

**Socioecology of the Midland Painted Turtle (*Chrysemys picta marginata*)**

by

Carter J. Rouleau

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**APPROVED/APPROUVÉ**

Thesis Examiners/Examineurs de thèse:

Dr. Jackie Litzgus  
(Co-Supervisor/Co-directeur(trice) de thèse)

Dr. Julia Riley  
(Co-Supervisor/Co-directeur(trice) de thèse)

Dr. Njal Rollinson  
(Committee member/Membre du comité)

Dr. Stephanie Godfrey  
(External Examiner/Examineur externe)

Approved for the Office of Graduate Studies  
Approuvé pour le Bureau des études supérieures  
Tammy Eger, PhD  
Vice President, Research (Office of Graduate Studies)  
Vice-rectrice à la recherche (Bureau des études supérieures),

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## **Socioecology of the Midland Painted Turtle (*Chrysemys picta marginata*)**

**Abstract:** The cryptic nature of sociality in many reptile species suggests that their social behaviour may indeed be more complex and varied than previous research supposes. I propose that the Midland Painted Turtle (MPT; *Chrysemys picta marginata*) demonstrates cryptic social organization in its nesting and basking behaviours. In this thesis, I aim to: i) describe patterns of social organization in MPT, and ii) investigate influences on the social behaviour of MPT. In Chapter I, I investigate the potential for kin-bias in the communal nesting behaviour of MPT. I show that this bias is not present in MPT, and suggest that environmental factors and body size largely govern MPT nesting behaviour. In Chapter II, I investigate social organization in basking aggregations of MPT. I show that kin-bias is present in this behaviour. Further, I demonstrate that sex, body size, and capture frequency of MPT affect their social behaviour.

**Keywords:** turtle, sociality, behaviour, basking, nesting, association, social network

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## General Introduction

Interaction between individuals is fundamental to the life history of all animals. When they occur between conspecifics, these interactions are often defined as social (Hofmann *et al.*, 2014; Kappeler, 2019). Social systems are commonly divided into three components – social organization, social structure, and the mating system – that all contribute to a species' social complexity (Kappeler, 2019). Social organization refers to the size, composition, and distribution of social groups (Struhsaker, 1969; Kappeler, 2019). Questions regarding social organization often address issues of social group size, group composition, or the nature of associations between individuals (Kappeler, 2019). Social structure defines interactions, communication, and social relationships (Silk *et al.*, 2013). Mating systems involve mating patterns, reproductive skew, and reproductive tactics (Kappeler & van Schaik, 2002). A species' social complexity can be defined as the frequency and variation of its social interactions (de Waal & Tyack, 2003). Social systems are incredibly varied and demonstrate a wide range of complexities across the animal kingdom (Smelser *et al.*, 2001).

Aggregation has been identified as a potential precursor to the evolution of social systems (Wey *et al.*, 2008; van Veelen *et al.*, 2010). However, alone it is insufficient to indicate intentional social associations and behaviour (Halliwell *et al.*, 2017). Individuals may congregate due to the shared need of a localized resource or environmental feature, in which case their grouped presence may be incidental (Brown, 1975; Graves & Duvall, 1995). This is a key distinction that limits the possibility of a group of conspecifics erroneously being characterized as a social group. It has been suggested that aggregations may provide the opportunity for natural selection to favour increased sociality if individuals benefit from those behaviours beyond their costs (Hamilton, 1964a,b; Halliwell *et al.*, 2017). Hamilton's (1964a,b) kin-selection theory

suggests that the benefits of social behaviour to the individual are measured in terms of both individual and inclusive fitness. In some species, social behaviour involves individuals sacrificing individual fitness for the sake of inclusive fitness. This is commonly manifested in the form of post-birth parental care (Gross, 2005; Klug & Bonsall, 2010). For example, young adults in many bird species will forego their own mating opportunities to act as an extra caregiver to the clutch of another pair of mates (Hatchwell, 2009; Jetz & Rubenstein, 2011). Hamilton's Inequality Rule (1964a,b) states that altruistic behaviours should be favoured when the product of the benefit provided and the relatedness between the actor and the recipient of the benefit is greater than the cost incurred by the actor. Thus, altruism is more likely to evolve between kin. Higher relatedness between the actor and the recipient increases the inclusive benefit to the actor.

In many species, social systems involve associations between kin (e.g., Hatchwell, 2009; Davis *et al.*, 2011; Clark *et al.*, 2012; Davis, 2012). Many studies support a hypothesis that kin groups are formed expressly through the delayed dispersal of offspring from parental territories (reviewed in Emlen, 1995). Delayed dispersal is commonly cited as critical in the evolution of social systems at large (Hamilton, 1964a,b; Doerr *et al.*, 2007). Specifically, it is hypothesized that extended contact between kin results in more opportunities for interaction and may promote the evolution of kin-based social systems (Queller, 1994; Lion & van Baalen, 2007; Halliwell *et al.*, 2017). However, sociality is not limited to strictly kin-based systems. Garcia and de Monte (2013) suggest that a simple tendency to form groups, even without assortative filters such as kin-bias or nepotism, is a sufficient precursor to the evolution of sociality in a species, provided fitness increases with group cohesion.

Social behaviour is extensively documented across the vertebrates (e.g., Tinbergen, 1953; Heatwole & Sullivan 1995; Doody *et al.*, 2013). Research on vertebrate sociality has

traditionally focused on mammals and birds but has more recently expanded to include studies on reptiles, a taxon long regarded as antisocial and largely solitary (Brattstrom, 1974). Yet there is still a lack of studies on reptile sociality, which may be rooted in the fact that reptile social behaviour and communication are less salient to humans than those of the other vertebrate groups (Shine, 1988; Bonnet *et al.*, 2002; Rivas & Burghardt, 2002). Though, when examined in ecologically relevant settings, reptiles can display similar cognitive functions to mammals and birds (Roth *et al.*, 2019). Reptiles often exhibit cryptic sociality, wherein the specific mechanisms through which they interact are difficult to observe (e.g., Shine *et al.*, 2004; Clark *et al.*, 2012). For example, juvenile Black Rock Skinks (*Egernia saxatilis*) share territory with their parents, which reduces their risk of infanticide by unrelated conspecifics (O'Connor & Shine, 2004). The risk is decreased solely through the presence of parents, who do not exhibit any active care or protective behaviours. However, there are examples of reptiles performing active parental care. Cunningham's Skinks (*Egernia cunninghami*) will behave aggressively towards potential predators when their offspring are in close proximity (Watson *et al.*, 2020). Further, the cognitive mechanisms of reptiles are not well understood (Kabelik & Hoffman, 2018), and their ectothermic nature can create wide phenotypic variation among individuals, particularly early in life (Matsubara *et al.*, 2017). Incubation and early life conditions can greatly influence phenotype and drive divergence in both physiological and life history traits of ectotherms (Richter-Boix *et al.*, 2015). Among reptiles, sociality is most thoroughly documented in squamates (reviewed in Gardner *et al.*, 2016; Bull *et al.*, 2017); however, the existence or absence of aggregations has been examined in less than 1% of all squamates (Gardner *et al.*, 2016). Squamates are known to live in stable social aggregations (Gardner *et al.*, 2016), exhibit parental care (O'Connor & Shine, 2004; Watson, 2020), and aggregate for thermoregulation (Shah *et al.*, 2003). The other

reptilian orders are even less studied, and in many cases have gained reputation as solitary, asocial animals (Doody *et al.*, 2013). In particular, evidence for turtle (Reptilia: Testudines) sociality is underreported.

Social interactions have been documented in the emydids (Testudines: Emydidae), primarily with respect to mating tactics (e.g., Thomas, 2002; Liu *et al.*, 2013) and intrasexual competition (e.g., Barzilay, 1980; Kaufmann, 1992; Rovero *et al.*, 1999). Evidence for interaction during performance of other behaviours is sparse, and many studies note an apparent general lack of interaction between individuals beyond mating forays or competitive encounters (Boyer, 1965; Ernst, 1971; Vogt, 1980). There have been recorded instances of interaction between basking emydids, all of which have been aggressive in nature. Bury and Wolfheim (1973) first described an apparently aggressive mouth-gaping behaviour performed by Western Pond Turtles (*Actinemys marmorata*), which has since been noted in a number of other species (*Chrysemys picta*: Lovich, 1988; 1990; *Glyptemys insculpta*: Barzilay, 1980; Kaufmann, 1992; *Emys orbicularis*: Rovero *et al.*, 1999; *Trachemys scripta*, *Pseudemys concinna*, *Graptemys pseudogeographica*, *Graptemys ouachitensis*: Lindeman, 1999), though there is evidence to suggest that this behaviour is not strictly aggressive. Lindeman (1999) reported that gaping elicited no response from conspecifics or heterospecifics sharing a basking structure. Other studies have reported that gaping regularly caused recipients to alter their basking posture or leave the basking site (Bury & Wolfheim, 1973; Lovich, 1988; Cordero *et al.*, 1999; Zagorski *et al.*, 2018).

Emydids are also known to physically displace conspecifics from basking sites (Pluto & Bellis, 1986; Lovich, 1988; Lindeman, 1999), and infrequently bite conspecifics (Bury & Wolfheim, 1973; Lindeman, 1999). It has been suggested that aggressive interactions can result

in quasi-stable social hierarchies within emydid populations (Kaufmann, 1992; Cordero *et al.*, 1999). In a number of emydid species, success in an aggressive encounter is dependent on body size (*T. scripta*: Auth, 1975; *G. geographica*: Pluto & Bellis, 1986; *T. scripta*, *P. concinna*, *G. pseudogeographica*, *G. ouachitensis*: Lindeman, 1999), and some species are known to avoid potentially aggressive interactions with larger conspecifics (*A. marmorata*: Bury & Wolfheim, 1973; *E. orbicularis*: Poschadel *et al.*, 2006; *Mauremys leprosa*: Ibáñez *et al.*, 2014).

Sociality of freshwater and sea turtles, if present, is likely quite cryptic, because they spend the majority of time in the water and hidden from an observer's eye. Despite this, their biology suggests that they have the capacity for social interaction. Turtles have well-developed visual (Wilkinson *et al.*, 2010), olfactory, and vomeronasal systems (Quinn & Graves, 1998; Muñoz, 2004), and there is evidence that they use these systems to interact with conspecifics. Poschadel *et al.* (2006) found that European Pond Turtles (*E. orbicularis*) use chemical cues to assess potential mates and competitors. Ibáñez *et al.* (2014) showed that Spanish Terrapins (*M. leprosa*) use chemosense to assess the size of a rival. Iverson *et al.* (2016) suggest that Painted Turtles (*C. picta*) may use olfactory cues left by conspecifics to inform nest site choice. Further, many turtle species live in high densities, and are often in extremely close proximity to conspecifics (Ernst & Lovich, 2009). A close proximity between individuals within a population could favour social association if it is advantageous (Halliwell *et al.*, 2017).

The Painted Turtle is recognized as a model system in ecology and human biology, and is receiving increasing interest in genetic and genomic studies (Valenzuela, 2009; Shaffer *et al.*, 2013). The Painted Turtle's life history is the most well-studied of all emydid turtles (Lovich & Ennen, 2013), making it an effective system for the study of emydid ecology and evolution. The nesting and basking behaviours of Painted Turtles have been thoroughly studied, and its

tendency to congregate is well known. However, to date, no study has examined the factors that might influence this tendency to aggregate, or the potential for a stable social system or hierarchy within Painted Turtle populations.

An ongoing life history study of Midland Painted Turtles (MPT; *C. p. marginata*) has been running in Algonquin Provincial Park, Ontario since 1978 (R. Brooks *et al.*; 45°34' N, 78°41' W), making wetlands in Algonquin Provincial Park ideal locales for study of MPT behavioural and social ecology. Further, both behaviours of interest in my study have been previously examined (albeit, not in a social context) at my study site, Wolf Howl Pond, a wetland within Algonquin Provincial Park. My research builds on the base of knowledge regarding MPT nesting and basking behaviours by examining them within a social context. Further, by collecting genotypic data, I address the potential for kin-bias in congregations of MPT, which has applications in elucidating the evolutionary mechanisms through which the social organization of MPT may have arisen.

By integrating behavioural and genotypic data, my research uses a novel approach to the study of turtle life history in a natural setting. Turtles are underrepresented in the sociality literature, and their social complexity has not been examined to the extent of other reptiles. My research aims to contribute towards reconciling the disparity between turtles and other reptiles in the field of reptile sociality, and to contribute a study on a taxon novel to the animal sociality literature. It is my hope that the genetic analyses conducted in my study will serve as a tool to further research on the life history of the Wolf Howl Pond population of MPT.

In this thesis, I aim to explain sociality in MPT through the use of behavioural observations and genotypic data. In Chapter I, I investigate social influences during communal nesting of female MPT. In Chapter II, I investigate patterns of cryptic social organization in basking aggregations

of MPT, with emphasis on the relative importance of environmental and social influences on social interactions during basking. Both of my chapters use genotypic data to estimate genetic relatedness between adult MPT in my focal population. These data are used in conjunction with social association data to examine patterns of kinship in the social organization of MPT.

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## **Chapter I: Social, kin-biased, and environmental influences on nesting behaviour of the Midland Painted Turtle (*Chrysemys picta marginata*)**

**Abstract:** Nesting site choice affects both maternal and offspring fitness in oviparous animals. Many oviparous species lack post-nesting parental care, so choosing a site that provides optimal conditions for their offspring is an important way of influencing developmental success and offspring phenotype. Turtles display nesting site selectivity, and often nest in areas where conspecifics have nested previously. In a number of other reptile groups, notably scincids (Squamata: Scincidae) and viperids (Squamata: Viperidae), congregations of this nature often include kin. If congregative nesting behaviour provides a survivorship or fitness benefit to nesters or their offspring, then females should cluster their nests in kin-based groups to maximize inclusive fitness. I predicted that nesting congregations would also be kin-biased in the Midland Painted Turtle (MPT; *Chrysemys picta marginata*) in Algonquin Provincial Park (Ontario, Canada). I monitored nest site location with high accuracy GPS and calculated genetic relatedness between all females in my focal population across two years. I constructed a matrix of pairwise nest distances, and correlated that with parallel matrices of pairwise relatedness and body size to determine the influence of these factors on nest placement. I found that female MPT exhibited no preference for nesting in close proximity to kin, and that nest timing was dependent on female size. This does not support my prediction that nesting female MPT congregate in kin-biased groups, and alternatively I propose that environmental factors may largely govern the nesting behaviour of MPT. These results suggest that the benefits of communal nesting are multi-faceted and may be gained irrespective of the relatedness of nesters. This finding has implications for the evolution of communal nesting behaviour and the use of social information.

## Introduction

Parental care can be a key component of animal development (Klug & Bonsall, 2010). Animals display a wide range of parental care behaviours with varying levels of individual investment (e.g., incidental guarding against infanticide in the Black Rock Skink, *Egernia saxatilis*: O'Connor & Shine, 2004; self-sacrificial maternal care in Crab Spiders, *Diaea ergandros*: Evans, 1998) and at varying times in a breeding cycle (e.g., pre-oviposition nesting site selection: Mappes *et al.*, 1994; Hoi *et al.*, 2012; post-birth provisioning: Nordlund & Barber, 2005; Neudorf *et al.*, 2013). Parental care in many species is complex, such that it often occurs throughout multiple stages of breeding and early offspring life (Gross, 2005; Klug & Bonsall, 2010). In these cases, the benefits gained from specific parental care behaviours can be difficult to separate (Wilson, 1998). Taxa that exhibit few or singular parental care behaviours are ideal study systems for research of this nature, since there are no confounding effects of multiple behaviours.

Nest site selection can be considered a type of parental care commonly observed in oviparous species, and it can affect the fitness and phenotype of offspring (Qualls & Andrews, 1999; Janzen & Morjan, 2002; Hughes & Brooks, 2006). Given that many oviparous species exhibit no post-nesting parental care, nest site selection is the only way they can influence the incubation conditions, and in turn, the hatching success of their eggs and phenotype of their offspring (e.g., morphology, performance, behaviour; Bernardo, 1996; Blouin-Demers *et al.*, 2004; Mitchell *et al.*, 2013). Thus, females should choose nesting sites that maximize fitness of their offspring (Schwarzkopf & Brooks, 1987), and indeed, many oviparous species do not choose their nesting sites randomly (Garmestani *et al.*, 2000; Freedberg *et al.*, 2005; Iraeta *et al.*, 2007; Mitchell *et al.*, 2013). Ideal nest site characteristics vary across and within species

depending on location (Morjan 2003), but are thought to be selected to create an ideal thermal incubation environment and decrease the likelihood of nest predation (Wilson, 1998).

Turtles are ideal study subjects for exploring the fitness benefits derived from nest site selection, given that post-nesting care is rare or absent in most turtle species (Congdon *et al.*, 1983a; Wilson, 1998; Marlen & Fischer, 1999; Ernst & Lovich, 2009; but see Ferrara *et al.*, 2013). Therefore, a female turtle's influence on her clutch's early life fitness is limited to maternal effects (Mousseau *et al.*, 2009; Wolf & Wade, 2009) and nest site selection (Bernardo, 1996; Mitchell *et al.*, 2013). Site selection can have substantial influence over multiple aspects of maternal and offspring fitness (reviewed in Refsnider & Janzen, 2010). Individual turtles within a population display some degree of coordination with respect to nest density (Painted Turtle, *Chrysemys picta*: Iverson *et al.*, 2016; Kell, 2018) and nesting timing (Common Snapping Turtle: *Chelydra serpentina*: Robinson & Bider, 1988; Olive Ridley Turtle, *Lepidochelys olivacea*: Eckrich & Owens, 1995). Yet, nest site selection is not an innate behaviour, because the heritability of nesting behaviour in turtles is low (McGaugh *et al.*, 2010). Thus, turtle nesting behaviour may, instead, be largely influenced by both environmental variables and the behaviour of conspecifics.

