to

inject the sample was standardized to approximately 6 seconds. The sample was monitored

continuously by the oxygen analyzer and me visually, until the pressure effect gradually

dissipated and stabilized. The stabilized Po_2 value (which is automatically averaged by the

Oxygen Analyzer) was recorded and the procedure was repeated for the same sample. The

average of two samples was used to calculate metabolic rate $\dot{M}_{O_2}$ ($\mu mole\ O_2 \cdot h^{-1}$) for each

snake (Equation 3). Sample results were also compared to a control (closed jar without snake) to

check against background bacterial respiration. The Po_2 corrected for background respiration was

used in the $\dot{M}_{O_2}$ calculation.

In addition to snake mass (g) and length (TL, SVL), the snakes' surface area and surface area

to volume ratio (SA:VOL) were estimated assuming the snake is composed of two cylinders

(head to vent and vent to tail) and added together. Area $= 2\pi rl + 2\pi r^2$; Volume $= \pi r^2 \times l$,

where r is half the average width taken from 8–10 cross sectional measurements from head to

vent, and 2–3 tail measurements and l = body length (SVL or tail length). Measurements were

made using scaled digital photos using Image J software™.

Experimental Design

All metabolism experiments were conducted in darkness, at constant lab air temperature of

5°C, and relative humidity (50 to 80%). All animals were held at 5°C in the dark at least four

weeks prior to the commencement of experiments and remained in these conditions until the

experiments were completed. Metabolic rate ($\dot{M}_{O_2}$) was assessed using closed system

respirometry and partitioned respiration methods (Tattersall et al., 2013). A snake was placed in

the respirometer and allowed a minimum of 1 hr to habituate with the stopper open. Then I added

a known amount of aerated water (partitioned experiment) or filled the jar to the top (forced

dive) and then closed the stopper (Fig. 3.1).

In 2013, preliminary trials were conducted first, at increasing time lengths (1 to 24 hr) with

small groups of Eastern Gartersnakes, to estimate the experimental length needed to discern

lowest activity to establish an individual's resting metabolic rate (control) (Fig. 3.3; Table A.1).

A resting metabolic rate value was assigned to each snake as the lowest value measured in air

following a trial time of 4 hours or longer. Similarly, a maximum value (Active_MR) was

assigned to each snake during the preliminary trials (Table A.1). Preliminary trials were also

conducted for partitioned respiration and force dive experiments to ascertain experiment length

to discern at least a 5point inflection in P_{O2} (Table 3.1; Fig. A.1A and Fig. A.1B). Metabolism

experiments were conducted on all 3 species using repeated measures in this order: (1) Resting

Metabolic rate (oxygen uptake in air only) which was the control, (2) Partitioned respiration

(oxygen uptake in air and water) including length of voluntary dives, (3) Full submergence

respiration (oxygen uptake in water only) followed immediately by (4) Post-submergence

recovery (oxygen uptake in air only; Fig. 3.3).

Experiment effects on respiration pathways were tested between the same media (water or air)

across experimental treatments. For example, partitioned respiration oxygen uptake from water

was compared to forced dive (water to water comparison). Dive recovery was compared to

resting metabolism (air to air comparison). Only the same individuals included in both experiments were used in the final model analyses (Fig. A.2 and A.3).

	Treatment	Media	Respiration Type	Measurement
	Resting (control)	100% air	pulmonary & cutaneous	Metabolic rate Activity
	Partitioned (stopper)	50% water	pulmonary & cutaneous	Metabolic rate Activity Dive Length
	Partitioned (screen)	50% water	--	Activity Dive Length
	Forced Dive	100% water	cutaneous	Metabolic rate Activity
	Forced Dive Recovery	100% air	pulmonary & cutaneous	Metabolic rate Activity

Fig. 3.3 Experimental treatments, media (air and water content), respiration pathways examined, measurements taken during respirometry and extended dive observations (screened lid). All experiments were completed in darkness and constant temperature 5°C, except voluntary dive experiments which were also conducted at 9°C.

Activity and diving behaviour

A high definition infra-red (night vision) closed-circuit digital camera and a digital video recorder computer system (DVR) were used to record snake behaviour (activity and diving) during experiments. Each experiment DVR recording was saved for later viewing and transcribed manually. Snakes moved freely within the respirometer throughout all experiments. Activity was scored from continuously recorded Camera DVR footage. A score of 1 was given if a snake's position changed from the previous minute. A score of zero was given if there were no changes in position. The total active intervals / total viewing time provided a proportion of time

spent active for each snake. In 2013 only, the Camera DVR system did not record during resting and dive recovery experiments.

Voluntary dives were quantified during all closed partitioned respirometry experiments and during several added extended viewing trials with open (screened) lids (Fig. A.6). Voluntary dive experiments were first conducted at 9°C each year and repeated at 5°C during and following the partitioned experiments. A voluntary dive was defined as the number of one-minute intervals when the snakes' nostrils were submerged below the water meniscus. Continuous dive length was scored each minute during the experiment and tallied. In 2013, voluntary dive length was scored and tallied for Eastern Gartersnakes and Red-bellied snakes at two temperatures (5° and 9°C). However, scoring Red-bellied snake dives at 9°C were problematic for short viewing trials due to high activity and therefore an extended viewing time was added in 2014 for Massasauga and Red-bellied snakes. Time constraints in 2014 prevented conducting extended viewing times for Eastern Gartersnakes (Table 3.1). Total dive time, proportion of time in a dive, and longest continuous dive were calculated for each snake. From the tabulated data, dive time interval (time gap between dives) was also calculated for each snake.

Energetics Estimates

A hibernation energy budget for neonatal snakes was estimated for each simulated habitat condition (state) by converting $\dot{M}_{O_2}$ (μmol O_2/h) into J/h (conversion factor = 0.44782 or 0.022391 mlO_2/ μmol O_2/h × 20 J/ml O_2). Based upon earlier research (Aleksiuk and Stewart, 1971; Derickson, 1976; Costanzo, 1985), I am assuming an equal use of the three energy stores (carbohydrate, protein and lipids) for juvenile snakes. I am also using a dry mass conversion of 30% (DBM) to estimate the depletion rate of DBM for neonatal snakes (mg/day) for each habitat

condition or state. This information will be used with hibernation habitat data (duration of water-levels, hibernation length) to create an estimate of energetic costs of hibernation (Equation 4).

$$Energy\ Costs\ (E) = n_1 C_1 + 2 \times (n_2 C_2) + n_3 C_3 + n_4 C_4 \qquad \text{(Equation 4)}$$

where;

n_1, n_2, n_3, n_4 = number of days in each state 1,2,3, or 4

C_1 = energy cost of snakes at rest (no activity) and no water present

C_2 = energy cost of snakes active by voluntary diving, increasing, and decreasing water levels both (water and air present)

C_3 = energy cost of snakes in forced submersion during a flood maximum event

C_4 = energy cost of snakes in post forced submersion flood levels have receded.

Problems encountered

Sample size for each species or across experiments was not equal (Table 9). Sometimes a sample was below detection (zero value) and therefore I assumed there was an equipment problem and removed the data. I also removed the data when a bubble was trapped against the oxygen analyzer probe creating an elevated value. For both instances, and if time permitted, I repeated the test. In 2013, metabolism experiments were delayed (February to March) due to procedure testing, equipment testing and preliminary trials. In 2014 experiments were conducted in early winter (November to January) to accommodate regulatory conditions imposed by the province which required the donation of Massasaugas to the Toronto Zoo by mid-December. Therefore, experiment length times were shortened in 2014. In 2013, to reduce stress on the

snakes, there was at least 1 week between repeated uses of an animal in an experiment, and two Red-bellied snakes and five Eastern Gartersnakes died during these intervals. In 2014, time between experiments was shortened (3 to 7 days). Due to mortality concerns and small sample size of Red-bellied snakes in 2013, they were not forcibly submerged. In 2014, all experiments were conducted on all species within same time period and without any snake mortality. However due to delays in acquiring permits, the sample sizes for each species were small and not equal.

Statistical Analysis

I used the program R to complete all statistical analyses, and graphing (version 3.6.1; R Core Team, 2019). Due to my concerns regarding the condition of the 2013 snakes, a t-test was used to compare between year differences in metabolism for each species and each experimental treatment. If there was no difference between years the data was included, if there were differences, the 2013 year was excluded from the model analyses.

Model residuals were tested for normality using a Shapiro-Wilk test and visually using qq-plots. Natural log transformations were completed when normality tests failed. Nonparametric Mann-Whitney Wilcoxon rank sum paired test was used to compare $\dot{M}_{O_2}$ (µmol O₂/h) between two species groups when model residuals were not normally distributed. Linear mixed effects model analysis and Akaike model testing were used to find the model of best fit. Fixed effects were species, body size using either mass, surface area (SA) or surface area : volume (SA:VOL); dive length, experimental treatment, time, year, and random effects were Snake ID. Fixed and random effects were analyzed with respect to the dependent variables using the lmer function from the lmerTest package (Table 3.2). A model selection analysis using the AICc function from the AICcmodavg package was used to assess the fixed and random effects that best predicted

$\dot{M}_{O_2}$ (µmol O_2/h) (Akaike, 1987). Type II Wald Chi square Analysis of Deviance tests were used to analyze the significance between groups within the top model. Beta regression analysis was used to assess differences in proportional data such as the proportion of cutaneous respiration $\dot{M}_{O_2}$ (oxygen depletion in water / total oxygen depletion in air + water) and proportion of time snake was active (Table 3.2).

The interval between voluntary dive events was calculated from a tally of one-minute observations from continuous recorded camera footage of snake position in the respirometer for the length of the experimental period. Continuous dive length was assessed in a linear mixed effects model, included the fixed effects of experiment length, experiment type and species with species identification (Snake ID) as the random effect. Analysis of deviance was conducted on the top model, according to the AIC approach outlined above.

Results

Age Determination

Eastern Gartersnakes (n= 139) and Red-bellied snakes (n= 134) were captured by hand in the study area throughout the summer and fall seasons of 2014, measured, and released. Total length frequency histograms were plotted to demonstrate the early summer gap in the population of small sized individuals that was eventually filled by newborn snakes observed in the late summer –fall season (Fig. 3.4). A comparison between field measurements of the snake population and those snakes collected for lab studies indicated that neonatal Eastern Gartersnakes were ≤ 260 mm TL and neonatal Red-bellied snakes were ≤ 200 mm TL by the late fall season. Although some overlap was expected between small 1-year snakes and larger neonatal snakes, a cluster analysis provided a basis for discriminating neonatal from juvenile year classes for this study (Fig. 3.5). Massasauga neonates captured in Parry Sound and brought to the lab, ranged from 191

to 242 mm (n=14) (Table 3.3), and Parry Sound neonates ranged from 150 to 255 total length

(n= 22 litters) (MNRF unpublished data).

Body Size

Massasauga neonates were significantly larger in mass than neonatal Eastern Gartersnakes

and Red-bellied snakes (Tukey post-hoc p < 0.001; Fig. 3.6). Massasauga snout to vent length

(SVL) was not different to Eastern Gartersnakes or Red-bellied snakes, however Red-bellied

snakes were significantly longer than Eastern Gartersnakes (Tukey post hoc; p<0.001; Table

3.3). E. Gartersnakes and Red-bellied snakes were not different by mass (p = 0.99). Skin surface

area (SA) was also different between species (Tukey post-hoc; p<0.001), with Eastern

Gartersnakes having the smallest surface area and Massasaugas the largest. Red-bellied snakes

were intermediate. Snakes with the highest surface area to volume ratio (SA:VOL) were Eastern

Gartersnakes followed by Red-bellied snakes and Massasaugas had the smallest SA:VOL. The

SA:VOL were significantly different by species (p<0.001; Table 3.3).

Partitioned Respirometry

Snakes moved freely up and down within the respirometer, conducted short or long voluntary

dives or remained partly submerged within both the air and water space during partitioned

experiments. Periodically snake breathing was observed on camera, however this information

was not consistently collected to establish a rate. Oxygen depletion occurred in both air and

water media, but much less oxygen uptake occurred from the water (Fig. 3.7). The top beta

regression model that explained the differences in the proportion of $\dot{M}_{O_2}$ uptake from water and

air media included the significant interaction between species and the proportion of time spent

voluntarily diving (p<0.001; Table 3.4 and 3.5).

Overall, the amount of oxygen depletion in water was a small fraction of the total oxygen consumed during this experiment (Fig. 3.8). Neonatal Eastern Gartersnakes obtained 24.8 % ± 0.05 (mean ± SE; n =10) of their oxygen from water compared to neonatal Massasaugas 13.3% ± 0.02 (mean ± SE; n =12), and Red-bellied snakes 7.7 % ± 0.2 (mean ± SE; n =10). Wilcoxon post-hoc pairwise species comparisons indicated significant differences between each species group in the proportion of oxygen uptake from water media (p < 0.01; Table 3.6).

Cutaneous Respiration during Forced and Voluntary Dives

Oxygen depletion $\log_e \dot{M}_{O_2}$ in water only media (cutaneous respiration) was significantly greater during forced submersion than during the partitioned respirometry experiment for all species (p<0.001; Fig. 3.9). The top model included the interaction of species and skin surface area plus the proportion of time spent submerged in a dive (Table 3.4). However only the proportion of time submerged had a significant effect on cutaneous respiration rate ($\log_e \dot{M}_{O_2}$; p<0.001; Table 3.7). Post-hoc pairwise comparisons between species using a Mann-Whitney Wilcoxon test indicated that forced dive cutaneous respiration rate $\log_e \dot{M}_{O_2}$ was not significantly different between species (p > 0.02; Bonferroni correction applied; Table 3.6). There were also no species differences in the overall increase in oxygen uptake from water (forced dive – voluntary dive; p > 0.02; Bonferroni correction applied; Fig. 3.10).

