

The effect of sex ratio on male reproductive success in painted turtles  
(*Chrysemys picta*)

by

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## ABSTRACT

### **The effect of sex ratio on male reproductive success in painted turtles**

*(Chrysemys picta)*

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Sex ratio theory suggests that the strength of intersexual selection will increase as a population more male-biased; reflecting increased selectivity in mate choice. Populations of pond turtle have varying adult sex ratios, in painted turtles (*Chrysemys picta*), reported sex ratios range from female biased (1:3) to male biased (3:1). I investigated the effect of sex ratio on male reproductive success (quantified as “fertilization success”) in painted turtles. I examined the mating system of painted turtles in a female-biased population using microsatellite paternity analysis, relating variation in male fertilization success to male phenotype and offspring survival, employed *ex situ* behavioural observation to clarify the mechanism behind the variation in male fertilization success and used agent-based modeling to simulate the effects of changing sex ratio, population density and proportions of male phenotype on male fertilization success. Small males contributed sperm to a greater number of clutches than did larger males, but were not more likely to reproduce in a season than larger males. There was no offspring fitness advantage related to male body size and no relationship between male claw length and fertilization success. Large male painted turtles courted at a higher frequency than small males. I found no relationship between male courtship behavior and claw length. Females showed no preference for males of any phenotype. Agent-based simulations were based on the distribution of best fit from the observed data; an amalgam of two Poisson distributions, each with its own probability of success and proportional representation in the final distribution. Increased female sex ratio bias, increased population density and increased proportions of “more successful” males all increased the mean and variance of male fertilization success, based on increased encounter rate among turtles. Small and large male painted turtles enjoy different fertilization success. It is uncertain whether this difference is based on active female choice, cryptic female choice, sperm competition or a combination of factors. Sex ratio simulations predict the opposite result as that

predicted by sex ratio theory. These contrary results should be compared to simulations manipulating choosiness and field data from painted turtle populations to clarify mechanisms influencing male reproductive success.

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## ***INTRODUCTION***

Sexually dimorphic traits are often thought to be maintained in a population not by natural selection, but by sexual selection (Andersson, 1982; Andersson, 1994; Arnold and Wade, 1984; Boake, 1986; Emlen and Oring, 1977; Ernst, 1972). Sexual selection may act through increased reproductive success as a result of competition with others of the same sex (intrasexual selection) or increased reproductive success as a result of being chosen as a mate (intersexual selection) (Andersson, 1994). Both intrasexual selection and intersexual selection may work overtly (e.g. physical competition wherein the victor mates, or acceptance of courtship leading to sperm transfer), or cryptically (e.g. sperm competition or oviductal sperm selection) (Andersson, 1994; Eberhard, 1996). Thus, while observational studies of mating behaviour may indicate an overt mechanism of sexual selection, possible cryptic mechanisms should not be discounted.

There is great variation in the strength of sexual selection and in the divergence of sexually dimorphic traits among closely related species as a result of differences in mating system and courtship behaviour (Andersson, 1994; Berry and Shine, 1980; Ims, 1988). Additionally, the strength of sexual selection varies among populations of the same species as a result of demographic factors, such as adult sex ratio and density, that change the magnitude of the fitness differential among males of differing phenotypes (Kwiatkowski and Sullivan, 2002, Møller *et al.*, 2006) and varies temporally within populations as a result of fluctuations in demographic factors over a single reproductive season (Kasumovic *et al.*, 2008 (operational sex ratio); Punzalan *et al.*, 2010 (adult sex ratio)). More drastically, differences in operational sex ratio have been related to a differences in the form of sexual selection in mealworms (*Tenebrio molitor*), from intersexual selection in female-biased populations to intrasexual selection in male-biased populations (Fairbairn and Wilby, 2001). Sex ratio thus has a strong effect on sexual selection over a range of spatial and temporal scales (Ims, 1988; Kasumovic *et al.*, 2008).

In discussions of mating and reproductive success, the ratio of greatest interest is the ratio of sexually receptive males to sexually receptive females (operational sex ratio).

Operational sex ratio is generally governed by the mating system of a species. Regardless of the adult sex ratio, if the mating system determines that one sex is a limiting factor for the other, then the operational sex ratio will be skewed (Partridge and Endler, 1987). Sexual selection is thus intense in highly polygamous species as many males are competing for few available females (Emlen and Oring, 1977). Females that have a large number of potential mates can be choosier than females that have few potential mates (Andersson, 1994), leading to stronger sexual selection and greater degree of sexual dimorphism in the former scenario and weaker sexual selection and a lesser degree of sexual dimorphism in the latter scenario. Similarly, males that have a large number of potential competitors will each be less likely to mate with a female (due to energy devoted to increased levels of competition and female occupation with other males) than will males with a small number of potential competitors, again leading to a greater degree of sexual dimorphism in the former scenario and a lesser degree of sexual dimorphism in the latter (Andersson, 1994).

Nonetheless, deviations in the adult sex ratio away from a 1:1 ratio can amplify or reduce the divergence in operational sex ratio. Fisher and Bennett (1999) hypothesized that in sexually reproducing species, the secondary sex ratio (at the end of parental investment) should always tend towards even, if there is an equal cost to raising either sex. If there is a space of sufficient time between the end of parental investment and the onset of sexual maturity, differences between the sexes, for example, in mortality, dispersal or behaviour, can skew the adult sex ratio away from 1:1. However, in sexually reproducing species, because the less-abundant sex has higher mean fitness than the more-abundant sex, the adult sex ratio should also tend towards even (Fisher and Bennett, 1999).

### ***Courtship and sexual dimorphism in *Chrysemys picta****

Adult male painted turtles (*Chrysemys picta*) initially follow and nose the cloacal region of potential mates; behaviour that is common during courtship in highly aquatic freshwater turtle species (Jackson and Davis, 1972*a,b*; Lovich *et al.*, 1990; Norris, 1996). After the initial approach, the male painted turtle positions himself face-to-face with a

female and vibrates his elongated foreclaws near the head of the female (Ernst, 1971*a*). The female turtle may respond by vibrating her foreclaws near the head and forelimbs of the male (Ernst, 1971*a*). The male then moves to mount the female, she drops to the bottom of the pond and copulation occurs (Ernst, 1971*a*).

Painted turtles are sexually dimorphic, the two most obvious characters are that males are generally much smaller than females (although in some populations, the body size of large males overlaps with the body size of small mature females (e.g. White Oaks, Pennsylvania, Ernst, 1971*b*)) and have highly elongated foreclaws as compared to females (Ernst and Lovich, 2009). Among freshwater turtle species, Berry and Shine (1980) found a significant relationship between the direction of sexual size dimorphism and the form of sexual selection. When males are the larger sex, sexual selection acts through male-male combat, however, when females are the larger sex, sexual selection acts through female choice. Since female *C. picta* are much larger than males, a system of intersexual selection is assumed (Berry and Shine, 1980). Large female sexual dimorphism likely the ancestral condition in the family Emydidae (Stephens and Weins, 2009). Additionally, natural selection should favour a larger female body size (to a point) because of restrictions placed on female turtles' reproductive potential by the presence of the shell (Congdon and Gibbons, 1987). If this is so, then the small body size of male painted turtles may be maintained through countervailing sexual selection (Congdon and Gibbons, 1987). Sexual maturity in painted turtles is governed by body size (Samson, 2003) and the species exhibits indeterminate growth (Ernst and Lovich, 2009). Generally, smaller males are younger males (Ernst and Lovich, 2009, but see Congdon and van Loben Sels, 1993), and so it is possible that female choice of smaller males may be simply choice of younger males; age being a strictly phenotypic trait. If females choose mates based on a phenotypic trait, then there should be variation in male reproductive success, but no evolutionary change should be observed as the trait is not heritable. In many reptiles with indeterminate growth, body size reaches an asymptote; growth continues, but change in size is practically undetectable (Andrews, 1982). The asymptotic size of different males may vary significantly and may constitute a heritable trait (there is a shortage of accurate age estimates with which to test this supposition) and female *C. picta* may indeed be choosing small mates. McTaggart (2000) suggests that

smaller males may have a fitness advantage in terms of ease of movement within the aquatic habitat.

The highly elongated foreclaws play an important role in courtship behaviour of male painted turtles (Ernst, 1971) and females may use some attribute of the claw as a cue for mate choice; length being the most obviously sexually dimorphic attribute. Additionally, because females often reciprocate the claw waving/stroking behaviour, long claws are likely not necessary to perform the behaviour, so the length of the foreclaws in male painted turtles may be maintained by intersexual selection.

The few studies that have investigated whether either body size and foreclaw length are used as cues for female painted turtles when choosing mates produced contradictory results. For example, in an Algonquin Park, Ontario female-biased population of painted turtles, male body size predicted the frequency of paternity (McTaggart, 2000), whereas in an Illinois population, no relationship between male reproductive success and phenotype was found (Pearse *et al.*, 2002; note that there are no published sex ratio estimates for this population).

The mating system of painted turtles is complex and poorly understood. Painted turtles are polygynandrous; both females and males copulate with many mates over a mating season (Ernst and Lovich, 2009). However, the reproductive output of females is generally restricted to from one to three clutches of four to 20 eggs each per season, partly depending on latitude (Ernst and Lovich, 2009), with only one or two males contributing paternity to each clutch (McTaggart, 2000; Pearse *et al.*, 2001). In addition, female painted turtles can store sperm in their oviducts over multiple seasons (Gist and Jones, 1989; anecdotally, up to three years, Ernst and Lovich, 2009) and use stored sperm to fertilize sequential clutches either within or between seasons (Pearse *et al.*, 2001: 2002). Thus, depending on the incidence of intraseasonal iteroparity and of stored sperm use, the operational sex ratio may be skewed as compared to the adult sex ratio.

### ***Painted turtle sex ratios***

Many freshwater turtle populations have skewed adult sex ratios, for example, in painted turtles, sex ratio ranges from highly female-skewed (this study) through even (Ernst, 1971*b*) to highly male-biased (Congdon et al, 1986). According to Gibbons (1990), a skewed adult sex ratio should be expected if one sex matures at an earlier age than the other. The timing of painted turtle maturity is size-based, with males maturing at a smaller size and thus at an earlier age than females (Samson, 2003), so a male skew is predicted. However, Freedberg and Wade (2001) suggest that in species with environmental sex determination, such as painted turtles, cultural inheritance of nest site choice will lead to a female skewed adult sex ratio. Female turtles are produced in nests of a specific thermal profile. If nesting females have a tendency to nest in a similar location to that in which they were hatched, then they will be more likely to produce female offspring rather than male (Freedberg and Wade, 2001). Alternatively, skewed sex ratios reported in many turtle species may be attributed to sampling bias, for example, if studies focus on nesting behaviour, differential dispersal patterns between sexes leading to differential mortality risk (McCulloch and Secoy, 1983; Rowe, 2003; Scribner *et al.*, 1993), or anthropomorphic effects, such as collection for the pet trade (Reeves and Litzgus, 2008), habitat alteration (Eskew *et al.*, 2010) or road density (Patrick and Gibbs, 2010). Regardless of the effects of polygynandry on the operational sex ratio in painted turtles, variation in adult sex ratio also results in variation in operational sex ratio, and influences the form and strength of sexual selection and thus, variation in male reproductive success among populations of *C. picta*. I propose to investigate the effects of variation in adult sex ratio on male reproductive success in painted turtles.

### ***Study population and site***

*Chrysemys picta marginata* (midland painted turtle, Emydidae) is a medium-sized, mainly aquatic freshwater turtle (Ernst and Lovich, 2009). This subspecies ranges from southern Ontario and Quebec south to Tennessee and Alabama (Ernst and Lovich, 2009). The population of interest is located at Wolf Howl Pond (WHP) and West Rose Lake (WRL) in Algonquin Park, Ontario (45°34' N 78°41'W). Teams of researchers have

observed nesting behaviour at this site over 28 of the past 33 years (1977-2010). Researchers find between 44 and 326 nests each summer, depending on seasonal variability and sampling effort. A recent study estimated a total population size of 313 painted turtles in these ponds (Samson, 2003). The adult sex ratio was estimated at 1:3.44 (Samson 2003).

Wolf Howl Pond and West Rose Lake are black spruce (*Picea mariana*) bogs, each divided by a sand railway embankment (built c. 1895) that is now a popular walking trail. The sides of the embankment face generally northeast and southwest in Wolf Howl Pond. In West Rose Lake, the slopes face more directly east and west. On the 240m embankment along Wolf Howl Pond, the steeply sloped northeast side rises 5m above the water and is dominated by white pine (*Pinus strobus*), balsam fir (*Abies balsamea*) and tamarack (*Larix laricina*). The southwest embankment has a gentler slope and is sparsely vegetated with grasses and shrubs (e.g. *Calamagrostis* sp., *Agrostis* sp., bush honeysuckle, *Diervilla lonicera*, blueberries, *Vaccinium angustifolium*, raspberries, *Rubus strigosus* and bracken fern, *Pteridium aquilinum*). The southwest slope rises 2m above the bog. The flat walking trail has no vegetative growth (about 3-4m wide). In West Rose Lake, the two sides of the trail are generally more similar to each other in angle and height from water. Along the 700m length of the embankment at this bog, the height and angle of the slope vary from less than a meter and about 15° slope at the south end, to 10m and about 50° slope at the north end. Vegetation at West Rose Lake is very similar in composition to that at Wolf Howl Pond; both sides of the embankment, however, support more mature tree growth and more abundant vegetation.

Painted turtles in Algonquin Park generally emerge from hibernation in late April and begin nesting in late May. Nesting continues until mid-July. At Wolf Howl Pond, painted turtles nest on the edges of the flat top of the embankment and on the gently sloped southwestern side. At West Rose Lake, painted turtles nest on the flat top of the embankment and in sandy, non-vegetated areas on either side of the embankment. The turtles return to hibernation in late October to late November (Rollinson *et al.*, 2008). Little courtship and mating behaviour has been observed in this population since the



focus of the long-term research has been demographics and nesting, however, males are often seen following females throughout the active season (pers. obs.)

In chapter 1, I use microsatellite analysis to investigate the mating system of a female-biased population of painted turtles in Algonquin Park, Ontario. Specifically, I address the relationship between male reproductive success and male phenotype in the context of female mate choice. In chapter 2, I use behavioural observation in artificial ponds and y-mazes to discriminate between female choice based on male phenotype and differences in male courtship behaviour as mechanisms leading to the observed variation in male reproductive success. In chapter 3, I use agent-based modelling to investigate the effect of variation in population sex ratio, density and proportions of male type on male reproductive success in painted turtles.

## **CHAPTER ONE**

### ***MATING AND PATERNITY OF PAINTED TURTLES IN ALGONQUIN PARK, ONTARIO***

Sexual dimorphism in a trait is often taken as evidence of the effect of sexual selection on the phenotype of a species (Darwin, 1874). Thus, any observed differences in the mean trait value between sexes is hypothesized to be due to differential reproductive success associated with that trait within one or both of the sexes (Arnold and Wade, 1984; Andersson, 1994). Reproductive success can vary when there is competition among members of one sex for access to reproductively active members of the other sex or to resources essential for mating (intrasexual selection) or when based on a preference for mates of a specific type (intersexual selection)(Andersson, 1994).

The mating system of painted turtles is generally characterized as one of female mate choice (Berry and Shine, 1980; Ernst and Lovich, 2009). Male painted turtles court by following females and then displaying, waving their elongated foreclaws on either side of the female's head. The female may first reciprocate by stroking the male's forelimbs with her claws or may simply drop to the bottom of the body of water, where copulation occurs (Ernst and Lovich, 2009). Extended male courtship using a sexually dimorphic character (enlarged foreclaws) in addition to sexual size dimorphism in *C. picta* (males smaller than females) implies a system of female choice (Berry and Shine, 1980) and thus, a certain degree of female control over male reproductive success.

Although painted turtles are polygynandrous (Ernst and Lovich, 2009), which is often associated with a lack of active choice (Ims, 1988), both male and female turtles can store viable sperm; females for up to four years (Gist and Jones, 1989; Pearse *et al.*, 2001) and males for at least 40 days (Gist *et al.*, 2001). Sperm storage may be a requirement for painted turtles, because spermatogenesis is completed and most sperm transfer occurs in fall, and ovulation occurs in spring (Gist *et al.*, 1990; 2001). Females retain the contributions of many males within their oviducts (Pearse and Avise, 2001), thus, female choice may be cryptic and observations of mating behaviour alone cannot demonstrate unequivocal paternity. The mechanisms of sperm storage and use in *C. picta* are as yet unknown (Kuchling, 1998), however there is some evidence to suggest that the

sperm from the last male to mate with a female is used to fertilize her offspring (“last-in, first-out”, Pearse *et al.*, 2001). A female may exert cryptic mate choice by remating if a prospective mate is of higher quality than her previous mates thus improving the quality of the last sperm contribution contained in her oviducts (Uller and Olsson, 2008). In addition, she may exert control over paternity of her clutch by allowing males to mate without allowing ejaculation (Note: Uller and Olsson (2008) suggest that the small quantity of sperm transferred in turtle copulation suggests a lack of sperm competition by volume).

Painted turtles are intraseasonally iteroparous; depending on the latitude of the population, females lay up to three nests in a single season (Ernst and Lovich, 2009). In the Algonquin population, no more than two nests for a single female in a single season have been recorded (Samson, 2003). In addition, multiple paternity of clutches has been observed in painted turtles (Pearse *et al.*, 2001; 2002), indeed in numerous reptile species (Uller and Olsson, 2008). Female painted turtles may exert further control over male reproductive success through use of stored sperm to fertilize consecutive clutches and/or use of sperm from multiple mates to fertilize a single clutch (Pearse *et al.*, 2002, Roques *et al.*, 2006, Refsnider, 2009). Regardless of the number of successful copulations by an individual male, a female may increase his reproductive success by using his sperm in multiple consecutive clutches or may decrease his reproductive success by using his sperm to fertilize only a portion of her clutch (Eberhard, 1996).

There are no direct benefits from mate choice in turtles, as males do not offer nuptial gifts or protection, nor do they contribute to care of offspring or provide nutritional benefit from ejaculate (Andersson, 1994; Uller and Olsson, 2008). There are possible indirect benefits to females from choosing a male of one phenotype over another; benefits due either to good genes or to sexy sons (Andersson, 1994). Either of these hypotheses could be applicable to painted turtles, however the life-history of turtles makes testing the latter hypothesis prohibitively time-consuming and expensive; it requires knowledge of mating success of sons of more-frequently-chosen males as compared to mating success of the sons of less-frequently-chosen males (Andersson, 1994). McTaggart (2000) found evidence to support the good genes theory in a previous

paternity analysis of the same population. Hence, I chose to focus on the good genes hypothesis; that females choose mates that increase the fitness of their offspring.

*C. picta* are most obviously sexually dimorphic both in body size (males are smaller than females) and foreclaw length (males have longer foreclaws than do females) (Ernst and Lovich, 2009), thus, foreclaw length and body size are both candidates as cues for female mate choice. Painted turtles continue to grow post-maturity (indeterminate growth), and it is difficult to tease apart whether females are choosing males based on age or based on body size. There is currently only a small sample of males with accurate age estimates in the study population (Samson, 2003), so I focus on body size and address age as a possible alternative in the discussion.