Female Midland Painted Turtles (MPT; *C. p. marginata*) are more likely to attempt nesting in an area where other turtles are nesting or have nested (Iverson *et al.*, 2016; Kell, 2018). Turtles are known to use visual (Davis & Burghardt, 2011) and chemosensory (Iverson *et al.*, 2016) cues in the interpretation of social information from conspecifics. Chemosensory cues have been implicated as important factors in location and recognition of the opposite sex in turtles (Cagle, 1950; Vogt, 1979). Also, a number of reptile species can discriminate between kin and non-kin based on chemoreception (e.g., Aragón *et al.*, 2001; Bull *et al.*, 2001; O'Connor &

Shine, 2006; Poschadel *et al.*, 2006; Pernetta *et al.*, 2008; Whitear *et al.*, 2016). Female MPT urinate during nesting, leaving a detectable scent (Mahmoud, 1968). Turtles may be able to determine where specific individuals have nested, and choose which individuals to nest near, based on the chemosensory cues within a female's urine. When assessing a potential nesting site, many turtle species perform a behaviour referred to as "ground-nuzzling", whereby they press their head and ventral neck against the substrate before choosing whether to initiate nesting (Morjan & Valenzuela, 2001). Ground-nuzzling has been implicated as a potential mechanism through which turtles search for chemosensory evidence of nesting activity (Iverson *et al.*, 2016; Kell, 2018). There is also evidence that turtles visually assess potential nesting sites to examine whether other females are using them (Escalona *et al.*, 2009; Kell, 2018).

Nests that are in close proximity can synchronously hatch and emerge, a phenomenon that can occur both within and between clutches (Spencer *et al.*, 2001; Colbert *et al.*, 2010; McGlashan *et al.*, 2012; McGlashan *et al.*, 2015). Hatching and emergence synchrony may provide a survivorship advantage for hatchlings through predator saturation or prey-switching (Darling, 1938; Ims, 1990). Painted Turtles are known to exhibit hatching synchrony (Colbert *et al.*, 2010). Further, after emergence, there is the possibility that hatchling turtles may follow scent trails of clutchmates or adult conspecifics (as seen in neonate Timber Rattlesnakes, *Crotalus horridus*: Brown and MacLean 1983; demonstrated in the Blanding's Turtle, *Emydoidea blandingii*: Butler & Graham, 1995; Paterson *et al.*, 2011; and the Yellow-Spotted River Turtle, *Podocnemis unifilis*: Ibáñez *et al.*, 2015) to overwintering locations. This type of behaviour is seen in hatchling Green Iguanas (*Iguana iguana*; Burghardt and Greene 1977), wherein hatchlings gain fitness benefits by navigating post-emergence in groups.

Close proximity between nests may confer survivorship advantages to nesting or hatchling turtles. Female turtles nesting gregariously may experience lower predation risk than solitary nesters (Hughes & Richard, 1974; but see Kell, 2018). Further, this aggregative behaviour could involve the use of public information left by other nesting females, which decreases the cost of assessment of a nesting site by a potential nester (Doody *et al.*, 2003b). There is evidence that predation of turtle nests is not density-dependent (Burke *et al.*, 1998; Doody *et al.*, 2003a), but that a high density of nests in a given area can dilute predation risk across an entire group of nests without lowering the probability of an individual nest being predated (Robinson & Bider, 1988; Eckrich & Owens, 1995; Spencer, 2002). This phenomenon could result in inclusive fitness benefits to the individual if the nests of kin are laid in close proximity to each other. Given that individual nests are predated equally regardless of nest density, kin selection theory suggests that inclusive fitness benefits could be maximized when nests are laid in kin groups (Hamilton 1964a,b).

The objective of this research was to test the hypothesis that MPT nest in kin-based groups. Previous research on my focal population of MPT has shown that nest placement is non-random, such that females are more likely to nest in areas where conspecifics have previously nested (Kell, 2018). Further, the distribution of nests across available nesting habitat is significantly clustered, and nests are laid in closer proximity to each other than would be expected with a random distribution across the nesting habitat (Kell, 2018). I observed nesting behaviour of MPT over two years. Genetic samples were collected from focal MPT and used to calculate genetic relatedness between individuals in order to test for a potential kin-bias to nesting behaviour. I hypothesized that if communal nesting provides an inclusive fitness benefit, then the distance between nest sites should be closer among related individuals than non-related

individuals. I predicted that distances between nests would decrease with increasing relatedness of females. Additionally, I examined the effect of maternal size on MPT nesting behaviour, with respect to both distance between nests and nest timing. Social interactions in turtles are often impacted by individual size (e.g., Lindemann, 1999; Ibáñez *et al.*, 2014), and thus body size may have an influence on aggregative nesting behaviour.

## **Methods**

### *Study site and population*

This research used, in part, data from ongoing studies of turtle life history in Algonquin Provincial Park, Ontario, Canada (R. Brooks *et al.*, unpubl. data; 45°34' N, 78°41' W). My research focused on a population of approximately 225 MPT living in Wolf Howl Pond, a Black Spruce (*Picea mariana*) wetland. This population size estimate is based on 42 years of mark-recapture data (R. Brooks *et al.*, unpubl. data; Samson, 2003). MPT occur at a density of 128.6 turtles/ha in Wolf Howl Pond (M. Keevil & S. Sanders, unpubl. data.). Wolf Howl Pond MPT nest along a 300 m abandoned railway embankment (built circa 1900) bordering the pond on the northeast side. The bank is comprised primarily of sand, and covered with a low shrub layer dominated by Lowbush Blueberry (*Vaccinium angustifolium*) and Alpine Strawberry (*Fragaria vesca*). A number of large Eastern White Pines (*Pinus strobus*) grow on the embankment, and the areas in which their needles fall are largely avoided by nesting MPT (C. Rouleau, pers. obs.). A sympatric population of Common Snapping Turtles (*Chelydra serpentina*) also use the Wolf Howl Pond embankment as their primary nesting habitat (C. Rouleau, pers. obs.).

Each turtle is individually marked with unique numeric or alphanumeric codes. These identities are based on one of three forms of marking: i) marginal scute notch codes (based on

Cagle, 1939), ii) aluminum ID tags affixed to posterior marginal scutes (described for *C. serpentina* by Loncke & Obbard, 1977), and iii) Passive Integrated Transponder (PIT) tags implanted into a turtle's right hind inguinal space (Smyth & Nebel, 2013). Turtles may bear one, two, or all of these markings, as each has been used at different times throughout the long-term study. In my study, turtles were captured by dip net or hand from canoes, and transported to a field lab at the Algonquin Wildlife Research Station for processing. Each turtle's midline carapace length (midCL) was measured with 20 cm Vernier calipers to the closest 0.01 cm. All turtles had their unique identities painted on their carapace using TREMCLAD<sup>®</sup> oil-based paint, to allow identification from a distance in the field. The painted markings are lost when turtles shed their scutes at the beginning of the active season.

#### *Observations of nesting behaviour*

Nesting surveys were conducted daily during the nesting season as a part of the long-term study (R. Brooks *et al.*, unpubl. data). The Wolf Howl Pond nesting embankment was patrolled daily at 20-min intervals from 1500 h until the cessation of nesting activity, typically between 2200 h and 0000 h. Surveys were stopped each day when no turtles had been observed attempting to nest for one hour. The end of surveys each season occurred after three consecutive survey days during which no turtles attempted to nest. Nest location and nesting time were recorded for each nest laid. All completed nests were marked with flagging tape on a stake placed 1 m from the nest chamber, as marking nests directly at the chamber has been shown to increase depredation rates (Rollinson & Brooks, 2007a). The location coordinates of each nest were recorded using a Trimble<sup>®</sup> R1 GPS receiver that is accurate to 30 cm.

### *Genetic data collection & relatedness analysis*

Blood samples were collected from the caudal vein of MPT during the 2018-2020 active seasons. Samples were drawn using sterilized BD Ultra-Fine™ 1 mL insulin syringes.

Approximately 100 µL of blood was sampled from each turtle. Samples were stored on Whatman® FTA® cards (Owen, 2011; Whatman, Inc.). DNA extraction and single-nucleotide polymorphism (SNP) genotyping of all samples was performed by Diversity Arrays Technology, Canberra, Australia (Jaccoud *et al.*, 2001), and yielded a dataset with 66,832 loci and mean call and reproducibility rates of  $82.65 \pm 0.20\%$ , and  $97.85 \pm 0.03\%$ , respectively.

Filtering of genotypic data was performed in R version 4.0.2 using the package ‘*dartR*’ (Gruber *et al.*, 2017; R Core Development Team, 2020), and the final filtered dataset contained 1015 loci. During filtering, I removed any monomorphic loci. When multiple polymorphisms were found within the same sequence, a single polymorphism was selected at random to minimize physical linkage of loci (Lemay & Rusello, 2015). I retained loci with a call rate and a reproducibility rate  $\geq 99\%$ . I calculated allelic coverage, and removed loci with read depths  $\leq 2$  and  $\geq 7$  (Lemay & Russo, 2015). Loci with minor allele frequencies  $\leq 2\%$  were filtered, as high minor allele frequency can bias genotypic data (Roesti *et al.*, 2012). When two loci had a Hamming distance (number of base differences)  $\leq 25\%$ , one locus was randomly selected to be included in the final dataset (Gruber *et al.*, 2017). I also filtered loci that differed significantly from Hardy-Weinberg Equilibrium ( $\alpha = 0.05$ ). The final filtered dataset had 1015 loci, and call and reproducibility rates of  $99.57 \pm 0.003\%$  and  $99.62 \pm 0.003\%$ , respectively.

Post-filtering, relatedness analyses were performed in the program COANCESTRY, which estimates pairwise relatedness between individuals using variation at marker loci (Wang, 2011). First, I simulated multilocus data based on allelic frequencies from my genotypic dataset

to select the appropriate relatedness estimator for the Wolf Howl Pond MPT population, because the accuracy of relatedness estimates depends on the genetic structure of a population (Van de Casteele *et al.*, 2001; Wang, 2011). I simulated 100 individuals with each of the following relatedness values:  $r = 0.000$  (unrelated),  $r = 0.031$  (second cousin),  $r = 0.125$  (first cousin),  $r = 0.250$  (half-sibling/avuncular/grandparent-grandchild),  $r = 0.500$  (full sibling), and  $r = 0.500$  (parent-offspring). I calculated pairwise relatedness estimates for each of these simulated individuals using seven relatedness estimators (Queller & Goodnight, 1989; Li *et al.*, 1993; Ritland, 1996; Lynch & Ritland, 1999; Wang, 2002; Milligan, 2003; Wang, 2007). I then calculated *Pearson's r* between actual relatedness of simulated individuals and estimated relatedness by each of the seven estimators. Triadic maximum likelihood relatedness estimates were most closely correlated to the true relatedness of the simulated individuals (*Pearson's r* = 0.996), and thus were used to estimate relatedness for the Wolf Howl Pond MPT population (Figure 1.1; Table 1.2; Wang, 2007).

For the depiction of my results (see Figures 1.2. and 1.3), I divided estimates into relationship categories based on four levels of relatedness:  $r = 0$  (unrelated),  $r = 0.125$  (first cousin),  $r = 0.250$  (half-sibling/avuncular/grandparent-grandchild),  $r = 0.500$  (full sibling/parent-offspring). Relatedness value ranges for each category were based on my simulated multilocus data (Figure 1.1). These ranges represent the spread of relatedness estimates that can be expected for each true relatedness value in my genotypic dataset. The “unrelated” bin was inclusive of second cousin relationships ( $r = 0.031$ ), as the triadic maximum likelihood method could not reliably distinguish between these two levels of relatedness.

### *Statistical analyses*

All statistical analyses were performed in R version 4.0.2 (R Core Development Team, 2020). In my analyses, I used data from 57 female MPT that nested in both study years. The pairwise distance between nests was significantly correlated across my two study years, such that the distances between all possible pairs of nests did not significantly differ between the two years ( $F_{1, 1594} = 22.39$ ,  $p < 0.05$ ,  $R^2_{adjusted} = 0.01$ ). However, this correlation was very weak, and thus data for each year were analyzed separately, though each year's dataset contained the same 57 individuals.

To test for a relationship between nesting location and kinship in MPT, I used a multiple regression quadratic assignment procedure with double semi-partialing from the R package 'asnipe' (MRQAP<sub>dsp</sub>; Krackhardt, 1988; Dekker *et al.*, 2007; Farine, 2013). MRQAP<sub>dsp</sub> is a permutation test for calculating multiple linear regression coefficients for data matrices. This method is commonly used in social network analyses, where parallel matrices of pairwise data for a set of focal individuals are correlated (Dekker *et al.*, 2007). All MRQAP<sub>dsp</sub> were run with 10,000 permutations. My data included a matrix of pairwise relatedness estimates between nesting female MPT ( $n = 57$ ). It also included matrices of pairwise Euclidean distances between nests for each study year that were constructed in R version 4.0.2 using the package 'raster', which converts latitude and longitude coordinates into a distance matrix in meters (Hijmans, 2020). I also constructed matrices containing pairwise differences in midCL and nesting Julian date between all turtles in my population. Values in these matrices were the absolute value of the difference in each attribute (nesting Julian data and midCL) for each possible pair of focal MPT.

In order to understand influences on nest proximity among female MPT, I performed two MRQAP<sub>dsp</sub> to calculate regression coefficients for each study year between pairwise nest

distances and three predictor attributes of nesting females: relatedness, size difference, and nesting date. MRQAP<sub>dsp</sub> does not calculate  $R^2_{\text{adjusted}}$  for each predictor in the model, rather an  $R^2_{\text{adjusted}}$  for the entire model is calculated. I therefore reported *Pearson's r* for correlations between each predictor attribute and pairwise nest distance from the R function '*cor*' (R Core Development Team, 2020). These correlation coefficients, however, do not take into account other predictor variables in the model, rather the correlation strictly between the response variable and a single predictor variable.

To account for environmental influence on nest timing, I also performed MRQAP<sub>dsp</sub> to calculate the relationship between nesting date and body size difference for each study year. I reported *Pearson's r* between the predictor and response variable using the R function '*cor*' (R Core Development Team, 2020). I performed a two-sample Welch's t-test using the R function '*t.test*' to determine if there was a significant difference in turtle nesting date across my two study years (R Core Development Team, 2020).

## Results

The mean pairwise relatedness between the 57 nesting female MPT included in my study was  $0.025 \pm 0.057$ , and median relatedness was 0. Relatedness estimates ranged from 0 to 0.53 (Figure 1.2). I detected 1496 unrelated dyads ( $r = 0$ ), 84 first cousin dyads ( $r = 0.125$ ), 12 half-sibling/avuncular/grandparent-grandchild dyads ( $r = 0.25$ ), and 4 half-sibling/parent-offspring dyads ( $r = 0.5$ ; Figure 1.2). The maximum distance between nests in 2018 was  $156.1 \pm 0.3$  m, and the minimum distance was a case where the coordinates of two nests were identical based on a GPS accuracy of  $\pm 0.3$  m. The maximum distance between nests in 2019 was  $159.1 \pm 0.3$  m, and the minimum distance was  $0.17 \pm 0.3$  m. Relatedness of nesting females was not

significantly correlated with nest distance in either sample year (2018:  $p = 0.99$ ,  $R^2_{adjusted} = -0.001$ ; 2019:  $p = 0.84$ ,  $R^2_{adjusted} = -0.001$ ; Table 1.3).

Difference in nesting date was positively and significantly correlated with distances between nests in 2019, such that nests laid at a greater temporal distance were also more physically distant from each other; the correlation was also positive in 2018, though not significant (2018:  $p = 0.43$ ,  $R^2_{adjusted} = 0.002$ ; 2019:  $p = 0.003$ ,  $R^2_{adjusted} = 0.01$ ; Table 1.3). Size difference was not significantly correlated with distance between nests in either sampling year (2018:  $p = 0.62$ ,  $R^2_{adjusted} = 0.0002$ ; 2019:  $p = 0.98$ ,  $R^2_{adjusted} = -0.0006$ ; Table 1.3). Nesting occurred significantly later in 2019 relative to 2018 ( $t = -14.99$ ,  $df = 107.64$ ,  $p = 0.02$ ). Body size was positively and significantly correlated with difference in nesting date in both sample years, such that larger MPT nested later in the season (2018:  $p = 0.006$ ,  $R^2_{adjusted} = 0.004$ ; 2019:  $p = 3.62 \times 10^{-8}$ ,  $R^2_{adjusted} = 0.012$ ; Figure 1.4; Table 1.4).

## Discussion

Euclidean distances between the nests made by 57 MPT across two nesting seasons were correlated with turtle body size and nesting date. Larger females nested later in the season in both study years (Table 1.4). Nests laid later in the 2019 season were at a greater Euclidean distance from previously laid nests (Table 1.3). There was no correlation between nest distance and relatedness such that related females did not nest closer to each other, suggesting that kin bias does not play a role in nest site choice in the MPT at Wolf Howl Pond. This result does not support my prediction that the distance between MPT nests should decrease with increasing relatedness of nesters, nor that inclusive fitness may be involved in female turtle nesting behaviour.

The pattern of nest placement I observed in MPT is in contrast with previous research on kin-bias in oviparous species. Social systems in reptiles often involve associations between kin (e.g., Davis *et al.*, 2011; Davis, 2012; Clark *et al.*, 2012). Accordingly, a number of reptile species are known to discriminate between kin and non-kin via chemoreception (e.g., Aragón *et al.*, 2001; Poschadel *et al.*, 2006; Pernetta *et al.*, 2008), including one turtle species (*Apalone spinifera*; Whitear *et al.*, 2016). Turtles are also known to discriminate individual characteristics of conspecifics by chemosense (Poschadel *et al.*, 2006; Ibáñez *et al.*, 2014). Given that the characteristic pre-oviposition ground-nuzzling behaviour performed by MPT has been implicated as a chemosensory mechanism (Iverson *et al.*, 2016), it is plausible that MPT could discern the identity or kin status of females who previously nested. Testing such a hypothesis was beyond the scope of my research, but my results do not support a kin-based nesting system in MPT (Figure 1.3). This, however, does not preclude the ability of nesting MPT to know the maternal identity of nests based on chemosense. My results suggest that kin selection is not a major influence on nest site choice in MPT. Given that MPT are known to nest at non-random densities, the influence of other factors should be considered to describe the nesting behaviour of MPT.