For all but one individual, there was an increase in cutaneous respiration rate during the short (average 1.5 hr) simulated flood event compared to cutaneous respiration rate when air was also present (Fig. 3.11A; Fig. A.2). Eastern Gartersnakes (n=18) averaged a 77% ± 3.4 SE increase, Massasaugas (n=12) averaged 64.5% ± 22.2 SE increase and Red-bellied snakes (n=5) averaged 77% ±15.9 SE increase in cutaneous respiration during a forced dive (Fig 3.11B). The larger variance with Massasaugas was attributed to an individual with a higher cutaneous respiration

rate during the partitioned respiration experiment than the forced dive (Fig. A.2). With that individual removed from the analysis, the rate of increase in cutaneous respiration was 87% ± 3.5 SE.

The proportion of time snakes were active during the forced dive was not significantly different than during the partitioned experiment (p=0.10). However, Red-bellied snakes (n=5) were more active than other species during the forced dive (χ^2 = 9.04, df =2, p = 0.01). Post-hoc pairwise comparisons between species using a Mann-Whitney Wilcoxon test indicated Red-bellied snakes had significantly higher activity during the forced dive then Eastern Gartersnakes and Massasaugas (Table 3.11; Fig. 3.12).

Post Dive Recovery

Dive recovery (i.e., post flood) metabolic rate was elevated after the short-forced dive event for all species, compared to control (i.e., resting; Fig. 3.13; Fig. A.3). The top model included the significant interaction of experiment type and species plus snake activity and body size (SA:VOL)(p < 0.001; Table 3.4). However, snake activity (p = 0.94), species (p=0.41) and body size effects were not significant (p=0.70) within the top model (Table 3.8). Post-hoc pairwise comparisons between species using a Mann-Whitney Wilcoxon test indicated there were no difference in snake activity between species during dive recovery or resting experiments (Table 3.11; Fig. 3.14).

Voluntary Dive Behaviour

The top model for continuous dive length included the species interaction with temperature plus experiment type (open or closed) and experiment length (Table 3.9). Analysis of deviance (Type II Wald chi-square test) indicated that species, experiment type, experiment length and temperature were significant, but the interaction of species and temperature was not significant

(p= 0.06; Table 3.12). Mann-Whitney Wilcoxon pairwise comparisons indicated each species was significantly different overall (p < 0.001; Table 3.13; Fig. 3.15). Experiment length had an important effect on continuous dive length with both Massasauga and Eastern Gartersnakes increasing dive length the longer the experiment was conducted (Fig. 3.16). Red-bellied snakes observed over lengthened time periods, did not conduct long dives (Fig. 3.17).

During the partitioned respirometry experiments, some individuals conducted voluntary dives and others did not. Red-bellied snakes were least likely to conduct long voluntary dives within the respirometer, whereas Massasaugas and Eastern Gartersnakes were more likely to conduct longer continuous dives (Fig. 3.17). Maximum length of continuous dives for Massasaugas was 405 minutes (97% of the experiment length). Maximum continuous dive for Eastern Gartersnakes was 381 minutes (26% of the experiment length) and for Red-bellied snakes was 11 minutes (< 3% of experiment length).

Additional voluntary dive trials with short and long continuous observation periods using an open screen lid instead of closed system respirometry provided further insight. The maximum continuous dive length was 579 minutes in a 909-minute period (64% of time), conducted by a Massasauga. Clearly, Massasaugas and Eastern Gartersnakes conducted long continuous dives voluntarily, however they did not remain underwater for the entire duration of the experiment. There was also a temperature effect on continuous dive length, with longer dives conducted at colder temperatures (5°C vs 9°C). The longest dive observed for a Massasauga at 9°C was 292 minutes (average 80 in 1279 minutes average total experiment time; n = 14). Snake activity was affected by temperature with Massasauga activity being too difficult to quantify at 9°C. Red-bellied snakes were also very active and did not conduct any long dives (> 1 min) at 9°C. Eastern

Gartersnakes were only observed for short experiments < 4 hrs at 9°C. The longest continuous dive was recorded for Eastern Gartersnakes was 23.3 minutes in a 2-hr experiment at 9°C.

Overwintering Energy Costs

Daily energy costs for neonatal snakes were estimated based upon measured metabolic rates within simulated habitat conditions (Table A.1 and Table 3.14). Estimated energy use for a 200-day hibernation period by neonatal Eastern Gartersnakes range from 626–5,904 joules. Massasaugas would use 2,240–12,986 joules and N. Red-Bellied snakes 1140–10,812 joules. Furthermore, if I assumed that energy needs were fueled equally amongst carbohydrate, protein and lipids, an estimate of total body mass loss would be determined depending on the length of hibernation. Given that dry body mass was approximately 30% of wet mass, Eastern Gartersnakes neonates had an average 1.1± 0.05 g dry body mass (DBM); Massasaugas 2.78 ± 0.11 g and Red-bellied snakes 1.18 ± 0.08 g (Table 23). Therefore, over a 200-day hibernation period Eastern Gartersnakes would lose from a minimum of 27 to a maximum of 253 mg DBM depending on hibernation habitat condition (Table 3.15). Comparatively, Massasaugas would lose 96 to 556 mg, and Red-bellied snakes 40 to 463 mg DBM (Table 3.15). The depletion of DBM was greatest for the Red-bellied snake, exceeding 10% DBM for all habitat conditions except resting. Eastern Gartersnakes and Massasaugas depleted energy reserves at similar rates for all habitat conditions. Habitat conditions that supported resting conditions provided the least depletion of energy reserves (2-3% DBM) for all three species. Although cumulative effects were not studied, all species would expend over 15% of their DBM in dynamic habitat with flood events or when snake activity remained elevated. Red-bellied snakes would deplete the most from activity and flooding events (16 to 39% DBM).

Discussion

In this chapter, I measured activity, behaviour and respiration strategies of three neonatal snake species during four water level states that simulate a flood event during winter. To my knowledge comparative wintering behaviour and cutaneous respiration abilities have not been previously studied in neonatal snakes. My results confirmed that oxygen use at rest was positively correlated to the size of snakes which was also correlated to species differences with all large snakes being Massasaugas. However, species and body size effects were not significant effects during forced dive and dive recovery experiments. The partitioned respiration experiment confirmed that all three species of neonatal snakes used both pulmonary and cutaneous respiration to meet their total metabolic needs. Metabolic needs under partitioned (i.e., partially flooded) conditions were greater overall than without the presence of water (i.e., resting control). This confirmed one of my predictions that the presence of water would increase overall metabolism. Higher metabolism during the partitioned experiment was largely driven by an increase in diving activity. Although total metabolic rate increased during the partitioned experiment, the amount of oxygen derived from the water was very low for all species. Therefore, my results may support my second prediction that when air and water are both present voluntary diving is sustained by pulmonary respiration. However, in the absence of air, such as during a flood event, the amount of oxygen derived from water increased significantly compared to when snakes were voluntarily diving. The ability to upregulate the amount of oxygen derived from water during a flood event supports my main hypothesis, that cutaneous respiration is a flood survival strategy. Since there were no species differences in the upregulation ability, cutaneous respiration may be an important strategy for small and neonatal snakes that hibernate in wetland ecosystems such as Eastern Gartersnakes, Massasaugas and Red-bellied snakes.

Finally, when the short term forced dive was completed, all species increased their oxygen uptake from air beyond control levels. This means that an oxygen debt occurred during the forced dive and snakes were compensating for not meeting their oxygen needs by increasing oxygen uptake during the dive recovery period. Since there were no species differences in metabolic rate during the recovery period, all species were affected by this oxygen debt. Therefore, cutaneous respiration is likely a short-term flood survival strategy for small snakes at cold temperatures under normoxic water conditions.

Most snakes engaged in voluntary diving during the partitioned and extended diving observations and the amount of time spent diving was different with Red-bellied snakes spending the least amount of time diving. Eastern Gartersnakes on the other hand spent more time partaking in long continuous dives. Eastern Gartersnakes periodically came to the surface, presumably to breathe air before returning below surface. Massasaugas also voluntarily dove for extended periods. Although this may be an artifact of the experimental design, some Massasaugas used a unique position with head under water and cloaca distended into the air space (Fig. A.4 and A.5). Perhaps Massasaugas can supplement oxygen uptake cutaneously through the cloacal skin and extend the length of their dives as well as minimize water loss from pulmonary respiration in this manner. Given that the Massasugas viewed in this experiment were from a Parry Sound population that hibernates exclusively in bogs, this shallow water dive position with cloacal gas exchange in the air space may provide for a greater oxygen diffusion gradient through the cloacal skin than if the snake was entirely submerged in hypoxic or anoxic bog water. Whether neonatal Massasugas from other populations, such as my study site use this shallow dive position is unknown.

Laboratory studies offer an opportunity to learn more about life history events that are challenging or impossible to study in the field within a subterranean hibernaculum. The application of closed system and partitioned respirometry combined with high definition continuous camera footage is novel and insightful. During partitioned respiration experiments, snake behaviour is not controlled, total respiration occurs by both pulmonary and cutaneous pathways in a mixed media (air and water) and represents conditions of a partially flooded hibernaculum. Respirometry in air represents a hibernaculum without changing water level disturbances and forced dive respirometry represents cutaneous respiration in normoxic water in a periodically flooded or dynamic hibernaculum. Finally, post dive recovery respiration and behaviour within a respirometer after a flood event, provides a follow up determination of how well a snake coped with a sudden hypoxic flood event.

The forced dive experiment confirmed that all three neonatal snake species can significantly upregulate their cutaneous respiration abilities during a short-term flood event. The increase in cutaneous respiration rate also coincided with an increase in snake activity which may be a stress response induced by the experimental conditions, or may also trigger an intrinsic physiologic response to flooding. Increased snake activity during a force dive may help prevent the formation of a hypoxic boundary layer along the skin surface which can limit cutaneous gas exchange (Pinder and Burggren, 1986; Feder and Booth, 1992). Longer submergence experiments may result in a lower rate of cutaneous oxygen uptake, however longer submergence experiments may also induce severe hypoxia or anoxia when conducted in a closed respirometer (Pinder and Burggren, 1986; Feder and Booth, 1992). The ability of a snake to upregulate oxygen during forced submersion was clearly demonstrated without asphyxiation (i.e., suffocation) or drowning of any snakes, which was an important consideration in the experimental design. Although there

were some species differences in activity during this experiment, the ability to increase cutaneous respiration was ubiquitous at cold ambient temperatures, in normoxic water and is an important flood survival strategy. Snake activity during the recovery period was not different from resting conditions, therefore the increase in oxygen uptake after the dive was not derived by an increase in snake activity. This means all three species incurred an oxygen debt and that the upregulation in cutaneous respiration is insufficient to meet their entire metabolic oxygen needs when submerged in normoxic water. Although I did not study hypoxia, all snakes in this study exhibited oxygen regulation in normoxic conditions and not oxygen conformity such as the overwintering frog (Tattersall and Boutilier, 1997, 1999; Tattersall and Ultsch, 2008; Jackson and Ultsch, 2010). The ability to increase cutaneous oxygen uptake when ambient oxygen levels are naturally low (i.e., hypoxic or anoxic) as in bog environments is unknown and requires further study. Although my objectives were to test species differences, we should avoid over-interpreting differences between species because of small sample sizes. The overall relationships of small neonatal snakes and how they responded to the various tests is the important outcome of this study.

The ability to upregulate cutaneous respiration at cold temperatures and normoxic conditions in snakes, is partly due to snake's ability to shunt blood flow within the heart to the systemic circulation via a right to left shunt (Hicks, 2002). By enabling this shunt, blood oxygen levels can remain lower than ambient to facilitate diffusion through the skin (Burggren and Moalli, 1984; Burggren 1987). Skin thickness plays a direct role in the ability to diffuse oxygen cutaneously (Fick's Law; Feder and Burggren, 1985). Neonatal skin before their first shed is likely the thinnest during their life span, presumably facilitating diffusion of respiratory gases and water within the uterine environment (Lillywhite, 2006). However, newborn snakes shed

within a few days of their birth and the function of the epidermis with respect to evaporative water loss, changes with ecdysis. The first ecdysis is likely important to decrease evaporative water loss while transitioning from a moist *in utero* existence to a dry environment (Tu et al., 2002). In Timber rattlesnakes, the first neonatal shed contains higher lipid content than in adult sheds (Agugliaro and Reinert, 2005). Therefore, neonatal snakes likely retain a high lipid content in their skin to aid in water balance (Agugliaro and Reinert, 2005). Considering the importance of the skin for water balance, and from this study where I confirmed that the skin of neonatal snakes also functions in respiration during hibernation, the epidermal layer must also be thin to facilitate gas diffusion (Fick's Law; Feder and Burggren, 1985). The thinner spaces between the scutes of snakeskin may facilitate this gas exchange function. A snake's body position in the hibernaculum may expose the thinner skin sections to aid respiratory diffusion and minimize water loss. For example, a tightly coiled position would expose only the outer parts of the snake to a higher diffusion rate, with less diffusion and water loss within the central coil. A relaxed stretched out position may have a higher cutaneous respiration rate. Moving within the hibernaculum would intermittently expose these thinner skin sections or disrupt hypoxic boundary layers as seen in submerged amphibians (Feder and Booth, 1992). Water loss may be greater with pulmonary respiration than cutaneous respiration. During partitioned respiration (i.e. like a partially flooded burrow), snakes kept their head under water and exposed only their tail and occasionally their cloaca to the air spaces. Additional research involving snake position, water level, cutaneous respiration and evaporative water loss is a next step in assessing skin functions during hibernation.