Active mate choice is difficult to study in painted turtles because copulation often occurs at the bottom of murky water bodies (Ernst and Lovich, 2009) and further may not be useful in determining paternity because of potentially cryptic mate choice (Pearse *et al.*, 2001). Thus, I use male fertilization success as a proxy for female mate choice. The concept of male fertilization success assumes *a priori* that a female accepts copulation from a given male and that his sperm is used to fertilize a portion of her offspring.

I hypothesized that differences in male fertilization success are related to sexually dimorphic traits and that having these traits is associated with greater offspring fitness. First, I assessed variation among male *C. picta* with regards to the two most obvious sexually dimorphic traits: body size and claw length. Second, I addressed two predictions relating to male fertilization success and fitness:

1. If male fertilization success is based on differences in male phenotype, then observed variation in male fertilization success will be related to either male body size or to male foreclaw length.
2. If males of a specific phenotype have higher fitness than males of other phenotypes, then observed variation in offspring hatch success, offspring survival and change in offspring size over hibernation (fitness proxies) will be related to either male body size or to male foreclaw length.

Third, I assessed the potential for female painted turtles to exert significant control over male reproductive success by use of stored sperm and through multiple paternity within a single clutch. I thus estimated the frequency of stored sperm use both within and between seasons and the frequency of multiple fathers within a single clutch.

## *Methods*

### **Field methods**

In May of 2004, 2005 and 2006, researchers used dip nets to catch as many painted turtles as possible in Wolf Howl Pond and West Rose Lake (see introduction for description of the sites). We transported the turtles to the Wildlife Research Station, Algonquin Park (WRS) to identify individuals and collect morphometric data (specifically, mid-plastron length, mass and 3<sup>rd</sup> claw length on both front feet). Turtles were away from the pond for a maximum of 24 hours. All turtles in this population are permanently marked with notches in the carapacial scutes (Cagle, 1939). Additionally, all mature turtles are identified by an alphanumeric code on an aluminum tag that is wired to the carapace. Upon capture each year, the females are marked with their individual alphanumeric code in white paint on the carapace. This allows identification of the turtles with a minimum of disturbance during nesting. Beginning in early June, researchers patrolled the nest areas (240m long in WHP and 700m long in WRL) at 45-minute intervals to locate nesting females and observe nesting behaviour. Upon completion of oviposition by the female turtles, researchers excavated the nest, and the eggs were transported to the WRS in plastic containers filled with moist vermiculite. We collected morphometric data (egg length, width and mass) and reburied the eggs within 24 hours.

### *Blood collection from adult turtles*

Researchers collected blood from all adult painted turtles caught in 2004, 2005 and 2006 (a single blood sample was taken from each turtle), by withdrawing 100 $\mu$ l of blood from the caudal artery where it runs through the tail distal to the cloaca using a 1ml syringe and a 27-gauge 1/4" bevel tip needle. The tail was washed with water and sterilized with an alcohol wipe before and after drawing blood. Blood was stored in 1ml

of Queen's lysis buffer (Seutin et al., 1991) in a 1.5ml microcentrifuge tube. All blood samples were moved to a  $-20^{\circ}\text{C}$  freezer as soon as possible following collection; samples remained at room temperature for a maximum of one week prior to freezing.

### *Nest selection*

In summers of 2004 and 2005, I chose a subsample of all nests laid to include in the paternity analysis. I aimed to collect data from 40 first clutches and 20 second clutches in each year. I defined a first clutch as the first recorded clutch attributed to a specific female in a season. I defined a second clutch as the second recorded clutch attributed to a specific female in a season. As a result, some clutches that were laid late in the season may have been incorrectly identified as first clutches because we may have missed a female's first clutch and so these late clutches were in fact second clutches. To allow comparison of paternity among multiple clutches of single females both within and between years, I used the 28-year Algonquin Park *C. picta* database (R.J. Brooks) to compile a list of 45 target turtles: mature females with a tendency for intraseasonal iteroparity (had laid two clutches in a single season in at least two years) and that had not been reported dead. In 2004, I chose all first clutches laid by target females each day of nesting and then used a random numbers table to choose between one and nine additional clutches each day to a maximum of 40 first clutches. I subsequently chose 20 second clutches laid by females from which I had already collected a first clutch in that year. In 2004, this method resulted in 12 out of 40 first clutches collected in a single day (see Table 1). Hence, I altered the methodology in 2005 to ensure that I selected clutches more evenly across the nesting season. In 2005, I chose all first clutches laid by females from which I had collected a clutch in 2004, I chose all first clutches laid by target females and then used a random numbers table to determine the maximum number of clutches chosen each day. I haphazardly selected additional clutches each day to bring the number of clutches collected each day up to the randomly determined total. I selected a total of 40 first clutches in 2005. Because fewer females produced double clutches in 2005 and because of my revised methodology, I only sampled eight second clutches laid by females from which I had collected a first clutch in 2005. See Tables 1 and 2 for a distribution of nests laid and chosen per day. To control for maternal effects of nest-site

choice, all clutches in both years were moved to the Lake Sasajewun dam at the Wildlife Research Station, Algonquin Park, Ontario and reburied by clutch in 9cm-deep nest cavities under one of three large chicken wire nest protectors (see Figures 1 and 2). All nests were reburied within 24 hours of being laid.

In early October 2004, I excavated all of the nests on the dam. Cold weather in August delayed hatching and so many of the hatchlings were still in their eggs or had died while hatching (see Appendix 1 for fate of eggs by clutch). I moved all live hatchlings and unhatched eggs to a climate-controlled room in the Aqualab facility, University of Guelph, Guelph, Ontario. Unhatched eggs were placed on moistened vermiculite in a 20.32cm x 30.42cm Tupperware box and maintained at 26°C in an incubator until hatched or pronounced dead. Live hatchlings were kept with their clutch mates on moistened vermiculite in the 20.32cm x 30.42cm Tupperware boxes at 15°C with a 12h:12h light:dark schedule. In mid-December, 2004, I reduced the room temperature by 2 degrees a day to 4°C and changed to a 24h dark schedule to induce hibernation. Throughout pre-hibernation and hibernation, I checked the hatchlings daily to ensure that the vermiculite remained moist. I ended hibernation at the end of March by increasing the room temperature by 2°C a day to 20°C and changing to a 12h:12h light:dark schedule. I then moved the hatchlings (retaining clutch identity) to 12.7cm x 12.7cm Tupperware boxes containing 1" of water and basking rocks. I suspended one broad spectrum basking light over each group of six boxes and rotated the boxes daily to ensure that no clutch was exposed to too much or too little heat. I changed the water in the boxes daily, added calcium supplement to the water and fed the hatchlings frozen bloodworms. Surviving hatchlings were released into their natal ponds in late April 2005 (N=71).

In 2005, exceptionally warm July and August temperatures resulted in hatchlings emerging from their nests into the common garden nest protector in the last week of August. Painted turtle hatchlings in Algonquin Park usually remain within the nest cavity until the spring (R.J. Brooks, pers. comm.). I excavated all hatchlings and unhatched eggs and moved them to the Aqualab Facility and maintained them using the method described above. I induced hibernation at the end of November, 2005 and ended

**Table 1 - Distribution of clutches by date laid, 2004**

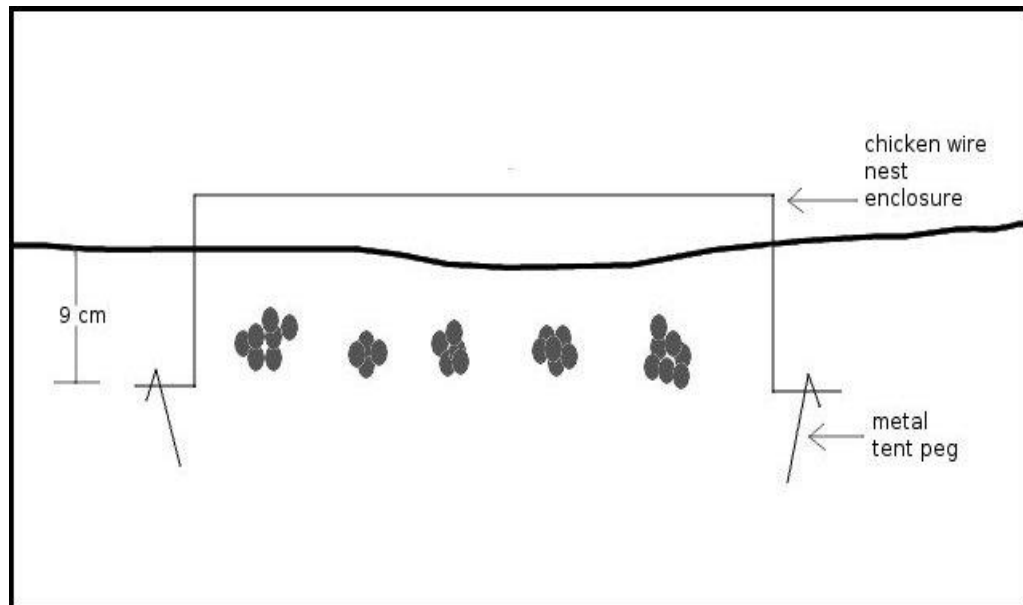
Nest date	Total 1 <sup>st</sup> clutches laid	Chosen 1 <sup>st</sup> clutches		Non-target clutches	Total 2 <sup>nd</sup> clutches laid	Chosen 2 <sup>nd</sup> clutches
		Target clutches				
05-Jun-04	1	0	0	0		
07-Jun-04	9	7	3	4		
08-Jun-04	10	4	3	1		
09-Jun-04	14	4	3	1		
10-Jun-04	8	6	3	3		
11-Jun-04	10	7	2	5		
12-Jun-04	19	12	4	8		
13-Jun-04	13					
14-Jun-04	33					
15-Jun-04	15					
16-Jun-04	15					
17-Jun-04	18					
18-Jun-04	8					
20-Jun-04	6				1	
22-Jun-04	9				1	1
23-Jun-04	7				4	4
24-Jun-04	3				0	
25-Jun-04	6				5	2
26-Jun-04	6				1	1
27-Jun-04	0				4	1
28-Jun-04	3				1	
29-Jun-04	5				0	
30-Jun-04	7				17	10
01-Jul-04	9				7	1
02-Jul-04	4				7	
03-Jul-04	4				11	
04-Jul-04	4				9	
06-Jul-04	4				4	
09-Jul-04	0				1	
10-Jul-04	1				2	
<b>Totals</b>	<b>179</b>	<b>40</b>	<b>18</b>	<b>22</b>	<b>75</b>	<b>20</b>



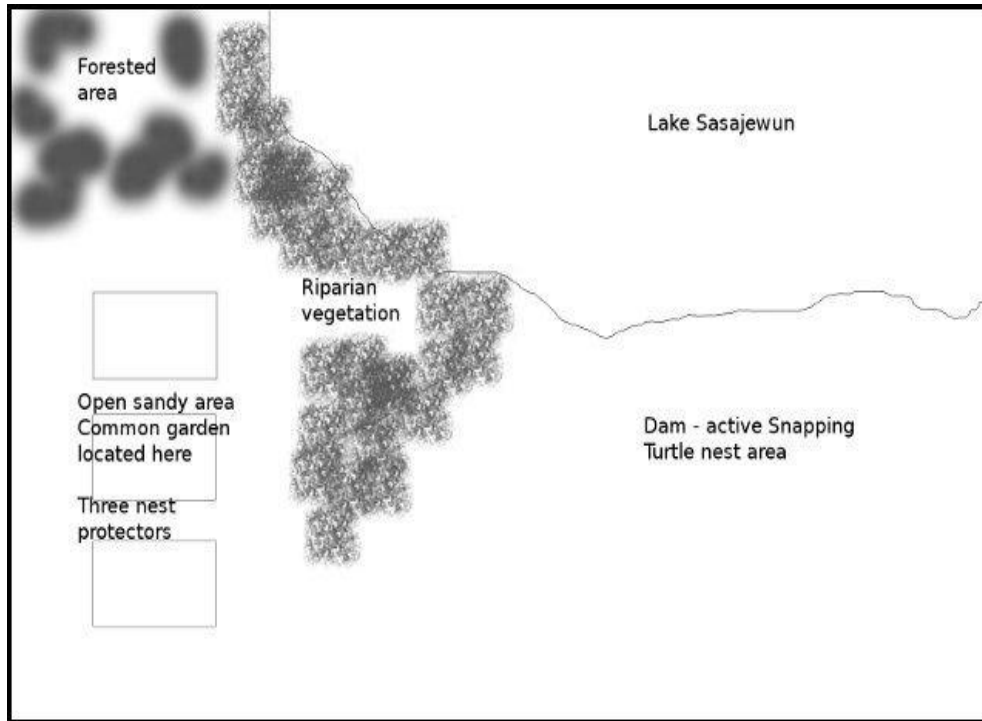
**Table 2 - Distribution of clutches by date laid, 2005.**

Nest date	Total 1 <sup>st</sup> clutches laid	Chosen 1 <sup>st</sup> clutches	Target clutches	Non-target clutches	Total 2 <sup>nd</sup> clutches laid	Chosen 2 <sup>nd</sup> clutches
06-Jun-05	6	2	2	0		
07-Jun-05	7	1	1	0		
08-Jun-05	19	6	6	0		
09-Jun-05	34	6	4	2		
10-Jun-05	24	3	3	0		
11-Jun-05	31	2	2	0		
12-Jun-05	28	5	2	3		
13-Jun-05	20	4	2	2		
14-Jun-05	17	4	2	2		
15-Jun-05	1	0	0	0		
17-Jun-05	1	2	0	2		
18-Jun-05	1	0	0	0		
19-Jun-05	4	4	2	2	1	1
20-Jun-05	8	1	1	0	3	
21-Jun-05	1				1	
22-Jun-05	4				6	1
23-Jun-05	2				0	
24-Jun-05	2				10	1
25-Jun-05	8				9	1
26-Jun-05	6				15	2
27-Jun-05	5				10	
28-Jun-05	1				9	
29-Jun-05	3				1	1
30-Jun-05	0				1	
01-Jul-05	1				1	1
04-Jul-05	3				0	
<b>Totals</b>	<b>237</b>	<b>40</b>	<b>27</b>	<b>13</b>	<b>67</b>	<b>8</b>

**Figure 1 – Lateral view of nest protector showing locations of five of the twenty nests buried beneath.**



**Figure 2 – Location of three nest protectors on the Lake Sasajewun dam, Wildlife Research Station, Algonquin Park, ON.**



hibernation at the end of March, 2006. In order to improve post-hibernation survival rates, I moved all hatchlings to the water-filled boxes before increasing the room temperature. I used the same hatchling maintenance protocol as in the previous year and surviving hatchlings were released to their natal ponds in late April 2006 (N=214).

In both years, I collected morphometric data (mid-plastron length, mid-carapace length, carapace height and mass) from the hatchlings before and after hibernation.

I used Pearson's  $r$  to correlate the number of offspring successfully hatched (hatch success) and hatchling survival to release with Julian lay date in each year. Additionally, I evaluated change in hatchling morphometrics (carapace length and height, plastron length and body mass) from the start to the end of hibernation using Student's  $t$ -tests.

#### *Tissue collection from hatchlings*

I collected toe clips from live hatchlings and tissue samples from dead hatchlings, partly-developed hatchlings and embryos, thus the condition of the tissue ranged from limbs or internal organs from recently deceased individuals to highly decomposed tissue or bones and scutes. In both years, I collected tissue from all dead hatchlings, partly-developed hatchlings and embryos upon excavation of the nests (early October 2004 and late August 2005) and then immediately upon the death of a hatchling while at the Aqualab Facility. I collected toe clips from live hatchlings in early January, 2005 and in late March 2006. I collected toe clips earlier in the first year to accommodate a concurrent study.

For all tissue samples, I used scissors and forceps sterilized with 70% ethyl alcohol to remove tissue from the hatchling. I stored the samples in Nalgene cryotubes in liquid nitrogen immediately following collection and moved samples to a  $-80^{\circ}\text{C}$  freezer as soon as possible. Tissue samples collected during excavation at the WRS remained in liquid nitrogen for up to a week before moving to a freezer, while samples collected at the Aqualab Facility remained in liquid nitrogen for a maximum of 24 hours. I used the same method to collect tissue samples from two adults (E50, female and 0310, male) found dead during the course of the study.

I collected tissue samples from live hatchlings before ending hibernation, while still at 4°C, to minimize pain and movement (Machin, 2001). I removed two hind toes from each hatchling; these marks also serve as an identifier of the year in which the turtles hatched. I used Bactine antiseptic and anaesthetic spray to clean the limb prior to incision and to bathe the limb following tissue collection and to reduce pain.

### **DNA extraction and amplification**

I extracted DNA from blood samples using the GenElute mammalian DNA miniprep kit (Sigma-Aldrich) and from tissue samples using a phenol-chloroform extraction. I amplified the DNA on five microsatellite loci, BTGA3, BTCA5 and BTCA7 (Libants *et al.*, 2004), BTCA11 (S. Libants, unpub. data) and EB11 (Osentoski *et al.*, 2002). PCRs were run in two different laboratories and visualized on two different machines. As such, I followed two different protocols for DNA amplification and visualization, however I used the same published PCR conditions in both labs and all PCRs were run on 96-well GeneAmp PCR systems (Applied Biosystems). At the University of Guelph laboratory (Guelph, ON), 1ml of DNA was amplified in a 20µl reaction, 5µl of PCR product was run on a 1mm thick 6% polyacrylamide gel for 2.5 hours or until the dye had migrated off the bottom of the gel and subsequently visualized on a Hitachi FMBioII multi-view scanner. At the Simon Fraser University laboratory (Burnaby, BC), 1ml of DNA was amplified in a 10µl reaction, 0.3µl of PCR product was run in a 1mm thick 6% polyacrylamide gel in a LiCor 4300 DNA analyzer for 1 hour or until all bands were visible on the computer readout. I used the resulting images to score the genotypes of all individuals. If a sample was unclear or the genotype was uncertain, I repeated the PCR reaction up to two times using a larger quantity of DNA for the unclear samples. If the image remained unclear in the visualization, I repeated the gel visualization up to three times, using more PCR product if the bands were very faint. Samples that remained unclear or were not visible were discarded from the subsequent analysis. See Appendix 2 for the fate of each sample.

After assigning genotypes to each individual, I identified all genotype mismatches between mothers and offspring (i.e. no shared alleles on at least one locus). I compared maternal and offspring genotype images to determine if the mismatch could be reconciled

based on an error in genotyping. If the mismatch could not be reconciled, I compared it with other mismatches on the same locus. If a number of individuals exhibited the same mismatch with the mother and if the mismatch was of two base pairs (e.g. 160bp instead of 162bp at locus BTCA7), I assumed that the mismatch was due to a null allele, a mutation or a scoring error. I discarded microsatellite BTGA11 because of a large number of unresolved mismatches of greater than two base pairs between mothers and offspring, indicating a lack of specificity in the primer. Seventy-four unresolved mother-offspring single locus mismatches (four identified as null alleles by NewPat) representing 24 females remained following this reconciliation. Each female-offspring pair of these 74 displayed a mismatch of only two base pairs on a single locus.

### **Paternity analysis**

I used NewPatXL V. 5 (Amos, 2000) to determine paternity of all hatchlings. I ran each paternity analysis twice, once “strict”, allowing no mismatches between offspring and potential fathers and once “relaxed”, allowing a single mismatch of 2 base pairs between offspring and potential fathers. In both analyses, I allowed a single mismatch of two base pairs between the offspring and the known mother. I first assigned paternity based on the strict output and only used the relaxed output if I was unable to identify a most-likely father using the strict output.