My results suggest that the timing of nesting is dependent on female body size in MPT, such that larger individuals nest later in a given season (Figure 1.4). Body size is correlated with a number of reproductive traits in turtles. Clutch size, egg mass, individual clutch mass, and annual clutch mass are all positively correlated with body mass in many turtle species (reviewed in Iverson, 1992). Accordingly, the energetic cost of clutch production should also follow this trend. Basking, the primary thermoregulatory behaviour of emydid turtles, is well-studied in the Wolf Howl Pond MPT population. Females in this population are known to bask more frequently

and for longer periods than males just prior to and during the nesting season (Krawchuck & Brooks, 1998). However, basking bout length in this population does not increase with body size (Lefevre & Brooks, 1995). In ectothermic animals, heating and cooling are dependent on body size, such that larger individuals experience greater thermal inertia (Grigg *et al.*, 1979). Further, a set amount of heat is required to successfully complete metabolic processes (Obbard & Brooks, 1987; Holt, 2000); therefore, larger MPT must bask for a longer cumulative duration to meet their energetic requirements. These requirements may be heightened due to the larger egg and clutch sizes these larger turtles produce (Iverson, 1992). My results suggest that larger MPT nest later in the season (Figure 1.4). This finding, coupled with the assertion of Krawchuck & Brooks (1998) that basking bout length does not depend on individual size, provide support for a hypothesis that the energetic requirements of nesting in larger MPT prevent them from nesting early in a given season. Larger MPT may bask more frequently, and into the start of a nesting season, to meet the energetic requirements of clutch development. This may prevent social effects (i.e., kin selection) from having substantial influence over the nesting behaviour of MPT, given that the time at which individual females are energetically prepared to nest may differ within a population.

Weather conditions varied greatly between the two nesting seasons, and the effects of these differences need to be considered in light of the observed behavioural patterns. Nesting occurred significantly later in the 2019 season, and the initiation of the 2019 nesting season (i.e., the first nest laid) was the latest on record since the long-term study began in 1978 (R. Brooks *et al.*, unpubl. data). The delay in nesting may have resulted from mean temperatures across the critical periods of energetic gain for reproductively active females being lower in the 2019 season (Government of Canada: Historical Weather and Climate Data). There are two periods

identified as critical in meeting the energetic costs of nesting: 1) the late spring (~1 May – ~ 10 June), just prior to nesting, when females store energy necessary to successfully lay a clutch (Congdon *et al.*, 1983b; Krawchuck & Brooks, 1998; Lovich *et al.*, 2012), and 2) the fall prior to nesting (~ 1 August – ~ 31 October), when it is hypothesized that the majority of follicular development occurs (Rollinson & Brooks, 2007b). Mean temperatures in these critical time periods were consistently lower in the 2019 season (including the fall of 2018, when development of follicles for 2019 reproductive activity took place; Government of Canada: Historical Weather and Climate Data).

Wolf Howl Pond MPT reside at the species' northern range limit (Ernst & Lovich, 2009; Gervais *et al.*, 2009; Barela & Olson, 2014). Thus, their active season is truncated relative to more southern populations, and their energetic expenditure is more constrained, particularly with respect to reproductive output (Ernst & Lovich, 2009). These constraints may further magnify the effects of suboptimal seasonal conditions, as were experienced by the population in 2019, and produce abnormal behavioural patterns. I acknowledge the possibility that the size-assortative nest timing I observed in the Wolf Howl Pond MPT may be an artefact of abnormal weather and temperature regimes across my sampled seasons. Painted Turtles have been observed previously exhibiting the opposite pattern to that which I observed. Larger females in two southern Painted Turtle populations are known to nest earlier in a season than smaller, inexperienced females (Gibbons & Greene, 1990; Bowden *et al.*, 2004). Further research is required to determine the biological relevance of the size-assortative temporal pattern I detected.

My observation of nesting behaviour in the Wolf Howl Pond MPT population presents a number of opportunities for further research. Firstly, additional mapping of nest placement by females is necessary. I acknowledge that the climatic conditions across one of my sample

seasons (2019) were suboptimal, and that this abnormality may have contributed to the temporal and spatial patterns I observed. Mapping of focal females' nesting activities across a longer timespan could elucidate behavioural patterns not detectable with only two years of data. The use of long-term trends will provide more concrete evidence about the influence of social and environmental factors on the nesting behaviour of MPT. Further, more comprehensive observation of pre-oviposition behaviour in MPT could describe the use of social information by nesting females. Researchers could track the location of all pre-oviposition nesting activity by focal females to determine if it is centered around areas where focal individuals' kin have nested. Future research could also examine the post-hatch behaviour of MPT. Hatchling MPT are known to exhibit hatching and emergence synchrony (Spencer *et al.*, 2001), though the impact of this phenomenon on post-hatch success or behaviour is unknown. Further, researchers should examine whether clutchmates remain grouped post-hatch, and if hatchlings of nests laid by close kin are more or less likely to interact once hatched. There is evidence of hatchling turtles remaining in conspecific groups post-hatch (Butler & Graham, 1995; Ibáñez *et al.*, 2015), and demonstrating post-hatch close-kin avoidance (Whitear *et al.*, 2016). This phenomenon has not been tested in MPT.

I acknowledge a limitation of my research is that nesting behaviour was not observed in real time. By utilizing only final nest placement as a measure of social coordination, it is possible that I missed informative social interactions pre-oviposition. Future research on nest placement in MPT should strive to observe and document the nesting behaviour of individual MPT in its entirety. I also acknowledge that I detected low numbers of highly related MPT. This low detection may have decreased my ability to discern a biologically relevant pattern of kin-biased nesting behaviour. However, the distribution of relatedness values in my focal females is similar

to the overall distribution of values in the entire Wolf Howl Pond MPT population (C. Rouleau *et al.*, unpubl. data). That being said, different populations of MPT and other turtles may hold higher numbers of closely-related individuals, as it is well-known that ecological features of the landscape can affect sociality of a species. Thus, although I did not find evidence for kin-based nesting behaviour in my population of MPT, this should not be extrapolated definitively across this species' range or to other turtles.

The nesting behaviour of MPT, while well-studied, is still not fully understood. Given the reptilian potential for kin-based social organization (reviewed in the order Squamata by Gardner *et al.*, 2016), reptiles should be examined more closely to determine if cryptic sociality may be an underlying influence on behaviour. I examined the potential for kin-bias in the nest placement of MPT. I have provided evidence that this bias is not present in MPT at Wolf Howl Pond (Algonquin Provincial Park, Ontario, Canada). Further, I proposed that environmental conditions, when suboptimal, may be the primary influence on nesting behaviour in MPT. Finally, I note that my findings should not be used to fully discount the use of social information by nesting MPT, and acknowledge that social information might be used during pre-oviposition nesting behaviours to inform nest site choice.

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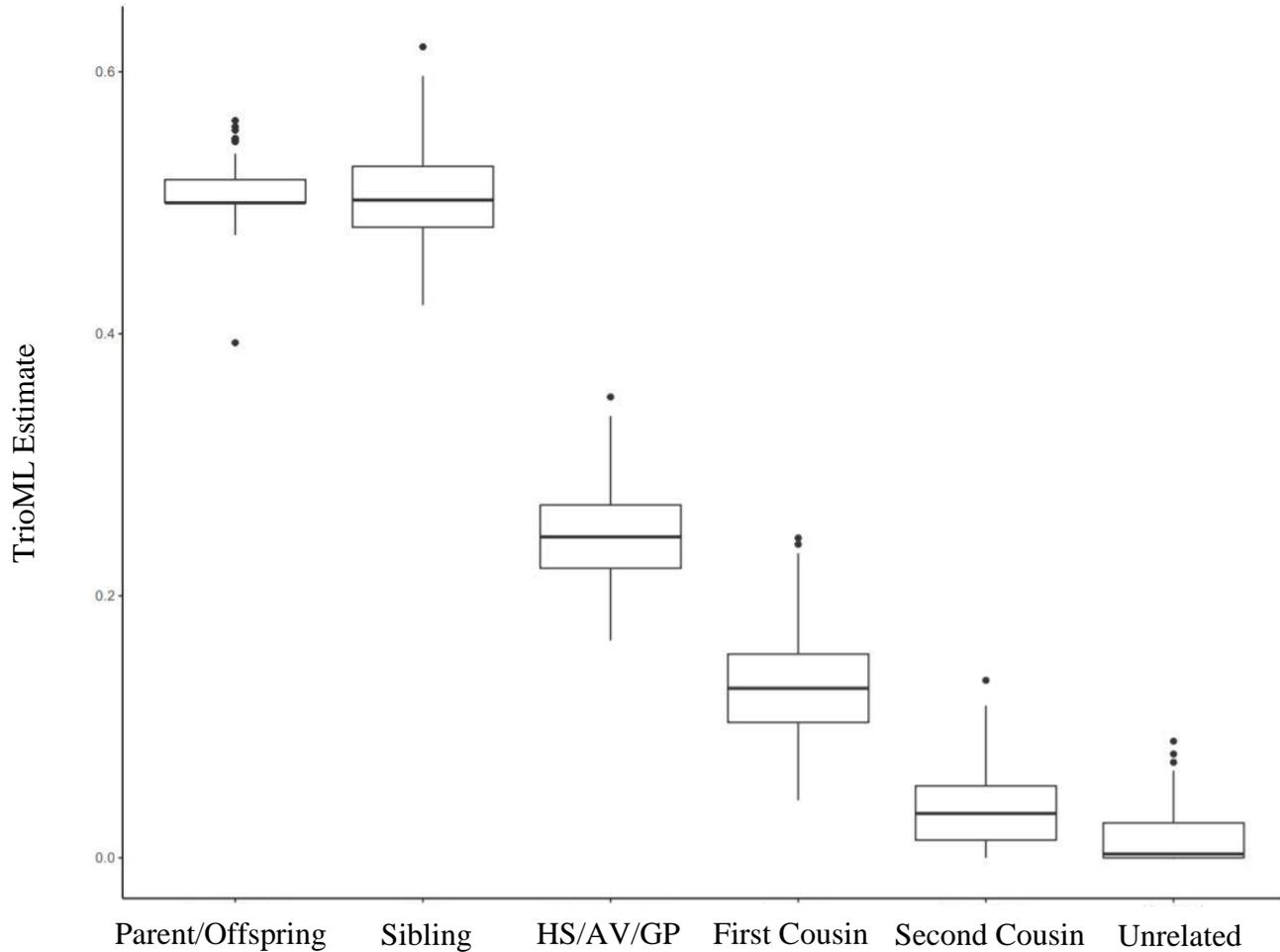
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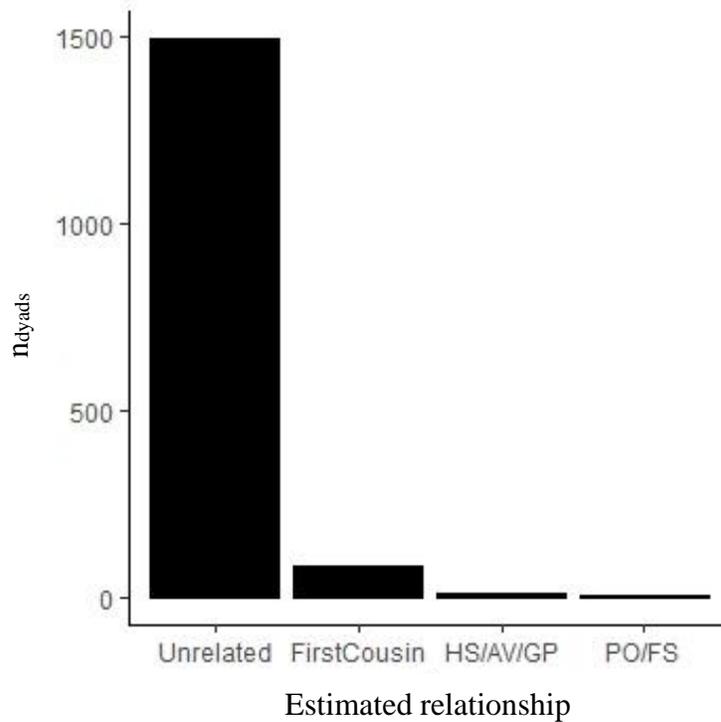
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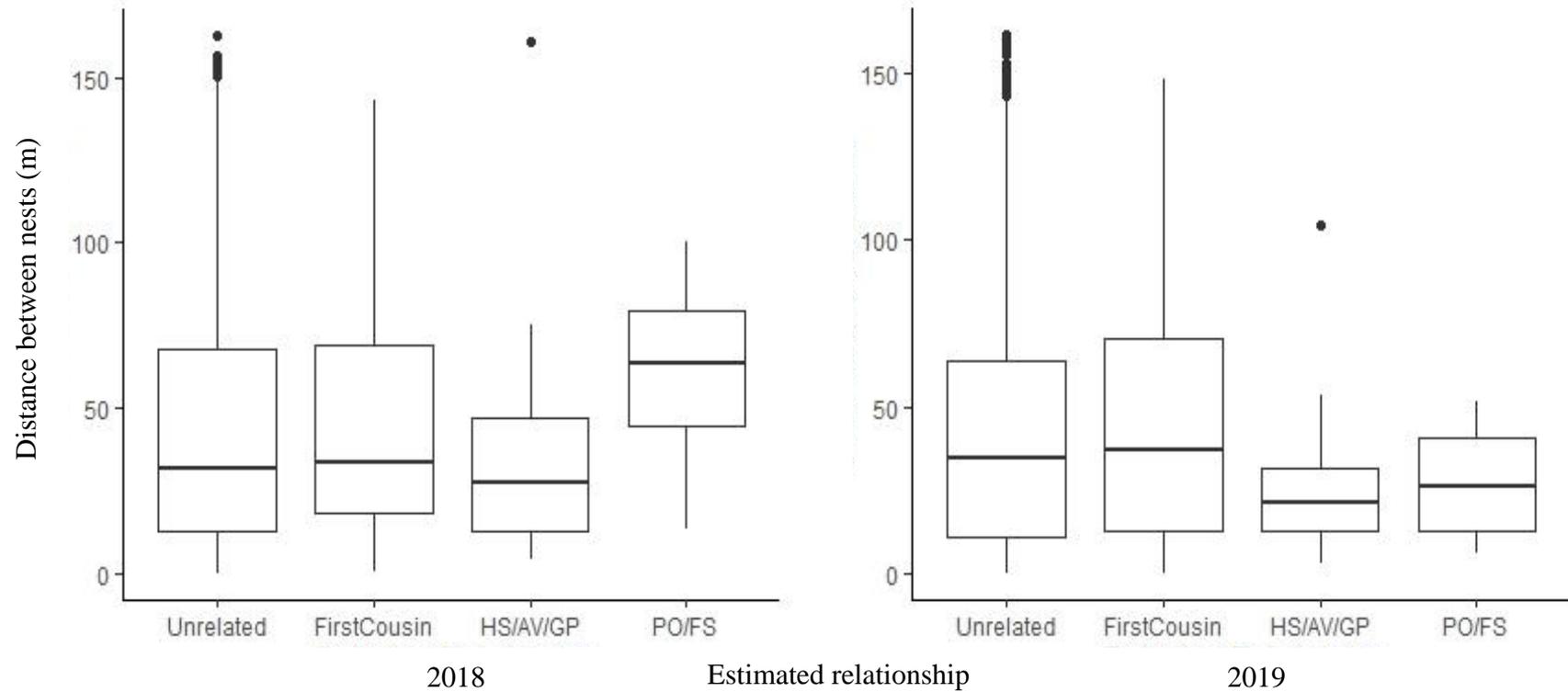
## Figures and Tables



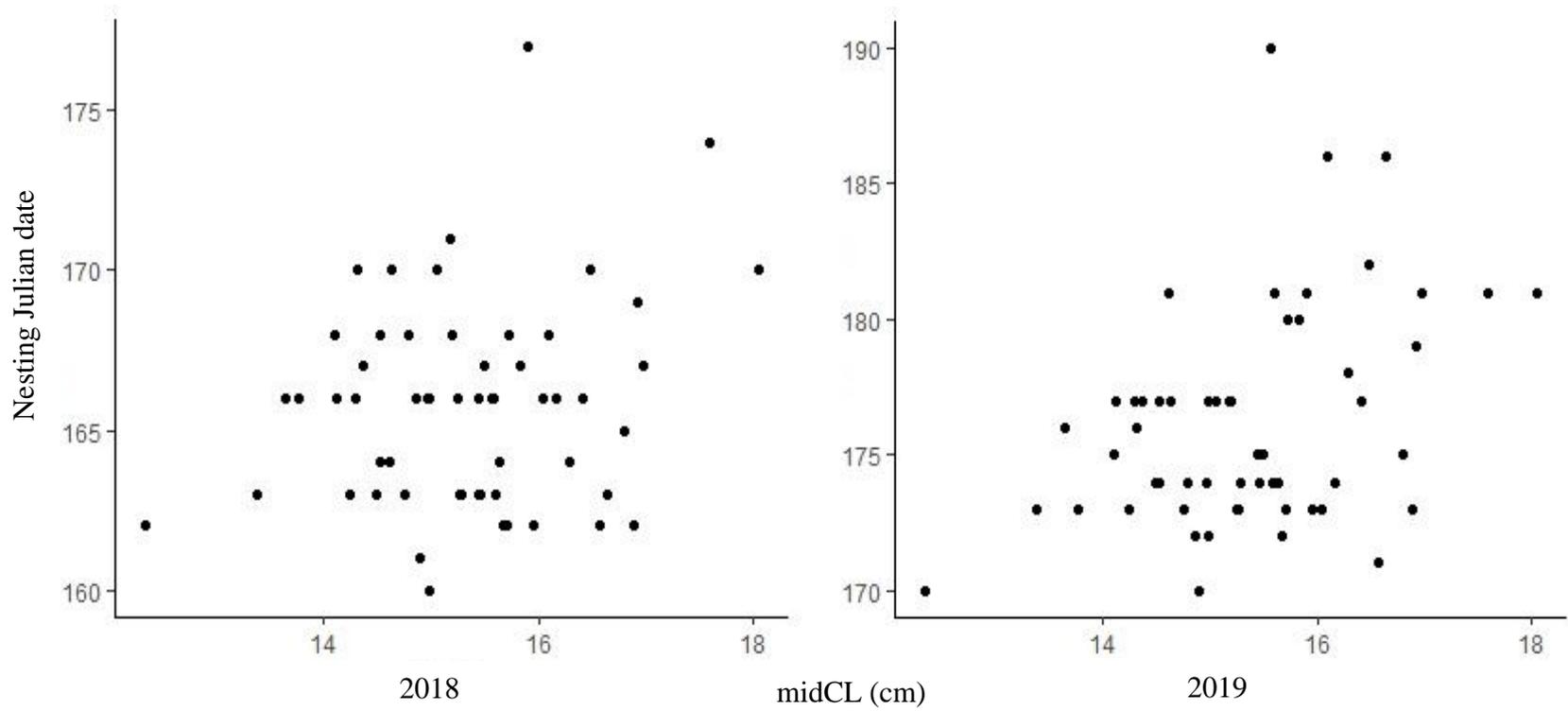
**Figure 1.1.** Density plots of triadic maximum likelihood (TrioML) relatedness estimates based on data simulated using allelic frequencies from the Wolf Howl Pond (Algonquin Provincial Park, Ontario, Canada) Midland Painted Turtle (*Chrysemys picta marginata*) population. Each bin represents the spread of TrioML values calculated for simulated individuals ( $n = 100$ ) whose relatedness values were given based on six levels of relationship: Parent/Offspring,  $r = 0.5$ ; Sibling,  $r = 0.5$ ; Half-siblings/Avuncular/Grandparent & Grandchild (HS/AV/GP),  $r = 0.25$ , First Cousin,  $r = 0.125$ ; Second Cousin,  $r = 0.0313$ ; Unrelated,  $r = 0$ . All simulated individuals in each bin have an actual relatedness exactly equal to their bin designation. Less overlap between bins indicates the ability of the TrioML method to distinguish between different relationships through numerical relatedness.