Although enhanced cutaneous respiration was measured during a short, forced dive for all species, the Red-bellied snakes did not demonstrate an affinity to voluntary diving and they were

the most active during the partitioned, and forced dive experiments. Therefore, they are likely more successful hibernating above the groundwater table in hibernacula that are not dynamic in terms of water levels and do not exhibit prolonged flooding. This species also exhibited a different response to forced dive with larger snakes by skin surface area having a lower metabolism. This may be related to low sample size, sample error, natural history differences or this may be related to the older age of the Red-bellied snakes. Older snakes may not have the same skin diffusivity for oxygen than younger snakes, putting them at a disadvantage during a flood event. Although this needs to be explored further by measuring older snakes from each species group, if older snakes cannot increase oxygen uptake during a flood event, this could explain the severity of the mortality event in my study area following the first flood event (Chapter 2). Eastern Gartersnake neonates, on the other hand, seem to be the best suited to the long-term presence of water in the hibernacula which confirms earlier research completed by Costanzo (1986, 1989a, 1989b). By exhibiting enhanced cutaneous respiration and long dive abilities, Massasauga neonates also seem well adapted to a range of water levels in the hibernacula. However, this study does not support a completely aquatic hibernation strategy for any snake species. Access to aerial oxygen is necessary for successful snake hibernation.

For this series of experiments, Massasaugas were collected from a natural bog ecosystem in Parry Sound ON and Eastern Gartersnakes and Red-bellied snakes were collected from a more southern and anthropogenically altered bog ecosystem near Port Colborne, ON. There may be genetic differences or adaptations that distinguish the two Massasauga populations, especially since populations are allopatric and contemporary geneflow is absent (Chiucchi and Gibbs, 2010). Additional research is needed to confirm Massasugas from the anthropogenically altered

ecosystem behave and respire similarly to snakes from a natural bog ecosystem with respect to cutaneous respiration and variable water levels.

Both a snake's behaviour and metabolism during hibernation and the quality of their wintering site contributes to their overall survival. Moving forward from this experiment, I suspect neonatal survival would be greatest in wintering sites where water levels do not change or change very little so that the snake does not increase activity and arousal frequency that increases metabolism and expends energy reserves. Frequent arousals may be a contributing factor for winter mortality in small and neonatal snakes. Additional research in the areas of winter survival within habitats with different water level dynamics is warranted.

Hibernation Energy Model for Neonatal Snakes

Resting metabolism represents minimum activity and reflects energetic costs for a neonatal snake in a stable hibernaculum without water. Active metabolism represents the energetic costs of snake movement activity, in a stable hibernaculum without water. Partitioned measurements represent energetic costs in a hibernaculum that is stable at a constant water level with an air space. Given that an oxygen debt was incurred following a short-forced dive, the energetic cost of flood and recovery are additive. More importantly in a dynamic flooding environment, the energetic costs of a full flood cycle (zero water to rising water to flood maxima to declining water back to zero water) would equal the sum of resting + partitioned × 2 + forced submersion + post dive recovery (Fig. 3.18). However, I did not measure metabolism in a partitioned experiment following a forced dive. Meaning Equation (4) may be an underestimate of the energetic costs of a flood event. Furthermore, the maximum activity range is likely not sustainable over a long winter, increases in snake activity likely occur during disturbances or dynamic changes to habitat such as a fluctuating water table caused by flooding or fluctuating

frost layer caused by freeze-thaw events. By applying estimates of energy use and the number of days a hibernaculum is in an environmental state (i.e., changing water levels, flooded, stable, dry), provides an opportunity to model the energetic costs of hibernation at 5°C in a flood prone hibernaculum. Temperature effects can also be incorporated into the energetic-habitat model. If temperature changes within the hibernacula during winter, the rate of energy loss can be estimated using the Q_{10} effect. I can infer from this study that energetic costs could at least double at 15°C. However, I was not able to measure metabolism during a continuous complete flood cycle. A complete flood event cycle would begin with no water, rising to a flood maximum and then receding. Therefore, my energetics model requires additional research to make it fully functional and to evaluate interactions of a dynamic water table and cumulative effects of flooding. However, in the meantime, I have compiled a conceptual model and an equation that can be used as a first approximation of the energy costs of a flood event on neonatal snakes (Fig. 34 and Equation 4). Future use involving field experiments with known snakes, known habitat and environmental factors and survival would provide a more informed assessment of energy costs of hibernation.

Conclusions

1. All snakes increase cutaneous respiration during short forced dives in normoxic water, but they all incurred an oxygen debt. This means cutaneous respiration is insufficient to meet the metabolic needs of overwintering snakes during a flood event.

2. There was no reduction in metabolism or energy savings during a forced dive which is contrary to Costanzo (1989a), and the energy costs of a full dive, dive recovery and changing water levels are additive. This may explain poor snake survival in stochastic environments.

3. Eastern Gartersnakes and Massasaugas perform long aerobic dives through breath holding (apnoea) and Red-bellied snakes do not perform long dives.

4. I found no evidence to support a complete aquatic hibernation strategy for small, neonatal snakes. If snakes hibernated in the central mined peatland in October 2006, they likely did not survive the flood cycle.

5. Therefore, the Life Zone of a snake hibernacula must include a non-freezing, non-flooding, aerobic air space to support snake survival.

Tables

Table 3.1: Sample sizes by experimental treatment (†repeated use up to 3 times per individual, #2013 only). Final model analyses compared oxygen depletion in water (partitioned vs forced dive) or air (resting vs dive recovery). Only the same individuals included in both experiments were used as follows; Eastern Gartersnakes (n = 6), Massasauga (n=12) and Red-bellied snake (n= 5).

	Eastern Gartersnake	Massasauga	Red-bellied snake
Preliminary Respirometry † (1–24 hr)	25 (†76)	14 (†38)	11 (†26)
Preliminary Partitioned Respirometry (3–24 hr)	#15	14	5
Preliminary Forced Dive and Dive Recovery (1–7.5 hr)	#13	0	0
Resting (Control) (2013 and 2014)	22	14	12
Partitioned Respirometry and Voluntary Dive (2013 and 2014)	10	12	10
Forced Dive (2014 only)	6	12	5
Dive Recovery (2014 only)	6	14	5
Voluntary Dive (9 °C)	#18	14	5
Voluntary Dive (5 °C)	0	14 (†9)	5

Table 3.2. Summary of Linear models including random and fixed effects for each experiment comparison.

Dependent Variable	Linear Model		Partitioned Respirometry	Experiment Comparisons		
				Forced dive vs Voluntary Dive	Post Dive vs Resting	Voluntary Dive Behaviour
Proportion cutaneous respiration = $\dot{M}_{O_2}water/\ \dot{M}_{O_2}\ total$	Bounded (0-1) variable	Beta regression	Species, Activity, Proportion voluntary dive time, Skin surface area (SA), SA:VOL			
Snake activity = proportion time active/ total time	Bounded (0-1) variable	Beta regression		Species, Experiment treatment	Species, Experiment treatment	
Metabolic rate ($\dot{M}_{O_2}$) (µmol O2/h)	Continuous variable	Linear mixed effect		Species, Activity, Dive length, Skin surface area (SA), Experiment treatment, Experiment length Species ID (random effect)	Species, Activity, SA:VOL Experiment type, Experiment treatment Species ID (random effect)	
Continuous voluntary dive time (minutes)	Continuous variable	Linear mixed effect				Species, Temperature, Experiment treatment, Experiment Length. Species ID (random effect)

Table 3.3. Summary of body size measurements by species used in lab experiments including total length (TL), snout to vent length (SVL) (mm), pre-hibernation weight (g), surface area and surface area to volume (SA:VOL). All values are mean (± 1 SE) and range (min to max).

Species	n	Age Estimate	TL (mm)	SVL (mm)	Pre-Hibernation Mass (g)	Surface area (mm^2)	SA:VOL
Eastern Gartersnake	25	Neonate	231 ± 3	182 ± 3	3.69 ± 0.13	3125 ± 63	0.87 ± 0.12
			198 –255	155 –210	2.41–5.09	2544 – 3824	0.75–0.97
Massasauga	14	Neonate	213 ± 3	190 ± 3	9.13 ± 0.35	5216 ± 195	0.52 ± 0.01
			194 –242	171–207	7.66 – 12.09	3661– 6512	0.45–0.65
Red-bellied snakes	12	Juvenile	253 ± 7	204 ± 6	4.07 ± 0.32	3895 ± 192	0.77 ± 0.02
			213 –299	166–237	2.07–5.49	2834 – 4753	0.66–0.90
Overall	51		231 ± 3	189 ± 2.4	5.27 ± 0.36	3880 ± 145	0.75 ± 0.02

Table 3.4 Akaike Model Selection Analysis showing the top five and null models for the dependant variables (Y). A) is a beta regression model for the proportion of oxygen uptake from water during partitioned respirometry B) Linear mixed effect model (LME), comparing $\log_e$ $\dot{M}_{O_2}$ $\mu mol\ O_2 \cdot h^{-1}$ uptake from water during partitioned and forced dive experiments C) LME $\log_e$ $\dot{M}_{O_2}$ $\mu mol\ O_2 \cdot h^{-1}$ for resting metabolism and dive recovery experiments conducted in air media. Fixed effects tested include species, experiment type, experiment length, snake activity, dive time and body size as either skin surface area (SA) or surface area: volume ratio (SA:VOL). Species ID is the random effect.

	Y	Model	AICc	dAICc	df	weight
A	Partitioned Pr $\dot{M}_{O_2}(water)/$ $total$	Species × Proportion time voluntary diving + $\log_e$ (SA) + Experiment length	-150.7	0	9	0.842
		Species × Proportion time active	-145.6	5.1	5	0.065
		Species + Proportion time voluntary diving + Proportion time active	-145.2	5.5	6	0.054
		Species × Proportion time active	-143.2	7.5	7	0.02
		Species × SA:VOL	-141.7	9	7	0.009
		Null	-114.9	35.8	2	<0.001
B	Cutaneous Respiration $\dot{M}_{O_2}$	Species × $\log_e$ (SA) + Proportion time diving	131.5	0	9	0.74
		Experiment length	134.5	3	4	0.17
		Species + Proportion time diving	137	5.5	6	0.05
		Species + Proportion time diving + Proportion time active	139	7.5	7	0.02
		Species × Proportion time diving	139.4	7.9	8	0.01
		Null	172.8	41.3	3	<0.001
C	Dive Recovery $\dot{M}_{O_2}$	Experiment type × Species + Proportion time active +body size (SA:VOL)	124.8	0	10	1
		Experiment type + Proportion time active	142.3	17.5	5	<0.001
		Species × Proportion time active	142.5	17.7	8	<0.001
		Experiment type	142.7	17.9	4	<0.001
		Experiment length	156.2	31.4	4	<0.001
		Null	164.5	39.7	3	<0.001

Table 3.5. Analysis of Deviance Table (Type II Wald chi square tests) for the top beta regression model relating the proportion of $\dot{M}_{O_2}$ $\mu mol\ O_2 \cdot h^{-1}$ uptake from water during the partitioned experiment.

Main Fixed Effects	Chisq	*df*	Pr(>Chisq)
Species	10.65	2	<0.01*
Proportion time voluntary diving	0.20	1	0.65
$\log_e$ (SA)	0.71	1	0.40
Species × Proportion time voluntary diving	14.88	2	<0.001*

Table 3.6. Mann-Whitney Wilcoxon post-hoc species pair wise comparison tests of $\dot{M}_{O_2}$ $\mu mol\ O_2 \cdot h^{-1}$ for each main metabolism experiment. Bonferroni correction applied such that a p-value < 0.017* indicates significant differences between the two species groups.

Species		Resting $\log_e \dot{M}_{O_2}$		Partitioned $\dot{M}_{O_2}$ (water) / $\dot{M}_{O_2}$ (air + water)		Forced Dive $\log_e \dot{M}_{O_2}$		Dive Recovery $\log_e \dot{M}_{O_2}$	
		W	p-value	W	p-value	W	p-value	W	p-value
Eastern Gartersnake	Massasauga	3	<0.01*	374	<0.01*	28	0.07	50	0.73
Eastern Gartersnake	Red-bellied snake	12	0.66	374	<0.001*	11	0.15	5	0.17
Massasauga	Red-bellied snake	70	<0.01*	388	<0.001*	35	0.65	17	0.22

Table 3.7. Analysis of Deviance (Type II Wald chi square tests) for the top linear mixed effect model relating the cutaneous oxygen uptake $\dot{M}_{O_2}$ $\mu mol\ O_2\ \cdot h^{-1}$ from water during the partitioned and forced dive experiments. * denotes significance

Main Fixed effects	Chisq	*df*	Pr(>Chisq)
Proportion time diving	100.35	1	<0.001 *
Species	0.03	2	0.99
Body size as log$_e$ (SA)	0.13	1	0.71
Species × log$_e$ (SA)	4.04	2	0.13

Table 3.8: Analysis of Deviance Table (Type II Wald chi square tests) for the top linear mixed effect model relating log$_e$ $\dot{M}_{O_2}$ $\mu mol\ O_2\ \cdot h^{-1}$ post dive recovery and resting (control) metabolism. Snake ID was set as the random effect. (* denotes significance)

Main Fixed effects	Chisq	*df*	Pr(>Chisq)
Experiment type	41.16	1	<0.001*
Species	1.77	2	0.41
Proportion time active	0.01	1	0.92
Body Size as SA:VOL	0.14	1	0.70
Species × Proportion time active	24.15	2	<0.001*

Table 3.9: Akaike Model Selection Analysis showing the top five and null models for the dependant variables snake activity (D) and continuous dive length (E). Snake activity is a beta regression model for the proportion of time snakes were active during respirometry experiments E) Linear mixed effect model (LME) for continuous dive time during partitioned and voluntary dive experiments. Fixed effects tested include species, experiment type, experiment length, temperature. Species ID is set as a random effect in the mixed model.

		Model	AICc	dAICc	df	weight
D	Snake activity/ Experiment time	Experiment type × Species	-295.8	0	13	0.962
		Species + Experiment type	-288.7	7.1	7	0.028
		Experiment type	-286.7	9.1	5	0.01
		Null	-259.7	36.1	2	<0.001
		Species	-259.3	36.4	4	<0.001
		Experiment Time	-258.6	37.2	3	<0.001
E	Continuous dive length	Species × Temperature + Experiment type + Experiment length	1324	0	10	0.996
		Species × Temperature + Experiment type	1336.2	12.3	9	0.002
		Species × Experiment type + Temperature	1336.5	12.5	9	0.002
		Species + Experiment type + Temperature + Experiment length	1344.6	20.6	8	<0.001
		Species + Temperature + Experiment type	1353.3	29.3	7	<0.001
		Null	1408.6	84.6	3	<0.001

Table 3.10: Analysis of Deviance Table (Type II Wald chi square tests) for the top beta regression model for the analysis of snake activity (proportion of time snake was active) during metabolism experiments (resting, partitioned, forced dive and dive recovery).