I analysed paternity by clutch, i.e. males were assigned whole or partial paternity of a clutch, not of an individual hatchling. I began, however, by assigning paternity to individual hatchlings. For each hatchling, I ranked the output relatedness scores. NewPat relatedness scores range from 0 to 1. NewPat also determines the number of randomly generated fathers (based on offspring and mother genotypes) that match the putative fathers, a high randomization number indicating a good match between father and offspring. If the output contained only a single possible male, or a single male with a much higher relatedness score than other candidates (i.e., no other relatedness score was within 0.1 points of the highest) and a high randomization number, then I attributed paternity of the hatchling to that male. If the output contained multiple males with equally high relatedness scores and randomization numbers, I compared the ranked fathers against the assigned fathers for other hatchlings in the clutch. If there was a

concordance between one of the highest ranked fathers for a target hatchling and the assigned father for others in the clutch, I awarded paternity of the target hatchling to the common father. If there was no concordance between the highest ranked fathers and the assigned father for others in the clutch, I awarded partial paternity of the clutch to multiple males. If there was no obvious male ranking for a specific hatchling, then I identified that hatchling as “no father”.

To compare male fertilization success and male phenotype, I categorized each male by the number of clutches to which he contributed paternity. Subsequently, I collapsed those categories into three “fertilization success” categories: 1) males that contributed to no clutches, 2) males that contributed to a single clutch and 3) males that contributed to two or more clutches. I used categories to allow comparison between this work and a previous study on the same population (McTaggart, 2000) and to allow the use of parametric statistics. I used ANOVA to investigate the relationship between male phenotypes (plastron length and claw length) as the independent variables and the three fertilization success categories as the dependent variable. To investigate the relationship between male phenotype (body size and claw length) and offspring hatch success I used a linear regression analysis with male phenotype as the independent variable, number of offspring hatched per clutch as the dependent variable and year and initial clutch sizes as covariates. To investigate the relationship between male phenotype and offspring survival to release, I used a linear regression analysis with male phenotype as the independent variable, number of offspring survived to release per clutch as the dependent variable and year and number of offspring hatched as covariates.

### **Multiple paternity and sperm storage**

I determined rates of multiple paternity using two methods. First, I used the results from the NewPat paternity analysis to determine actual rates of multiple paternity (paternity in a single clutch could be attributed to more than one male). I identified cases of potential multiple paternity when a clutch contained a single known father and also hatchling(s) for which paternity was inconclusive (“no father”). Second, I used Gerud 2.0 (Jones, 2005) to reconstruct paternal genotypes based on known maternal and hatchling genotypes.

I categorized sperm storage by the number of clutches fathered and sequence of clutches. When a single male was found to contribute to more than one clutch by the same female, it was considered potential evidence for sperm storage. When the two clutches were consecutive within a single season, the most parsimonious explanation for similar paternity is stored sperm, since there is a very short period of time between laying the two clutches (14-16 days, Samson, 2003). When the clutches were separated by a season, evidence in favour of sperm storage is equivocal since a female is highly likely to encounter courting males between laying the two clutches and, in a female-biased population, has a fair probability of encountering the same male in two consecutive seasons. Thus the latter cases were not considered evidence of sperm storage. I classified all incidences of within-season sperm storage as 1) one male fathered both clutches, 2) two males fathered both clutches, 3) one male fathered all of first clutch and part of second clutch, 4) inconclusive, i.e. male fathered one clutch and paternity is inconclusive in other clutch, and 5) no evidence of sperm storage. I used these same categories for the paternal reconstruction data from GERUD.

## **Statistics**

All statistics were performed using Microsoft Excel 2009 (Microsoft Inc.) and PASW Statistics 18 (SPSS Inc., 2009).

## ***Results***

### **Blood and tissue sampling and morphometrics**

I collected blood from 79 males, representing 72% of the total population of active males (those males in the long-term database that were caught between 2000 and 2006, were not identified as a juvenile in 2005 or later and were not identified as deceased in 2004 or earlier). Of these 79 males, two were discarded because they were successfully genotyped on only one locus. Of the remaining 77 males, 18 were genotyped on 3 loci and 59 were genotyped on 4 loci. Sample males were representative of the total population of active males (N=110) in terms of mid-plastron length (sample mean=12.41cm, population mean=12.40cm, Student's  $t=0.40$ ,  $p=0.968$ ) and right front 3<sup>rd</sup> (RF3) claw length (sample mean=1.20cm, population mean=1.20cm, Student's  $t=$



-0.341,  $\rho=0.734$ ). Note that three sample males had a broken RF3 claw, so I used the left front 3<sup>rd</sup> claw length (LF3) in all statistics and a single male was missing all front claws and was thus excluded from claw length analyses.

I sampled blood and clutches from 56 females, representing 13.7% of the total population of active females (those females in the long-term database that were caught between 2000 and 2006, were not identified as a juvenile in 2005 or later and were not identified as deceased in 2004 or earlier). Of these 56 females, one female (E50) was not successfully genotyped on any loci, and was dropped from the analysis. Sample females were representative of the greater population in terms of mid-plastron length (sample mean=14.6cm, population mean=14.3cm, Student's  $t=1.776$ ,  $\rho=0.079$ ).

I sampled a total of 60 clutches in 2004 and 48 clutches in 2005, representing 23.9% of all clutches laid in 2004 (22.7% of all first clutches and 26.7% of all second clutches) and 20.3% of all clutches laid in 2005 (24.7% of all first clutches and 10.8% of all second clutches). The 60 clutches from 2004 represented 421 eggs and I sampled tissue from 364 hatchlings (the remainder were unfertilized or unaccounted). The 48 clutches in 2005 represented 369 eggs and I sampled tissue from 291 hatchlings. Within each clutch, I classified each egg's fate as "live hatchling" (hatchling successfully released to natal pond), "dead hatchling" (hatchling emerged from egg and died before release), "dead in egg/partly developed" (hatchling or remains in egg), "infertile/no tissue" (no evidence of embryo on yolk), "depredated" (empty shell with evidence of insect depredation or inundation with grass roots), "unaccounted" (discrepancy between number of eggs excavated in the fall and the known clutch size). See Appendix 1 for the fate of eggs by clutch. In 2004, later laid nests had both higher hatch success (Pearson's  $r=0.646$ ,  $\rho<<0.05$ ) and higher survival to release (Pearson's  $r=0.419$ ,  $\rho=0.001$ ) than earlier laid nests, while there was no such relationship in 2005; hatch success (Pearson's  $r=0.044$ ,  $\rho=0.768$ ) and survival to release (Pearson's  $r=0.097$ ,  $\rho=0.511$ ). In both years, hatchlings generally decreased in all size measurements and in mass over hibernation, and hatchlings in 2004 decreased to a greater degree than did hatchlings in 2005 (see Table 3).

## **Paternity analysis**

The majority of adult males and adult females were successfully genotyped on four loci (76% and 98%, respectively). The four loci, BTGA3 (eight alleles), BTCA5 (three alleles), BTCA7 (eight alleles) and EB11 (16 alleles) produced a combined exclusion probability of 0.94 (the probability of accurately excluding non-candidate males).

A combination of factors (including seasonal temperatures and date of nest excavation) resulted large variation in the quality of hatchling tissue samples (see Appendix 2 for sample quality by clutch). Tissue samples ranged from tissue sampled from a live hatchling, tissue sampled from a dead hatchling, tissue sampled from a small embryo, tissue sampled from a partly decomposed/ fully decomposed hatchling and samples containing only scutes and bones (no tissue). A good sample was quantified as tissue from a live hatchling, dead hatchling or embryo. Generally, the quality of tissue samples was higher in 2005 than in 2004 (2004 proportion good samples=0.41, 2005 proportion good samples=0.72, Mann-Whitney  $Z=-5.226$ ,  $p<<0.05$ ). Differences in tissue sample quality translated into differences in the quality of DNA extracted from these samples and also differences in successful genotyping and assignation of paternity. As a result of poor sample quality, a number of samples, and also a number of clutches, could not be included in the paternity analysis. Individuals were excluded from paternity analysis if they were successfully genotyped on only one locus. Additionally, individuals genotyped on two loci were excluded if BTGA5 (three alleles) was one of the two. Two hundred and thirty-one hatchling samples were excluded based on these criteria (24.7% of total hatchling samples); 182 out of 364 (49.9%) samples in 2004 and 49 out of 291 samples (16.8%) in 2005. Of the remaining samples in 2004, 14.3% (26) of hatchlings were genotyped on two loci, 56.0% (102) were genotyped on three loci and 36.8% (67) were genotyped on four loci. In 2005, 2.5% (six) of hatchlings were genotyped on two loci, 22.3% (54) were genotyped on three loci and 83.5% (202) were genotyped on four loci. Including those hatchlings genotyped on fewer than four loci reduced my total exclusion probability for individual hatchlings; however, my clutch-wise method of assigning paternity increased the likelihood of correctly attributing clutch paternity.

**Table 3 – Change in hatchling morphometrics over hibernation**

	Mean change 2004	Mean change 2005	t ( $\rho$ )
Plastron length (mm)	-0.77	-0.51	-2.516 (0.012)*
Carapace length (mm)	-0.84	-0.50	-1.743 (0.082)*
Carapace height (mm)	-0.17	-0.07	-0.466 (0.641)
Carapace width (mm)	-0.74	-0.36	-3.628 (<<0.05)*
Mass (g)	-1.05	-0.16	-28.311(<<<0.05)*

Mean change = post-hibernation - pre-hibernation.

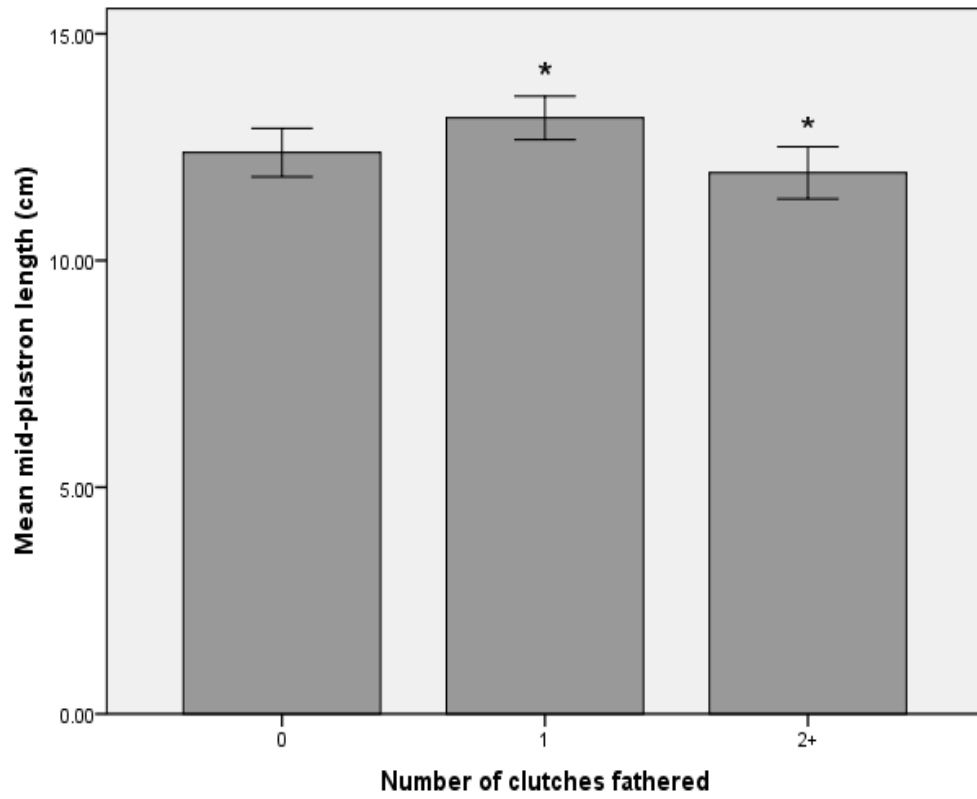
Significance indicated by asterisk,  $\rho=0.05$ .

Twenty out of 426 (4.7%) of hatchlings generated no paternity output, indicating that paternity for these individuals may be found outside of the sample of male turtles included in this study. Additionally, 43 out of 406 hatchlings (10.6%) for which a paternity output was generated had inconclusive paternity and so were designated as “no father”. Thus, the following paternity results are based on 362 hatchlings (2004-149 hatchlings, 2005-213 hatchlings) representing 90 clutches (2004-46 clutches, 2005-44 clutches) and 52 females.

Number of clutches attributed to each male were distributed as follows: 28 males fathered no clutches, 20 fathered a single clutch, 19 fathered two clutches, six fathered three clutches, four fathered four clutches, one fathered five clutches and two fathered six clutches. There was no correlation between the number of clutches fathered in 2004 and 2005 for each male (Pearson’s  $r = -0.132$ ,  $p = 0.362$ ). I subsequently collapsed the number of clutches fathered by each male into three categories, based on McTaggart’s (2000) study of the same population: 1) fathered no clutches, 2) fathered a single clutch, and 3) fathered two or more clutches. Males that fathered more clutches were generally smaller than males that fathered fewer clutches ( $F = 4.56$ ,  $p = 0.014$ ), specifically, males that fathered two or more clutches were significantly smaller than those that fathered one clutch (Figure 3), however, there was no difference in body size between males that fathered no clutches and either those that fathered one or those that fathered more than two clutches. There was no difference in claw length between males of differing fertilization success ( $F = 0.261$ ,  $p = 0.771$ ). One male (ID 0603) contributed sperm to two clutches despite lacking all front claws.

Neither father’s mid-plastron length ( $\beta = 0.015$ ,  $p = 0.883$ ) nor claw length ( $\beta = 0.081$ ,  $p = 0.439$ ) was related to hatch success. Neither father’s mid-plastron length ( $\beta = -0.074$ ,  $p = 0.401$ ) nor claw length ( $\beta = 0.099$ ,  $p = 0.258$ ) was related to survival to release. A greater number of eggs hatched in 2005 than in 2004 ( $\beta = 0.330$ ,  $p < 0.05$ ) and a greater number of hatchlings survived to release in 2005 than in 2004 ( $\beta = 0.368$ ,  $p < 0.05$ ).

**Figure 3 – Number of clutches fathered by male body size.**



Error bars represent 95% confidence intervals.

Significance indicated by asterisk.

### **Multiple paternity and sperm storage**

I analysed the 70 clutches with more than two hatchlings genotyped for evidence of multiple paternity. According to NewPat, 13 out of 70 clutches (18.6%) had multiple fathers represented, with potential multiple paternity (one father identified, other father(s) equivocal) in a further 16 clutches totalling 39 out of 70 clutches (41.4%). According to the GERUD analysis, 16 out of 70 clutches (22.9%) could be attributed to multiple fathers.

A total of 16 pairs of within-season clutches were successfully genotyped. Evidence of sperm storage was found in 31.2% of the clutch pairs in the NewPat analysis, while GERUD found evidence of sperm storage in 43.8% of the clutch pairs. Inconclusive evidence of sperm storage was found in 6.3% of clutch pairs in the NewPat analysis and 31.3% of clutch pairs in the GERUD analysis. Of clutches with conclusive evidence of sperm storage, NewPat assigned paternity of two full clutches to a single male (two pairs of clutches, 12.5%), assigned paternity of two full clutches to two males (one pair of clutches, 6.3%) and assigned paternity of all of the first clutch and part of the second clutch to one male (one pair of clutches, 6.3%). These four pairs of clutches represent five males, with mean mid-plastron length of 12.53cm (range 10.2cm to 14.08cm). GERUD assigned paternity of two full clutches to two males (two pairs of clutches, 12.5%) for which there was conclusive evidence of multiple paternity

A total of 25 pairs of between-season clutches were successfully genotyped for both the GERUD and the NewPat analyses. In both analyses, four of the 25 clutch pairs (16%) contained evidence of common paternity. No evidence of common paternity was found in 68% (GERUD) to 72% (NewPat) of the clutch pairs, the remainder was inconclusive.

### ***Discussion***

#### **Morphometrics and hatchling fitness**

The distribution of phenotypes (body size and claw length) in my sample of 72% of the reproductively active males was representative of the distribution of male phenotypes found in the greater population. The distribution of body sizes in my sample of 13.7% of

reproductively active females was representative of the distribution of female body sizes in the broader population. I preferentially selected clutches from females that had shown a tendency towards intraseasonal iteroparity and thus, the sample females were slightly more likely to produce multiple clutches in a single season than the broader population. Environmental factors have been found to influence the frequency of intraseasonal iteroparity, such as longer growing season (Ernst et. al, 1994). There is little published research on factors intrinsic to the turtles that may contribute to the frequency of multiple clutching, however, older females are more likely to be intraseasonally iteroparous than younger females (Bowden *et al.*, 2011). Older females are generally larger than younger and have more energy to allocate to reproduction than younger females (Congdon and Gibbons, 1985, Bowden *et al.*, 2011), increasing the probability of a successful second clutch. Based on 15 years of data from the Algonquin Park population, however, there was no relationship between size of female and likelihood of a second clutch (Student's  $t=0.134$ ,  $p=0.893$ ,  $N=2430$ ) and the correlation between body size and Julian lay date of the first nest was non-significant (Pearson's  $r=0.017$ ,  $p=0.394$ ,  $N=2430$ ).

Hatch success and hatchling survival to release were both higher in 2005 than in 2004. There were vast differences in late summer temperatures in 2004 and 2005. In 2004, the temperature turned relatively cold in mid-August and none of the eggs had hatched by the start of October at which point I moved the eggs to an incubator (maintained at 26°C) to encourage hatching. All eggs had completed hatching by mid-November. In 2005, all eggs had completed hatching by mid-September and indeed had begun to emerge from the nests by mid-August. Hatchling painted turtles emerge from the eggs with a yolk sac external to the body and within a few days, the yolk sac is drawn into the abdominal cavity. Throughout hibernation the first year, the hatchling is sustained on the energy reserves contained in the yolk sac (Ernst and Lovich, 2009), as such, I did not provide hatchlings with food between hatching and the commencement of hibernation in either year. In 2004, while the hatchlings remained in the egg for the extra month as compared to 2005, it is possible that rather than dedicating energy solely to maintenance, the hatchlings also expended energy on attempts to hatch, despite the low temperatures (Congdon, 1989). Hatchlings in 2004 may have depleted their yolk energy

stores more rapidly than they would have had they already hatched and were devoting energy to maintenance only.

Hatchlings in 2004 lost a greater proportion of body size (likely due to desiccation) and mass over hibernation than those in 2005. A lack of environmental moisture may have resulted in the hatchlings depleting internal water supplies and led to the reduced survival rates over hibernation in 2004 as compared to 2005 (Gregory, 1982).

Clutches laid later in 2004 had higher hatch success and higher survival to release than clutches laid earlier in the season. No such relationship was observed in 2005. First clutches contain more lipids and proteins than second clutches (Harms *et al.*, 2005). Painted turtles do not emerge from the nest until spring, thus offspring produced in a first clutch require maintenance from egg lipids and proteins for a longer period of time than offspring from second clutches. The increased parental investment in the first clutches should accommodate this additional maintenance requirement and thus, survival of the two clutches should be equal (Harms *et al.*, 2005). Clutches laid later in the season in 2004 spent less time within the egg during the cold August temperatures and thus depleted a smaller proportion of their maintenance lipids prior to hatching as compared to clutches laid earlier in the season.