**Figure 1.2.** Estimated relationships of Wolf Howl Pond (Algonquin Provincial Park, Ontario, Canada) Midland Painted Turtles (*Chrysemys picta marginata*;  $n = 57$ ) based on triadic maximum likelihood relatedness estimates of relatedness. Relationships were based on relatedness estimates: unrelated ( $r = 0$ ), first cousin ( $r = 0.125$ ), half-sibling/avuncular/grandparent-grandchild (HS/AV/GP;  $r = 0.25$ ), parent-offspring/full sibling (PO/FS;  $r = 0.5$ ). All individuals are replicated 56 times, such that counts represent all possible pairwise combinations of focal MPT ( $n = 1596$  dyads).



**Figure 1.3.** Density plots of pairwise distance between nests based on estimated genetic relationships of nesting Wolf Howl Pond (Algonquin Provincial Park, Ontario, Canada) Midland Painted Turtles (*Chrysemys picta marginata*,  $n = 57$ ). Estimated relationships are based on triadic maximum likelihood relatedness estimates: Unrelated ( $r = 0$ ;  $n = 2912$ ), First Cousin ( $r = 0.125$ ;  $n = 168$ ), Half-sibling/Avuncular/Grandparent-Grandchild (HS/AV/GP;  $r = 0.25$ ;  $n = 24$ ), Parent Offspring/Full Sibling (PO/FS;  $r = 0.5$ ;  $n = 8$ ). All individuals are replicated 56 times, such that data points represent all possible pairwise combinations of focal MPT ( $n = 1596$  dyads).



**Figure 1.4.** Nesting date by midline carapace length (midCL) in Wolf Howl Pond (Algonquin Provincial Park, Ontario, Canada) Midland Painted Turtles (*Chrysemys picta marginata*;  $n = 57$ ).

**Table 1.1.** Filtering criteria for raw single-nucleotide polymorphism genotyping data on the Wolf Howl Pond (Algonquin Provincial Park, Ontario, Canada) population of Midland Painted Turtles (*Chrysemys picta marginata*). Any loci meeting the filtering value criteria for each step were removed from the analysis. All filtering was performed in R using the package ‘dartR’ (Gruber *et al.*, 2019; R Core Development Team, 2020).

| Step no. | Filter                   | Function  | Filtering value  | Number of loci        |
|----------|--------------------------|---|--|-----------------------|
| 0        | -                        | -   | -  | 66832 (pre-filtering) |
| 1        | Monomorphism             | Removes loci for which there is no variation across the entire dataset                          | All monomorphic or duplicated loci                         | 43349                 |
| 2        | Coverage                 | Removes loci with read depths below the lower threshold and above the upper threshold           | Lower limit – 2<br>Upper limit – 7                         | 34406                 |
| 3        | Repeatability            | Removes loci with repeatability lower than filtering value                                      | 99%  | 13210                 |
| 4        | Call Rate                | Removes loci with a higher proportion of missing values than the filtering value                | 99%  | 1431                  |
| 5        | Hamming Distance         | Removes loci with lower base pair variation than the filtering value                            | 25% of base pairs showing variation across all individuals | 1394                  |
| 6        | Minor Allele Frequency   | Removes loci with lower minor allele frequency than the filtering value                         | 2%   | 1015                  |
| 7        | Hardy-Weinberg Deviation | Removes loci for which allelic frequencies differ significantly from Hardy-Weinberg Equilibrium | $\alpha = 0.05$  | 1015                  |

**Table 1.2.** *Pearson's r* comparing known and estimated relatedness from simulated genotypic data ( $n = 100$  simulated individuals) based on allelic frequencies at target loci from the Wolf Howl Pond (Algonquin Provincial Park, Ontario, Canada) population of Midland Painted Turtles (*Chrysemys picta marginata*).

| <b>Relatedness Estimator</b>               | <b><i>Pearson's r</i></b> |
|--|---------------------------|
| Triadic Maximum Likelihood (Wang, 2007)    | 0.99582                   |
| Dyadic Maximum Likelihood (Milligan, 2003) | 0.99579                   |
| Lynch & Ritland (1999)                     | 0.99310                   |
| Queller & Goodnight (1989)                 | 0.99060                   |
| Wang (2002)                                | 0.98950                   |
| Li <i>et al.</i> (1993)                    | 0.98947                   |
| Ritland (1996)                             | 0.98393                   |

**Table 1.3.** Summary statistics of correlations by multiple regression quadratic assignment procedure with double semi-partialing (MRQAP<sub>dsp</sub>) of Euclidean distance between nests and attribute matrices (relatedness, size difference [midCL], difference in nesting date) for female Wolf Howl Pond (Algonquin Provincial Park, Ontario, Canada) Midland Painted Turtles (*Chrysemys picta marginata*;  $n = 57$ ). MRQAP<sub>dsp</sub> for both sampling years were run with 10000 permutations. *Pearson's r* values for each predictor variable were calculated using the base R function 'cor'. Superscript (\*) denotes a significant correlation ( $\alpha < 0.05$ ).

|              | 2018                            |       |                    | 2019                           |       |                    |
|--------------|---------------------------------|-------|--------------------|--------------------------------|-------|--------------------|
|              | $\beta$                         | $p$   | <i>Pearson's r</i> | $\beta$                        | $p$   | <i>Pearson's r</i> |
| Intercept    | 45.28                           | 0.00* | -                  | 38.33                          | 0.00* | -                  |
| Relatedness  | 1.00                            | 0.99  | -0.001             | -4.12                          | 0.84  | -0.001             |
| midCL        | -1.10                           | 0.62  | 0.0002             | -0.25                          | 0.98  | -0.0006            |
| Nesting date | 0.22                            | 0.43  | 0.002              | 1.07                           | 0.03* | 0.01               |
|              | Model $R^2_{adjusted} = -0.003$ |       |                    | Model $R^2_{adjusted} = 0.007$ |       |                    |

**Table 1.4.** Summary statistics of correlations by multiple regression quadratic assignment procedure with double semi-partialing (MRQAP<sub>dsp</sub>) of size difference (midCL) and difference in nesting Julian date for female Wolf Howl Pond (Algonquin Provincial Park, Ontario, Canada) Midland Painted Turtles (*Chrysemys picta marginata*;  $n = 57$ ). MRQAP<sub>dsp</sub> for both sampling years were run with 10000 permutations. *Pearson's r* values for each predictor variable were calculated using the base R function 'cor'. Superscript (\*) denotes a significant correlation ( $\alpha < 0.05$ ).

|           | 2018    |        |                    | 2019    |                         |                    |
|-----------|---------|--------|--------------------|---------|-------------------------|--------------------|
|           | $\beta$ | $p$    | <i>Pearson's r</i> | $\beta$ | $p$                     | <i>Pearson's r</i> |
| Intercept | 3.35    | 0.00*  | -                  | 3.66    | 0.00*                   | -                  |
| midCL     | 0.22    | 0.006* | 0.004              | 0.56    | 3.62x10 <sup>-8</sup> * | 0.02               |

## Chapter II: Social organization in basking aggregations of the Midland Painted Turtle (*Chrysemys picta marginata*)

**Abstract:** Basking is extensively documented in reptiles, and in some reptile groups, notably scincids (Squamata: Scincidae), basking congregations are representative of repeatable and stable social groups. Evidence for sociality in the basking behaviour of other reptilian taxa is less reported. If aggregated basking provides a survivorship or fitness benefit, then basking reptiles should organize themselves in a manner that maximizes individual fitness. I predicted that basking congregations in the Midland Painted Turtle (MPT; *Chrysemys picta marginata*) would be kin-biased, and that social behaviour would differ based on individual body size. I observed basking behaviour in a population of MPT over two active seasons, and used social network analysis to construct matrices of social association between basking individuals, and explored possible predictors of their social affinity (i.e., genetic relatedness, body size, spatial overlap, and sex). I also calculated four metrics of social behaviour for individual MPT: i) binary degree, a count of the number of associations an individual maintained, ii) weighted degree, a sum of the strengths of all associations an individual maintained, iii) coefficient of variation of edge weights, a measure of homogeneity and interconnectedness of social groupings, and iv) betweenness, a measure of social connectivity, and statistically examined whether body size and a boldness proxy explained this trait on the individual-level. I found that MPT associate with close-kin while basking, and that basking behaviour differed between the sexes. I found evidence that body size affects the number of associations an individual turtle maintains, such that larger individuals maintain fewer social associations which is suggestive of a potential size-based social hierarchy. I also found evidence that male MPT associate with a higher number of individuals than females, which may be related to sex-specific differences in mating behaviour. My results support both my prediction of kin-bias in the basking behaviour of MPT, and of size differences. My findings have implications for the evolution of cryptic sociality, and call for more thorough examination of social organization in a wider range of taxa.

## Introduction

Basking, or sunning, has long been recognized as a critical component of the life history of ectothermic animals, and in particular, reptiles. Bogert (1949) and Cagle (1950) proposed that, among other purposes, basking is a primarily thermoregulatory practice. Support has since become widespread for thermoregulation being the primary purpose of basking (e.g., Boyer, 1965; Auth, 1975; Crawford *et al.*, 1983; Schwarzkopf & Brooks, 1985; Carrière *et al.*, 2008; Bulté & Blouin-Demers, 2010a; Sears *et al.*, 2016), though it is still suggested that some species do not use basking in this capacity (Manning & Grigg, 1997). Basking has also been implicated in the performance of non-thermoregulatory functions (reviewed completely in Congdon, 1989) including digestion (Harwood, 1979; Hennemann, 1979; Sturbaum, 1982; Chessman, 1987; Hammond *et al.*, 1988), removal of external parasites and growths (Cagle, 1950; Neill & Allen, 1954; Boyer, 1965; Ryan & Lambert, 2005), vitamin D synthesis (Moll & Legler, 1971; Ferguson *et al.*, 2003; Peterman & Ryan, 2009), infection fighting (Monagas & Gatten, 1983) and reproduction (Congdon & Tinkle, 1982; Whittow & Balasz, 1982; Schwarzkopf & Shine, 1991; Wapstra, 2000; Bulté & Blouin-Demers, 2010b).

Basking has been extensively documented in the emydid turtles (Testudines: Emydidae), who are known as frequent atmospheric (i.e., terrestrial) and aquatic baskers (e.g., Lindeman, 1999; Peterman & Ryan, 2009; *Chrysemys picta*: Schwarzkopf & Brooks, 1985; Lovich, 1988; 1990; Lefevre & Brooks, 1995; *Graptemys geographica*: Ben-Ezra *et al.*, 2008; Bulté & Blouin-Demers, 2010a,b; *Trachemys scripta*: Cagle, 1950; Crawford *et al.*, 1983; Spotila *et al.*, 1984; Hammond *et al.*, 1988; Cadi & Joly, 2003; *Glyptemys insculpta*: Harding & Bloomer, 1979; Dubois *et al.*, 2009). Emydids atmospherically bask on a wide range of substrates, most notably fallen logs and vegetation mats that allow them to fully remove themselves from water (Boyer,

1965; Moll & Legler, 1971; Lindeman, 1999; Peterman & Ryan, 2009). Many emydids live in high densities, and basking space can be limited (Lindeman, 1999). Accordingly, turtles are often seen in large basking congregations (e.g., Cagle, 1950; Boyer, 1965; Schwarzkopf & Brooks, 1985; Lindeman, 1999).

The benefits of communal basking in reptiles have been most thoroughly examined in Egernia-group (Squamata: Scincidae: Egerniinae) skinks. Communally basking Egernia-group skinks have enhanced vigilance during basking (Lanham & Bull, 2004), more consistent access to higher quality basking sites (Duffield & Bull, 2002; Gardner *et al.*, 2007; Michael *et al.*, 2010), and higher juvenile survivorship (O'Connor & Shine, 2004) relative to solitary baskers. Maximizing basking efficiency, through both habitat selection and time spent basking, can impact the fitness of basking ectotherms, and have significant effects on their energy budgets (Bulté & Blouin-Demers, 2010b). When these benefits are gained through communal basking, kin-selection theory suggests that they should be maximized when basking aggregations are kin-biased, thus maximizing inclusive fitness benefits to the individual (Hamilton, 1964a,b).

While the benefits of communal basking in non-Egernia-group reptiles are not known, the act of communal basking in itself can be considered socially tolerant behaviour (Réale *et al.*, 2007), and thus may be representative of cryptic sociality (Kappeler, 2019). A number of turtle species are noted as being tolerant of conspecifics sharing a basking structure (*Terrapene carolina carolina*: Dolbeer, 1969; Madden, 1975; *Terrapene coahuila*: Brown, 1974; *Graptemys geographica*: Flaherty & Bider, 1984; *Glyptemys insculpta*: Kaufmann, 1992). In some instances, group basking is thought to occur as a result of limited suitable basking habitat (e.g., Bury & Wolfheim, 1973; Lindeman, 1999), as per the ecological constraints hypothesis, which has been invoked to explain patterns of association in communally basking Cordylid (Squamata:

Cordylidae) lizards (Visagie *et al.*, 2005). These ecologically-driven associations can facilitate the evolution of sociality in a species if its grouped presence is selectively advantageous (Halliwell *et al.*, 2017). Further, the distinction between ecologically-based associations and grouping strictly for social purposes is key, as the evolution and maintenance of both are notably different (Graves & Duvall, 1995; Wey *et al.*, 2008; van Veelen *et al.*, 2010; Halliwell *et al.*, 2017).

Body size is the main known influence on basking activity in turtles. When basking habitat is limited, it is likely that body size is a key determinant of basking frequency, given that success in potentially antagonistic interactions is often dependent on body size (*T. scripta*: Auth, 1975; *G. geographica*: Pluto & Bellis, 1986; *T. scripta*, *Pseudemys concinna*, *G. pseudogeographica*, *G. ouachitensis*: Lindeman, 1999). Further, some emydid species are known to avoid potentially aggressive encounters with larger conspecifics (*Actinemys marmorata*: Bury & Wolfheim, 1973; *Emys orbicularis*: Poschadel *et al.*, 2006; *Mauremys leprosa*: Ibáñez *et al.*, 2014). However, basking habitat is not always a limited resource in turtle populations. Examining a population too small to occupy all possible high quality basking sites, Flaherty & Bider (1984) suggested that social factors are likely responsible for the observed distribution of individuals across basking sites.

The objective of my research was to investigate patterns of aggregation in the basking behaviour of the Midland Painted Turtle (MPT; *Chrysemys picta marginata*). To address these objectives, the basking habits in a population of MPT were observed over a three-week period at the beginning of their active season in two consecutive years. Genetic samples were collected from focal turtles to examine potential patterns of kin association in basking behaviour. I predicted that individual MPT would preferentially associate with close-kin, due to inclusive

fitness benefits. Further, I predicted that the social behaviour of MPT would differ based on individual body size.

## Methods

### *Study site and population*

My focal group is a population of approximately 225 MPT living in Wolf Howl Pond, a wetland within Algonquin Provincial Park (45°34' N, 78°41' W). This population size estimate is based upon 42 years of mark-recapture data (R. Brooks *et al.*, unpubl. data; Samson, 2003). Wolf Howl Pond is a Black Spruce (*Picea mariana*) bog containing partially submerged logs and floating mats of Sphagnum (*Sphagnum sp.*), Bladderwort (*Utricularia vulgaris*), Bog Leatherleaf (*Chamaedaphne calyculata*), Marsh Spike-Rush (*Eleocharis smallii*) and White Beakrush (*Rhynchospora alba*). These structures are common basking habitat for turtles (Schwarzkopf & Brooks, 1985; C. Rouleau pers. obs.). MPT occur at a density of 128.6 turtles/ha in Wolf Howl Pond (M. Keevil & S. Sanders, unpubl. data.). Wolf Howl Pond MPT are sympatric with populations of Common Snapping Turtles (*Chelydra serpentina*) and Blanding's Turtles (*Emydoidea blandingii*), and have been observed basking, feeding, and swimming in close proximity to, and occasionally in physical contact with, both species (J. Kentel & C. Rouleau, pers. obs.).