Main fixed effects	Chisq	*df*	Pr(>Chisq)
Experiment	37.90	3	<0.001*
Species	8.11	2	0.017*
Experiment × Species	20.82	6	0.002*

Table 3.11: Mann-Whitney Wilcoxon post-hoc tests species pair wise comparisons of snake activity (proportion of time active) during each metabolism experiment. Bonferroni correction applied such that p- value < 0.017* indicates significant differences between the two species groups.

Species		Resting		Partitioned		Forced Dive		Dive Recovery	
		W	P-value	W	P-value	W	P-value	W	P-value
Eastern Gartersnake	Massasauga	187	0.15	186.5	0.15	144.5	0.89	66	0.05
Eastern Gartersnake	Red-bellied snake	68	0.17	68	0.17	5	0.002*	15	1
Massasauga	Red-bellied snake	131	0.84	131	0.84	0	0.001*	18	0.13

Table 3.12: Analysis of Deviance Table (Type II Wald chi square tests) for the linear mixed effect top model for continuous dive length set as a function of species, temperature (5°C and 9°C), experiment length, experiment type (closed vs screen lid), and the interaction of species × temperature. Species_ID is set as the random effect. * denotes significance

Main Fixed Effects	Chi Sq	df	P value	
Species	17.77	2	<0.001	*
Experiment type	4.00	1	0.045	*
Experiment length	12.53	1	<0.001	*
Temperature	16.66	1	<0.001	*
Species × Temperature	5.53	2	0.06	

Table 3.13: Mann-Whitney Wilcoxon post-hoc species pairwise comparison tests of continuous dive length during voluntary dive behaviour experiments. Bonferroni correction applied such that, p- value < 0.017* indicates significant differences between the two species groups.

Species		All temperatures (5°C and 9°C)		5°C only	
		W	P-value	W	P-value
Eastern Gartersnake	Massasauga	497	<0.001 *	311.5	<0.01 *
Eastern Gartersnake	Red-bellied snake	633	<0.001 *	282.5	<0.001 *
Massasauga	Red-bellied snake	1060	<0.001 *	693	<0.001 *

Table 3.14: Estimated daily energy use (joules / day) mean ± SE by neonatal snakes for each

hibernacula habitat type at 5°C calculated from each metabolism experiment.

Species	n	Resting	Active	n	Voluntary Diving	n	Forced Dive + Dive Recovery
Eastern Gartersnakes	25	3.13 ± 0.53	29.52 ± 3.98	20	10.00 ± 1.64	20	19.81 ± 3.29
Massasaugas	14	11.20 ± 0.90	64.93 ± 10.61	14	30.31 ± 6.87	14	56.59 ± 9.11
Red-bellied snakes	12	4.62 ± 0.65	21.85 ± 5.29	10	22.79 ± 3.53	5	54.06 ± 8.82
Overall	51	5.70 ± 0.62	37.44 ± 4.37	44	19.37 ± 2.75	39	37.41 ± 4.76

Table 3.15: Estimated daily rate of depletion (mg/day) mean ± SE assuming equal use of three of

energy substrates (carbohydrate, protein and lipid) by neonatal snakes for each hibernacula

habitat type at 5°C.

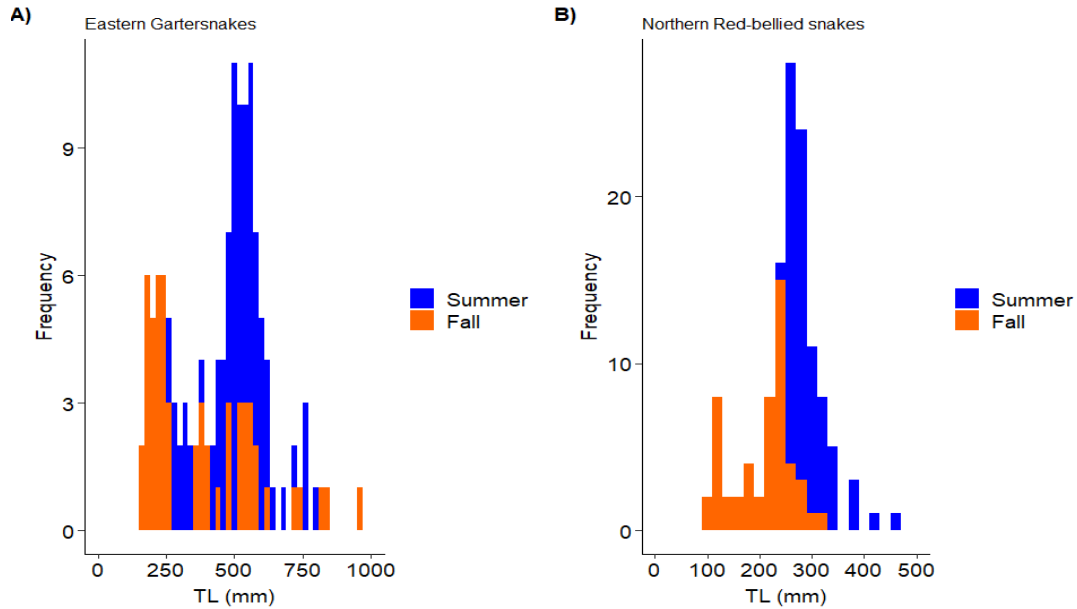| Species	n	Resting	Active	n	Part water filled	n	Flood + Dive Recovery
Eastern Gartersnakes	25	0.13 ± 0.02	1.27 ± 0.17	20	0.43 ± 0.07	20	0.85 ± 0.14
Massasaugas	14	0.48 ± 0.04	2.78 ± 0.45	14	1.3 ± 0.29	14	2.42 ± 0.39
Red-bellied snakes	12	0.20 ± 0.03	0.94 ± 0.23	10	0.98 ± 0.15	5	2.32 ± 0.38
Overall	51	0.24 ± 0.03	1.6 ± 0.19	44	0.83 ± 0.12	39	1.6 ± 0.2

Figures

Fig. 3.4. Size Frequency histograms of (A) Eastern Gartersnakes and (B) Red-bellied snake total length (TL) measured in the study area during 2014 active season to establish neonatal size classes by fall
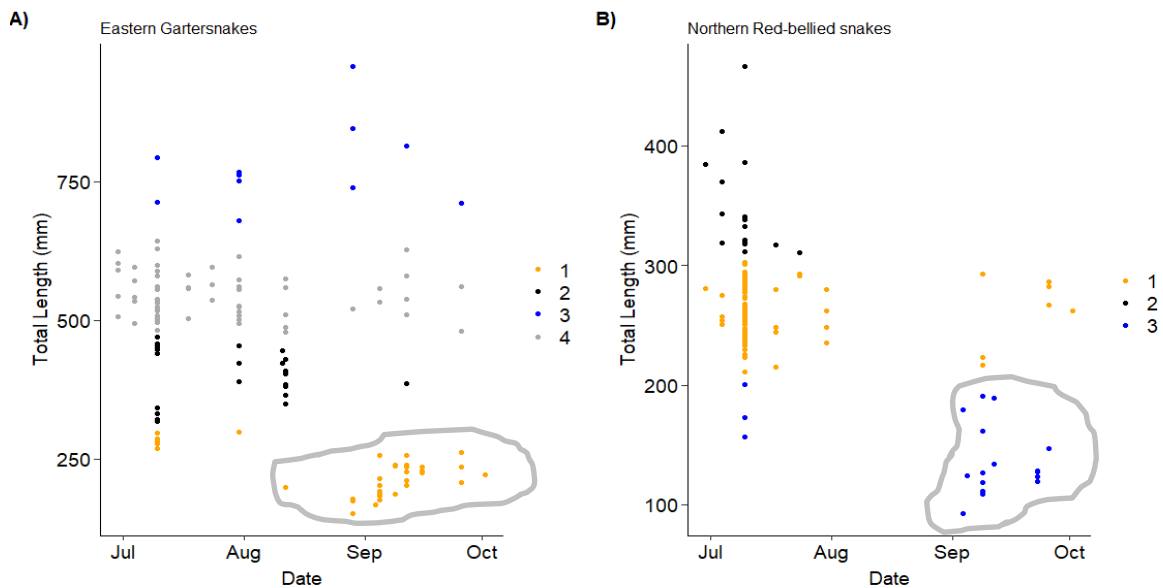

Fig. 3.5. Cluster analysis on total length by species and date. In 2014, neonatal snakes were first observed in middle to late August (circled). Neonatal Eastern Gartersnakes were ≤ 260mm, and Red-bellied snakes were ≤ 200 mm by the fall season.