Differences in seasonal temperatures and differences in methodology between the two years also influenced the quality of tissue samples collected. Many turtles in 2004 failed to successfully hatch, however, they remained in incubation within the eggs until mid-November prior to tissue harvesting in order to ensure that they were not going to hatch. Thus, many of the unhatched, fully-developed turtles were in stages of mild to advanced decomposition when tissue was sampled. In 2005, very few turtles failed to successfully hatch and so most tissue samples showed minimal decomposition.

### **Paternity analysis**

Males that fathered two or more clutches were significantly smaller than males that fathered only one clutch. There was no relationship between claw length and male fertilization success. A previous study on the same population also found that smaller male painted turtles had higher fertilization success than larger and that there was no



relationship between claw length and fertilization success (McTaggart, 2000). We found no fitness advantage for smaller fathers over larger fathers in terms of either hatch success or survival to release. McTaggart (2000), however, found greater survival in the clutches of males fathering more than one clutch than in clutches of males fathering a single clutch and tentatively suggested support for the good genes hypothesis of sexual selection. Despite studying the same population, I found no such relationship and thus found a lack of support for the good genes hypothesis. A larger sample both of males and of offspring in the current research and/or the poor quality of my hatchling samples and subsequent unsuccessful genotyping of hatchlings, particularly in 2004, may partly explain this lack of concordance between the two studies.

It is unlikely that female painted turtles in this population are trading up by remating when encountering a male of higher quality than their previous mate(s) (Uller and Olsson, 2008). The highly female-skewed sex ratio in the Algonquin population may mean that females are not likely to encounter many males during the active season and thus do not have the opportunity to trade up by remating. Interestingly, there was no difference in body size between the males that fathered no clutches and either the males that fathered one clutch or males that fathered two or more clutches. This result also corroborates McTaggart's (2000) results from the same population and further suggests that the paucity of males in the population restricts the female turtles' ability to exert mate choice. Females in a female-biased population may mate indiscriminately with all males they encounter to ensure fertilization of their eggs, trading up only when the rare opportunity arises, thus there should be little to no phenotypic difference between those males that were reproductively successful as compared with those that were not reproductively successful. That successful males in 2004 were not necessarily successful males in 2005 in terms of the number of clutches fathered also suggests that females are not particularly discriminating in their choice of mate.

I found no relationship between the numbers of clutches fathered and claw length. Claw length has very low variation in this population and discrimination among males on the basis of claw length may not be possible. Highly variable sexually dimorphic traits are associated with variation in reproductive success, while traits with low variability

may be a prerequisite for mating, but do not contribute to variation in reproductive success (Andersson, 1994). Since female *C. picta* employ reciprocal waving as part of courtship, long claws are perhaps not necessary for the behaviour or for successful mating, but only serve to enhance the visibility of the male's display in a murky environment. One of the known fathers in this study is missing all front claws and yet fathered all of the offspring in one clutch and fathered a portion of the offspring the clutch of a different female.

### **Sperm storage and multiple paternity**

I estimated that between 19% and 23% (conservatively) and 41% (liberally) of all clutches had more than one father. My method of awarding paternity within the clutch may have produced a bias towards single paternity rather than multiple paternity, however, my values are within the ranges reported by Pearse et al. (13%, 2001; 33%, 2002) and only slightly higher than those reported by McGuire *et al.* (16.3%, in press) for *C. picta*. My estimates are much higher than those previously reported for the Algonquin population (4%, McTaggart, 2000), possibly because of larger sample size. Reported levels of multiple paternity range widely, however, both among turtle species (0-100%, Uller and Olsson, 2008) and within turtle species from study to study (e.g., *Caretta caretta*, 31.4%-95%, *Lepidochelys olivacea*, 20%-92%, Uller and Olsson, 2008). Differences in study population in terms of demographics and ecology (Pearse and Avise, 2001), and differences in methodology among studies (Uller and Olsson, 2008) both contribute to this observed variation.

My estimates of 12.5% (conservatively) to 25% (liberally) of clutches with evidence of within-season use of stored sperm are much lower than other estimates for *C. picta* (100%, Pearse et al., 2002; 97.5%, McGuire *et al.*, in press) and other turtle species (*Chelonia mydas*, Fitzsimmons, 1998; *Lepidochelys kempii*, Kichler et al., 1999; *Emys orbicularis*, Roques et al., 2006). The Algonquin population has a very highly female-skewed sex ratio and females in this population run the risk of not reproducing if they are too selective about their mates. The population studied by McGuire *et al.* (in press) has a male-skewed sex ratio (1.9:1) and females may be more selective about which males father their clutches, using sperm from a single high-quality male over a number of

sequential clutches. Previous research suggests that the inter-nesting period is too short for remating, on account of time needed to recover physiologically from prior nesting (Congdon and Gatten, 1989), however, female mating behaviour prior to laying the first clutch may vary depending on the sex ratio of the population. In a population with few potential mates, the impetus for females would be successful fertilization of her full complement of eggs, rather than a successful mating with a high-quality male, thus, females may mate with many males before laying their first clutch and so may have sperm from a large number of males stored. Although preliminary evidence suggests a method of last in-first out sperm selection (Pearse *et al.*, 2001), the sperm of younger males may have a competitive advantage over that of older males or female *C. picta* may be able to control the sperm contributions from different mates by restricting the length of copulation for lower quality males and allowing longer copulation with higher quality males. Conversely, females in a male-skewed population will be more likely to encounter a high quality male during the mating season than females in a female-skewed population and hence, we should expect to see a greater use of stored sperm from a high-quality male in the former case. The observed high levels of multiple paternity in the Algonquin population support the hypothesis that females in a female-skewed population mate with many males and may use their sperm in a less discriminating fashion than do females in a male-skewed population.

The five males that contributed sperm used in sequential clutches according to the NewPat analysis were slightly larger, though non-significantly, than the average male both in the sample and in the larger population. Female painted turtles in the Algonquin population fertilize their clutches preferentially with sperm from smaller males, as such, this result is contradictory and suggests a possible alternative strategy for larger males to ensure fertilization. Thomas (2002) found that older/larger male slider turtles (*Trachemys scripta*), a close relative of the painted turtle, used an alternate mating strategy than that employed by younger/smaller males. The older males included biting in their courtship, whereas the younger males exclusively relied on display. Painted turtles in the Algonquin population have been observed with circular injuries on their heads and necks and females have these injuries at a higher frequency than do males (R.

J. Brooks, unpub. data), suggesting a similar use of biting during courtship to that described by Thomas (2002).

In a female-biased population, females have a fair probability of encountering the same male in multiple seasons and thus, the low levels of common paternity observed in between-season clutches (four of 25 clutch pairs or 16%) may be explained by remating with the same male. There is insufficient evidence to conclude that the similarities are due to stored sperm. These levels are also lower than those reported by other authors both for painted turtles (4 of 9 pairs or 44%, McGuire *et al.*, in press) and for other turtle species (*Emys orbicularis*, 58%, Roques *et al.*, 2006).

There are differences amongst male *C. picta* of differing phenotypes in terms of the number of clutches to which they contribute (and also the number of offspring they father). Small males contribute sperm to a greater proportion of clutches than do large males. Female choice is generally considered to be the mechanism driving differences in fertilization success of male painted turtles; however, differences in male behaviour may determine the proportions of large and small males with which a female has the opportunity to mate. I address this possibility in chapter 2. Regardless, female painted turtles may have a degree of control over male reproductive success despite the polygynandrous mating system. Females increase male reproductive success by using a male's sperm to fertilize multiple clutches and decrease male reproductive success by using multiple males' sperm to fertilize a single clutch.

The sex ratio of the population, however, influences both the rates of stored sperm use and of multiple paternity. The (female-biased) Algonquin population has lower rates of stored sperm use and higher rates of multiple paternity than does a population with a marked male bias (McGuire *et al.*, in press). I investigate the effects of sex ratio (and other demographic factors) on male fertilization success, stored sperm use and multiple paternity using modeling in chapter 3.

On the topics of painted turtle (indeed, freshwater turtle) mating, male fertilization success and female control over male reproduction more remains to be studied. In depth investigations into the mechanisms of sperm storage and the conditions

that increase or decrease the likelihood of intraseasonal iteroparity, the use of multiple sperm contributions in a single clutch, and the use of stored sperm will be invaluable. However, most importantly, the study of turtle reproductive success requires the development of a larger library of polymorphic genetic markers to make genetic paternity analyses more accessible to all researchers and thus increase the body of information regarding reproductive success in multiple populations.

## CHAPTER TWO

### *EX SITU PAINTED TURTLE COURTSHIP BEHAVIOUR AND FEMALE CHOICE*

Assignment of paternity in wild populations of highly aquatic freshwater turtles (such as painted turtles, *Chrysemys picta*) is challenging because courtship and mating are difficult to observe since the turtles tend to drop to the bottom of the bottom of water prior to intromission (Ernst, 1971a), as well as the typically murky nature of their aquatic habitat. Although maternity of hatchlings can be unequivocally assigned by observation of nesting behaviour and nest excavation prior to hatchling emergence, paternity in a natural population cannot be determined by observation of successful copulation and hence researchers must use alternative measures of paternity, such as genetic analysis (McTaggart, 2000, Pearse *et al.*, 2001). However, genetic analysis can only reveal patterns of differential paternity, it cannot be used to make inferences about the reasons underlying the observed variation in male reproductive success. Questions remain regarding whether this variation is due to intersexual selection, intrasexual competition or differential patterns of spatial distribution and movement among males.

In specific reference to pond turtles (Emydidae), first, females may choose mates based on a preference for a specific male phenotype as an indicator of male quality. Second, males of differing phenotypes may behave in ways that increase their reproductive success, for example, courting vigorously, mate guarding or forcing copulations. Thomas (2002) found that in the closely related Red-Eared Slider (*Trachemys scripta*), larger males employed biting as part of their courtship, whereas smaller males courted by waving their foreclaws near the females' heads. McTaggart (2000) suggested that small males may be at an advantage in terms of finding potential mates in an aquatic habitat because of increased agility. And third, males of differing phenotypes may be spatially distributed, or may have patterns of movement such that some males will encounter reproductive females more frequently than other males. In a river population of painted turtles, males migrated farther than females, who migrated farther than juveniles (McCulloch and Secoy, 1983). If this pattern reflects an increased risk of predation for juvenile turtles, thus contributing to reduced movement, then young

(and hence small, because of indeterminate growth, Ernst and Lovich, 2009) males may also have reduced movement patterns. The latter two alternatives may manifest either through the male behaviour itself or through a female preference for that behaviour.

Female choice is generally considered to be the mechanism driving differences in male reproductive success in painted turtles, because of the form of sexual size dimorphism (males smaller than females) and because of the pre-mounting form of courtship where males display to females using a sexually dimorphic trait, i.e. their elongated foreclaws (Berry and Shine, 1980). I investigate alternative explanations for the patterns of male fertilization success observed in chapter 1, specifically, I focus on differences in male courtship behaviour among phenotypes. “Male fertilization success” assumes *a priori* that the sperm contribution of a given male fertilizes a portion of a given female’s reproductive output.

I used *ex situ* observations of courtship and mating behaviour to study whether females show a preference for males of a specific phenotype and/or whether males of different phenotypes behave differently during courtship. I varied the two most obvious sexually dimorphic characters in my trials, body size and foreclaw length.

I first identified which behaviours comprise the courtship ritual of male painted turtles. I considered a behaviour to be part of the courtship ritual if a) males engaged in these behaviours at a higher frequency than females and b) these behaviours were directed towards females more frequently than towards males. I also quantified the frequency of these behaviours according to time of year and time of day. I then quantified whether males of differing phenotypes court differently (different form, frequency or duration).

I subsequently addressed two predictions regarding variation in male fertilization success:

- 1) If the observed variation in fertilization success among males (see chapter 1) is a result of female choice based on preference for a male phenotype, rather than on the tenacity of courting males, then males of the phenotype that father more off-

spring will court at the same or lower frequency, and for the same or lesser duration than males of the phenotype that father fewer offspring.

- 2) If female painted turtles choose their mates based on phenotype, then a) females will respond differently to males of different phenotypes and b) females will accept courtship and copulation more frequently from the male phenotype that fathered a greater proportion of the offspring (from chapter 1).

### ***Methods***

Previous studies indicate that many species of pond turtle (Emydidae) will court and mate freely in artificial environments, in fact some of the earliest observations of pond turtle courtship occurred under such conditions (Brumwell, 1940; Davis and Jackson, 1973; Ernst, 1981; Taylor, 1933). I used a modified version of the artificial pond methodology used by Thomas (2002) in his study of courtship behaviour in Red-eared Sliders (*Trachemys scripta*).

Because of a lack of female responses in the artificial pond trials in the first year, I further employed y-mazes to address whether females respond differently to males of different phenotypes. Y-mazes have been used to answer questions of mate choice in a variety of species (Diaz & Thiel, 2004 (rock shrimp, *Rhynchocinetes typus*); Kavaliers et al., 2003 (mouse, *Mus musculus domesticus*); Riechert & Singer, 1995 (spider, *Agelenopsis aperta*); Voss & Voigt, 1997 (American lobster, *Homarus americanus*); Westermann & Beuerlein, 2005 (nautilus, *Nautilus pompilius*)) with the assumption that a female will choose to associate with males with whom she would also choose to mate. Most frequently, the stimulus of interest is chemical, but y-mazes may also be used to investigate responses to visual cues (Diaz & Thiel, 2004). Available materials to construct the y-maze limited the design to one with no flow-through system. Hence, I make the assumption that although, in a natural setting, a combination of visual, chemical and tactile cues may be necessary for complete courtship and fertilization in painted turtles, female *C. picta* will rely mainly on visual cues (based on sexually dimorphic characters) to choose her mate.



## **Artificial ponds**

### *Description of trials*

I conducted 26 artificial pond observation trials in the summers of 2005 and 2006. Trials were distributed as shown in Table 4. Note that no trials were run during nesting season in either year; late May to early July. The variation in the number of trials run in each observation session was due to equipment limitations and a shortage of naïve candidate males for the latter trials in each year.

Trials were run in two 1m (depth) x 2m (diameter) plastic pools located within a chain link enclosure at the Wildlife Research Station (WRS) in Algonquin Park, Ontario. Pools were lined with white tarpaulins to facilitate viewing of the turtles and were filled 0.5 m deep with untreated water from Lake Sasajewun (this lake supports a population of snapping turtles (*Chelydra serpentina*) and a small number of painted turtles). Pools were emptied and rinsed with untreated water between observation sessions. Each pool was illuminated with two full-spectrum fluorescent strip lights on a 14:10 (l:d) schedule. Turtles used in each trial were introduced into the artificial ponds and allowed to acclimate for at least 8 hours prior to the start of observation. Trials were recorded on VHS tape, using cameras suspended above each pool. Each trial was recorded for 2 hours, during which time no humans were present within the enclosure and activity was minimized outside the enclosure. In 2005, the pools were empty save for the turtles. Due to a lack of courtship displays in the 2005 trials, I made a number of alterations to my 2006 methods in an attempt to facilitate full courtship displays. First, I ran some trials in the afternoon to allow time for the water to heat up from overnight temperatures (in 2004 all trials began at 09:00h). Second, I added a heat lamp suspended above a cinder block basking platform to each pool. The heat lamp was on during the acclimation period and was off while videotaping. Third, I reduced the number of male turtles in each trial. In 2005, I used four males (two of one phenotype, e.g. large, and two of the other, e.g. small) and two females in each trial. In 2006, I used two males (one of each phenotype) and two females in each trial. And fourth, because preliminary results from chapter 1 suggested that body size was a more important female choice cue than claw length, I

**Table 4 - Artificial pond trials divided by date, time of day and male phenotype.**

Dates	# morning trials		# afternoon trials	
	Body size	Claw length	Body size	Claw length
May 24-26, 2005	3	3	0	0
July 19-20, 2005	2	1	0	0
Aug 15-17, 2005	1	2	0	0
May 3-5, 2006	3	0	3	0
Sept 13-15, 2006	0	0	4	4

reduced the number of claw length trials in 2006 in favour of more body size trials to maximize statistical power.

### *Selection of turtles*

Painted turtles used in the artificial ponds were captured in Wolf Howl Pond (WHP) and West Rose Lake (WRL) by dipnet from canoes and in hoop traps baited with cat food (few turtles were caught by the latter method) (see introduction for a description of the site). I attempted to reduce the number of turtles that were used multiple times, but limitations in the number of available turtles made some repeats unavoidable.

Females were captured opportunistically from WHP and WRL and were paired in trials according to size.

I generated a short-list of male turtles to satisfy the requirements for males of differing phenotypes. The most recent plastron length (PL) and claw length measurements were gleaned the 28-year Algonquin painted turtle database (R. J. Brooks). Plastron length measurements of males were divided into three groups of large (>13cm PL), medium (11-13cm PL) and small (<11cm PL). Male turtles were preferentially used if they fell into the large and small groups, however because of the limited number of males in the population (numbers of male turtles caught in each of the last 10 years ranged from 46 to 90, mean = 61) and the increasing difficulty of capture later in summer, I used small “medium” turtles with large and large “medium” turtles with small.

Claw length measurements in this population are based on the 3<sup>rd</sup> digit on each front foot (RF3 and LF3), measured using digital callipers. Males were discarded from the candidate list if the measured right and left claw lengths were highly asymmetrical, i.e. variation greater than 0.05cm from a perfect 1:1 relationship. This reduced list did, however, include turtles that may have had deformities on claws other than the 3<sup>rd</sup>. If such a turtle was identified upon capture, it was also excluded from trials. Additionally, because of the potential variability inherent in measuring a claw (i.e. how much of the cuticle to include), I assessed the variability in claw measurements for each turtle over the last 5 captures. Among-year variability within individuals was never greater than 0.05mm, so I did not discard any individuals. Mean length of RF3 claw did not differ

significantly from mean length of LF3 claw (RF3=1.23mm, LF3=1.21mm,  $t=1.11$ ,  $p=0.23$ ); hence I used only RF3 measurements for the remainder of the analyses. Claw length was then divided into three groups, “short” (<1.15mm RF3 length), “medium” (1.15-1.25mm RF3 length) and “long” (>1.25mm RF3 length) to identify individuals for preferential capture. As with the body size trials above, many turtles from the medium group were used to maximize the number of possible trials.

### *Analysis of trials*

Tapes were viewed once for each turtle in the trial and behaviour was coded for 30 minutes. I discarded the first 15 minutes of each trial to allow time for the turtles to recover from my presence while I started the recording. Low ceiling height in the enclosure meant that I could not suspend the cameras at a great enough distance to capture the entire artificial pond in the video image. This created blind spots at the edges of the ponds where the turtles were not visible on tape. I coded 30 minutes of behaviour for each turtle while they were within the viewable area of the pond and the total amount of tape that I viewed for each individual thus varied from 45 minutes to 2 hours.

For each individual, I recorded duration of each behaviour performed, identity of the turtle towards which the behaviour was directed and response of that turtle. I described five initiating behaviours and three response behaviours. Initiating behaviours were: 1) approach and follow (a&f) – one turtle makes continuous directed movements towards another turtle, even if other turtle moves away; neck is usually stretched out, 2) nose – one turtle touches another turtle with its nose, 3) aggress – one turtle snaps or gapes at another turtle, 4) bite – one turtle bites another turtle on the head, neck or limb and holds on for at least a count of two and 5) display – one turtle vibrates its foreclaws on either side of another turtle's head. Response behaviours were: 1) no response – target turtle does nothing, 2) move away – target turtle makes a directed move away from the instigating turtle and 3) aggress – target turtle snaps or gapes at the instigating turtle.