Wolf Howl Pond MPT are participants in a long-term mark-recapture study, and have unique numeric or alphanumeric identities. These identities are based on one of three forms of marking: i) marginal scute notch codes (based on Cagle, 1939), ii) aluminum ID tags affixed to posterior marginal scutes (described for *C. serpentina* by Loncke & Obbard, 1977), and iii) Passive Integrated Transponder (PIT) tags implanted into a turtle's right posterior inguinal space

(Smyth & Nebel, 2013). Turtles may bear one, two, or all of these markings, as each has been used at different times throughout the long-term study. For my study, turtles were captured by dip net or hand from canoes in April/May of 2019/2020, and transported to the Algonquin Wildlife Research Station to collect morphometric data and visually-mark each individual. Midline carapace measurements (midCL) were taken with 20 cm Vernier calipers to the closest 0.01 cm. All turtles had their unique identities painted across their carapace in TREMCLAD® oil-based paint, to allow for identification at a distance with binoculars in the field. These markings are lost when turtles shed their scutes at the beginning of their active season each year, but remained on all turtles throughout my observations of their basking behaviour.

### *Basking surveys*

Basking surveys began each season when turtles in Wolf Howl Pond had been exhaustively sampled (Table 2.1; M. Keevil & S. Sanders, unpubl. data). Two surveys were conducted daily, at 1000 h and 1600 h, in the late spring of 2019 and 2020. During surveys, the pond was visually scanned using binoculars from its perimeter, and then in a canoe by a pair of researchers. Surveys lasted approximately one hour, and researchers left the site directly after completing morning surveys, and did not return until beginning afternoon surveys. Researchers were consistent between all surveys within a year; CJR and Jenna Kentel conducted all surveys in 2019, and CJR and Brooke Carroll conducted all surveys in 2020. This allowed all possible basking locations to be observed while minimizing disturbance to the turtles. The identity and location of each turtle basking at the time of the survey were recorded. Turtles who left one basking location and began basking at another during the survey were only noted at their initial location; this approach minimized pseudoreplication. A turtle was considered to be basking when

it was stationary with its carapace completely or partially out of the water, as is consistent with a definition of atmospheric basking (Chessman, 1987).

All known basking structures ( $n = 118$ ) in Wolf Howl Pond were marked with numbered pin flags in the spring of 2019. When turtles were observed basking on an unmarked structure, the structure's location was noted to allow for consistent identification. A total of 77 additional, unmarked structures were used over the two years of surveys. Basking structures in Wolf Howl Pond vary greatly in size and shape. Many structures are large enough to support multiple individuals or groups of basking turtles who could plausibly be unaware of each other's presence (J. Kentel & C. Rouleau, pers. obs.) by virtue of obstructed sightlines or excessive distance between individuals. The visual capabilities of emydid turtles are largely unknown, although Tortoises (Testudines: Testudinidae) and Sea Turtles (Testudines: Cheloniidae) have highly adept visual perception (Mäthger *et al.*, 2007; Wilkinson *et al.*, 2010). Wilkinson *et al.* (2010) found that Red-Footed Tortoises (*Geochelone carbonaria*) could follow the gaze of a conspecific within an 80 cm-long enclosure. This suggests a confirmed sight range of 80 cm in Testudines. Basking structures were thus conservatively subdivided into 60 cm quadrats. Turtles sharing a structure (or quadrat, when structure division was necessary) were considered to be basking in a group. Turtles more than 60 cm apart were considered to be basking alone. I considered two turtles basking at a distance greater than 60 cm to be socially independent of each other, such that they were not exhibiting tolerance of each other.

#### *Genetic data collection & relatedness analysis*

Blood samples were collected from the caudal vein of turtles during the 2018, 2019 and 2020 active seasons. Samples were drawn using sterilized BD Ultra-Fine™ 1 mL insulin

syringes. Approximately 100  $\mu$ L of blood was sampled from each turtle. Samples were stored on Whatman<sup>®</sup> FTA<sup>®</sup> cards (Owen, 2011; Whatman, Inc.). DNA extraction and single-nucleotide polymorphism (SNP) genotyping of all samples was performed by Diversity Arrays Technology, Canberra, Australia (Jaccoud *et al.*, 2001), and yielded a dataset with 66,832 loci and mean call and reproducibility rates of  $82.651 \pm 0.200\%$  and  $97.854 \pm 0.025\%$ , respectively.

Filtering of genotypic data was performed in R version 4.0.2 using the package ‘*dartR*’ (Table 2.2; Gruber *et al.*, 2017; R Core Development Team, 2020). During filtering, I removed any monomorphic loci. When multiple polymorphisms were found within the same sequence, a single polymorphism was selected at random to minimize physical linkage of loci (Lemay & Rusello, 2015). I retained loci with a call rate and a reproducibility rate  $\geq 99\%$ . I calculated allelic coverage, and removed loci with read depths  $\leq 2$  and  $\geq 7$  (Lemay & Russo, 2015). Loci with minor allele frequencies  $\leq 2\%$  were filtered, as high minor allele frequency can bias genotypic data (Roesti *et al.*, 2012). When two loci had a Hamming distance (number of base differences)  $\leq 25\%$ , one locus was randomly selected to be included in the final dataset (Gruber *et al.*, 2017). I also filtered loci that differed significantly from Hardy-Weinberg Equilibrium ( $\alpha < 0.05$ ). The final filtered dataset had 1015 loci, and call and reproducibility rates of  $99.57 \pm 0.0031\%$  and  $99.62 \pm 0.0032\%$ , respectively.

Post-filtering, I estimated pairwise relatedness between individuals in the program COANCESTRY (Wang, 2011). First, I simulated multilocus data based on allelic frequencies from my genotypic dataset to select the appropriate relatedness estimator for the Wolf Howl Pond MPT population, because the accuracy of relatedness estimates depends on the genetic structure of a population (Van de Casteele *et al.*, 2001; Wang, 2011). I simulated 100 individuals with each of the following relatedness values:  $r = 0.000$  (unrelated),  $r = 0.031$  (second cousin),  $r$

= 0.125 (first cousin),  $r = 0.250$  (half-sibling/avuncular/grandparent-grandchild),  $r = 0.500$  (full sibling), and  $r = 0.500$  (parent-offspring). I calculated pairwise relatedness estimates for each of these simulated individuals using seven relatedness estimators (Queller & Goodnight, 1989; Li *et al.*, 1993; Ritland, 1996; Lynch & Ritland, 1999; Wang, 2002; Milligan, 2003; Wang, 2007). I then calculated Pearson correlation coefficients (*Pearson's r*) between actual relatedness of simulated individuals and estimated relatedness by each of the seven estimators. Triadic maximum likelihood relatedness estimates were most closely correlated to the true relatedness of the simulated individuals (*Pearson's r* = 0.996), and thus were used to estimate relatedness of turtles in my study population (Figure 2.1; Table 2.3; Wang, 2007).

### *Social network construction*

Before network construction, I examined whether data from my two study years should be combined or not. I, indeed, found that associations between individuals were significantly and positively correlated across study years ( $\beta = 0.070$ ,  $p = 0.042$ , *Pearson's r* = 0.005). I tested for this correlation using a multiple regression quadratic assignment procedure with double semi-partialing (MRQAP<sub>dsp</sub>) using the function '*mrqap.dsp*' in the R package '*asnipe*' (Krackhardt, 1988; Dekker *et al.*, 2007; Farine, 2013). MRQAP<sub>dsp</sub> is a permutation test for calculating multiple linear regression coefficients for data matrices, and can be interpreted similarly to a multiple regression, such that one variable can control for the effect of another. This method is commonly used in social network analyses, where parallel matrices of pairwise data for a set of focal individuals are correlated (Dekker *et al.*, 2007). Accordingly, all social networks were constructed using pooled data from both years of basking surveys.

So, from both years of basking behavioural data, I constructed three networks of social associations between Wolf Howl Pond MPT (Farine & Whitehead, 2015). One network contained all turtles observed eight or more times during basking surveys ( $n = 88$ ). This network was constructed because spatial overlap estimations (as described below) were not accurate for turtles observed less than eight times, and used for testing relationships between social association and four predictor variables (as described below). The other two networks contained all observed individuals of a single sex ( $n = 157$  females;  $n = 32$  males). These networks were used for calculating four descriptive social network metrics and examining relationships between these metrics and two predictor variables (as described below). Female turtles in Wolf Howl Pond are significantly larger than males (analyzed using a Student's  $t$ -test:  $t = -6.420$ ,  $df = 46.642$ ,  $p < 0.001$ ), so the sexes were analyzed separately to allow for the inclusion of body size as a predictor. Juveniles, defined as turtles with a midCL  $< 9$  cm or for whom sex could not be accurately determined, were not included in any social networks.

Within each network, association strength for each possible dyad (pair of turtles) was calculated using the Half-Weight Association Index (HWI), which ranges from 0 (pair never in a group during a basking survey) to 1 (pair in a group during all basking surveys). HWI is commonly used to analyze grouping data where the complete population is unlikely to be observed in a sampling period (Cairns & Schwager, 1987; Whitehead, 2008), as is the case with my study population. Networks were weighted and undirected, such that nodes were connected when  $HWI > 0$  between them. Networks were constructed in the R environment using the package '*asnipe*' using the functions '*get\_group\_by\_individual*' and '*get\_network*' (Farine 2013; R Core Development Team, 2020).

I also calculated preferred social associations for the network including both sexes ( $n = 88$ ). To calculate preferred associations, 10,000 permutations of a random group-by-individual matrix containing the same dyads as the observed matrix, but where group membership was randomly assigned in all cases, were run (Farine, 2013). Then, I calculated 95% quantiles for each dyad in the random networks. The quantile value for each dyad was subtracted from its observed HWI, and differences  $> 0$  were considered preferred associations, since they were occurring at a significantly higher frequency ( $\alpha = 0.050$ ) than would be expected with random association between all individuals. All differences  $< 0$  were assigned a 0 value in the resultant matrix of preferred social associations. I calculated relationships between HWI and four predictor variables using matrices of overall associations and preferred associations (see details below).

### *Spatial overlap analysis*

I quantified space use by turtles based on location observations of basking in nine discrete clusters of basking habitat across Wolf Howl Pond (Figure 2.3; Appendix 1.2.). This approach was used because the turtles appeared to exhibit low site-specific fidelity, but high fidelity to a general area with a number of suitable basking structures (J. Kentel & C. Rouleau, pers. obs.). Calculations of spatial overlap were not accurate for turtles observed less than 8 times during basking surveys. Accordingly, spatial overlap was examined only in individuals of both sexes observed eight or more times during basking surveys ( $n = 88$ ). Overlap was calculated using a Bray-Curtis dissimilarity matrix ( $BC$ ; Bray & Curtis, 1957), such that:

$$BC_{xy} = 1 - \frac{2C_{xy}}{S_x + S_y}$$

where  $C$  is the number of clusters in which both individuals  $x$  and  $y$  were observed, and  $S$  is the total number of clusters in which each individual was observed. Using  $\text{MRQAP}_{\text{dsp}}$  to explore this data preliminarily, the correlation between space use and HWI was weak, but significant in one sample year (2019:  $R^2_{\text{adjusted}} < 0.001$ ,  $p = 0.823$ ; 2020:  $R^2_{\text{adjusted}} = 0.010$ ,  $p < 0.010$ ). Thus, I decided not to control for spatial overlap between individual turtles in any calculations of HWI, and, instead, I included the effect of spatial overlap as a factor in the analyses examining the predictors of MPT social associations (see below for details).

### *Drivers of MPT social associations*

$\text{MRQAP}_{\text{dsp}}$  was performed to test for relationships between HWI and four predictor variables: genetic relatedness, spatial overlap, sex, and midCL (Krackhardt, 1988; Dekker *et al.*, 2007; Farine, 2013). All  $\text{MRQAP}_{\text{dsp}}$  were run with 10,000 permutations. The sex difference matrix was constructed using a binary code, such that dyads consisting of individuals of the same sex were coded as “1” and dyads of different sexes were coded as “0”. The midCL matrix was constructed using the absolute value of the difference in midCL between the two individuals in each dyad. I also ran  $\text{MRQAP}_{\text{dsp}}$  to test the relationships between predictors. Regression coefficients in the  $\text{MRQAP}_{\text{dsp}}$  were calculated based on networks of overall associations and preferential associations (HWI) only.  $\text{MRQAP}_{\text{dsp}}$  does not calculate  $R^2_{\text{adjusted}}$  for each predictor in the model, rather an  $R^2_{\text{adjusted}}$  for the entire model. I therefore reported *Pearson’s r* for correlations between each predictor attribute and the appropriate HWI from the R function ‘*cor*’ (R Core Development Team, 2020). These correlation coefficients, however, do not take into account other predictor variables in the model. Instead, they estimate a correlation strictly between the response variable and a single predictor variable. I also ran a linear model using the

base R function *'lm'* to examine the relationship between midCL and basking frequency, defined as the proportion of surveys in which an individual was observed basking, in order to inform my interpretation of the results of these MQRAP<sub>dsp</sub> (R Core Development Team, 2020).

### *Network metric analysis*

Linear models were used to determine how individual attributes (i.e., body size, sex, and boldness) affect individual social behaviour in MPT. These were run using the base R function *'lm'* (R Core Development Team, 2020). One linear model was run with each of my four network metrics as a response variable (response variables; Table 2.4), and midCL and boldness were included in each. Boldness was estimated by calculating the proportion of years in which an individual turtle was captured over the long-term study (i.e., capture frequency) based on the assumption that turtles who spend more time basking or are higher in the water column are bolder and are therefore more likely to be captured. Sampling effort of Wolf Howl Pond has remained consistent since the formalization of long-term mark-recapture effort at the site in 1990, so capture effort did not influence this proxy (see Appendix 1.1). Thus, turtles who have been captured more frequently across the long-term study were considered to be bolder. MidCL and capture frequency were determined to be non-collinear, suggesting that an increase in capture frequency (i.e., boldness) was not due to an increase in body size or age. An interaction between midCL and capture frequency was found to be likely during data exploration (Zuur *et al.*, 2010), and thus was also included in models initially. When this interaction was not significant, models were re-run without it to allow interpretation of main effects. The network metric dataset was examined prior to modelling to ensure normality, lack of outliers, and lack of

collinearity. Assumptions of residual normality and homogeneity of variance were ensured before model interpretation for all linear models (Zuur *et al.*, 2010).

Significance testing of data generated from social networks is based on comparison of observed data to random permutations of a null model (Farine & Whitehead, 2015). This is necessary due to the non-independence of data generated from social association networks, so 10,000 permutations were run on a group-by-individual matrix containing the same set of dyads as the observed matrix where group membership in each permutation of the model was randomly assigned to individuals. For each permutation of the random matrix, the same network metrics that were calculated with the observed data were also calculated with the random matrices (see Table 2.4), and the same linear models were run. *P* values ( $p_{\text{rand}}$ ) were calculated by comparing observed model coefficients for each predictor variable to the range of coefficients for all random permutations of its corresponding model (Farine & Whitehead, 2015; Leu *et al.*, 2016). Effects were considered significant if the observed coefficients were outside the 95% range of the random model coefficient distributions (Farine & Whitehead, 2015).

## **Results**

### *Demographics of basking aggregations*

I observed 4138 instances ( $n = 3521$  females;  $n = 621$  males) of 189 ( $n = 157$  females;  $n = 32$  males) unique individual turtles basking. Basking aggregations ranged in size from 2 to 24 individuals, with a mean of  $2.002 \pm 1.854$  (mean  $\pm$  standard deviation) and a median of 1.000. I observed 929 total basking aggregations, and 1138 instances of solitary basking. I observed 352 female-only basking aggregations, 16 male-only aggregations, and 561 mixed-sex aggregations. Mixed-sex aggregations ranged from six females per male to two males per female. No complete

basking aggregation containing more than two individuals was repeated, but pairs of individuals were observed in the same aggregation in a maximum of 52 of a possible 79 surveys (66.67%). Unique pairs of turtles were found aggregating between 0.00% and 35.74% of surveys, with a mean of  $1.17 \pm 3.13$  % (mean  $\pm$  standard deviation) and a median of 2.96%.

#### *Summary of turtle attribute data and MQRAP<sub>dsp</sub> results*

Genetic relatedness: Mean pairwise genetic relatedness between the 88 MPT included in my analyses was  $0.022 \pm 0.054$  (mean  $\pm$  standard deviation). Relatedness estimates ranged from 0.000 to 0.500 (Figure 2.2). I detected 22 parent-offspring/full sibling dyads ( $r = 0.500$ ), 46 half-sibling/avuncular/grandparent-grandchild dyads ( $r = 0.250$ ), 192 first cousin dyads ( $r = 0.125$ ), and 3557 unrelated dyads ( $r = 0.000$ ). The unrelated designation is inclusive of relationships defined as second cousin ( $r = 0.0313$ ), as the triadic maximum likelihood estimator could not reliably distinguish between these two levels of relatedness (Figure 2.1). Genetic relatedness was a significant predictor of overall HWI ( $p = 0.005$ ) and preferred HWI ( $p = 0.0005$ ), such that the more related a dyad, the stronger their social association (Figure 2.4; Table 2.6; Table 2.7; Table 2.8).

Size difference: Mean size difference (midCL) between the 88 MPT included in my analyses was  $1.67 \pm 1.26$  cm (mean  $\pm$  standard deviation). Differences ranged from 0.00 cm to 6.94 cm. Size difference was not a significant predictor of overall HWI ( $p = 0.212$ ) or preferential HWI ( $p = 0.660$ ) (Table 2.6; Table 2.7). Body size significantly affected basking frequency, such that larger individuals were observed in a higher proportion of basking surveys (Figure 2.5). Body

size was a significant predictor of space use dissimilarity, such that individuals of similar size showed a higher degree of spatial overlap ( $\beta = 0.038$ ,  $p < 0.001$ , *Pearson's r* = 0.031).