110
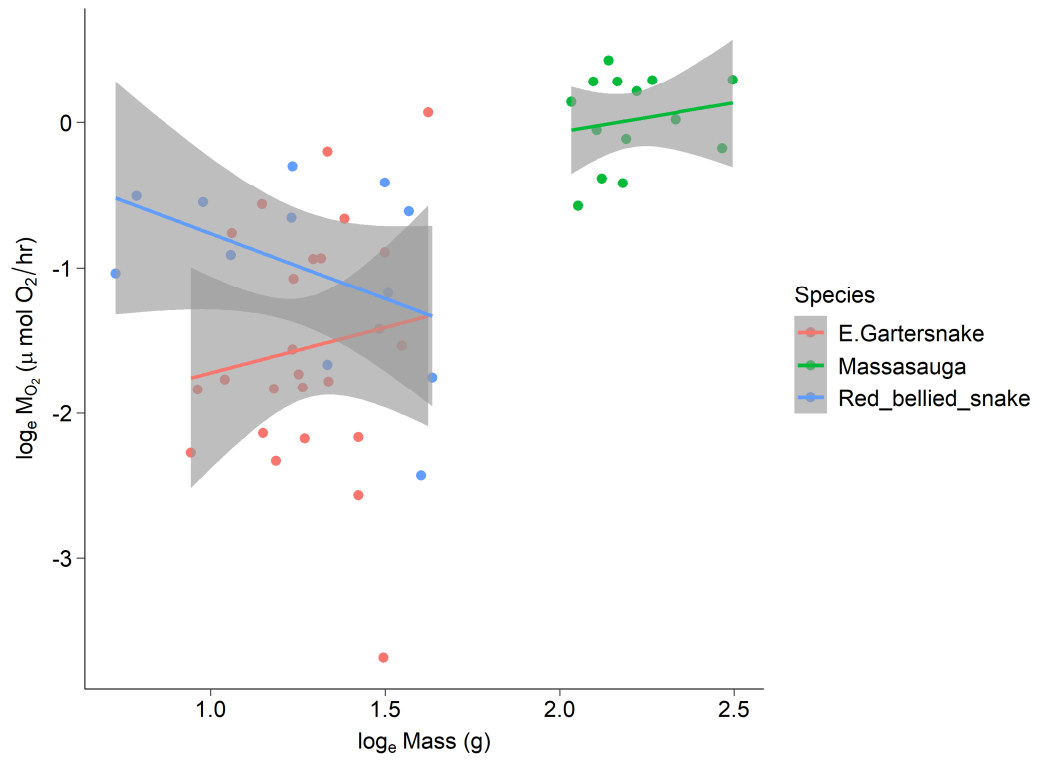
Fig. 3.6 Body size effects using mass (g) on resting metabolism $\dot{M}_{O_2}$ (μmol· h^{-1}) grouped by species, n=51, r^2 = 0.64.
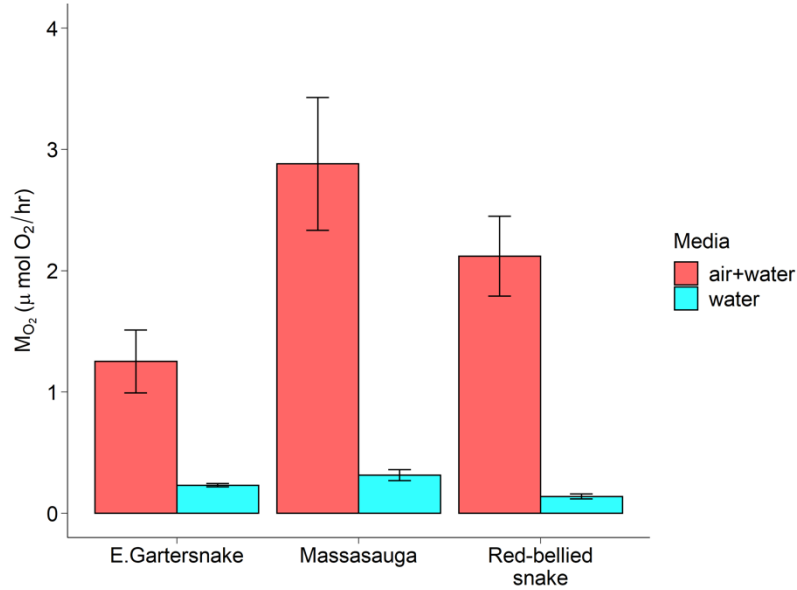
Fig. 3.7 Partitioned respirometry experiment showing oxygen uptake $\dot{M}_{O_2}\ \mu mol\ O_2 \cdot h^{-1}$ data (mean ± SE) from water and total (air + water) for each species.
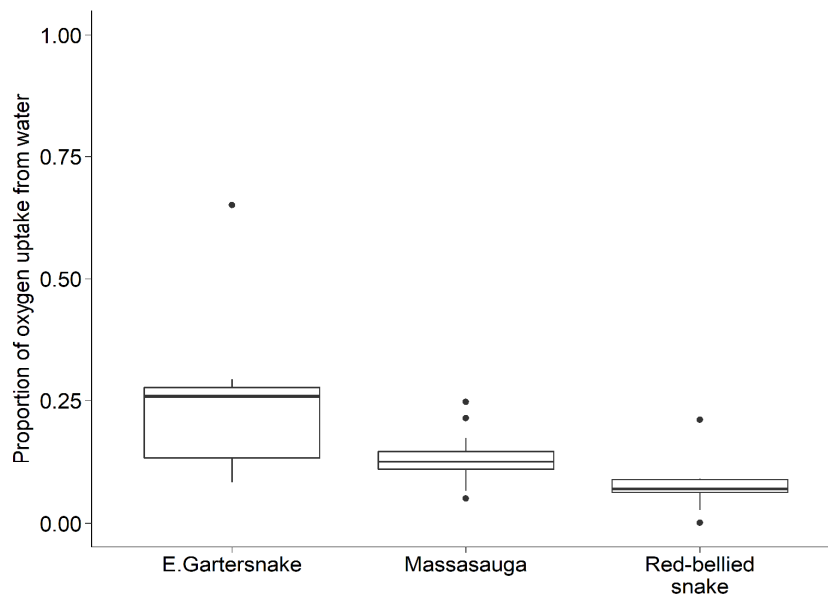
Fig. 3.8 Proportion of oxygen uptake from water by each species during the partitioned respirometry experiment
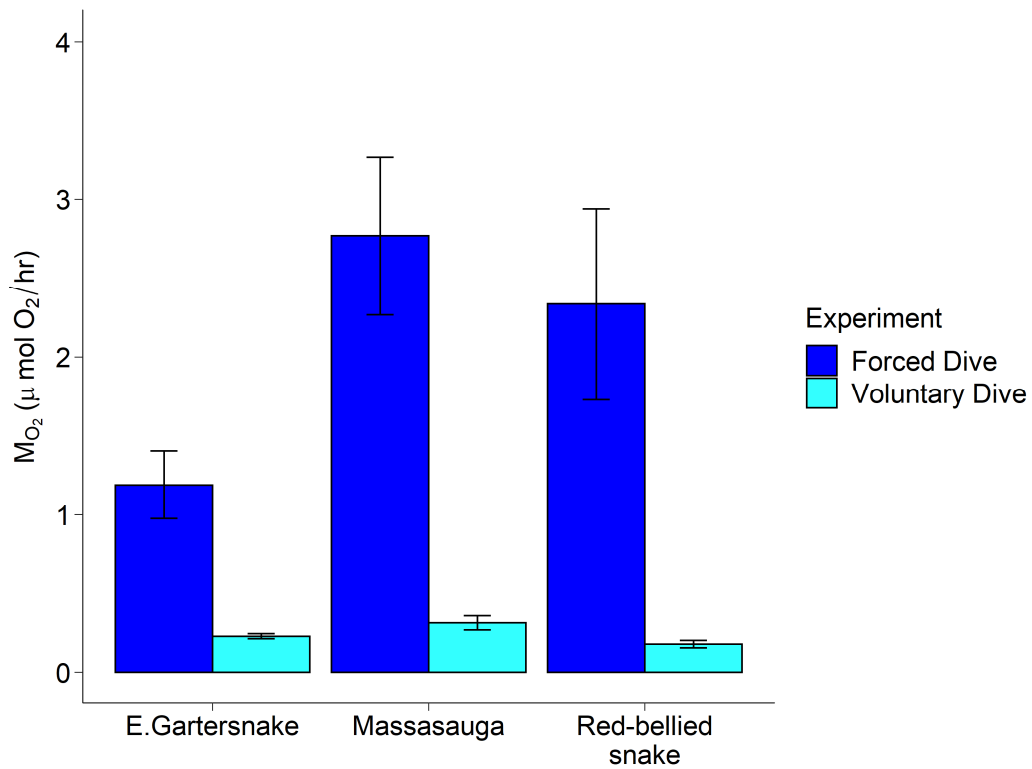
Fig. 3.9 Cutaneous respiration rate $\dot{M}_{O_2}$ $\mu mol\ O_2 \cdot h^{-1}$ (mean ± SE) for three snake species during a short forced dive (100% water), compared to cutaneous respiration during partitioned respirometry (50% water, 50% air). Both experiments were conducted in 5℃ normoxic water. Forced dives were short duration (1 to 2 hrs, average 1.5 hr). No snakes drowned during any experiment.
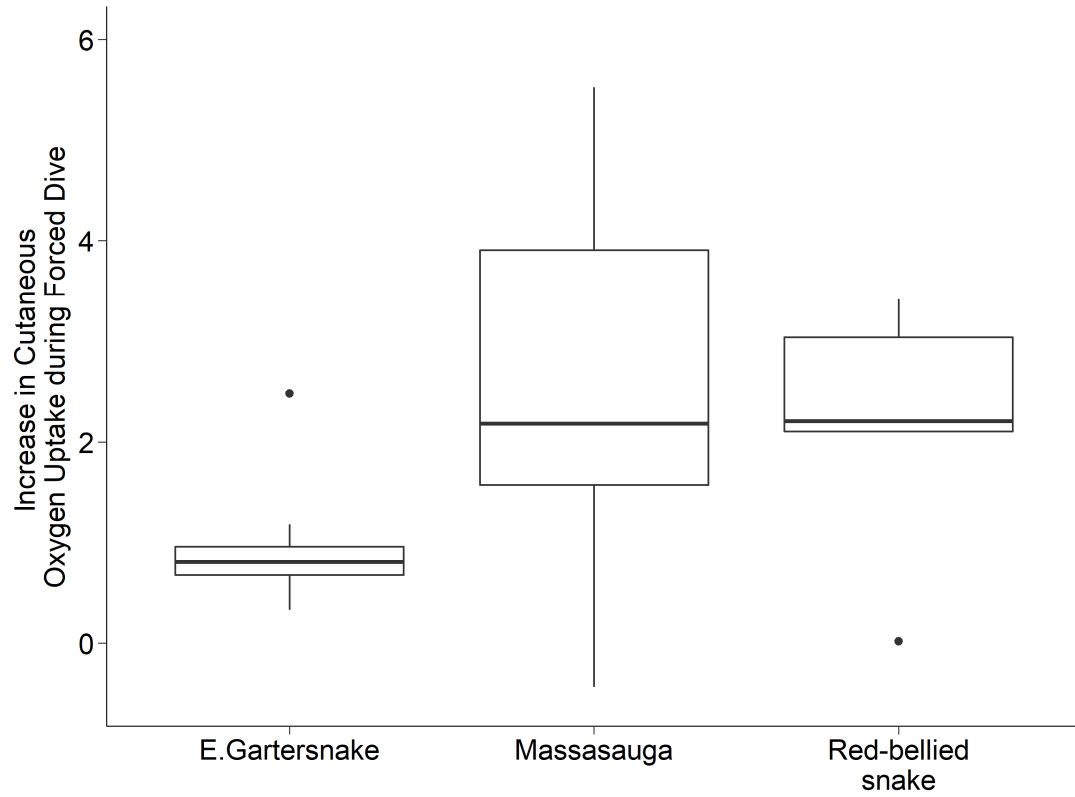
Fig. 3.10 Increase (forced dive oxygen uptake from water minus oxygen uptake from water during the partitioned experiment (i.e., voluntary diving)) in in cutaneous oxygen uptake $\dot{M}_{O_2}\ \mu mol\ O_2\ \cdot h^{-1}$ during a short forced dive (average 1.54 hr) (forced dive – voluntary dive).
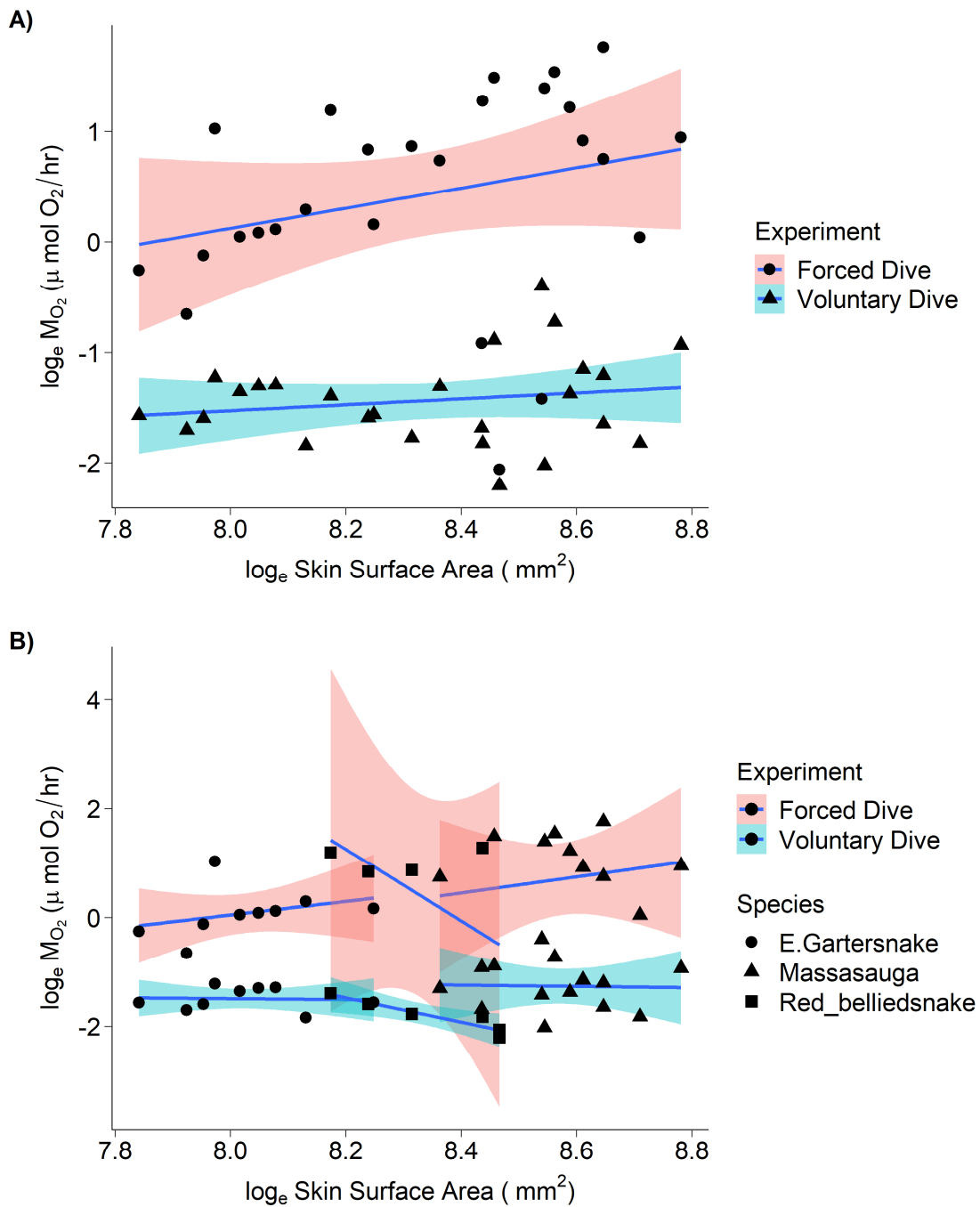
Fig. 3.11 Cutaneous respiration linear models A) during a full and voluntary dive as a function of skin surface area. B) represents the same linear models grouped by species. Shaded areas are standard error.
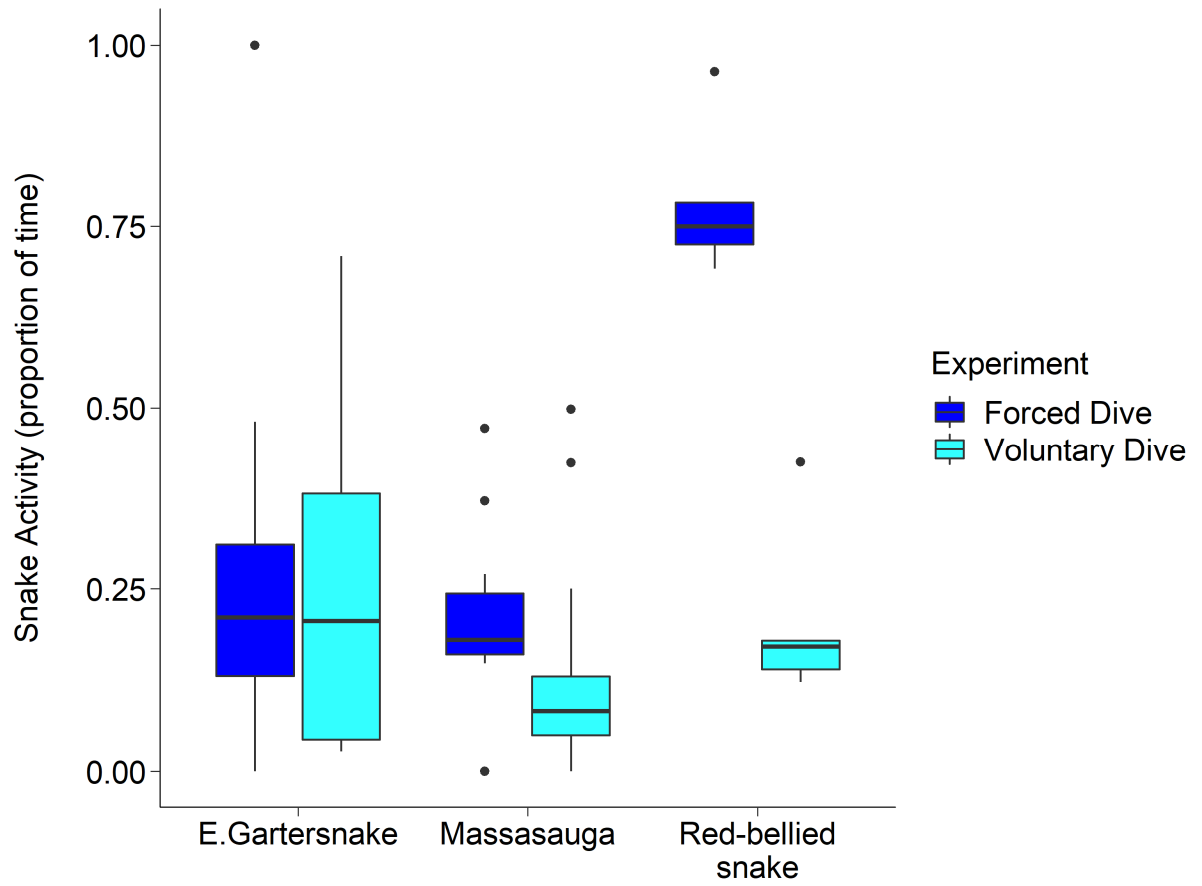
Fig. 3.12 Snake Activity by species during forced and voluntary dive experiments