I used hourly temperatures from the Environment Canada, Algonquin Park East Gate weather station ([http://climate.weatheroffice.ec.gc.ca/climateData/canada\\_e.html](http://climate.weatheroffice.ec.gc.ca/climateData/canada_e.html)) to determine daily low temperature and average temperature over each trial.

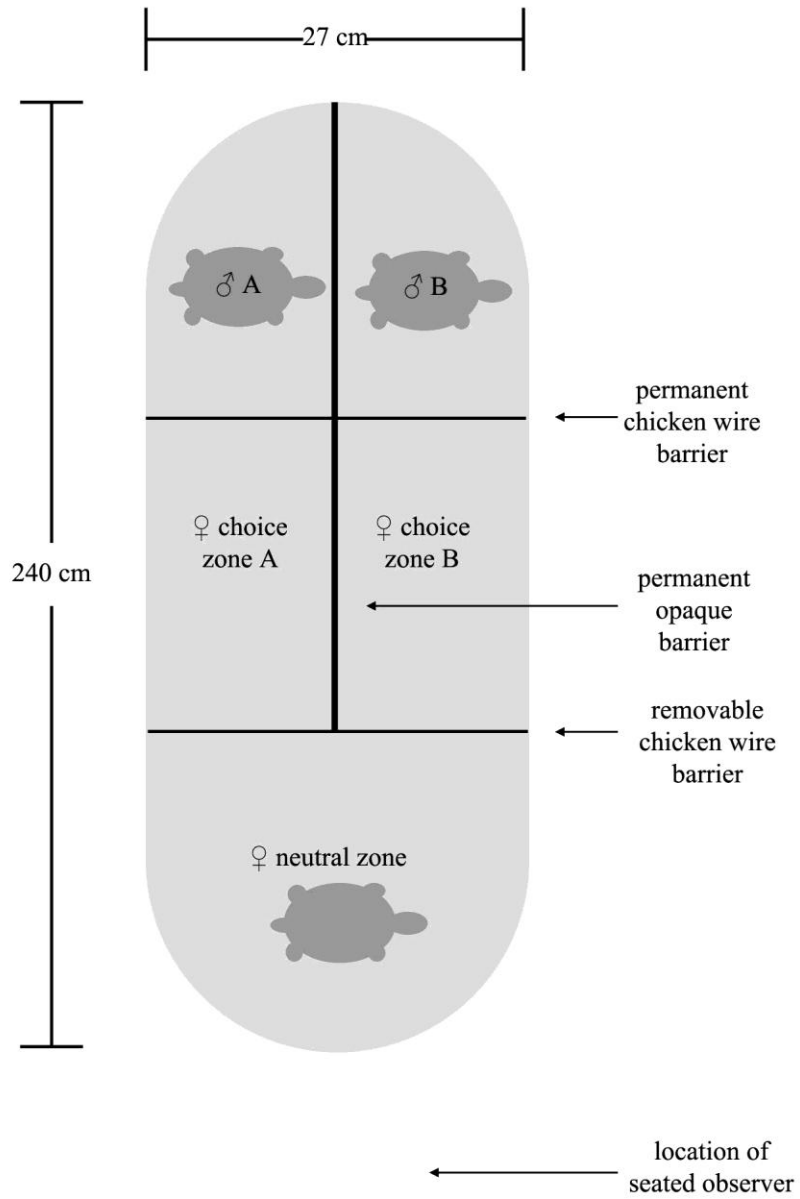
### *Statistics*

I used Student's t-tests to compare frequencies of behaviours (dependent variables) between years and times of day (independent variables) and ANOVAs to compare frequencies of behaviours (dependent variable) among months (independent variable). I assessed the relationship between frequencies of behaviours and temperatures using Pearson's  $r$ . I applied Chi-square tests to assess differences between males and females and between males of differing phenotypes in frequency and duration of initiating behaviours. I also used Chi-square tests to assess differences in the sex of the target turtle. For trials in 2005, with two males of each phenotype, I used the mean frequency of behaviours for each pair of males in all comparisons. Statistics were performed using Microsoft Excel 2010 (Microsoft Inc.) and PASW Statistics 18 (SPSS Inc., 2009).

### **Y-mazes**

I ran 12 y-maze trials from September 14-16, 2006: five trials on September 14, four trials on September 15 and three trials on September 16. In seven of these trials, I varied body size and in the remaining five, I varied claw length. I chose to run y-maze trials in early Autumn to coincide with the period of highest sperm transfer (Gist et al., 1990). I conducted all trials in the turtle laboratory at the Wildlife Research Station, Algonquin Park, Ontario. Trials were run throughout the day between 08:00h and 20:00h. The laboratory was maintained at 18°C throughout the trials. I constructed the y-maze using a galvanized metal tub 240cm x 27cm x 50cm (see Figure 4); dividers were made of chicken wire on a wooden frame and black garbage bags on a wooden frame. The y-maze tank was filled 25cm deep with water from Lake Sasajewun. Laboratory windows were covered to reduce differentials in lighting and no personnel save the observer were in the room during trials. For each trial, I placed a male turtle of one phenotype in one of the male zones, a male turtle of the other phenotype in the other male zone and a female turtle in the neutral zone. The female turtle could make visual contact with both males; however, the two males could not make visual contact with one another.

**Figure 4 – Y-maze apparatus.**



I alternated in which side of the tank I placed the male phenotypes to reduce any “side-of-tank” effects in the female’s response. The turtles were allowed to acclimate for 30 minutes with no humans present. I then entered the room, removed the temporary chicken wire barrier to allow the female to move out of the neutral zone, sat at the end of the tank nearest the neutral zone and remained motionless for the duration of the trial. Each trial lasted for 60 minutes, during which time I recorded the length of time the female spent in each of the male choice zones and any interactions between the female and the males. Movement of a female into a male choice zone was considered “choice” and the duration was recorded if the female moved up to the permanent chicken wire barrier. I also noted if the male and female touched one another through the permanent chicken wire barrier, if any of the turtles remained immobile, if the male moved away from the female at the chicken wire barrier and if turtles attempted to climb the chicken wire barriers.

I used a subset of male turtles also used in the artificial pond trials in September 2006. Males were used in the artificial pond trials prior to the y-maze trials to reduce any effects of previous experimental experience on male courtship behaviour in the artificial ponds. Eleven males were used in two trials each to maximize the number of trials that could be run with the few males available in September; a further two males were used in one trial each. Experimentally naïve females were captured opportunistically from WHP and WRL.

I used paired t-tests to determine if there was effect of side of tank (independent variable) on female behaviour (dependent variable) and Pearson’s correlation coefficient to investigate the effect of time of day on female behaviour. I also employed paired t-tests to determine if females entered one male choice area more frequently than the other and to determine if females remained in one male choice area for a longer duration than the other, with male choice area as the independent variable and female behavior as the dependent variable in both cases. All statistics were performed using PASW Statistics 18 (SPSS Inc., 2009)

## **Results**

### **Artificial Ponds**

Female *C. picta* used in the artificial pond trials ranged in size from 12.75cm to 16.68cm mid-plastron length (PL) (mean = 15.27cm, N=52). Plastron length of females used in 2005 did not differ significantly from PL of females used in 2006 (2005=15.22cm, 2006=15.31cm, Student's  $t=0.39$ ,  $p=0.70$ ). There was also a high correlation in PL between the two females used in each trial (Pearson  $r=0.86$ ,  $p <<0.05$ ). Five females were used in both 2005 and 2006.

Large males used in 2005 did not significantly differ in PL from large males used in 2006 (2005=13.68cm, 2006=13.81cm, Student's  $t=0.46$ ,  $p=0.65$ , N=22). Similarly, small males used in 2005 did not differ in PL from small males used in 2006 (2005=10.88cm, 2006=11.4cm, Student's  $t=1.82$ ,  $p=0.08$ , N=22). I subsequently pooled body size data from both years in the remainder of the analyses. Large and small turtles used in each trial were significantly different in PL from one another (large=13.76cm, small=11.21cm, paired Student's  $t=18.30$ ,  $p <<0.05$ , N=16).

Long-clawed males used in 2005 did not differ in RF3 length from long-clawed males used in 2006 trials (2005=1.27mm, 2006=1.34mm, Student's  $t=0.54$ ,  $p=0.60$ , N=16). Short-clawed males used in 2005 had slightly shorter RF3 claw length than short-clawed males used in 2006 (2005=1.11mm, 2006=1.13mm, Student's  $t=2.21$ ,  $p=0.04$ , N=16). Since this difference was close to non-significant, however, I combined the claw length data from the two years for the remainder of the analyses. Long-clawed and short-clawed turtles used in each trial were significantly different from one another (long-clawed=1.30cm, short-clawed=1.18cm, paired Student's  $t=10.91$ ,  $p <<0.05$ , N=10).

Eighteen male turtles were used in both 2005 and 2006. Three males were used twice in 2006; one of these was used once in a body size trial and once in a claw length trial. The remaining two turtles were used in body size trials twice; the data from their two trials were therefore pooled in the remaining analyses.



I was unable to code 30 minutes of “in picture” activity for two individuals as they remained out of the frame for the majority of the trial: one female only reached 25 minutes of “in picture” activity in the 2-hour recording and one male remained out of the picture for the whole 2 hours. I thus excluded these two individuals from the analyses. I also discarded one body size trial in May 2006 and one claw length trial in September 2006 because the tapes were damaged.

Trials had different numbers of turtles depending on the year; six turtles in each trial in 2005 and four turtles in each trial in 2006. For the following frequency results, I divided the total frequency of each behaviour in each trial by the total number of turtles in the trial to calculate the average activity per turtle per trial. There was no difference in the frequency of all behaviours from one year to the next, or in the frequency of initiating behaviours (Table 5). There was also no difference in frequency of all initiating behaviours from month to month or in the frequencies of a&f or aggress from month to month. Turtles were slightly less likely to nose in May as compared with July and August, however this result was close to not significant (Table 6). Bites and displays were observed rarely; one bite in each of May and August 2005 and three in September 2006, and one display in each of May and June 2005 and five displays in September 2006 (one individual). There was no difference in frequency of all behaviours between morning and afternoon trials, mean morning=5.29 and mean afternoon=4.96 (Student’s  $t=0.572$ ,  $p=0.573$ ). There was no correlation between either daily low temperature or average temperature over the 2 hour trial and the mean number of behaviours in each trial (daily low Pearson’s  $r=-0.106$ ,  $p=0.622$  and average temperature Pearson’s  $r=0.344$ ,  $p=0.099$ ).

Males were much more likely than females to initiate contact with another individual (595 vs 49,  $X^2=462.193$ ,  $p<<0.05$ ) and target individuals were more likely to be female than male, regardless of the sex of the instigating turtle (499 vs 143,  $X^2=614.159$ ,  $p<0.05$ ). Additionally, males were more likely to initiate contact with females than with other males ( $X^2=637.848$ ,  $p<0.05$ , 481 vs 112), whereas females were as likely to initiate contact with males as with females ( $X^2=3.449$ ,  $p=0.063$ , 31 vs 18).

**Table 5 - Mean frequency of initiating behaviours per turtle per trial by year.**

Behaviour	Mean (sd) 2005	Mean (sd) 2006	t (p)
Total	5.85 (3.23)	4.19 (2.67)	1.373 (0.184)
A & F	3.42 (1.88)	2.71 (1.63)	0.984 (0.366)
Nose	1.88 (1.49)	1.19 (1.03)	1.313 (0.204)
Aggress	0.50 (0.59)	0.13 (0.29)	1.984 (0.060)
Bite	0.03 (0.06)	0.06 (0.11)	-0.923 (0.369)
Display	0.03 (0.06)	0.10 (0.36)	-0.722 (0.478)

Note: The total frequency of behaviours in each trial was divided by the total number of turtles in that trial to give the average frequency for each trial (divided by six in 2005 and by four in 2006).

**Table 6 – Mean frequency of initiating behaviours per turtle per trial by month.**

Behaviour	Mean (sd) May 2005/2006	Mean (sd) July 2005	Mean (sd) August 2005	Mean (sd) September 2006	F (p)
Total	4.35 (2.81)	6.50 (2.96)	7.61 (2.25)	4.32 (3.36)	1.342 (0.389)
A & F	2.96 (2.12)	3.56 (1.17)	4.00 (0.58)	2.61 (1.74)	0.494 (0.691)
Nose	0.95 (0.70)	2.67 (1.88)	2.83 (1.59)	1.39 (1.24)	3.232 (0.044) *
Aggress	0.40 (0.60)	0.22 (0.10)	0.72 (0.59)	0.04 (0.09)	1.742 (0.191)

Note: The total frequency of behaviours in each trial was divided by the total number of turtles in that trial to give the average frequency for each trial (divided by six in 2005 and by four in 2006).

As noted above, males performed more discrete behaviours than females in general. Certain behaviours were more frequently directed towards females than males. Females were more often the target for a&f (329 vs 63,  $X^2=463.57$ ,  $p<<0.05$ ), nose (147 vs 44,  $X^2=55.55$ ,  $p<0.05$ ) and display (6 vs 1,  $X^2=3.57$ ,  $p=0.059$ ). Biting behaviour was directed equally at males and females, though sample size was very small (4 vs 1,  $X^2=1.8$ ,  $p=0.180$ ) and aggress was more likely to be directed at males (13 vs 34,  $X^2=9.38$ ,  $p=0.002$ ). Certain behaviours were also more likely to be initiated by males than females: a&f (380 vs 14,  $X^2=339.99$ ,  $p<<0.05$ ) and nose (173 vs 18,  $X^2=125.79$ ,  $p<<0.05$ ). Males exclusively performed display (7) and bite (5) behaviours. Males were also more likely to perform aggress behaviours (30 vs 17,  $X^2=3.57$ ,  $p=0.058$ ) because males were more active than females in general.

The following male phenotype results are based on combined data from all trials in both years. There was a difference in the frequency of all behaviours between large and small males, large males performed a higher number of discrete behaviours than small males (Table 7). Large males exclusively engaged in strike, aggress, bite and display behaviours, and also, large males were more likely to engage in a&f and nose behaviours than small males (Table 7, Figure 5). There was no difference in the frequency of all behaviours by long- and short-clawed males, however, long-clawed males exclusively performed the two observed display behaviours (Table 8, Figure 6). Duration of behaviours did not differ between large and small males (Table 9, Figure 7), nor did duration differ between long and short-clawed males (Table 10, Figure 8). The long mean duration of biting is the result of a single individual in both cases.

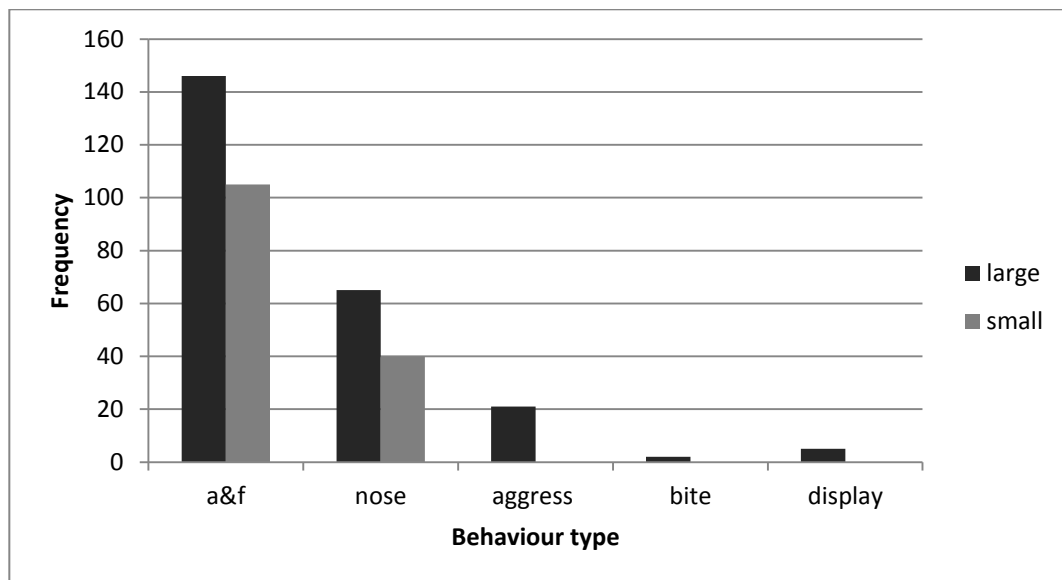
Female painted turtles were far more likely to move away from a male turtle than either show no response or aggress (399 vs 87 vs 2,  $X^2=537.25$ ,  $p<<0.05$ ). Since large males performed significantly more behaviours (234 in all trials) than small males (145 in all trials), the following results are based on the proportion of total male behaviours that elicited each type of response. A similar treatment was applied to the long- and short-clawed male data to ensure consistency. Female turtles moved away from 164 out of 234 large male behaviours (68.6%) and 92 out of 145 small male behaviours (63.4%) and thus were no more likely to move away from large or small males (68.6% vs 63.4%,  $X^2=2.133$ ,  $p=0.144$ ), showed no response to 26 out of 234 large male behaviours (11.0%)

and 27 out of 145 small male behaviours (18.6%) and thus were no more likely to respond to either large or small males (11.0% vs 18.6%,  $X^2=0.188$ ,  $p=0.665$ ). Only a single female aggressed once towards a large male. Female turtles showed no difference in response towards long- (104 total behaviours) and short-clawed (107 total behaviours) males: no response (14% vs 14%,  $X^2=0$ ,  $p=1.00$ ), move away (63% vs 59%,  $X^2=0.131$ ,  $p=0.717$ ). Again, a single female aggressed once towards a long-clawed male.