Spatial overlap: Mean space use dissimilarity between the 88 MPT included in my analyses was  $0.394 \pm 0.271$  (mean  $\pm$  standard deviation). Values ranged from 0.000 to 0.982. Spatial overlap predicted overall HWI ( $p < 0.001$ ) and preferential HWI ( $p = 0.002$ ), such that individuals who used the same area of Wolf Howl Pond more frequently showed stronger levels of association (Table 2.6; Table 2.7).

Sex similarity: The sample of 88 MPT included in my analyses contained 73 females and 15 males. Sex similarity was a significant predictor of overall HWI ( $p = 0.003$ ), such that same-sex dyads had higher levels of association than mixed-sex dyads (Table 2.6). Yet, sex similarity was not a significant predictor of preferred HWI ( $p = 0.270$ ; Table 2.7). Sex did not impact how frequently individuals were observed during basking surveys ( $t = -1.221$ ,  $df = 59.620$ ,  $p = 0.227$ ).

#### *Analysis of individual social behaviour*

Binary degree: Binary degree was significantly affected by midCL ( $p_{rand} < 0.001$ ), boldness ( $p_{rand} < 0.001$ ), and their interaction ( $p_{rand} < 0.001$ ) in females, such that the number of individuals with which a focal turtle associated was lower for turtles with a larger midCL, and the strength of this negative relationship lessened with increasing boldness (Figure 2.6; Table 2.9). Female turtles that were larger and bolder associated with fewer turtles than turtles that were smaller and shyer. In males, binary degree decreased significantly with increasing body size ( $p_{rand} = 0.050$ ; Table 2.10).

Weighted degree: Weighted degree was significantly affected by body size ( $p_{rand} < 0.001$ ), boldness ( $p_{rand} < 0.001$ ), and their interaction ( $p_{rand} < 0.001$ ) in females such that weighted degree decreased with increasing body size, but the strength of this negative relationship lessened with increasing boldness (Table 2.9). In males, weighted degree decreased significantly with increasing body size ( $p_{rand} = 0.007$ ; Table 2.10).

CV: CV was significantly affected by boldness ( $p_{rand} < 0.001$ ) in females such that CV decreased with increasing boldness (Table 2.9). In males, CV was significantly affected by body size ( $p_{rand} < 0.001$ ), boldness ( $p_{rand} < 0.001$ ), and their interaction ( $p_{rand} < 0.001$ ), such that CV decreased with increasing body size, but the strength of this negative relationship lessened with increasing boldness (Table 2.10).

Betweenness: Betweenness was significantly affected by body size ( $p_{rand} < 0.001$ ) in female turtles, such that betweenness decreased with increasing body size (Table 2.9). In males, betweenness was not significantly affected by body size, boldness, or their interaction (Table 2.10).

## **Discussion**

Social networks using HWI were created to examine patterns of association during basking in a population of MPT. Patterns of association in all networks differed significantly from random associations between individuals, which suggests that this population of turtles has preferred relationships in their social interactions during basking. HWI was correlated with four

attributes of individual turtles: genetic relatedness, body size, space use, and sex. HWI increased significantly with increasing genetic relatedness of baskers, higher spatial overlap between baskers, and between same-sex basking pairs relative to mixed-sex pairs (Table 2.6; Table 2.7). Four descriptive social network metrics were calculated for individual MPT, and effects of body size and a boldness proxy on these metrics were examined. Social network metrics were significantly affected by body size in both sexes (Table 2.9; Table 2.10). These metrics described the number and strength of associations a turtle maintained, as well as individual social connectivity, and the tightness of social groupings (as described in Table 2.4). These results support my prediction that the basking behaviour of MPT is kin-biased, such that individuals are more likely to share basking structures with close kin. Further, analyses of social network metrics support my hypothesis that social interactions while basking are dependent on body size, such that larger individuals secure more frequent basking opportunities, and maintain fewer social associations, suggesting a social hierarchy in MPT.

The pattern of kin-biased association I observed among turtles in Wolf Howl Pond has been observed in other reptile species (e.g., Bull *et al.*, 2001; Clark, 2004; O'Connor & Shine, 2004; Galoyan, 2013), including hatchlings of one turtle species when raised in captivity (*Malaclemys terrapin*; MacDonald & Rife, 2007). Tree-crevice Skinks (*Egernia striolata*) congregate in retreats in close-kin groups when habitat availability is not a limiting factor (J. Riley *et al.* unpubl. data). Flaherty and Bider (1984) suggest that social factors may be responsible for arrangement of basking individuals in a population of Northern Map Turtles (*Graptemys geographica*) where suitable habitat is not limited. Basking habitat in Wolf Howl Pond does not appear limited, as no more than 40 of a possible 195 known basking locations in the pond were ever in use simultaneously at the time of a basking survey. Basking is critical for

meeting the energetic needs of ectotherms, and it has been suggested that alterations to basking behaviour can affect individual fitness (Bulté & Blouin-Demers, 2010b). Thus, ectotherms should bask in a manner which maximizes their individual fitness. Basking in groups provides benefits to both the individual through increased access to higher quality habitat (Duffield & Bull, 2002; Gardner *et al.*, 2007; Michael *et al.*, 2010), and the congregation at large through increased group vigilance (Lanham & Bull, 2004). Accordingly, kin selection theory suggests that individual fitness should be maximized when groups are formed between kin (Hamilton, 1964a,b). MPT in Wolf Howl Pond bask more frequently with kin than non-kin, suggesting that there are inclusive fitness benefits to communal basking. Given that the benefits of group basking are likely to increase the fitness of the individual, gaining these benefits through association with kin should maximize individual and inclusive fitness. For example, a kin-group securing more consistent basking habitat access may be able to allocate more energy towards a reproductive bout, since basking is known to affect reproductive behaviour of ectotherms (Congdon & Tinkle, 1982; Whittow & Balasz, 1982; Schwarzkopf & Shine, 1991; Wapstra, 2000; Bulté & Blouin-Demers, 2010b). I acknowledge that I detected low levels of close-kinship in Wolf Howl Pond MPT. Other MPT populations may hold higher numbers of close-kin, and those relationships may affect the social structure of those populations differently than in the Wolf Howl Pond population.

Binary and weighted degree of Wolf Howl Pond MPT were significantly affected by body size in both sexes (Table 2.9; Table 2.10). A decrease in binary degree shows a lower number of social associations in a focal individual, while a decrease in weighted degree can represent a decrease in either the number or strength of associations (Whitehead, 2008). Given that size difference between individuals did not affect HWI, I suggest that the strength of

association between individuals is similar across body sizes (Table 2.6; Table 2.7). Together these results suggest that larger turtles have fewer social associations than smaller individuals. Given that body size is often a determinant of competition success in turtles (Auth, 1975; Pluto & Bellis, 1986; Lindeman, 1999), it is plausible that larger MPT may preferentially bask in high quality habitat more consistently. By virtue of this preference, larger MPT may maintain fewer social associations, since the same dominant individuals will repeatedly bask at the same high quality locations. Indeed, similarly-sized individuals showed a higher degree of spatial overlap than individuals with large size disparities. Further, MPT may have evolved to be tolerant of association with similarly sized conspecifics, particularly highly related individuals, to reinforce and maximize the benefits of communal basking. Halliwell *et al.* (2017) suggest that close proximity between individuals could favour higher levels of social association if it is selectively advantageous.

Analysis of social network metrics suggested that the factors influencing social behaviour of Wolf Howl Pond turtles differ between the sexes. The Wolf Howl Pond MPT population is heavily female-biased, with approximately three females per male (R. Brooks *et al.*, unpubl. data). Accordingly, the majority of basking observations made for my research are of females and female-dominated basking congregations. Mixed-sex dyads ranged from six females per male to two males per female. However, mixed-sex dyads had significantly lower levels of association than same-sex dyads (Table 2.6) despite individual males and females being observed at similar frequencies throughout sampling. The predictors used in analysis affected CV more strongly in males than females (Table 2.10). CV decreased with increasing male body size and boldness. A decrease in CV suggests less variability in an individual's social associations (Brandl *et al.*, 2019), indicating that larger and bolder male turtles were more

consistent in their social behaviour than smaller males. I propose that this sexual difference in basking social behaviour may be related to mating tactics.

Spring is the primary mating season for MPT (Krawchuk & Brooks, 1998; Ernst & Lovich, 2009; but see Moldowan *et al.*, 2020), and larger male MPT in the Wolf Howl Pond population are known to interact with females in aggressive courtship and pre-mating capacities more frequently (Moldowan *et al.*, 2020). Further, female MPT are less willing to mate with larger males (Moldowan *et al.*, 2020). This pattern is also seen in Sleepy Lizards (*Tiliqua rugosa*), where more aggressive males associate less strongly with females (Godfrey *et al.*, 2012). My results suggest that large male MPT may exhibit variable social behaviour as they regularly move through and between social groupings to maximize their mating opportunities. They may also preferentially associate with social groups containing smaller females, since coercive mating success by males decreases with female body size (Moldowan *et al.*, 2020). Smaller females were observed to bask in larger groups (Table 2.9), which may contribute to the decrease in CV observed for males.

Preliminary testing on three social network metrics revealed a significant interaction between the effects of body size and capture frequency (binary degree and weighted degree in females; Table 2.9; CV in males; Table 2.10). Capture frequency had a consistent alleviating effect on body size, such that the effect of decreasing body size on social behaviour was lessened by the capture frequency of the individual (Figure 2.6). Boldness (i.e., capture frequency) in reptiles is known to vary among individuals in a population (Cooper, 2009; Carazo *et al.*, 2014; Li *et al.*, 2016; Michelangeli *et al.*, 2016). The Spanish Terrapin (*Mauremys leprosa*) demonstrates differential response to conspecific chemical cues based on individual boldness (Ibáñez *et al.*, 2014). Further, boldness is a trait for which individual turtles can vary (Réale *et*

*al.*, 2007). Boldness may affect an individual's predisposition for engaging in potentially antagonistic behaviours, and for their probability of success in the encounter (Sundstrom *et al.*, 2004; López *et al.*, 2005; Colleter & Brown, 2011; Ibáñez *et al.*, 2014).

I propose that increased capture frequency in female MPT results in less discrimination of the social or environmental quality of basking habitat. This is shown in the alleviating effect of capture frequency on binary degree and weighted degree in female MPT. Individuals with higher capture frequencies (i.e., bolder) have higher binary and weighted degrees than less frequently captured individuals of a similar size, meaning that these more frequently captured individuals maintain more social associations (Whitehead, 2008). Therefore, I suggest that frequently captured individuals will be more likely to bask in a given area regardless of the social grouping present. Further, these individuals may associate with higher numbers of individuals due to flight responses and rearrangement of individuals on basking structures. Increased boldness is known to result in a less sensitive flight response in reptiles (Cooper, 2009; Cooper, 2012). Accordingly, boldness in Wolf Howl Pond MPT is likely to affect the willingness of an individual to terminate a basking bout due to a perceived risk. Wolf Howl Pond turtles did not exhibit strong individual site fidelity, and often reinitiated basking on structures near their initial location after disturbance (J. Kentel & C. Rouleau, pers. obs.). This may result in highly bold individuals associating with a higher number of individuals if they do not terminate basking as willingly as less bold individuals. By remaining on a basking structure through a perceived risk, bold turtles may associate with different groupings of less bold individuals.

My observations of basking behaviour in MPT present many opportunities for continuing research. Future researchers could conduct surveys over a larger per-survey timeframe, and focus on particular areas of basking habitat in Wolf Howl Pond to observe interactions between

individuals. Further, researchers could conduct behavioural trials to directly test the patterns of hierarchical social structure and kin-bias I have proposed. Trials could be run to examine interactions between kin and non-kin, as well as between pairs of varying body sizes and sexes. Further research could also attempt to examine the benefits of communal basking in MPT. Researchers could examine the vigilance of grouped versus solitary baskers, as well as the potential for protection against antagonism. By explaining these benefits, researchers could more accurately hypothesize about the social structure of MPT and its evolution.

My observations of the basking behaviour of MPT represent a novel approach to the study of sociality in an emydid turtle. I have provided evidence for kin-bias in social associations between basking individuals, and suggest that their social behaviour is affected by body size. My study shows that differences in body size, sex, and individual boldness affect the social behaviour of MPT during basking. My findings suggest that a common aggregative behaviour in a taxon largely regarded as asocial is, in fact, reflective of sociality that is driven by a complex suite of individual and population level factors. This study provides first insights into the sociality of an understudied taxonomic group, the turtles, and should be encouraging for an examination of sociality in additional testudine species, to understand the diversity of sociality in this group and the diversity of vertebrate sociality more generally.

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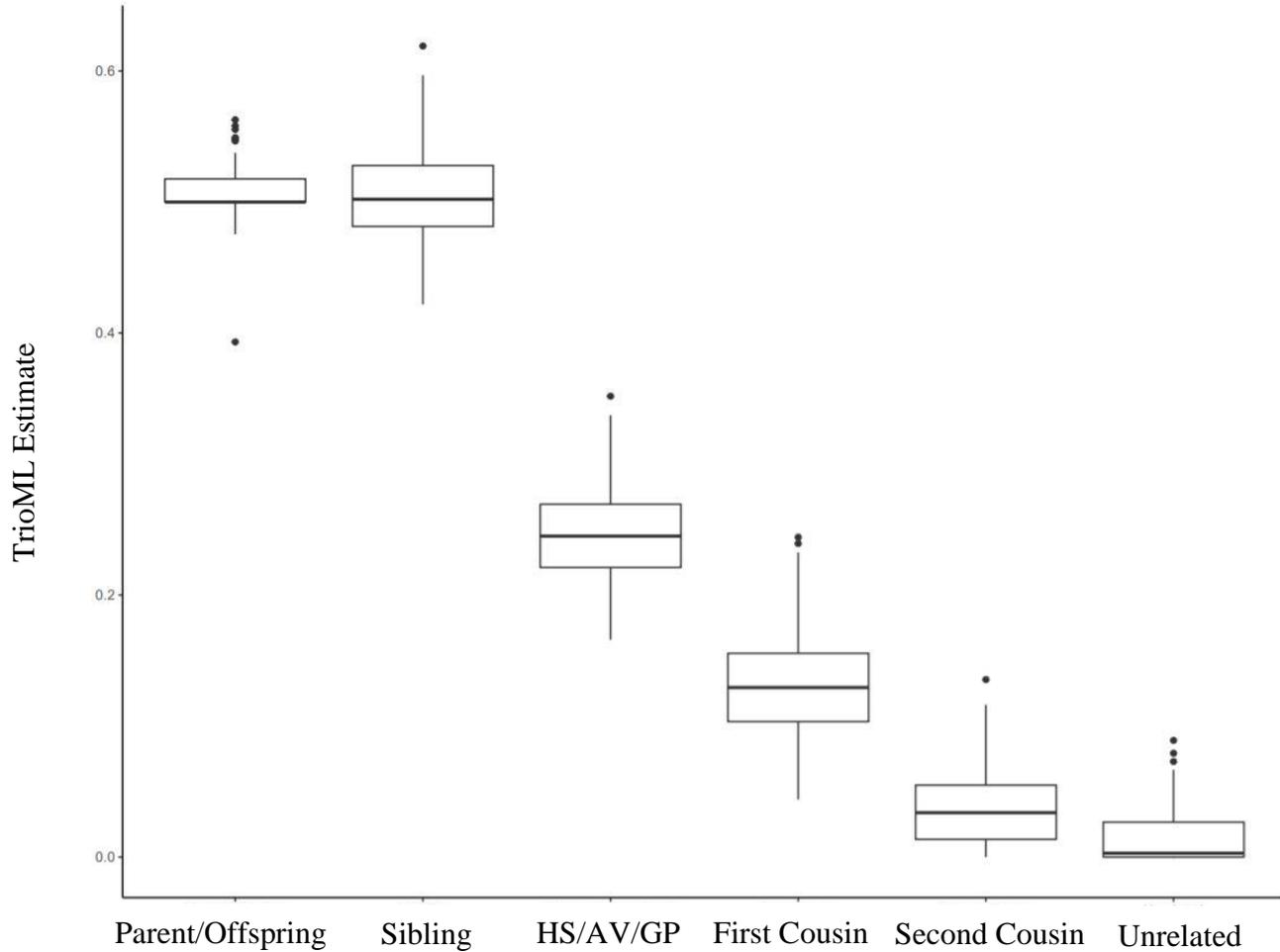
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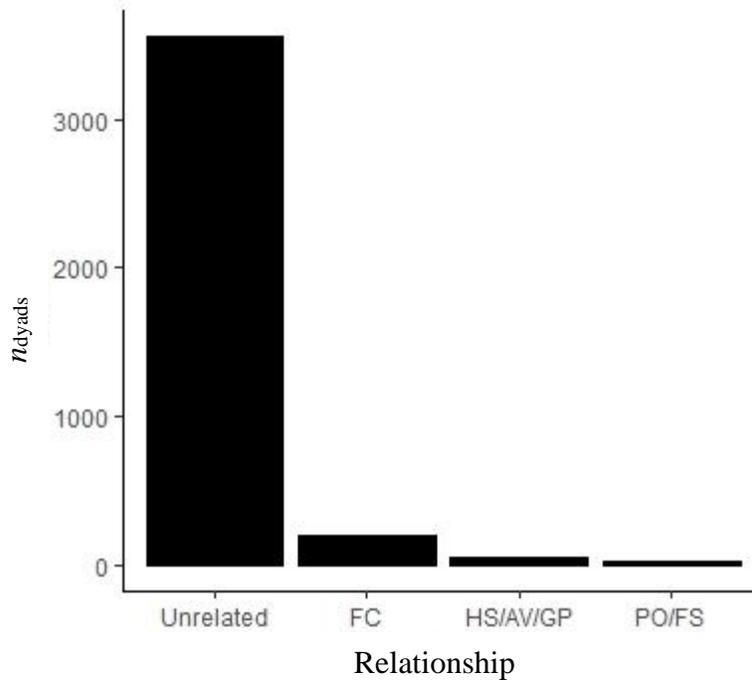
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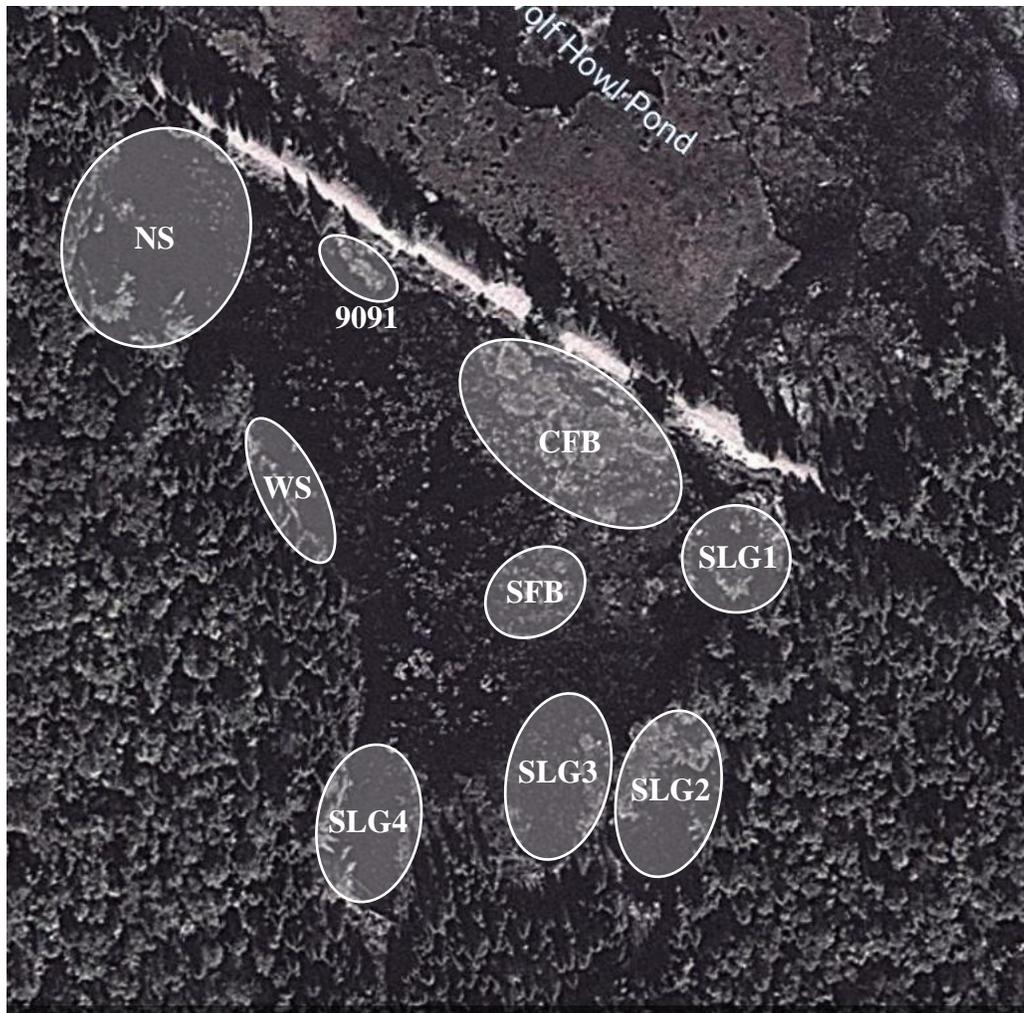
## Figures & Tables