Fig. 3.13 Post forced dive recovery and resting metabolic rate $\dot{M}_{O_2}\ \mu mol\ O_2 \cdot h^{-1}$
Mean ± SE for each species during simulated winter conditions (5°C).
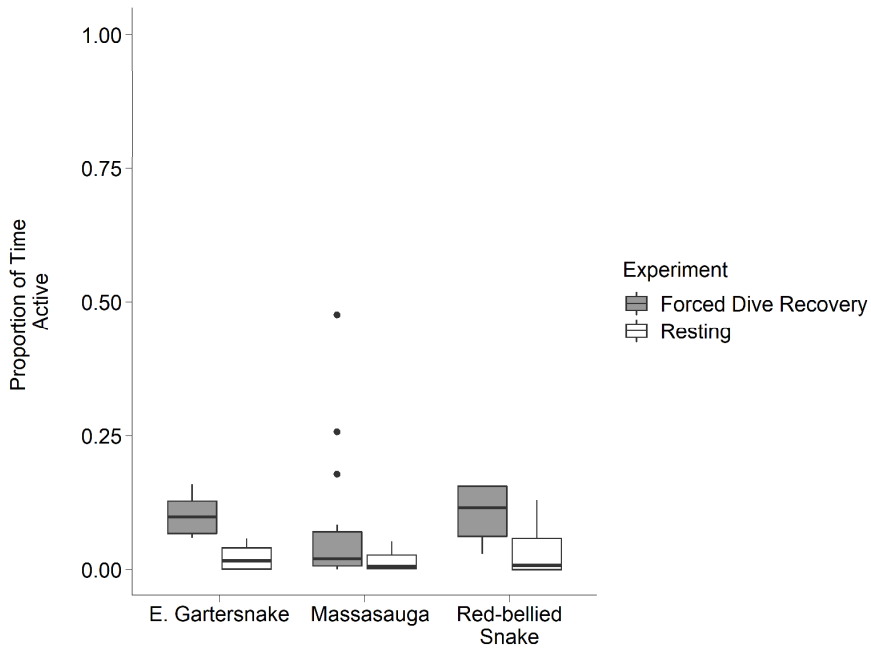
Fig. 3.14 Snake activity during post dive recovery and resting experiments by species during simulated winter conditions (5°C).
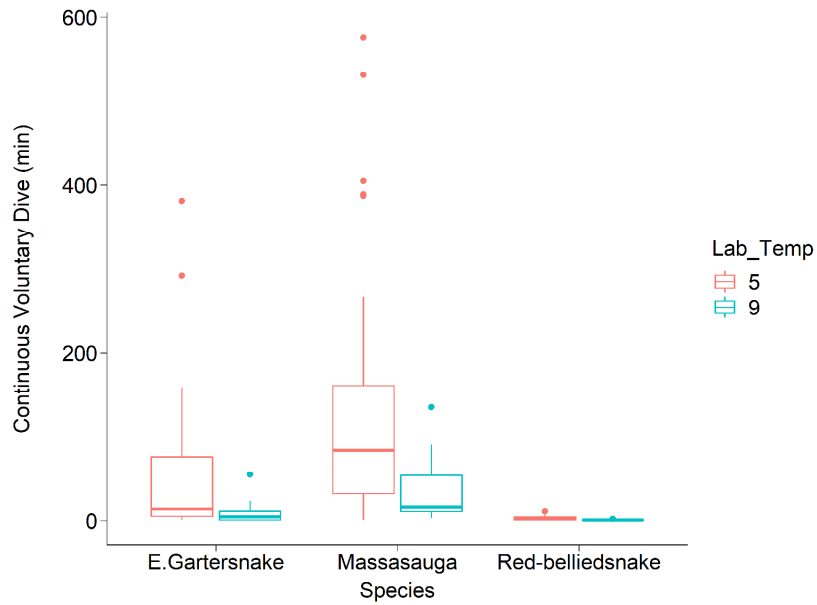
Fig. 3.15 Temperature effects (5 and 9 ℃) on continuous dive length for each species.
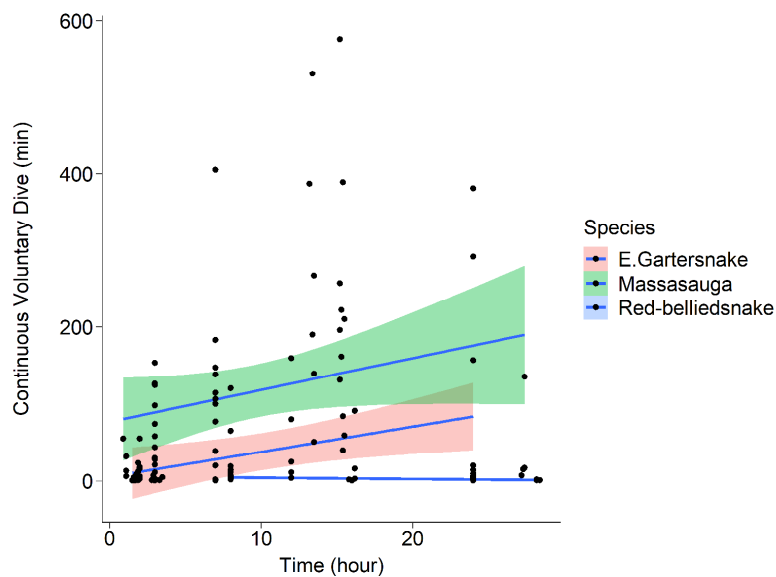
Fig. 3.16 Experiment time effects on continuous voluntary dive length for each species. Data from all voluntary dive experiments for each species. Repeated measures are included with an estimated line of best fit. Shaded area is the standard error estimate. Red-bellied snakes did not show a difference or a trend in continuous dive length over time.
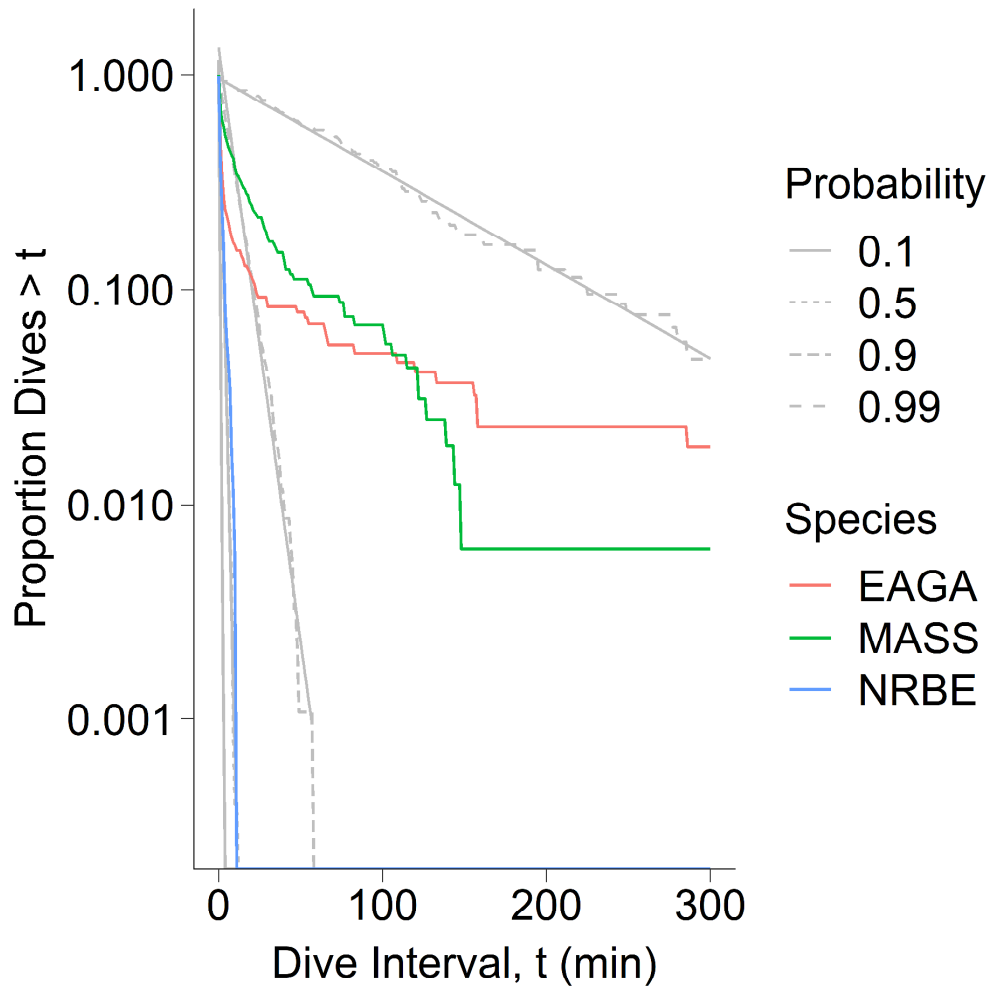
Fig. 3.17 Proportion of dives greater than the dive interval for each species are shown with theoretical probability curves generated from randomized data where dive status was modelled as a binomial response variable every 30 second observation period. Graph abbreviations used were Eastern Gartersnake (EAGA), Massasauga (MASS) and Red-bellied Snakes (NRBE).
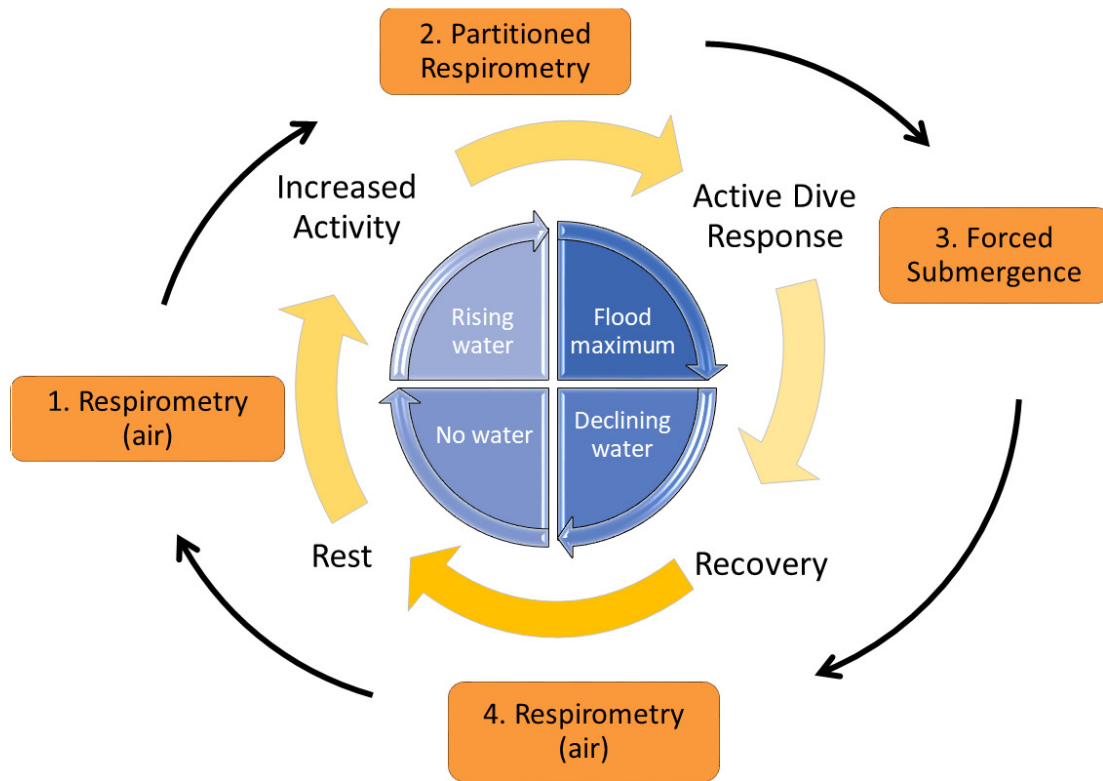
Fig. 3.18 A conceptual model relating the four experiment treatments or states (orange), and snake behaviour responses (yellow) within a complete flood event cycle (blue). Energy used during dry conditions (C_1) is represented by metabolism experiments conducted in air only media, during including rising water and declining water were represented by partitioned respirometry experiments (C_2), flood maximum energy use (C_3) was represented by a forced dive experiment and post flood was represented by dive recovery experiments (C_4). The equation for a hibernation period relates the number of days (n_x) and the energy use during each flood cycle event as; $Energy\ Costs\ (E) = n_1 C_1 + 2 \times (n_2 C_2) + n_3 C_3 + n_4 C_4$

Chapter 4: General Discussion and Conclusions

Environmental stochasticity is an unpredictable change in the environmental conditions brought about by factors such as habitat loss and weather events (Fujiwara and Takada, 2017). Large scale losses of animals can be attributed to stochastic weather events especially during vulnerable life stages or during certain parts of their annual life cycle such as hibernation and may contribute to population declines and extinction (Shine and Mason, 2004; Harvey et al., 2014). Population losses at a global, national and local level are a conservation concern (Parks Canada, 2015; IUCN, 2018).