**Table 7 - Frequency of behaviours by large and small males showing Chi-square statistic and significance.**

		Type					
		a&f	nose	aggress	bite	display	Total
Phenotype	large	146	65	21	2	5	239
	small	105	40	0	0	0	145
X <sup>2</sup> (p)		6.697 (0.010)	5.592 (0.015)	n/a	n/a	n/a	18.764 (0.002)

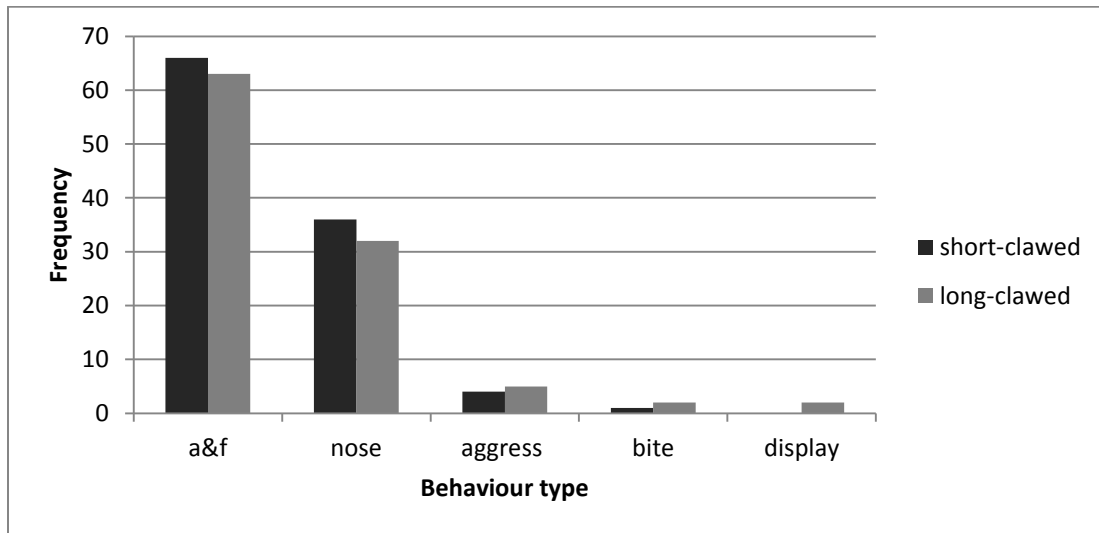
**Figure 5 – Frequency of initiating behaviours by male phenotype: small vs. large.**



**Table 8 - Frequency of behaviours by long- and short-clawed males showing Chi-square statistic and significance.**

		Type					
		a&f	nose	aggress	bite	display	Total
Phenotype	long	63	32	5	2	2	104
	short	66	36	4	1	0	107
X <sup>2</sup> (p)		0.070 (0.792)	0.235 (0.628)	0.111 (0.739)	0.333 (0.564)	n/a	0.043 (0.806)

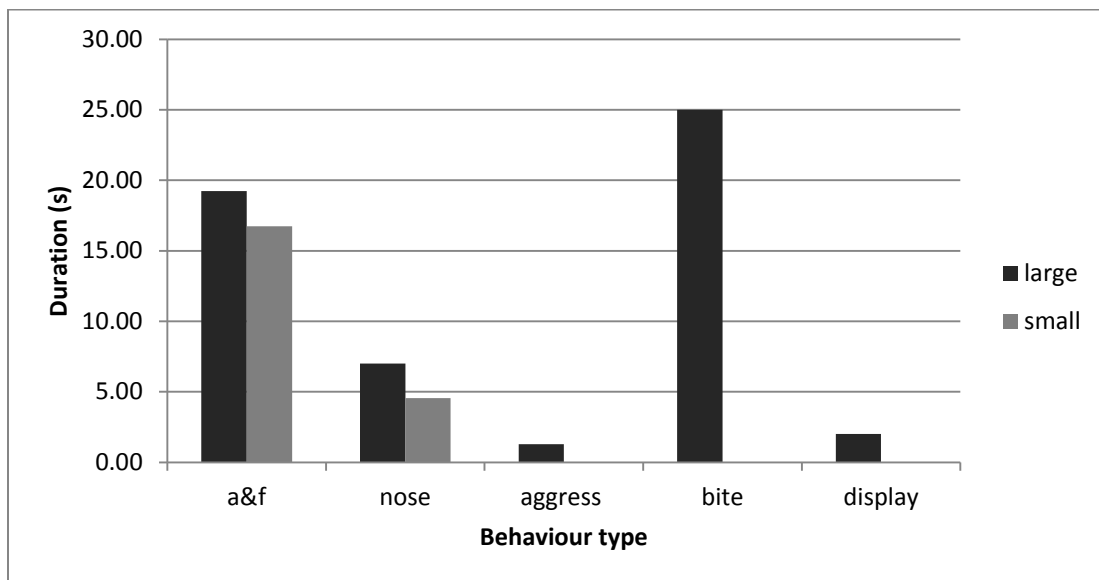
**Figure 6 – Frequency of initiating behaviours by male phenotype: short-clawed vs. long-clawed.**



**Table 9 - Mean duration (seconds) of behaviours by large and small males showing Student's t statistic and significance.**

		Type					
		a&f	nose	aggress	bite	display	Total
Phenotype	large	19.23	7.00	4.00	25.00	2.00	14.02
	small	16.74	4.55	0	0	0	13.38
t (p)		1.005 (0.316)	1.279 (0.204)	n/a	n/a	n/a	0.342 (0.732)

**Figure 7 – Duration of initiating behaviours by male phenotype: small vs. large.**



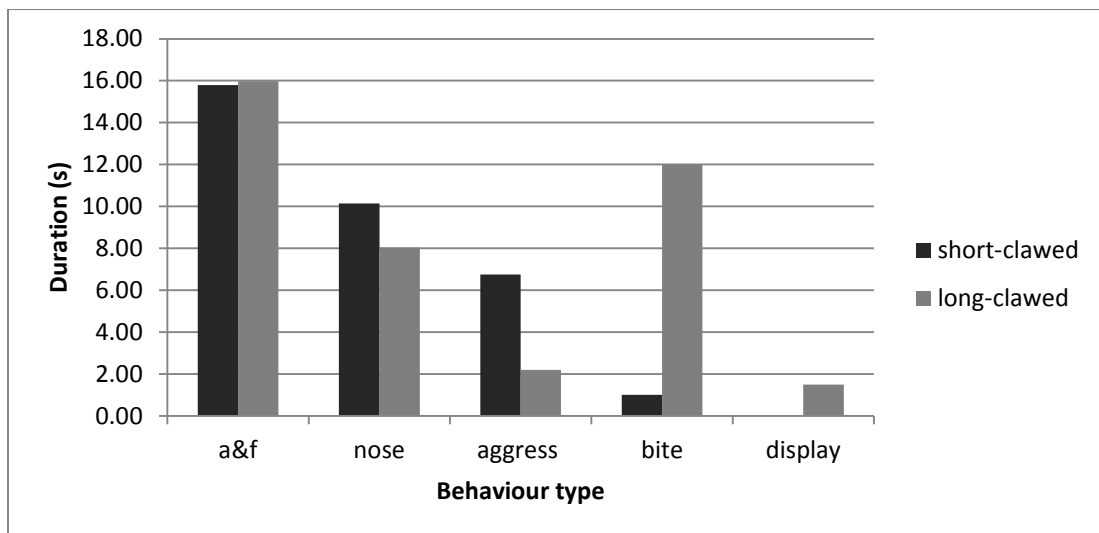


**Table 10 - Mean duration (seconds) of behaviours by long- and short-clawed males showing Student's t statistic and significance.**

		Type					
		a&f	nose	aggress	bite	display	Total
Phenotype	long	15.98	8.03	2.2	12	1.5	12.52
	short	15.79	10.14	6.75	1	0	13.41
	t (p)	0.079 (0.937)	-0.632 (0.530)	-0.832 (0.465)†	n/a	n/a	-0.460 (0.646)

† unequal variances

**Figure 8 – Duration of initiating behaviours by male phenotype: short-clawed vs. long-clawed.**



## Y-mazes

As a result of a lack of support for claw length as a potential sexually selected trait (see chapter 1) and to increase the statistical power of this experiment, I chose not to continue with the analysis of the claw length trials and augmented the seven body size trials with one claw length trial in which the two males differed by more than 2cm in mid-plastron length (total N=8). Large males (mean PL = 13.83 cm) were significantly larger than small males (mean PL = 11.48 cm) in each trial (paired Student's  $t = 8.706$ ,  $p < 0.01$ ).

I used all 12 trials to investigate the effects of side of tank and time of day. Females showed no preference for one side of the tank over the other, either in terms of frequency of entering a male choice area (mean right=2.8, mean left=4.2, paired Student's  $t=1.087$ ,  $p=0.300$ ) or duration of time spent in that area (mean right=16.34s, mean left=18.52s, paired Student's  $t=0.305$ ,  $p=0.766$ ). Additionally, there was no effect of time of day (start time of trial) in terms of either frequency of entering a male choice area (Pearson's  $r=0.138$ ,  $p=0.157$ ) or duration of time (Pearson's  $r=0.044$ ,  $p=0.327$ ) spent in that area. The remainder of the results are based on the seven body size trials. Females did not enter one male phenotype choice area more frequently than the other (mean number of entries into large male choice area=3.5, mean small=3.5, Student's  $t=0.000$ ,  $p=1.00$ ), nor did they spend more time in one male phenotype choice area than the other (mean large=14.83s, mean small=19.71s, Student's  $t=-0.784$ ,  $p=0.446$ ).

I identified and recorded four specific behaviours exhibited by females during the trials: 1) Went up to chicken wire (WUTW) – a female enters the male choice area and moved to the chicken wire divider nearest to the male turtle, 2) Interact – the female and male interacted through the chicken wire divider, one touched a body part of the other with its nose, 3) Aggress – one turtle gaped or snapped at the other turtle and 4) Climb – the female turtle climbed up the chicken wire divider. There was no difference in female behaviour towards large or small male turtles, WUTW (mean number of times WUTW in large male choice area=3.63, mean small=2.75, paired Student's  $t=0.918$ ,  $p=0.389$ ), interact (mean number of times interacting with large male=2.62, mean small=3.5, paired Student's  $t=-0.813$ ,  $p=0.443$ ). I only observed large male turtles behaving aggressively.

I recorded seven incidences of aggression and six of those incidences were attributed to a single male. I did not observe any female aggression towards male turtles. Female turtles exclusively climbed the chicken wire divider, generally falling back into the water within seconds, however one female climbed 12 times during a single trial and achieved the top of the chicken wire divider on one attempt, remaining suspended for 17 minutes.

### *Discussion*

Male painted turtles courted with equal frequency in each of the months in which trials were run. This corroborates many reports of male painted turtles observed courting throughout the active season, leading to disagreement regarding the phenology of mating in this species (Ernst, 1971a; Gibbons, 1968; Gist *et al.*, 1990). Additionally, there was no indication that time of day or water temperature influenced the activity level of the turtles. Cagle (1946) reports a “preferred activity temperature range” in the closely related slider turtle (*Trachemys scripta*) of 18°C-30°C (body temperature) whereas Spotila *et al.* (1990) report a much more restricted ideal range of temperature for activity in slider turtles at 25°C-30°C. However, both studies observed activity in the field over the range of air temperatures measured in this study (range 12.4°C to 25°C, mean 18.5°C). Air temperatures over the trials were generally warmer in 2005 (mean=20.8°C) than in 2006 (mean=16.5°C), but the addition of a basking light and platform in 2006 may have allowed the turtles to raise their body temperature sufficiently despite colder ambient air temperatures. The seven displays observed in the artificial ponds were at two different times during the active season, also corresponding with many reports of male painted turtles courting throughout the active season in natural populations. The timing of courtship behaviours in this study (i.e. throughout the active season) supports the contention that male courtship behaviour is not a good indicator of fertilization; Gist *et al.* (1990) found the largest quantity of sperm transferred in the fall.

Three initiating behaviours were more likely to be performed by males and were also more likely to be directed towards females and thus may be considered as courtship display behaviours of painted turtles. Indeed, approach and follow, nose, and display behaviours are well-accepted courtship behaviours of painted turtles (Ernst, 1971a).

Biting and aggressive behaviours were also more likely to be performed by males, but they were directed at males and females equally. The greater likelihood of males biting and performing aggressive behaviour may be attributed to the fact that male painted turtles were generally more active than females, and thus had more encounters with other turtles than did female turtles.

Long- and short-clawed males performed all behaviours at equal frequency except that two long-clawed males displayed to females, while no short-clawed males displayed. The claw length result corresponds with the results of chapter 1 in which there was no difference in male reproductive success related to claw length.

Large males performed more of the specified behaviours overall than did small males and performed more courtship behaviours than did small males. This result is contrary to what might be expected considering the results from chapter 1 where small males had higher fertilization success than large males. Greater frequency of courtship behaviour is associated with higher reproductive success if females mate with the most convenient or the most tenacious males. Thomas (2002) found that larger/older male sliders added biting to their courtship behaviour (thus improving their chances of mating) despite the fact that females accepted copulation more frequently from small/young males. In painted turtles, large males may also attempt to compensate for female choice by courting frequently and vigorously. Since I did not observe any full courtship displays culminating in intromission (or even apparent female acceptance as reported by Thomas (pers. comm.)), I am unable to confirm that the increased activity levels of the large males do, indeed, pay off in terms of increased mating opportunities as compared to small males.

Female painted turtles did not respond differently to any of the studied male phenotypes. The only response behaviours observed were move away, no response and a single aggressive behaviour. R. Brent Thomas (pers. comm.) observed an additional female response behaviour to male displays; some females dropped to the bottom of the artificial pond, perhaps signalling acceptance of copulation. The lack of full courtship displays in the current research may have been an indication that females did not receive

appropriate cues to drop to the bottom of the pond. Similarly, if male painted turtles require specific cues from the females, such as reciprocal displays, in order to advance courtship then the lack of response from females may have contributed to the lack of full courtship. There was also no difference in female response to different male phenotypes in the y-maze experiment, however, more trials are required to gain enough power to detect an effect (G\*Power 3.1.2 recommends a minimum of 18 samples to detect a large effect). Future research should also allow the turtles more time to acclimate to the y-maze apparatus and use clear plastic dividers rather than chicken wire to deter climbing behaviour.

The observed lack of female response to males of differing phenotype suggests that female painted turtles may not exert overt mate choice, but may employ cryptic mate choice based on preferential use of stored sperm or based on trading up by subsequently mating with a higher quality male (Uller and Ollson, 2008). Pearse *et al.* (2001) provide evidence of a “last-in, first-out” method of stored sperm use in painted turtles, which supports the latter mode of cryptic mate choice. If so, then large males could increase their reproductive success by copulating with as many females and as frequently as possible, thus increasing the likelihood that their sperm contribution is the last in and also the first out to fertilize offspring in order to counteract any possible mating advantage (either through mate choice or male behavior) for small male turtles. That large males have increased activity levels as compared to small males provides preliminary support for this potential sexual conflict between female painted turtles and large males. Further research is required to determine first if the increased frequency of courtship behaviour in large males results in increased copulations, second, if increased copulation translates into increased fertilization and third, if an increased-courtship-frequency tactic of large males results in higher fitness as compared to large males that do not employ the alternative mating tactic.

### **CHAPTER THREE**

#### ***AGENT-BASED MODELING OF THE EFFECT OF SEX RATIO, DENSITY AND MALE TYPE ON MALE REPRODUCTIVE SUCCESS IN CHRYSEMYS PICTA***

Modelling has long been used as an aid to understand ecological systems, particularly those that are difficult to study in nature. With the increase in processing power in personal computers, simulations of the dynamics of systems have become accessible tools for all modellers (DeAngelis and Gross, 1992). A particularly powerful and interesting method of modelling is the agent-based model, in which the behaviour of individual agents is simulated and system-level phenomena emerge as a consequence of the behaviour of those individual agents (DeAngelis and Gross, 1992, DeAngelis and Mooij, 2005). In this way, long-held assumptions about the basis of system-level patterns may be tested and challenged.

The effects of sexual selection on phenotype are difficult or impossible to measure directly in many taxa because the measurement requires observation of mating in a natural setting, unequivocal determination of parentage and observation of fitness over multiple generations (Arnold and Wade, 1984; Andersson, 1994; Boake, 1986). Evolutionary ecologists thus use many techniques to approximate sexual selection and investigate its effects, including observation of mating in artificial settings, genetic parentage analysis, study of fitness surrogates over one or more generations, cross-sectional research of parentage and fitness, and modelling (Andersson, 1994; Arnold and Wade, 1984; Congdon *et al.*, 1999; Pearse and Avise, 2001, Thomas, 2002). Once data have been gathered from an ecological system based on the former techniques, models can be used to test hypotheses about how sexual selection might act in a population, observe the outcome of parameter modifications over multiple iterations and compare the results with data collected from natural populations (DeAngelis and Mooij, 2005). Using agent-based modelling, changes in gene frequency due to sexual selection (a population-level trait) may be observed as the product of the behaviour of individuals within the population.

Turtle researchers have long theorized that painted turtles have a mating system characterized by female choice (Berry and Shine, 1980; Ernst and Lovich, 2009) based on observations of the courtship of painted turtles whereby male turtles display for females using their elongated foreclaws (Ernst, 1971*a*) and based on the form of sexual size dimorphism whereby females are much larger than males (Berry and Shine, 1980). Hence, it is assumed first that sexual size dimorphism in painted turtles is maintained by sexual selection and second, that the small size of male *C. picta* compared to females precludes intrasexual selection or mate coercion, thus leaving intersexual selection as the only alternative (Berry and Shine, 1980).

The results from chapter 1 provide cautious support for the first of these assumptions as smaller male painted turtles contribute paternity to more clutches than larger painted turtles. If body size is a heritable trait in painted turtles, then this greater fertilization success enjoyed by smaller males can be expected to maintain the divergence in body size in painted turtles. Chapter 2, however, casts some doubt on the first assumption suggesting that females may not exert much choice in mating (at least not observable choice). However, the males with most success in chapter 1 (smaller males) were found in chapter 2 to court less vigorously than the larger males, so differences in male fertilization success between the sizes is likely not due to greater persistence on the part of one size of male.

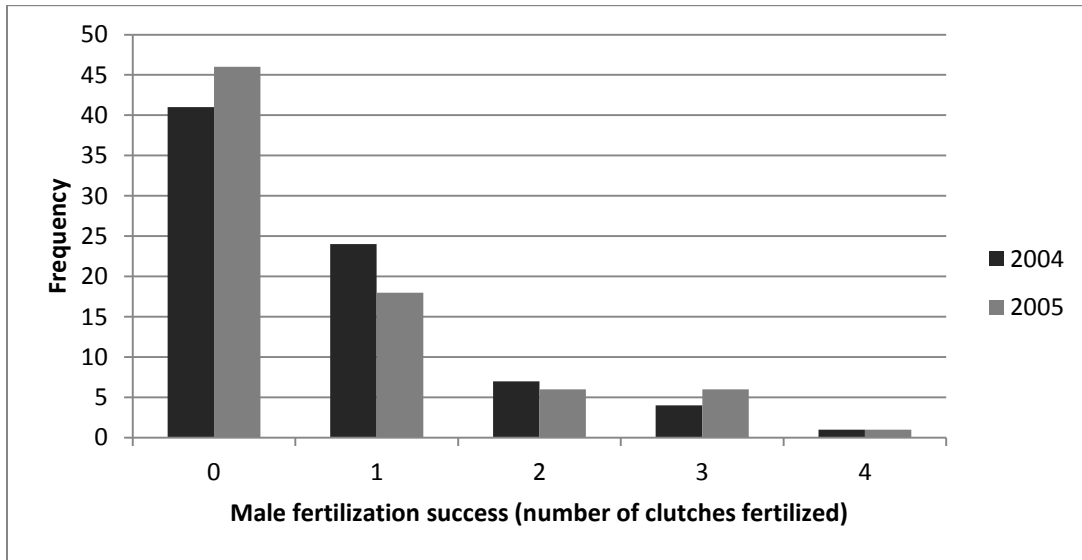
The mating system of painted turtles is an ideal candidate for modelling because of the difficulties inherent in directly studying courtship, mating, paternity and fitness in such a long-lived and cryptic species. Courtship has been observed in many natural populations (Christens and Bider, 1986; Gibbons, 1968; Gist *et al.*, 1990; Iverson and Smith, 1990) and in captivity (Ernst, 1971*a*; Taylor, 1933); however, the timing of courtship and mating remain the subject of debate (Gibbons, 1968; Gist *et al.*, 1990, Taylor, 1933). Paternity analyses are rare (but see Pearse and Avise, 2001; Pearse *et al.*, 2002) as direct observation of fertilization in natural populations is difficult and may not be a good indicator of paternity since both male and female painted turtles store sperm (Gist *et al.*, 2001; Pearse *et al.*, 2001). Additionally, there are few genetic markers published for painted turtles (but see Pearse *et al.*, 2001; 2002), making genetic paternity

analyses challenging. More markers have been developed for other freshwater turtle species (Hauswaldt and Glenn, 2003; King and Julian, 2004; Libants *et al.*, 2004; Mantziou *et al.*, 2005; Rafeliasoa *et al.*, 2006; Schwartz *et al.*, 2003 and many others published since 2006), but few amplify with *C. picta* DNA and are sufficiently polymorphic for paternity analysis. Freshwater turtle fitness is also relatively unstudied; a few fitness surrogates have been suggested and studied (survival and growth, Boby and Brooks, 1994; body size, Congdon *et al.*, 1999; Janzen *et al.*, 2000; locomotor performance, Janzen, 1993 and dispersal, Kolbe and Janzen, 2001), but most researchers focus solely on the hatchling life stage. Determination of fitness over the lifespan of a turtle is prohibitive because of the long life and late maturation typical of the taxon (Ernst and Lovich, 2009). Few research groups have collected long-term morphometric and nesting data on painted turtles (but see R. J. Brooks' work in Algonquin Park, Ontario, J. Congdon's work at the E. S. George Reserve, Michigan and F. Janzen's work at the Thomson Causeway, Thomson, Illinois).