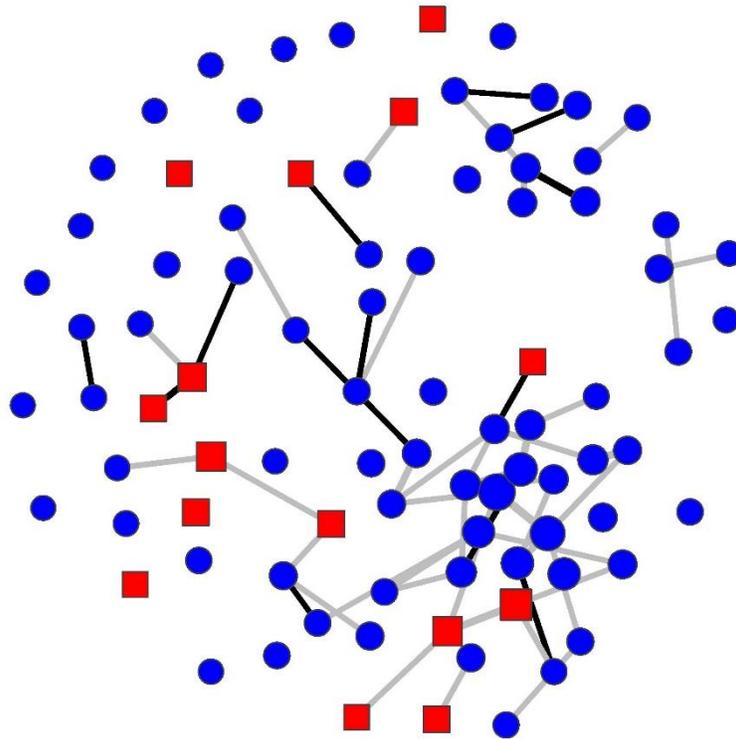
**Figure 2.1.** Density plots of triadic maximum likelihood (TrioML) relatedness estimates based on data simulated using allelic frequencies from the Wolf Howl Pond (Algonquin Provincial Park, Ontario, Canada) Midland Painted Turtle (*Chrysemys picta marginata*) population. Each bin represents the spread of TrioML values calculated for simulated individuals ( $n = 100$ ) whose expected relatedness values were based on six levels of relationship: Parent/Offspring,  $r = 0.5$ ; Sibling,  $r = 0.5$ ; Half-siblings/Avuncular/Grandparent & Grandchild (HS/AV/GP),  $r = 0.25$ , First Cousin,  $r = 0.125$ ; Second Cousin,  $r = 0.0313$ ; Unrelated,  $r = 0$ . All simulated individuals in each bin have an expected relatedness exactly equal to their bin designation. Less overlap between bins indicates the ability of the TrioML method to distinguish between different relationships through numerical relatedness.



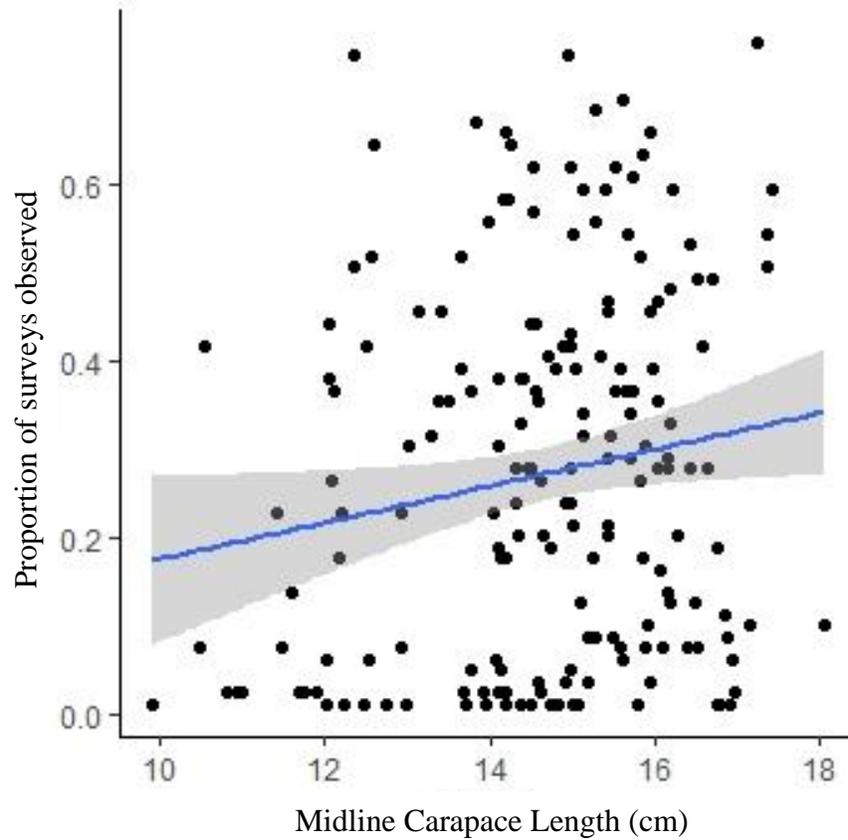
**Figure 2.2.** Estimated relationships of Midland Painted Turtles (*Chrysemys picta marginata*;  $n = 88$ ) in Wolf Howl Pond (Algonquin Provincial Park, Ontario, Canada) based on triadic maximum likelihood estimates of relatedness. Relationships were based on relatedness estimates: unrelated ( $r = 0$ ), first cousin (FC;  $r = 0.125$ ), half-sibling/avuncular/grandparent-grandchild (HS/AV/GP;  $r = 0.25$ ), parent-offspring/full sibling (PO/FS;  $r = 0.5$ ). All individuals are replicated 87 times, such that counts represent all possible pairwise combinations of focal Midland Painted Turtles ( $n = 3817$  dyads).



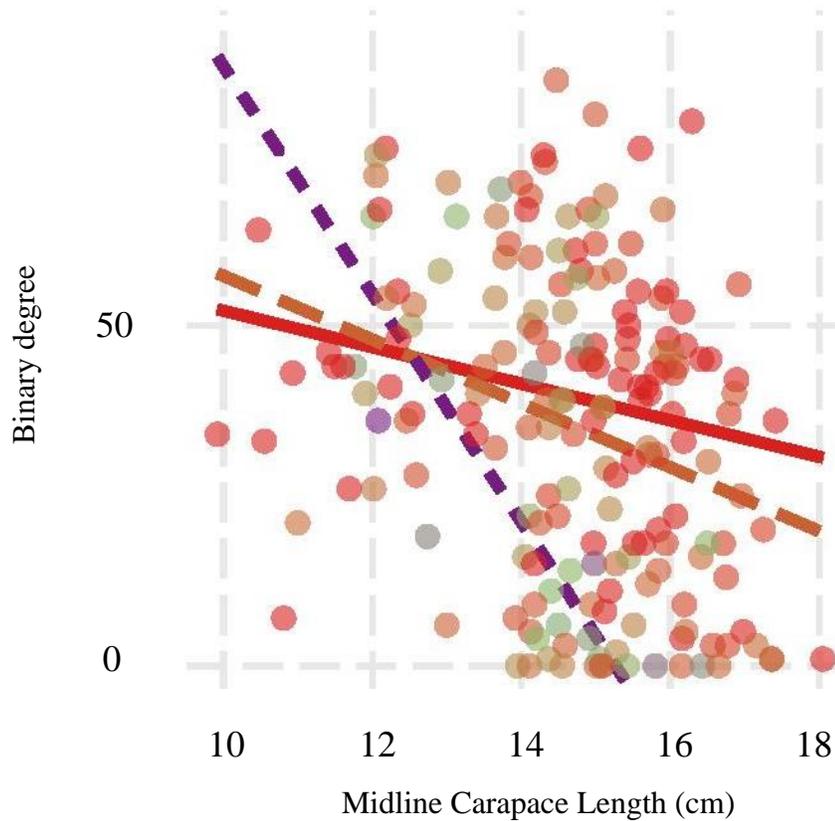
**Figure 2.3.** Distribution of basking structure clusters used in spatial overlap analysis of basking Midland Painted Turtles (*Chrysemys picta marginata*) in Wolf Howl Pond (Algonquin Provincial Park, Ontario, Canada). Clusters are designated as follows: northern shore (NS), marked bogmats 90 & 91 (9091), central floating bog (CFB), solitary floating bogs (SFB), southern log jams (SLG1-4), western shore (WS). Each cluster contains a number of marked and unmarked basking structures, all of which were used as basking habitat by turtles Source: Google Earth. See Appendix 1.2 for cluster descriptions.



**Figure 2.4.** Social network diagram of preferential social associations among Midland Painted Turtles (*Chrysemys picta marginata*;  $n = 88$ ) in Wolf Howl Pond (Algonquin Provincial Park, Ontario, Canada). Females are denoted by blue circles ( $n = 73$ ), and males by red squares ( $n = 15$ ). Nodes are sized relative to the individual's number of associations. Edges are sized based on the strength of association between their terminal nodes. Edges are colored based on estimated relatedness between associating Midland Painted Turtles, such that  $r > 0.125$  between two individuals results in a black edge, and  $r < 0.125$  results in a grey edge.



**Figure 2.5.** Relationship between body size (midline carapace length) and the proportion of basking surveys in which an individual was observed for Midland Painted Turtles (*Chrysemys picta marginata*;  $n = 189$ ) in Wolf Howl Pond (Algonquin Provincial Park, Ontario, Canada). A linear model was fitted using the base R function 'lm' ( $t = 2.14$ ,  $p = 0.03$ ,  $R^2_{adjusted} = 0.02$ ).



**Figure 2.6.** Relationship between binary degree (the number of observed associations) and the interaction between body size (midline carapace length) and boldness in female Midland Painted Turtles (*Chrysemys picta marginata*;  $n = 157$ ) in Wolf Howl Pond (Algonquin Provincial Park, Ontario, Canada). Regression lines represent an estimation of the effect of body size at a specified level of boldness. The three boldness levels included are the minimum value (0.382; broken purple regression line), the mean (0.911; broken orange regression line), and the maximum (1.000; solid red regression line) capture frequencies of females. Regression lines were estimated using the base R function ‘*lm*’, and plotted in the package ‘*interactions*’ (Long, 2019).

**Table 2.1.** Sampling effort of Midland Painted Turtles (*Chrysemys picta marginata*) in Wolf Howl Pond (Algonquin Provincial Park, Ontario, Canada) before the commencement of basking surveys. An exhaustive sample is defined as approximately 180 turtles (90% of the estimated population; M. Keevil & S. Sanders, unpubl. data).

| <b>Year</b> | <b>No. of turtles captured</b> | <b>Date of first basking survey</b> | <b>Date of last basking survey</b> | <b>No. of basking surveys</b> |
|-------------|--------------------------------|-------------------------------------|------------------------------------|-------------------------------|
| 2019        | 176                            | 24 May                              | 18 June                            | 40                            |
| 2020        | 182                            | 17 May                              | 8 June                             | 39                            |

**Table 2.2.** Filtering criteria for raw single-nucleotide polymorphism genotyping data on the Wolf Howl Pond (Algonquin Provincial Park, Ontario, Canada) population of Midland Painted Turtles (*Chrysemys picta marginata*). Any loci meeting the filtering value criteria for each step were removed from the analysis. All filtering was performed in R using the package ‘dartR’ (Gruber *et al.*, 2019; R Core Development Team, 2020).

| Step no. | Filter                   | Function  | Filtering value  | Number of loci        |
|----------|--------------------------|---|--|-----------------------|
| 0        | -                        | -   | -  | 66832 (pre-filtering) |
| 1        | Monomorphism             | Removes loci for which there is no variation across the entire dataset                          | All monomorphic or duplicated loci                         | 43349                 |
| 2        | Coverage                 | Removes loci with read depths below the lower threshold and above the upper threshold           | Lower limit – 2<br>Upper limit – 7                         | 34406                 |
| 3        | Repeatability            | Removes loci with repeatability lower than filtering value                                      | 99%  | 13210                 |
| 4        | Call Rate                | Removes loci with a higher proportion of missing values than the filtering value                | 99%  | 1431                  |
| 5        | Hamming Distance         | Removes loci with lower base pair variation than the filtering value                            | 25% of base pairs showing variation across all individuals | 1394                  |
| 6        | Minor Allele Frequency   | Removes loci with lower minor allele frequency than the filtering value                         | 2%   | 1015                  |
| 7        | Hardy-Weinberg Deviation | Removes loci for which allelic frequencies differ significantly from Hardy-Weinberg Equilibrium | $\alpha = 0.05$  | 1015                  |

**Table 2.3.** Pearson correlation coefficients (*Pearson's r*) comparing simulated and expected relatedness from simulated genotypic data ( $n = 600$  simulated individuals) based on allelic frequencies at loci from the Wolf Howl Pond (Algonquin Provincial Park, Ontario, Canada) population of Midland Painted Turtles (*Chrysemys picta marginata*).

| <b>Relatedness Estimator</b>               | <b><i>Pearson's r</i></b> |
|--|---------------------------|
| Triadic Maximum Likelihood (Wang, 2007)    | 0.99582                   |
| Dyadic Maximum Likelihood (Milligan, 2003) | 0.99579                   |
| Lynch & Ritland (1999)                     | 0.99310                   |
| Queller & Goodnight (1989)                 | 0.99060                   |
| Wang (2002)                                | 0.98950                   |
| Li <i>et al.</i> (1993)                    | 0.98947                   |
| Ritland (1996)                             | 0.98393                   |

**Table 2.4.** Network metrics of individual social behaviour measured in the Wolf Howl Pond (Algonquin Provincial Park, Ontario, Canada) population of Midland Painted Turtles (*Chrysemys picta marginata*). Metrics were calculated based on definitions from Whitehead (2008) manually, or using the R packages ‘*raster*’ and ‘*sna*’ in the R Environment (Csardi & Nepusz, 2006; Butts, 2019; R Core Development Team, 2020).

| <b>Metric</b>                                 | <b>Formula</b>  | <b>Definition</b>   | <b>Source</b>  |
|---|---|---|--|
| Binary degree                                 | $bd_x = n_{edges}(x)$   | Number of edges connected to a focal node. Individuals with higher binary degrees have known associated with a higher number of individuals.  | Coded manually (based on the definition from Whitehead, 2008). |
| Weighted degree                               | $s_x = \sum_x \alpha_{xy}$  | Sum of HWI of all edges connected to a focal node. Individuals with higher weighted degrees can a combination of stronger and more numerous associations with other individuals.                      | Coded manually (based on the definition from Whitehead, 2008). |
| Coefficient of variation of edge weights (CV) | $c_{xy} = \frac{\sum_x \sum_z \alpha_{xy} \alpha_{xz} \alpha_{yz}}{\max \alpha_{yz} \sum_y \sum_z \alpha_{xy} \alpha_{yz}}$ | Heterogeneity of edges connected to a focal node. Individuals with higher CV demonstrate more variability in their social associations.   | <i>raster::cv</i>  |
| Betweenness                                   | $B_x = \sum_{y,z:y \neq z, y \neq x, z \neq x} \frac{g_{xyz}}{g_{yz}}$  | The number of most parsimonious paths between non-directly connected nodes that pass through a focal node. Individuals with higher betweenness are more socially connected within the social network. | <i>sna::betweenness</i>  |

$x$ ,  $y$ , and  $z$  are individuals (nodes) in a social association network.  $\alpha_{xy}$  is the half weight association index (HWI) value between individuals  $x$  and  $y$ .  $g$  is the index of the graph for which betweenness is being calculated.