Wetlands aid in dampening the effects of climate variability through flood attenuation, groundwater recharge and discharge functions (Russi et al., 2013). Wetlands also provide ecosystem functions of nutrient assimilation, surface water filtering, carbon sequestering, biodiversity, specialized habitats, resources, recreational and research opportunities (Ducks Unlimited, 2010; Russi et al., 2013). The loss of wetlands is a global concern and is one of the major causes of decline in global biodiversity. Locally we are still losing wetlands (Ducks Unlimited, 2010). Wetland restoration is needed to help reverse that trend (Erwin, 2009; McDonald et al., 2016). Wetlands are important features that many species depend on directly or indirectly to complete their life cycle. Reptiles and amphibians are examples of ectothermic vertebrates that are often associated with wetlands. Some species move between terrestrial and wetland features during the active season and return to wetlands or nearby for hibernation. This is part of their annual lifecycle where they remain sequestered within their hibernacula longer than the active season.

Hibernation is a vulnerable life stage for snakes and mortality events are largely undetected because they occur out of our view and only survivors are found. Overwintering mortality is also

not limited to species at risk (SAR) populations and not always attributed to stochastic weather events. Although hibernacula environmental data was not presented, one of the suspected causes of post-hibernation mortality of two telemetered ratsnakes (*Elaphe obsoleta*) from a communal den in Ontario was attributed to excessive dryness in the den (Prior and Shilton, 1996). Snake mortality at a communal den in Utah was attributed to excessive weight loss especially in smaller individuals (Hirth, 1966). Researchers found 34 to 50% over winter mortality of 3 species, Striped whipsnake (*Masticophis t. taeniatus*); Great Basin rattlesnake (*Crotalus viridis lutosus*); Western Racer (*Coluber constrictor mormon*) (Hirth, 1966). Other researchers found very little winter mortality at this den site (3.6%) over five different winters (Parker and Brown, 1974). The largest recorded winterkill in Canada was more than 60,000 Red-sided Gartersnakes (*Thamnophis sirtalis parietalis*), and was attributed to stochastic weather events, freezing during midwinter due to poor snow cover and or flooding in late fall due to heavy rains (Shine and Mason, 2004). Sudden changes to a snakes' burrow during hibernation may decrease overwinter survival. Our knowledge of how well snakes adapt to change especially freezing, flooding or hypoxic conditions during hibernation is not well understood. For example, an experimental attempt to release rattlesnakes into a prairie did not work (Harvey et al., 2014). During a recent snake repatriation attempt, all 27 Massasauga rattlesnakes perished during their first winter (Harvey et al., 2014) and all snakes were young (age 3) naïve hibernators that overwintered in crayfish burrows near a watercourse which flooded its banks during a stochastic winter storm. In addition to the Massasaugas, several Eastern Gartersnakes were also found dead in these same burrows. It is unknown whether the snakes died from flooding, freezing, hypoxia, or a combination of these factors. Clearly water levels or moisture content within a snake hibernaculum are important considerations in their survival.

With respect to life history, the neonatal life stage generally has the highest natural mortality in most vertebrate species. Winter survival of Western rattlesnake (*Crotalus virdis*) neonates was estimated at 55% (Charland, 1989) and 76.5–100% adults at communal den sites in, British Columbia (Charland, 1989). An experimental division of 115 neonatal western rattlesnakes (*Crotalus oreganus*) into 2 den sites measured 55% overwinter mortality and found no difference attributed to birth weight or condition at birth (Charland, 1989). However, no habitat measurements were reported. Increasing neonatal winter survival may increase recruitment of juveniles into the adult breeding population and thereby determine the overall success of a population. Excessive mortality that exceeds recruitment causes populations to decline over time. For species at risk populations such as the Massasauga rattlesnake, increasing the population to a sustainable level is the main recovery objective (Parks Canada, 2015). Natural causes of neonatal mortality are complex and may be related to predation, body condition, genetic factors, winter severity and habitat quality. In chapter 3, I demonstrated that neonatal snakes could use cutaneous respiration during a short-term flood event, and their ability to upregulate oxygen exchange cutaneously may be related to thin neonatal skin. The ability of older snakes to use cutaneous respiration similarly requires further study.

In Chapter 3, I studied flood tolerance of neonatal and small sized snake species that represent two common species (Eastern Gartersnake and Red-bellied snake) and one endangered species (Massasauga) also native to my study area. The study site was an ombrotrophic bog ecosystem formed postglacially about 3600ybp and the raised bog formed about 370 years ago (Nagy and Warner, 1999). Ombrotrophic bogs are precipitation driven ecosystems, which means the annual water budget is entirely dependant upon precipitation. Ombrotrophic bogs store water and carbon

in the form of organic matter largely from sphagnum moss accumulations and are inherently low energy environments.

Today the Wainfleet bog is a remnant bog and swamp ecosystem that is highly altered from past drainage and peat mining activities (Frohlich, 1997; Yagi and Frohlich, 1998; Nagy and Warner, 1999; Yagi and Litzgus, 2012 and 2103; Browning, 2015). Following a bog restoration plan and beaver naturalization, the central peat mined areas are showing signs of bog vegetation recovery. Yet the central peatland still exhibits frequent cycles of flooding, drought, and wildfires. These cycles are likely brought about in part by an increasing human intervention to control beavers and remove their dams from municipal controlled drains during wet weather cycles. Followed by peatland wildfires in 2012 and 2016 during periods of drought which are invariably followed by flood events with renewed beaver activity during wet weather cycles (2017 to 2019 inclusive). Surrounding the mined areas are successional peatlands (tree-shrub dominant) situated at higher elevations and were not peat-mined, yet have a lower than expected water table due to ongoing drainage. I suspect these higher elevation areas are refugia habitats. How snakes manage to hibernate successfully within extreme water levels changes will aid in our understanding of snake flood survival and population viability especially in habitats with controlled or altered natural hydrology.

In chapter 2, I explained that we did not find direct evidence of flood mortality, yet we did find mounting indirect evidence that the pre-flood group survival declined, and annual reproduction was initially poor following the first flood event of the mined peatland. I suspect snakes with site fidelity to the mined hibernation sites died off and the remaining snakes survived because they were in hibernation areas that did not completely flood (not-mined habitats). Then gradually over time the population began to rebound with new recruits that

hibernated similarly. The central mined area remained flooded for five years which gave the recruits time to build up in size and the population began to increase. However, ongoing human intervention and manipulation of the water elevations to decrease the flood levels in the mined portions of the peatland will increase stochasticity and harm to the population which may re-initiate the ecological trap cycle. Using pre-flood radio telemetry data, Jones et al., (2012) estimated adult survival to be 86% over winter and 79% overall for Massasaugas in my study area. First winter survival rates of neonatal snakes in the study area is estimated during the same period to be (number recaptures from known litters / total neonates) 4.3%. However, this estimate is from a small sample of 7 litters. Given that Massasaugas are a cryptic species, neonatal snake winter survival is likely higher. Realizing that each captured snake represents a neonate that survived its first winter, by applying a snake's age we can back calculate first winter annual survival and by using average litter size (10 neonates/litter) we can estimate the pre-hibernation pool of neonates. In this way I have estimated neonatal first winter survival as 11.4%. In a Massasauga population from a grassland habitat in Michigan, neonatal survival was estimated at 38% and the population trend was stable (Hileman et al., 2018). For my study site neonatal snake survival must increase to facilitate an increasing population trend. *In situ* snake survival experiments in dynamic and stable habitats, with and without a water table would help answer these questions. In my study site the centrally mined peatland is a large open canopy area with proximity to surface water with a patchy distribution of cover. This area remains attractive to gestating females yet contains poor quality hibernation habitat that may flood during winter and is likely an ecological trap for naïve, neonatal snakes. By aiding neonatal dispersal through a technique, I developed called "assisted hibernation", into areas that maintain a life zone, it may

be possible to increase neonatal survival, and is the focus on my ongoing research in the study area.

Ecological restoration is a method of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed (McDonald et al., 2016). Literature suggests that a higher groundwater table would have a positive effect on restoring the bog vegetation community (Frohlich, 1997; Haapalehto et al., 2011; Browning, 2015). Some snake hibernation studies also suggest snake populations could benefit from more water, and snakes can survive prolonged inundation (Costanzo, 1985, 1986, 1989a, b; Kingsbury and Coppola, 2000; Vitt and Caldwell, 2014). Even though aquatic hibernation in snakes is largely untested (Ultsch, 1989), the concept of successful aquatic hibernation in Eastern Gartersnakes seems well nested in herpetology (Vitt and Caldwell, 2014). There are no examples of bog restoration projects that also coincide with rare reptile populations. Therefore, a precautionary approach is needed to guide wetland restoration activities in the long-term, to ensure resident reptile populations can adapt and recover from ecosystem level changes, such as flooding, over time. By keeping flood prone areas flooded and not used by snakes for hibernation is an ecological restoration approach toward both bog restoration and Massasauga recovery.

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Appendix

Resting Metabolism

For all snake respirometry results in air media, $\log_e (\dot{M}_{O_2})$ (μmol$\cdot h^{-1}$) declined with longer

duration measurement periods, with the steepest decline occurring at the three hour deflection

point (Fig. 36A). Snake activity was measured for 2014 snakes only and followed the same trend

as $\dot{M}_{O_2}$ with the greatest decline in activity occurring after 3 hrs with the inflection point

measured at 4.5hrs (Fig. 36B). Activity generated $\dot{M}_{O_2}$ was controlled by conducting experiments

> 3-4 hrs.

Resting $\dot{M}_{O_2}$ (control) was lowest in E. Gartersnakes ($0.29 \pm 0.05\ \mu mol\ O_2 \cdot h^{-1}$), followed

by Red-bellied snakes $0.43 \pm 0.06\ \mu mol\ O_2 \cdot h^{-1}$ and Massasaugas $1.04 \pm 0.09\ \mu mol\ O_2 \cdot h^{-1}$

(Table 24). A Tukey post-hoc test identified Massasaugas as having a significantly higher resting

$\dot{M}_{O_2}$ than E. Gartersnakes ($p < 0.001$) and Red-bellied snakes ($p<0.01$). Mass and species are

highly correlated with resting $\dot{M}_{O_2}$, with large snakes (i.e., Massasaugas) also having a

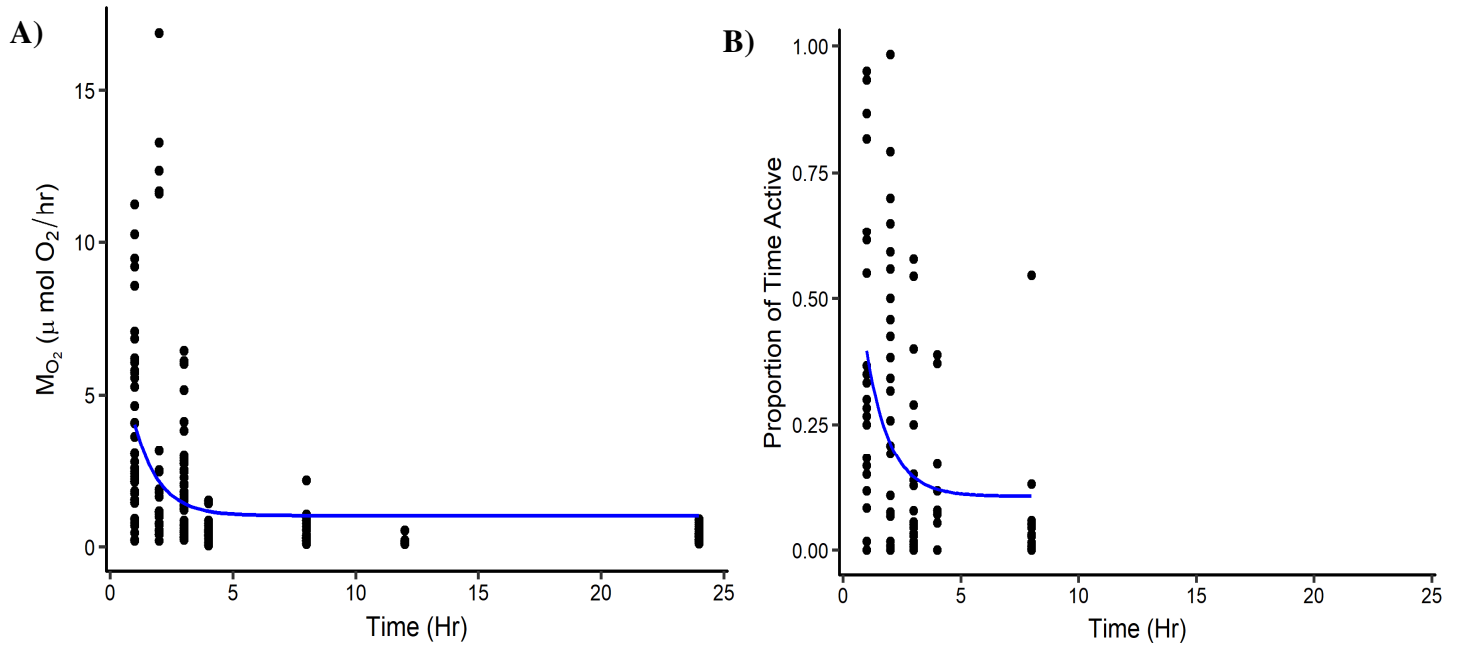significantly higher resting $\dot{M}_{O_2}$ ($r^2 = 0.64$; $F_{3,21} = 12.48$; $p<0.001$; Fig. 3.6).

Fig. A.1 A) The effect of time on $\dot{M}_{O_2}$ (μmol$\cdot h^{-1}$) and snake activity (proportion of time snake was active) in an air-filled respirometer. $\dot{M}_{O_2}$ declined after 3 hrs which was confirmed as the inflection point. B) Snake activity in an air-filled respirometer (all species in 2014 only) was reduced to low levels after 4 hrs. The inflection point in the raw data was calculated at 4.5 hrs.