In this chapter, I investigate the individual-based forces that produce a distribution of male fertilization success as was observed in chapter 1. The observed data, grouped into three categories of male fertilization success (zero clutches fertilized, one clutch fertilized and two or more clutches fertilized), suggest that smaller males are more likely to contribute paternity to individual clutches than larger males; however, the distribution of male fertilization success as a whole resembles a Poisson distribution (Sokal and Rohlf, 1995; Figure 9). As such, the observed pattern could arise from random encounters between males and females and a low probability of a female using the sperm from any male to fertilize her clutch, without requiring females to discriminate among potential mates. I compare the fit of this basic Poisson distribution to a distribution generated with the assumption that males are of different quality and females will respond to one group of males differently than another group of males. Since there are only five categories of male fertilization success, the degrees of freedom available for statistical testing (pooled g-test) limit my investigation to only two male categories (Sokal and Rohlf, 1995).



**Figure 9 – Observed distribution of fertilization successes from chapter 1.**



Despite a statistical fit to the two-male-type distribution, the observed data deviates from both the basic Poisson distribution and the two-male-type distribution in the right-hand tail (Figures 10, 11). I use agent-based simulations to estimate the probability that a result as extreme or more extreme as the right-hand tail of the observed data could be generated by either the basic Poisson distribution or the modified Poisson two-male-type distribution over 10000 hypothetical reproductive seasons.

Using the two-male-type distribution (the best fit and the one more likely to produce the extreme values observed in the right-hand tail of the observed distribution), I also use agent-based modelling to simulate the outcome of adjusting some of the parameters based on published data, specifically, sex ratio, turtle density and proportions of male types. Natural populations of painted turtles have widely varying adult sex ratios, from strongly female-biased (Algonquin Park, this study) to strongly male-biased (E.S. George Reserve, Michigan, Congdon *et al.* 1986; Scribner *et al.* 1993). The reasons for this variation in adult sex ratio may be due to a number of factors, acting either independently or in conjunction with other factors. Freedberg and Wade (2001) suggest that in species with environmental sex determination, such as painted turtles, cultural inheritance of nest site choice will lead to a female skewed primary sex ratio. However, skewed adult sex ratio should be expected if one sex matures at an earlier age than the other (Gibbons, 1990). Male painted turtles mature at a smaller size than females and thus at an earlier age (Samson, 2003), so a male-skewed adult sex ratio is predicted. Alternatively, skewed adult sex ratios reported in many turtle species may be attributed to sampling bias, for example, if studies focus on nesting behaviour, differential mortality risk (due to dispersal, McCulloch and Secoy, 1983; Rowe, 2003; Scribner *et al.*, 1993; due to habitat alteration, Eskew *et al.*, 2010; due to road density, Patrick and Gibbs, 2010; due to body size, Tucker *et al.*, 1999), or collection for the pet trade (Reeves and Litzgus, 2008). Having more males per female in the population increases the number of males that each female encounters throughout the mating season and thus increases her opportunity to discriminate amongst potential mates (Emlen and Oring, 1977). As females become choosier the more preferred males will generally fertilize a larger number of clutches and the less preferred males will generally fertilize a smaller number

of clutches, thus increasing the mean variance of male fertilization success in a male-biased population as compared to female-biased.

Painted turtles live in populations with widely varying adult densities. Such differences in density may be due to climatic variation (and thus water level variation) from one year to the next (Bowne *et al.*, 2006), how recently turtles have immigrated to a specific body of water (Eskew *et al.*, 2010) and/or other ecological factors, such as pond productivity, the presence of nest sites, the presence of predators of adult turtles, proximity to other wetlands or proximity to roads (Marchand and Litvaitis, 2004). Higher turtle density increases the encounter rate among turtles overall allowing all males to potentially contribute sperm to more clutches, thus resulting in a higher mean of male fertilization success. Higher densities of turtles also increase the variance of male fertilization success because as turtles move through the model space, each male encounters a random proportion of males and females and some males encounter more females than other males. The random nature of male success in the agent-based model ensures that some males have low-to-no fertilization success while others enjoy very high fertilization success, increasing the variance of male fertilization success in the high-density scenario as compared to the low-density scenario.

Although there are no data regarding actual proportions of “more successful” and “less successful” males from natural populations, such changes in the proportions may be produced by increased predation on one category of male over the other, if differences in predation risk are based on movement patterns (female pond turtles, *Emydura macquarii*, Spencer, 2002; Spencer and Thompson, 2003) or phenotype (hatchling scincid lizards, Downes and Shine, 1999), or differences in recruitment rate of different phenotypes, e.g. male attainment of sexual maturity at a smaller size in more southern populations as compared to northern populations (Ernst and Lovich, 2009; Samson, 2003). Since the numbers of male and female turtles remain consistent in all three scenarios, there is no difference in overall encounter rate. However, the greater proportion of “more-successful” males as compared to “less-successful” males will result in increases in both the mean and the variance of male fertilization success across the simulation. When there is a higher proportion of “more-successful” males in the population than “less-

successful” males, females have a higher likelihood of encountering a “more-successful” male and subsequently, a higher probability of using the sperm from the “more-successful” male to fertilize a portion of her clutch.

In all three simulations, increases in mean male fertilization success will be related to increases in the number of male sperm contributions that females use to fertilize their clutches and thus higher estimates of multiple paternity and (tangentially) higher rates of intraseasonal iteroparity.

## ***Methods***

### **Poisson distribution**

I generated a Poisson distribution based on a 45-day mating season (allowing for mating to occur both in the late summer/fall and early spring, see agent-based model methods for rationale) with each male encountering a female 67 times over the mating season (hereafter called the basic Poisson model). I estimated the mean encounter rate (number of other turtles encountered over each hypothetical mating season) of 67 using NetLogo 4.1.2 over 10000 simulations (see the methods for the agent-based model in this chapter for a full description of the parameters used to create the simulation). I also generated a modified Poisson distribution based on the assumption that there may be two groups of males in the population, each with its own probability of fertilization success (hereafter called the two-male-type modified Poisson model). To estimate the fertilization success rate parameter (in the basic Poisson model) and the two fertilization success rate parameters and proportions of each male type (in the two-male-type modified Poisson model), I fit each predicted distribution to the observed distributions of male mating success in each of the two years using a least squares method (see Tables 11 and 12 for parameter values). The fertilization success rate assumes *a priori* that the female accepted courtship, allowed insemination and subsequently used that male’s sperm to fertilize a portion of her reproductive output that season. I tested the goodness of fit for each of the expected distributions against the two years of observed data using pooled g-tests with a Bonferroni adjustment for ten *post hoc* tests. A lack of degrees of

**Table 11 – Parameter values in basic Poisson model simulation.**

# Females	# Males	Success critical value	Plot size, density
300	100	81/1000	148 x 148, 180 turtles/ha

Note: I converted the proportional critical values generated by the least squares analysis to a value out of 1000 to create a whole number to enter into the NetLogo code

**Table 12 – Parameter values in two-male-type Poisson model simulation.**

# Females	# Males1	Success critical value1	# Males2	Success critical value2	Plot size, density
300	85	66/1000	15	361/1000	148 x 148, 180 turtles /ha

Note: I converted the proportional critical values generated by the least squares analysis to a value out of 1000 to create a whole number to enter into the NetLogo code

freedom limited my exploration to two male types. All statistics were performed using Microsoft Excel 2010 (Microsoft, Inc.).

### **Agent-based model simulations**

Protocol for description based on Grimm et al., 2006

#### *Overview*

##### **Purpose**

I first used simulations to determine the probability that the extreme values in the right-hand tail of my observed distribution of male fertilization success could be generated by either the basic Poisson model or the two-male-type modified Poisson model. Second, using the two-male-type modified Poisson model, I investigated the effects of altering model parameters (sex ratio, turtle density and proportion of male types) on the mean and variance of male fertilization success and on the predicted rates of multiple paternity and intraseasonal iteroparity in females. I used NetLogo 4.1.2 (Wilensky, 1999) as the platform for my agent-based modelling and ran simulations using the BehaviorSpace tool.

##### **State variables and scales**

Agents are divided into males and females in the basic model and males are further subdivided into males1 and males2 in the two-male-type model. Agents are randomly placed within the model space at a specific density. All agents move randomly throughout the model space at a rate of one plot per time unit. The model space does not wrap around; when an agent reaches the edge of the model space, it must turn around in the next time step to remain active in the model. When a male agent and a female agent end up on the same plot in a specific time unit, the male agent is awarded +1 encounter. Successful fertilization occurs if a randomly chosen number falls below the success rate determined in the Poisson analysis. The male agent is awarded +1 success and the female agent is awarded +1 fertilization. This process continues for a specified number of time steps and then male success, male encounters and female fertilizations are tallied for each agent.

### Process overview and scheduling

The model advances in discrete time steps, the sum of which represents the painted turtle mating season. Each time step represents a unit of real time in which a turtle is likely to move a single metre. The density of turtles within the simulation is based on the size of the water body and the density of turtles at Wolf Howl Pond, Algonquin Park. Male success, male encounters and female fertilization are updated at each time step. Set up for the model includes the creation of female agents and male agents (or two types of male agent, if applicable) and the random placement of the agents on plots within the model space. The command “run” then begins with the process “move”. Each agent moves once in each time step. Agents turn a random number of degrees to the right (minimum  $0^\circ$ , maximum  $360^\circ$ ) and move forward a single plot in each time step. If a plot is inhabited by more than one agent in a single time step, two other processes are invoked; males “court” and females “meetfem”. For each male in the model space, the process “court” asks if any of the other agents inhabiting the same plot are female. If so, the male agent gains +1 encounter and a random number from 1 to 1000 is generated that determines whether or not the male is successful in fertilizing a part of the female’s clutch. If the random number is below the critical value, the male agent gains +1 success. This process is repeated if there are multiple females on the plot. For each female in the model space, the process “meetfem” asks if any of the other agents inhabiting the same plot are male. If so, a random number is generated from 1 to 1000 that determines whether or not the female uses the male’s sperm contribution to fertilize a portion of her clutch. If the random number is below the critical value (the same critical value as for the male), then the female agent gains +1 fertilization. This process is repeated if there are multiple males on the plot. In the basic model, the critical value is the same for all males, in the two-male-type model, the critical value depends on the identity of the male. The “move”, “court” and “meetfem” processes are continued for the specified number of time steps, at which point all processes end and male successes, male encounters and female fertilizations are tallied. Each simulation consists of 10000 iterations (model runs) of the above procedure. See Appendices 1 and 2 for NetLogo code.

### *Design concepts*

Emergence – Population-level patterns of male fertilization success and number of fertilizations that each female allows are the emergent phenomena that are expected to be generated by the agent-based model. The encounter rate (number of female turtles that a single male encounters over the course of the iteration) is an additional emergent phenomenon as a consequence of the size of the model space, the density of agents in the model and the sex ratio of agents.

Fitness – Male agents within these models accrue fitness according to the number of clutches to which they contribute sperm. The fitness of female agents is not directly measured, however, highly conservative estimates of female intraseasonal iteroparity can be made based on the number of fertilizations the female agent “allows”.

Sensing – Agents know their own sex and are able to sense the sex of other agents with which they interact. In the two-male-type model, male agents also know to which male type they belong and females sense the male type of the males with which they interact.

Interaction – Male agents interact with female agents through the process “court”, but do not interact with other male agents. Female agents interact with male agents through the process “meetfem”, but do not interact with other female agents. Agents do not interact with the environment.

Observation – Tallies of the number of successes for each male and the number of fertilizations for each female are made at the completion of each iteration in the simulation. In addition the means and the variances for male success, male encounter rate and female fertilization rate are reported for each iteration in the simulation.

### *Details*

#### Initialization

Each simulation begins with 100 male agents. The number of female agents is based on the sex ratio of the population in the simulation and the density of the population (see Tables 3, 4 and 5 for numbers of female turtles). Each iteration of each



simulation runs for 5000 time steps, representing the duration of the painted turtle mating season. Rowe (2003) reported that painted turtles move an average of 102 m/day. The exact timing of the painted turtle mating season is still under debate, so I chose to allow mating to occur both in the early autumn (Gist *et al.*, 1990; Gist *et al.*, 2000; approximately 30 days) and the late spring, once water temperatures were high enough to support feeding and mating but before nesting season (Gibbons, 1968; Ernst, 1971*a, b*; approximately 15 days). Hence, an average painted turtle may be expected to complete 4590 1-metre movements over the nesting season (102 m/day x 45 days). I rounded this value to 5000 time steps for the purposes of the model. Turtle density in the basic model is based on the size, density and sex ratio of Wolf Howl Pond, Algonquin Park (see Chapter 1 for a detailed description of the site). Wolf Howl Pond is 1.7 ha and supports a population of 228 female painted turtles and 78 male painted turtles (Bailey's triple catch for 2004, 2005 and 2006; Bailey, 1952). The density of painted turtles in Wolf Howl Pond is thus 180 turtles/ha and the sex ratio is 1:2.92. I converted the size of the pond to square metres and used that value to govern the size of the model space in NetLogo 4.1.2. For the purposes of the model, I rounded the population size to 400 turtles and the sex ratio to 1:3, hence I used 100 male turtles and 300 female turtles as my initial values. A density of 180 turtles/ha or 0.018 turtles/square metre with 400 turtles requires total area of 22,222 square metres, which translates into a model space of 148 plots each horizontally and vertically (each plot representing a single square metre). Sex ratio of the population is based on published values for painted turtles. I chose a female-biased ratio of 1:3 (this study, Algonquin Park population), an even ratio of 1:1 (Ernst 1971*b*) and a male-biased ratio of 3:1 (Congdon *et al.* 1986; Scribner *et al.* 1993). Population density is also based on published values of *C. picta* density. Alterations to turtle density were accomplished by adjusting the size of the model space while keeping the total number of turtles within the simulation constant. I chose a low density value of 20 turtles/ha (Eskew *et al.* 2010), a medium density of 180 turtles/ha (this study, Algonquin Park population) and a high density of 300 turtles/ha (Bowne *et al.* 2006). I excluded a higher density estimate of 2000 turtles/ha reported by Bowne *et al.* (2006) because it was recorded in a year with extreme climatic conditions. Since there are no published values for proportions of two male types in a population of painted turtle, I chose to use the

predicted proportions, even proportions and opposite proportions. The modified Poisson specified 85% less-successful males and 15% more-successful males. The even proportion scenario was 50%-50% and the opposite proportion scenario was 15% less-successful and 85% more-successful males. See Tables 13, 14 and 15 for the numbers of male and female turtles that were input into each simulation.

#### Input

Male fertilization success rates, based on the results of the Poisson and modified Poisson analyses of the observed data, were input into the model. Male agents in the model that met with a female agent in a specific time step achieved fertilization success if a random number fell below the critical value for success. NetLogo 4.1.2 uses a pseudo random number generator. I chose to allow NetLogo to set the random seed for each simulation based on the date and time of the simulation run.

#### Submodels

For each model, I ran 10000 iterations comprising 5000 time steps each, simulating 10000 possible mating seasons. See Tables 13, 14 and 15 for initial parameter values input into the models

#### Analysis

To address whether either the basic model or the two-male-type model could generate a right-hand tail as extreme or more extreme than the observed data, I ran each model through 10000 iterations, tallied the total number of males in each iteration that were awarded three or more successes and compared each of these values to the number of males awarded three or more fertilization successes in each year of the observed data. I summed all iterations that produced an equal or greater number of males with three or more fertilization successes. I transformed this sum into a probability and multiplied the probabilities of the two years to produce the probability that the model could generate values as extreme or more extreme as the observed values over two reproductive seasons.

To address what effect manipulating the model parameters has on the mean and variance of male fertilization success and on the mean and variance of the number of sperm contributions a female uses to fertilize her reproductive output, I ran each scenario

**Table 13 – Parameter values in simulations varying population density.**

Density	# Females	# Males1	Success critical value1	# Males2	Success critical value2	Plot size
Low 20 turtles /ha	300	85	66	15	361	448x448
Medium 180 turtles /ha	300	85	66	15	361	148x148
High 300 turtles /ha	300	85	66	15	361	116x116

**Table 14 – Parameter values in simulations varying sex ratio.**

Sex ratio	# Females	# Males1	Success critical value1	# Males2	Success critical value2	Plot size, density
Female biased 1:3	300	85	66	15	361	148 x 148, 180 turtles/ha
Even 1:1	100	85	66	15	361	106x106, 180 turtles/ha
Male-biased 3:1	33	85	66	15	361	86x86, 180 turtles/ha

**Table 15 – Parameter values in simulations varying proportions of two male types.**

Proportion	# Females	# Males1	Success critical value1	# Males2	Success critical value2	Plot size, density
85:15	300	85	66	15	361	148 x 148, 180 turtles/ha
50:50	300	50	66	50	361	148 x 148, 180 turtles/ha
15:85	300	15	66	85	361	148 x 148, 180 turtles/ha

through 10000 iterations and visualized the output using bar graphs. Starting with the two-male-model (the best fit for the observed data), I manipulated sex ratio, density and proportions of male types.

## ***Results***

### **Poisson modelling**

In the basic Poisson model, the fertilization success rate parameter that minimized the difference between the observed distribution and the expected distribution was  $p=0.0081$ ; that is, 0.81% of encounters resulted in the successful fertilization of a portion of a female's reproductive output (see Table 16 for parameter values). The observed data diverged from the expected Poisson distribution significantly (total g-test for goodness of fit, Bonferroni adjusted for 10 *post hoc* tests). The two years were homogenous in their goodness of fit; however, the 2005 data were significantly different from the Poisson distribution (Table 17 and Figure 10). For the two-male-type model, the parameters that minimized the difference between the observed and expected distributions were one male type comprising 85.2% of the population with a success rate of 0.0066 and a second male type comprising 14.8% of the population with a higher success rate of 0.0361 (see Table 18 for parameter values). A pooled g-test for goodness of fit found no statistically significant difference between the modified two-male-type Poisson distribution and the observed data (Table 19 and Figure 11). The g-test for heterogeneity between years was also non-significant, as were the g-tests for each year independently, thus, the two years were homogenous in their goodness-of-fit and the observed data did not differ significantly from the predicted distribution generated by the modified Poisson distribution (Table 19).