**Table 2.5.** Sample sizes and summary statistics of sex-specific grouping datasets used in social network metric analyses of Wolf Howl Pond (Algonquin Provincial Park, Ontario, Canada) Midland Painted Turtles (*Chrysemys picta marginata*). HWI is the half weight index of association strength (Cairns & Schwager, 1987; Whitehead, 2008). Observation counts are by individual, and HWI estimations are by dyad ( $n = 496$ ). Values are reported as mean  $\pm$  standard deviation.

| <b>Matrix</b> | <i>n</i> <sub>individuals</sub> | <i>n</i> <sub>observations</sub> | <b>Observations</b> |               |              | <b>HWI</b>      |               |              |
|---------------|---------------------------------|----------------------------------|---------------------|---------------|--------------|-----------------|---------------|--------------|
|               |                                 |                                  | <b>Mean</b>         | <b>Median</b> | <b>Range</b> | <b>Mean</b>     | <b>Median</b> | <b>Range</b> |
| Male          | 32                              | 617                              | 19.28 $\pm$ 12.23   | 21.50         | 1-49         | 0.02 $\pm$ 0.04 | 0.00          | 0.00-0.24    |
| Female        | 157                             | 3521                             | 22.43 $\pm$ 17.641  | 22.00         | 1-60         | 0.01 $\pm$ 0.03 | 0.00          | 0.00-0.67    |

**Table 2.6.** Output of the multiple regression quadratic assignment procedure with double semi-partialing (MRQAP<sub>dsp</sub>) analyzing overall half weight association indices (HWI) and attribute matrices (genetic relatedness, size difference [midCL], spatial overlap, and sex similarity) for Midland Painted Turtles (*Chrysemys picta marginata*;  $n = 88$ ) from Wolf Howl Pond (Algonquin Provincial Park, Ontario, Canada). MRQAP<sub>dsp</sub> was run with 10000 permutations. *Pearson's r* values were calculated using the base R function 'cor'. Superscript (\*) denotes a significant correlation ( $\alpha < 0.05$ ).

|                      | $\beta$ | $p$     | <i>Pearson's r</i> |
|----------------------|---------|---------|--------------------|
| Intercept            | 0.031   | 0.00*   | -                  |
| Genetic Relatedness  | 0.042   | 0.005*  | 0.003              |
| Body Size Difference | -0.0007 | 0.210   | 0.0009             |
| Spatial Overlap      | -0.010  | <0.001* | 0.010              |
| Sex Similarity       | 0.005   | <0.001* | 0.003              |

Model  $R^2_{adjusted} = 0.01$

**Table 2.7.** Output of the multiple regression quadratic assignment procedure with double semi-partialing (MRQAP<sub>dsp</sub>) of preferential half weight association indices (HWI) and attribute matrices (genetic relatedness, size difference [midCL], spatial overlap, and sex similarity) for Midland Painted Turtles (*Chrysemys picta marginata*;  $n = 88$ ) from Wolf Howl Pond (Algonquin Provincial Park, Ontario, Canada). MRQAP<sub>dsp</sub> was run with 10,000 permutations. *Pearson's r* values were calculated using the base R function 'cor'. Superscript (\*) denotes a significant correlation ( $\alpha < 0.05$ ).

|                      | $\beta$ | $p$     | <i>Pearson's r</i> |
|----------------------|---------|---------|--------------------|
| Intercept            | 0.004   | 0.00*   | -                  |
| Genetic Relatedness  | 0.030   | 0.0005* | 0.005              |
| Body Size Difference | 0.0002  | 0.660   | 0.0003             |
| Spatial Overlap      | -0.003  | 0.0002* | 0.002              |
| Sex Similarity       | 0.001   | 0.271   | 0.0005             |

Model  $R^2_{adjusted} = 0.005$

**Table 2.8.** Summary of relatedness estimates between preferentially associating Wolf Howl Pond (Algonquin Provincial Park, Ontario, Canada) Midland Painted Turtles (*Chrysemys picta marginata*;  $n = 186$  dyads). Each dyad represents a preferential relationship between two individual turtles. Relatedness estimates are based on the triadic maximum likelihood method. Preferential associations are based on the half-weight index of association (HWI). Proportions of genetic relationships represent the number of preferred associations at each relatedness level divided by the total number of relationships at that level. Genetic relatedness estimates are defined as: unrelated ( $r = 0.000$ ), first cousin ( $r = 0.125$ ), half-sibling/avuncular/grandparent-grandchild (HS/AV/GP;  $r = 0.250$ ), and full sibling/parent-offspring (FS/PO;  $r = 0.500$ ). Estimates are categorized based on the range of estimates of simulated relatedness for each level (Figure 1.1).

| <b>Relatedness estimate</b> | <b>Number of preferential associations</b> | <b>Proportion of relationships</b> |
|-----------------------------|--|------------------------------------|
| Unrelated                   | 172  | 0.05                               |
| First cousin                | 9  | 0.05                               |
| HS/AV/GP                    | 2  | 0.04                               |
| PO/FS                       | 2  | 0.10                               |

**Table 2.9.** Summary statistics of significance testing of social network metric data for female Midland Painted Turtles (*Chrysemys picta marginata*;  $n = 157$ ) in Wolf Howl Pond (Algonquin Provincial Park, Ontario, Canada). The effects of body size (midCL), boldness, and the interaction between these two variables were examined. I generated 10,000 permutations for each random network. Coefficient ranges that are presented for random network permutations span 95% of randomized coefficients. Effects are considered significant if the observed coefficient is outside the 95% range of randomized coefficients ( $\alpha = 0.05$ ). When the interaction variable was non-significant, it was removed from reported models and they were re-run. This is denoted using “---”. Significant effects are denoted by superscript (\*).

| <b>Metric</b>                            |                      | <b>Observed <math>\beta</math></b> | <b>Random <math>\beta</math> Range</b> | <b><math>p_{rand}</math></b> |
|--|----------------------|------------------------------------|--|------------------------------|
| Binary degree                            | Intercept            | 267.35*                            | -2.40 to 117.55                        | <0.001                       |
|  | Body Size            | -19.15*                            | -7.07 to -3.91                         | <0.001                       |
|  | Boldness             | -214.14*                           | -7.07 to -3.91                         | <0.001                       |
|  | Body Size * Boldness | 17.64*                             | -6.82 to -5.32                         | <0.001                       |
| Weighted degree                          | Intercept            | 23.17*                             | -1.81 to -0.02                         | <0.001                       |
|  | Body Size            | -1.58*                             | -0.37 to -0.23                         | <0.001                       |
|  | Boldness             | -20.31*                            | -0.37 to 0.23                          | <0.001                       |
|  | Body Size * Boldness | 1.51*                              | -0.35 to 0.23                          | <0.001                       |
| Coefficient of variation of edge weights | Intercept            | 86.50                              | -365.22 to 255.83                      | 0.172                        |
|  | Body Size            | 35.15                              | -6.29 to 43.20                         | 0.104                        |
|  | Boldness             | -308.25*                           | -6.29 to 43.20                         | <0.001                       |
|  | Body Size * Boldness | ---                                | ---                                    | ---                          |
| Betweenness                              | Intercept            | 78.41*                             | 295.41 to 639.83                       | <0.001                       |
|  | Body Size            | -7.94*                             | -32.38 to -10.63                       | <0.001                       |
|  | Boldness             | 68.91                              | -138.00 to 130.40                      | 0.503                        |
|  | Body Size * Boldness | ---                                | ---                                    | ---                          |

**Table 2.10.** Summary statistics of significance testing of social network metric data for male Midland Painted Turtles (*Chrysemys picta marginata*;  $n = 32$ ) in Wolf Howl Pond (Algonquin Provincial Park, Ontario, Canada). The effects of body size (midCL), boldness, and the interaction between these two variables were examined. I generated 10,000 permutations for each random network. Coefficient ranges that are presented for random network permutations span 95% of randomized coefficients. Effects are considered significant if the observed coefficient is outside the 95% range of randomized coefficients ( $\alpha = 0.05$ ). When the interaction variable was non-significant, it was removed from reported models and they were re-run. This is denoted using “---”. Significant effects are denoted by superscript (\*).

| <b>Metric</b>                            |                      | <b>Observed <math>\beta</math></b> | <b>Random <math>\beta</math> Range</b> | <b><math>p_{rand}</math></b> |
|--|----------------------|------------------------------------|--|------------------------------|
| Binary degree                            | Intercept            | 21.64                              | 0.32 to 26.11                          | 0.122                        |
|  | Body Size            | -0.70*                             | -0.79 to 0.91                          | 0.051                        |
|  | Boldness             | -3.19                              | -0.79 to 0.91                          | 0.564                        |
|  | Body Size x Boldness | ---                                | ---                                    | ---                          |
| Weighted degree                          | Intercept            | 2.23*                              | -0.20 to 1.84                          | < 0.001                      |
|  | Body Size            | -0.08*                             | -0.07 to 0.08                          | 0.007                        |
|  | Boldness             | -0.55                              | -0.07 to 0.08                          | 0.232                        |
|  | Body Size x Boldness | ---                                | ---                                    | ---                          |
| Coefficient of variation of edge weights | Intercept            | 2132.75*                           | -4064.05 to 1218.75                    | < 0.001                      |
|  | Body Size            | -152.98*                           | -87.57 to 319.90                       | < 0.001                      |
|  | Boldness             | -2071.54*                          | -87.57 to 319.90                       | < 0.001                      |
|  | Body Size x Boldness | 164.05*                            | -363.45 to 112.99                      | < 0.001                      |
| Betweenness                              | Intercept            | -4.22                              | -58.10 to 110.85                       | 0.692                        |
|  | Body Size            | 0.58                               | -4.47 to 4.39                          | 0.432                        |
|  | Boldness             | 10.97                              | -55.85 to 33.79                        | 0.233                        |
|  | Body Size x Boldness | ---                                | ---                                    | ---                          |

## General Conclusion

In Chapter I, I showed that social associations during nesting do not have a kin-bias in the Wolf Howl Pond population of Midland Painted Turtles (MPT; *Chrysemys picta marginata*). I demonstrated the importance of body size in determining nest timing, and suggested how the energetic requirements of increased body size in reproductively active MPT may be the preeminent factor that governs the species' nesting behaviour. Further, I showed how variable climatic conditions across years can alter the timing of nesting, and may prevent other factors from exerting influence over turtle nesting behaviour. Lastly, I suggested that my findings should not be used to discount the potential for kin-bias to social associations or social learning in the nesting behaviour of other populations of MPT, or, indeed, in other testudine species. The Wolf Howl Pond MPT population resides near the species' northern range limit, and accordingly is under greater thermal constraints than many other MPT populations, where non-environmental factors may play a larger role in governing nesting behaviour.

In Chapter II, I studied the social organization of basking aggregations of MPT. I demonstrated that individuals exhibit kin-bias to social associations during basking, specifically that an individual is more likely to associate with kin than non-kin while basking. I proposed that communal basking in MPT offers fitness benefits to the individual, and that any benefits may be enhanced through inclusive fitness. I, thus, called for further research to examine a number of potential benefits (e.g., more consistent access to high quality basking habitat, increased vigilance while basking), and how they may be enhanced through a bias towards associating with kin. I also showed that body size influences social associations during basking of MPT with respect to the number of associations an individual maintains, and the frequency with which they bask. I suggested that larger turtles may preferentially bask in higher quality habitat, and

dominant individuals may maintain social associations by repeatedly sharing these sites. I also demonstrated that influences on social association during basking in MPT may differ between the sexes. I proposed that these differences are a result of mating tactics. Male MPT maintained a higher quantity of weak associations relative to females, and I suggested that this sex-specific disparity may be caused by males attempting to maximize mating opportunities. I provided evidence that higher capture frequency of individuals results in less strict assessment of the social and environmental quality of basking habitat. Further, I suggested that bolder individuals (as indicated by capture frequency) may maintain more social associations by remaining static on a basking structure regardless of a perceived risk. This chapter represented a novel approach to the study of freshwater turtle behaviour and provided the first evidence of kin-bias to social associations during basking in a wild population of adult turtles.

The apparent social organization demonstrated by MPT through my research speaks to the importance of a taxonomic breadth of study in the field of ethology. Further, my findings contribute to the already extensive evidence for the importance of body size in governing the life histories of ectothermic animals. The evolution and maintenance of social systems in turtles, and indeed the many other animal taxa whose social behaviours are yet to be examined, is an area that offers great potential for future research. I provided evidence that mechanisms of social organization can vary by behaviour within a species, shown by the varying importance of kin-bias in associations during MPT nesting and basking aggregations. This finding has implications for the evolution of animal social systems at large. I also suggested that the social behaviour of MPT may have a direct impact on individual fitness. This hypothesis should be examined further, as it has implications for a number of conservation techniques. In particular, reintroduction efforts of at-risk species may be less successful if the social system of the focal

species is not taken into account. By ensuring an optimal social environment for introduced individuals (i.e., reintroduction in kin groups), these recovery efforts may enjoy greater success in the future. Finally, it is my hope that ethological research will continue to become more taxonomically diverse in the future, and I have no doubt that many species whose social capacities are currently underappreciated will demonstrate truly fascinating social complexities.

## Appendices

**Appendix 1.1.** Annual total number of Midland Painted Turtles (*Chrysemys picta marginata*) captured in Wolf Howl Pond (Algonquin Provincial Park, Ontario, Canada) during spring (May and June) sampling. The estimated proportion of the population captured is based on known population size estimates (M. Keevil & S. Sanders, unpubl. data).

| <b>Year</b> | <b>No. of turtles captured</b> | <b>Estimated proportion of population captured</b> |
|-------------|--------------------------------|--|
| 1990        | 201                            | 0.618  |
| 1991        | 183                            | 0.563  |
| 1992        | 230                            | 0.708  |
| 1993        | 219                            | 0.674  |
| 1994        | 232                            | 0.714  |
| 1995        | 236                            | 0.726  |
| 1996        | 216                            | 0.665  |
| 1997        | 207                            | 0.637  |
| 1998        | 236                            | 0.726  |
| 1999        | 278                            | 0.855  |
| 2000        | 302                            | 0.929  |
| 2001        | 257                            | 0.791  |
| 2002        | 310                            | 0.954  |
| 2003        | 308                            | 0.948  |
| 2004        | 290                            | 0.892  |
| 2005        | 317                            | 0.975  |
| 2006        | 272                            | 0.837  |
| 2007        | 263                            | 0.809  |
| 2008        | 282                            | 0.868  |
| 2009        | 180                            | 0.554  |
| 2010        | 218                            | 0.671  |
| 2011        | 259                            | 0.797  |
| 2012        | 256                            | 0.788  |
| 2013        | 271                            | 0.834  |
| 2014        | 253                            | 0.778  |
| 2015        | 244                            | 0.751  |
| 2016        | 245                            | 0.754  |
| 2017        | 262                            | 0.806  |
| 2018        | 254                            | 0.782  |
| 2019        | 271                            | 0.834  |
| 2020        | 265                            | 0.815  |

**Appendix 1.2.** Coordinates and descriptions of basking habitat clusters used to calculate the Bray-Curtis dissimilarity matrix for basking Midland Painted Turtles (*Chrysemys picta marginata*) in Wolf Howl Pond (Algonquin Provincial Park, Ontario, Canada).

| <b>Cluster</b>               | <b>Description</b>   | <b>Coordinates</b>  |
|------------------------------|--|---------------------|
| Northern shore (NS)          | A grouping of floating logs and <i>Sphagnum sp.</i> mats concentrated along the northeast shore.   | 45.5786°, -78.6905° |
| Marked bogs 90 & 91 (9091)   | A solitary free-floating <i>Sphagnum sp.</i> mat divided into two quadrats.  | 45.5785°, -78.6903° |
| Central floating bog (CFB)   | A network of interconnected <i>Sphagnum sp.</i> mats free-floating centrally in the pond.  | 45.5781°, -78.6894° |
| Solitary floating bogs (SFB) | A cluster of small free-floating <i>Sphagnum sp.</i> mats peripheral to, but distinct from, CFB.   | 45.5777°, -78.6981° |
| Southern log jam 1 (SLG1)    | A dense cluster of floating logs and small free-floating <i>Sphagnum sp.</i> mats in an isolated bay along the southern shore.   | 45.5776°, -78.6882° |
| Southern log jam 2 (SLG2)    | An isolated bay along the southern shore dominated by a large free-floating <i>Sphagnum sp.</i> mat with peripheral floating logs.   | 45.5774°, -78.6887° |
| Southern log jam 3 (SLG3)    | A large, dense cluster of floating logs in an isolated bay along the southern shore.   | 45.5772°, -78.6888° |
| Southern log jam 4 (SLG4)    | A large fallen tree still anchored to the southwestern shore with small peripheral free-floating <i>Sphagnum sp.</i> mats in an isolated bay at the junction of the southern and western shores. | 45.5768°, -78.6897° |
| Western shore (WS)           | A cluster of fallen trees and small free-floating <i>Sphagnum sp.</i> mats in a bay along the western shore.   | 45.5775°, -78.6898° |