Table A.1. Metabolic rate $\dot{M}_{O_2}$ $\mu mol\ O_2 \cdot h^{-1}$ (mean ± SE) of neonatal E. Gartersnake and Massasauga and juvenile Red-bellied snakes at 5°C during resting (100% air), partitioned (50% water and air), flood (100% water) and dive recovery (100% air). (updated with experiment length and activity biases removed).

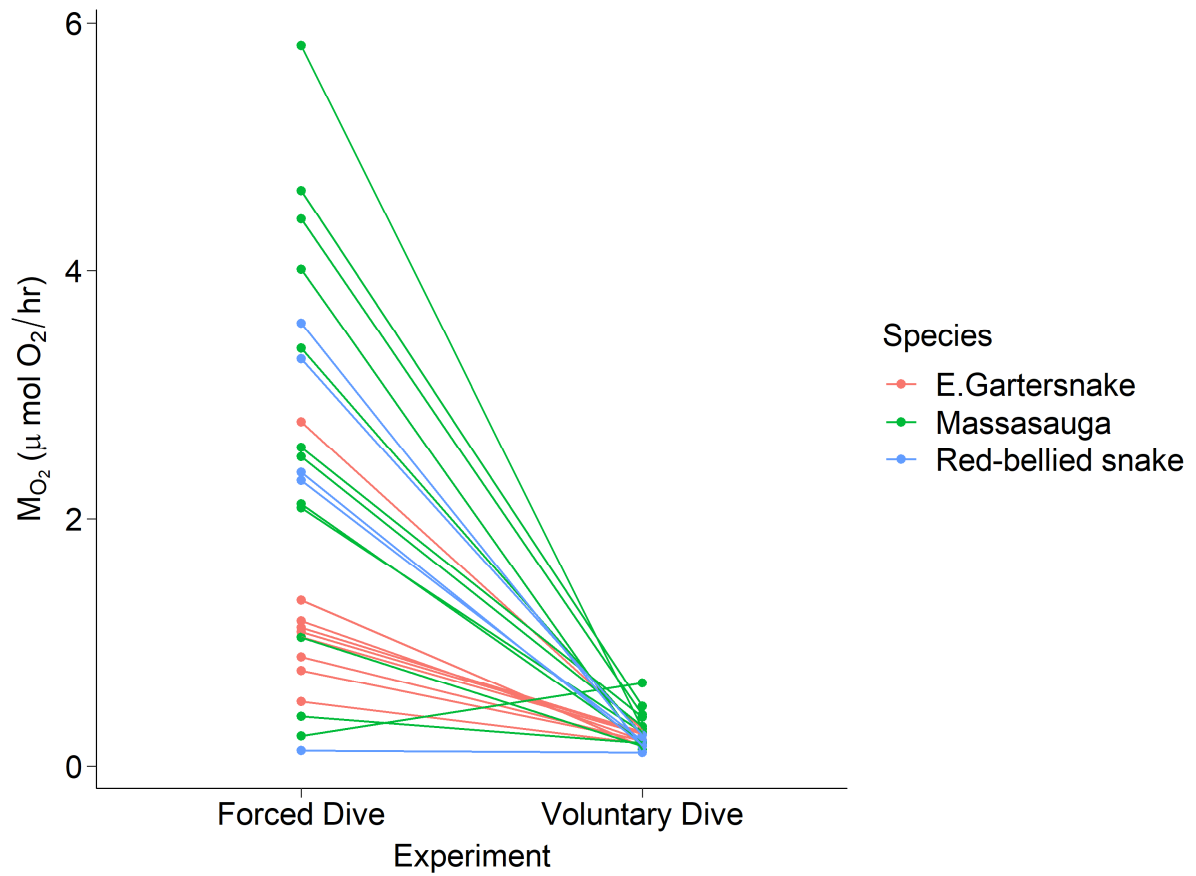| Species	n	Resting	n	Partitioned Total	n	Forced dive	n	Forced dive Recovery
E. Gartersnakes	25	0.29 ± 0.05	10	1.25 ± 0.26	6	1.32 ± 0.31	6	2.28 ± 0.29
Massasaugas	14	1.06 ± 0.08	12	2.88 ± 0.55	12	2.77 ± 0.50	14	2.59 ± 0.55
Red-bellied snakes	12	0.43 ± 0.06	10	2.12 ± 0.33	5	2.33 ± 0.60	5	2.70 ± 0.37
Overall	51	0.53 ± 0.06	32	2.13 ± 0.27	23	2.13 ± 0.30	25	2.53 ± 0.32

Fig. A.2 Oxygen uptake from water represents the cutaneous respiration abilities of three species of small snakes during a forced and voluntary dive at 5°C. Individual snakes are linked by the lines. Forced dive experiments averaged 1.5 hours (1 -5 hrs) and voluntary dives experiments using partitioned respirometry averaged 8 hrs (7 – 12 hrs) in length. One Massasauga had a higher $\dot{M}_{O_2}$ (μmol· h^{-1}) during the voluntary dive and this individual remained voluntarily submerged during full 7 hr partitioned experiment.
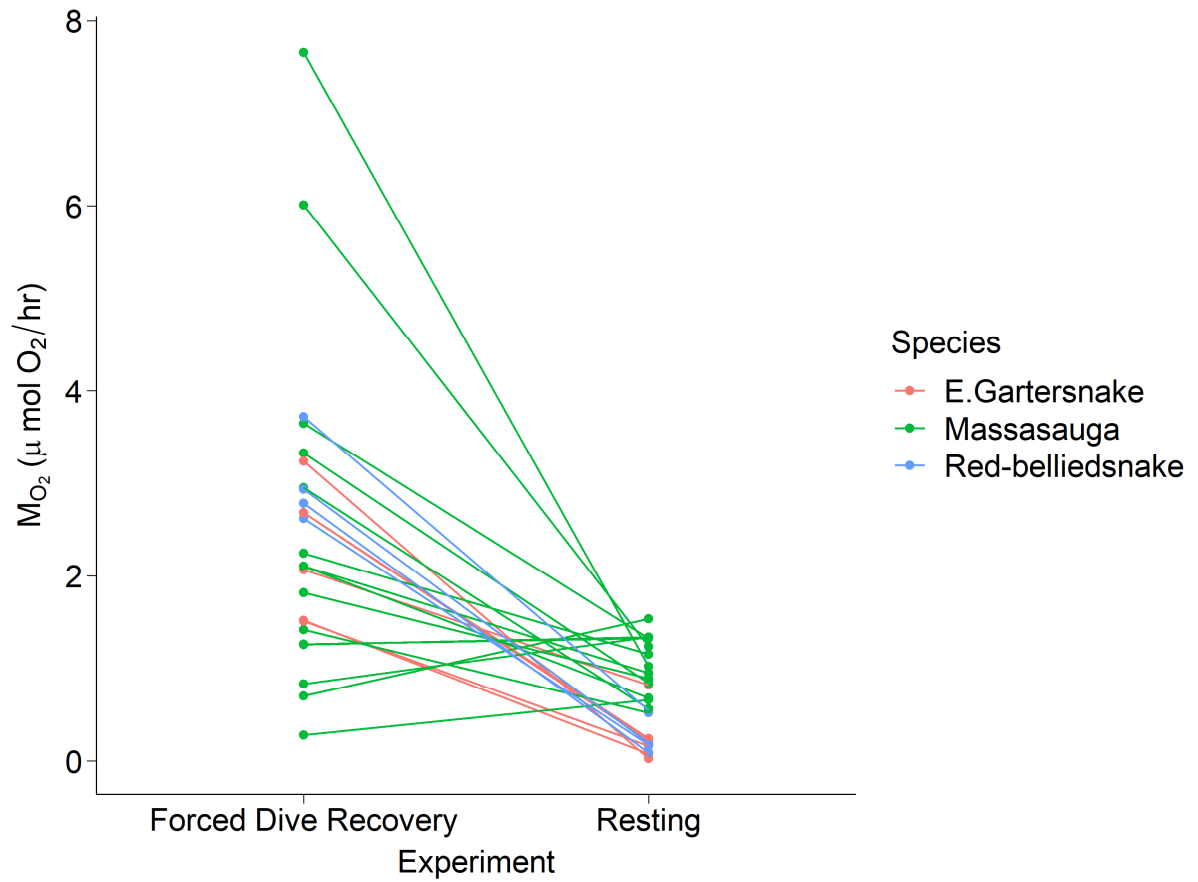
Fig. A.3 Post Dive Recovery and Resting $\dot{M}_{O_2}$ (µmol· h^{-1}) for three small snake species. Individual snakes are linked by the lines. Forced dive recovery experiments averaged 5.1 hrs (4 – 6 hrs) and Resting experiments averaged 7.3 hrs (3 to 8 hrs).

Additional observations include Massasaugas with cloacal distended position within the air space (Fig. A.4, A.5). This position of head down and cloaca distended in the air space was only seen in Massasugas.
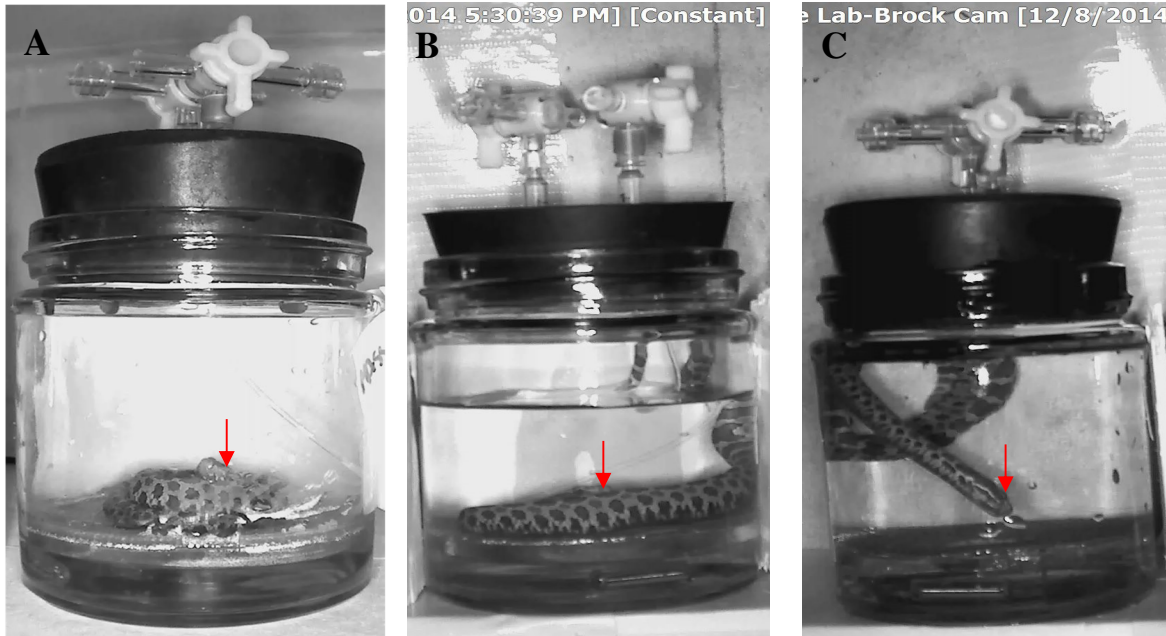

Fig. A.4 Camera views of snake positions within respirometer at various water depths. Respirometer (A) Air only (B) Air + Water (C) Water only. Red arrow indicates position of the snakes' head.
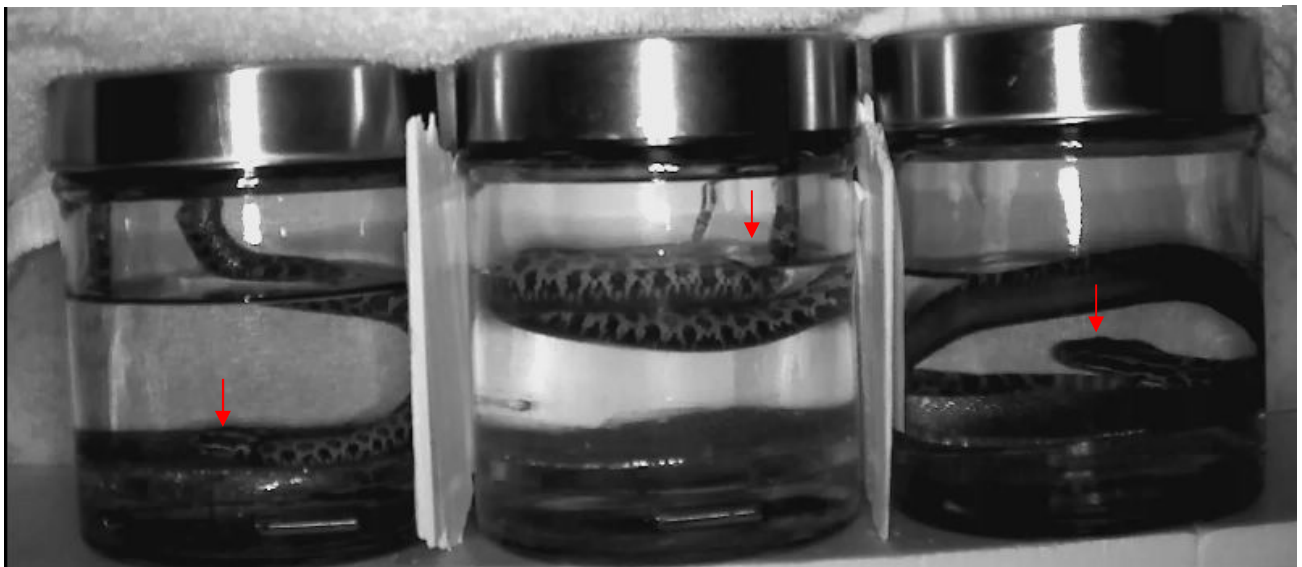

Fig. A.5 Camera view of snake dive positions during dive experiment. A towel was placed over the screen to decrease water loss and minimize wind generated by lab fans. The red arrow is snakes' head position.