**Table 16 – Parameter values for best-fitting Poisson distribution, basic model.**

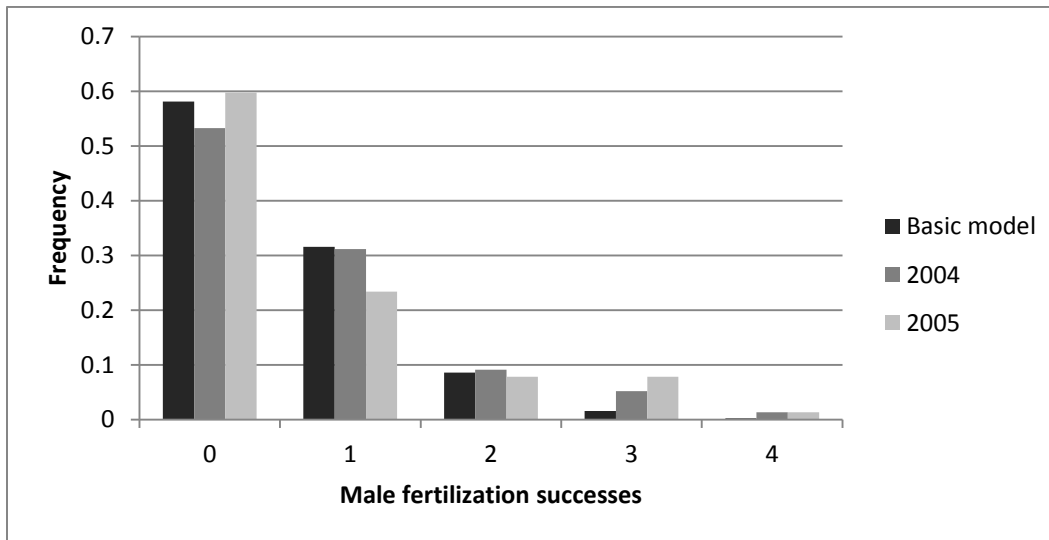
r	n	t	p
10	67	241200	0.0081

**Table 17 – Pooled g-test for goodness of fit, basic model.**

	g-statistic	df	p
Heterogeneity	1.6273	4	0.8039
Pooled	18.458	3	0.0004*
2004	6.426	3	0.1065
2005	13.66	3	0.0034*
Total	20.086	8	0.01

\* significant when evaluated using a Bonferroni correction for 10 post-hoc tests  $p=0.005$

**Figure 10 – Predicted male fertilization success (basic model) and actual male fertilization success data from 2004 and 2005.**





**Table 18 – Parameter values for best-fitting Poisson distribution, two male type model.**

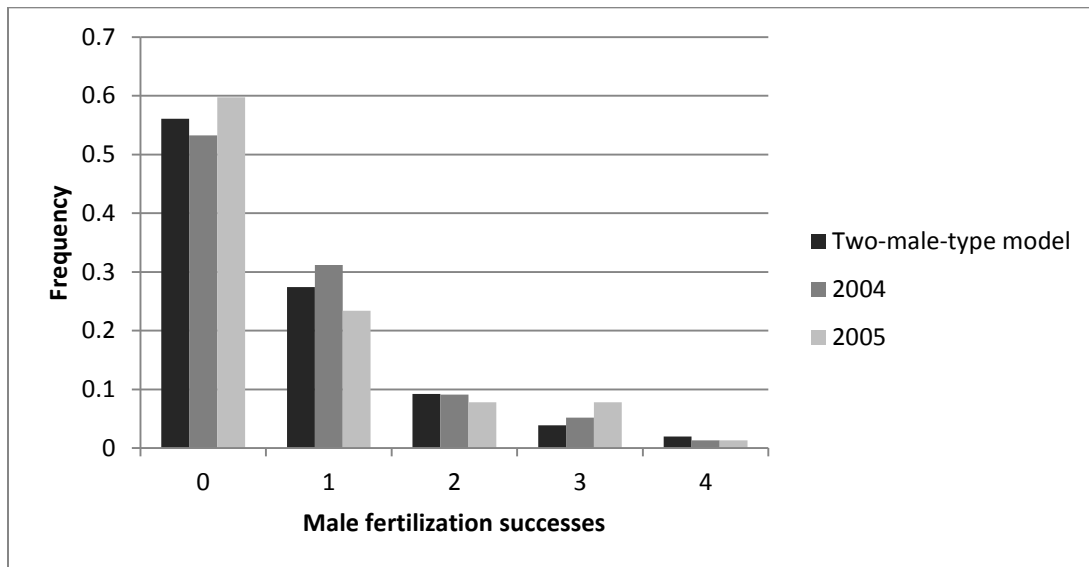
r	n	t	p male1	p male 2	proportion 1	proportion 2
10	67	241200	0.0066	0.0361	0.852	0.148

**Table 19 – Pooled g-test for goodness of fit, two male type model.**

	g-statistic	df	p
Heterogeneity	1.6273	4	0.8039
Pooled	7.2223	1	0.0072
2004	3.2462	1	0.0716
2005	5.6037	1	0.0179
Total	8.8498	8	0.3551

\* significant when evaluated using a Bonferroni correction for 10 post-hoc tests  $p=0.005$

**Figure 11 – Predicted male fertilization success (two-male-type model) and actual male fertilization success data from 2004 and 2005.**



## **Agent-based modelling**

### *Fitting the right-hand tail of the observed distribution*

The simulation of 10000 iterations of the basic model produced 34/10000 iterations that matched or exceeded the number of observed males contributing sperm to three or more clutches in 2004 (six males) and 0/10000 iterations that matched or exceeded the number of observed males contributing sperm to three or more clutches in 2005 (nine males). This resulted in a combined probability of 0 that values as extreme as the observed data could be generated by the basic model in two mating seasons. The simulation of 10000 iterations of the two-male-type model produced 1938/10000 iterations that matched or exceeded the number of observed males contributing sperm to three or more clutches in 2004 (six males) and 148/10000 iterations that matched or exceeded the number of observed males contributing sperm to three or more clutches in 2005 (nine males). This resulted in a combined probability of 0.002868 that values as extreme as the observed data could be generated by the two-male-model in two mating seasons.

### *Female reproduction – basic model vs. two-male-type model*

The basic model predicted that 16.5% (mean, range 9.3% - 24.3%) of females reproduce in a season, while the two-male-type model predicts that on average, 19% (mean, range 11.7%-27.7%) of females reproduce in a season. Between 1999 and 2006, 71.6% (mean, range 62.6% to 77.0%) of Algonquin Park females reproduced in a season, thus both models underestimate the number of reproductively active females in a single season. If we assume that a female uses a maximum of two male sperm contributions in a single clutch (i.e. there is only double paternity, not triple or higher paternity), then the basic model further predicts that among those females that do reproduce, 0.1% (conservatively - female uses sperm from three or more males) to 1.6% (generously - female uses sperm from two or more males) produce more than one clutch in a season. The two-male-type model predicts that 9.1% (conservatively) to 11.4% (generously) of all reproductive females produce more than one clutch in a season. Between 1999 and 2006, 19.9% (mean, range 9.4% - 29.8%) of Algonquin Park females produced more than one clutch in a season, thus both models underestimate the number of females that

produce multiple clutches. Additionally, the basic model predicted that four females would use sperm contributions from five males, which would mean that with two clutches produced in a season, one of the clutches included sperm contributions from three different males. The two-male-type model predicted 31 females would use sperm contributions from five or six males.

## **Simulations**

### *Sex ratio*

Varying the sex ratio from female-biased through even to male-biased resulted in a decrease in both the mean and the variance of male fertilization success (Figure 12*a,b,c*). In addition, both the mean and the variance of encounter rate decreased (Figure 12*d,e*) and the mean and the variance of the number of fertilizations for each female increased across the three categories (Figure 12*f*).

### *Density*

Increasing the density of turtles from 20 turtles/ha to 180 turtles/ha to 300 turtles/ha resulted in an increase in both the mean and variance of fertilization success (Figure 13*a,b,c*). Additionally, both the mean and variance of encounter rate increased from the low-density to high-density models (Figure 13*d,e*). Both the mean and the variance of female fertilization rate increased from low-density to high-density models (Figure 13*f*).

### *Male type proportions*

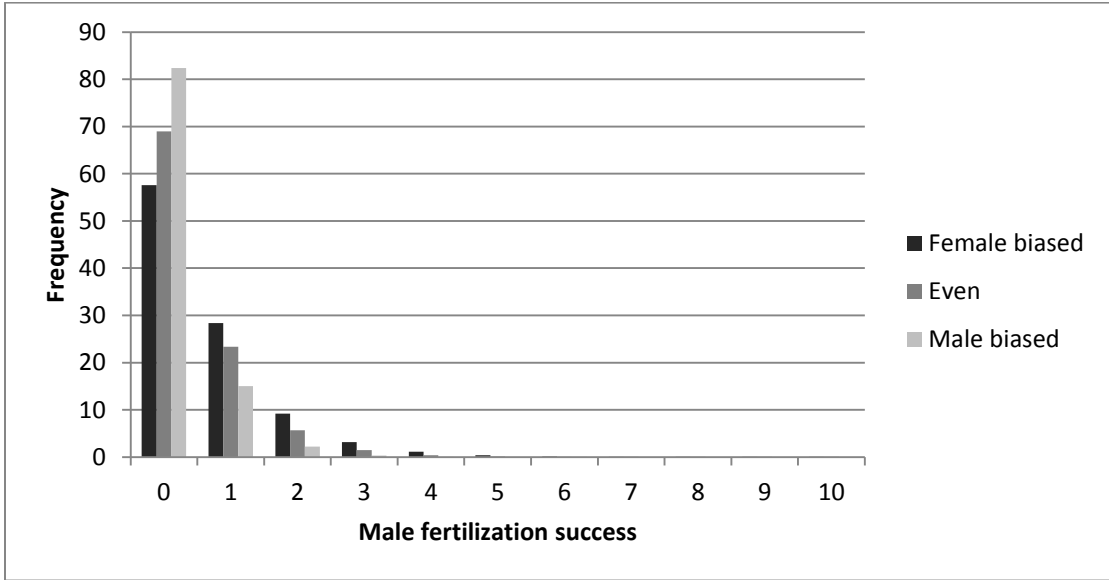
Varying the proportions of the two types of male resulted in an increase in both the mean and the variance of male fertilization success as the proportion of “more-successful” males increased (from 85:15 to 50:50 to 15:85, less-successful:more-successful) (Figure 14*a,b,c*). Neither the mean nor the variance of encounter rate changed with varying proportions of the two male types (Figure 14*d,e*). The mean and variance of female fertilization rates both increased with increasing numbers of more successful males (Figure 14*f*).

*Female reproduction – simulations*

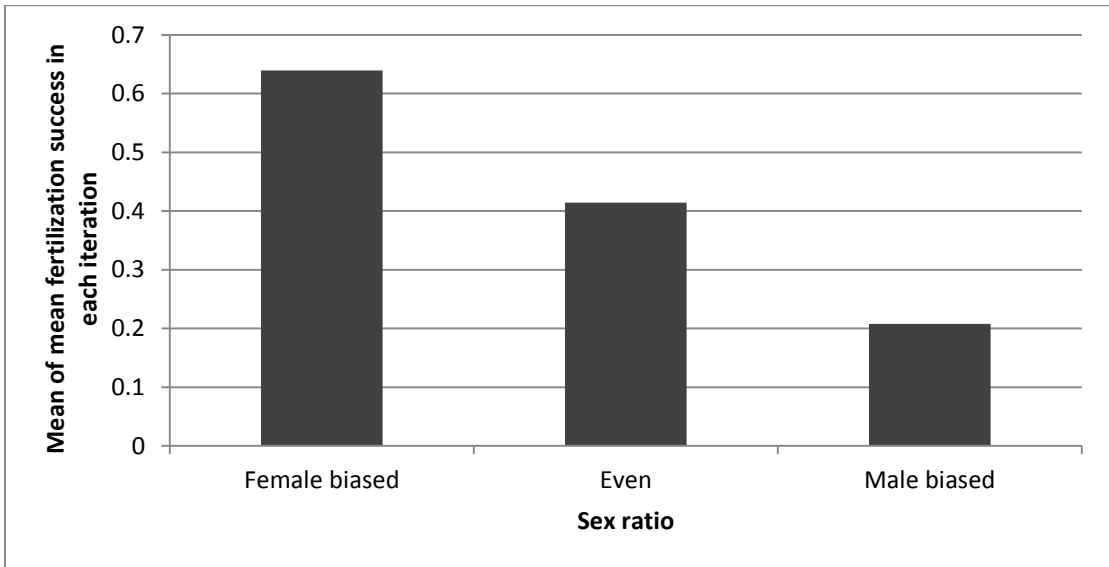
In all scenarios, rates of female reproduction within a season were predicted to be low. Most females in the simulations used the sperm contributions from no males (i.e. did not reproduce) (Figure 15*a,b,c*) and of those females that did reproduce, the predicted number that likely produced more than one clutch (fertilizations by three or more males) was also low (mean 2.58%, range 0.08% to 6% ). All simulations predicted low numbers of females likely to use sperm contributions from more than four males in a single season (mean 0.03%, range 0 to 0.17%).

**Figure 12 – Results of simulations varying sex ratio.**

a Mean frequencies of male fertilization success across 10000 iterations, b Mean of mean male fertilization success in each iteration.



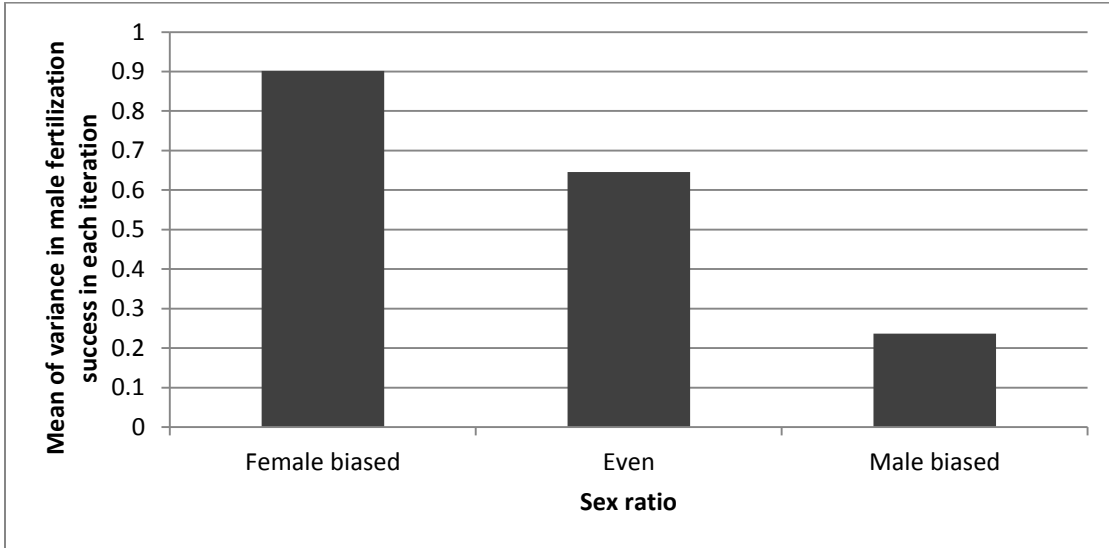
*a*



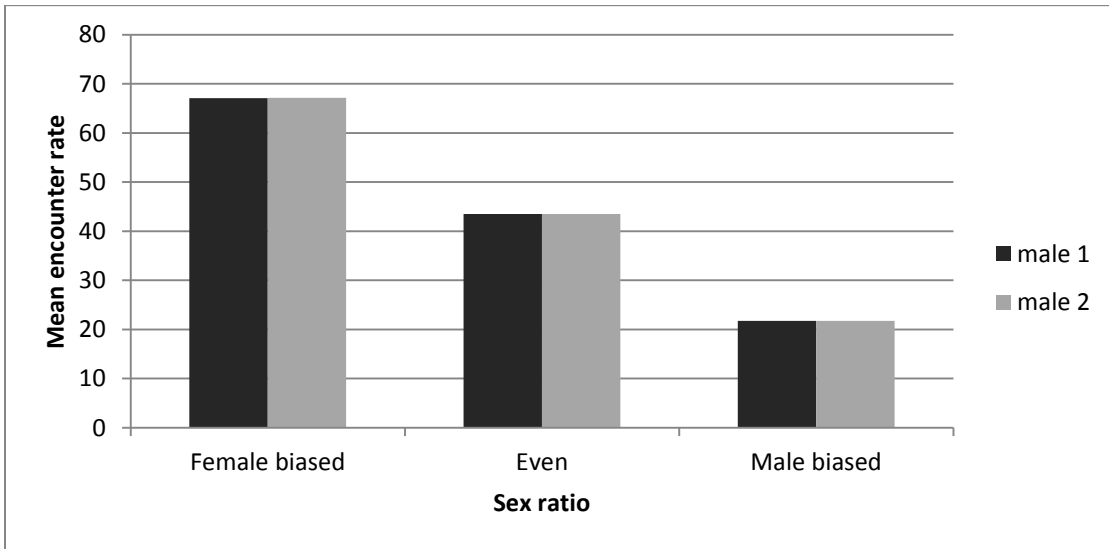
*b*

Figure 12 continued – Results of simulations varying sex ratio.

*c* Mean of variation in male fertilization success in each iteration, *d* Mean of the mean male encounter rate in each iteration.



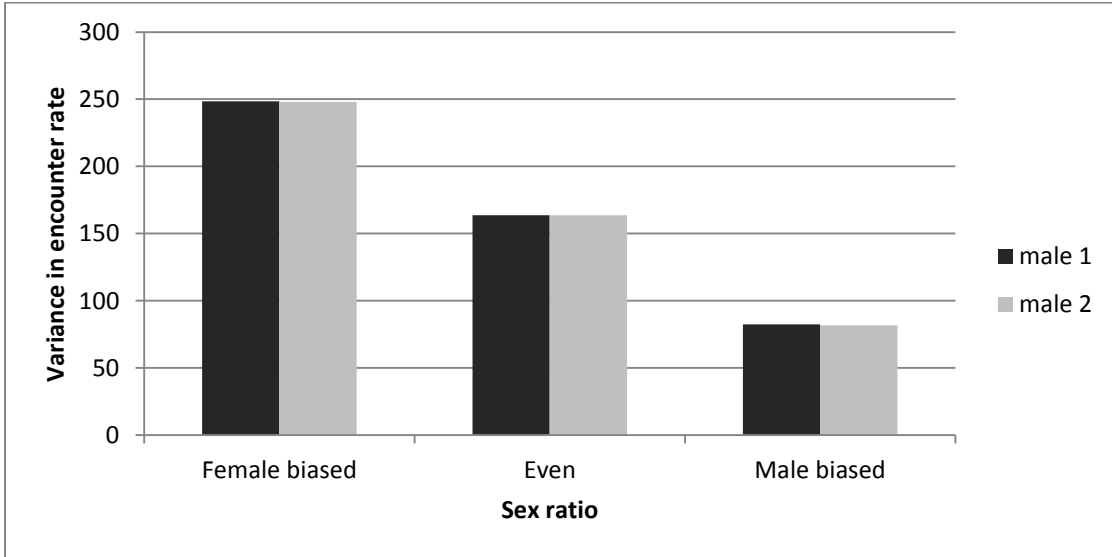
*c*



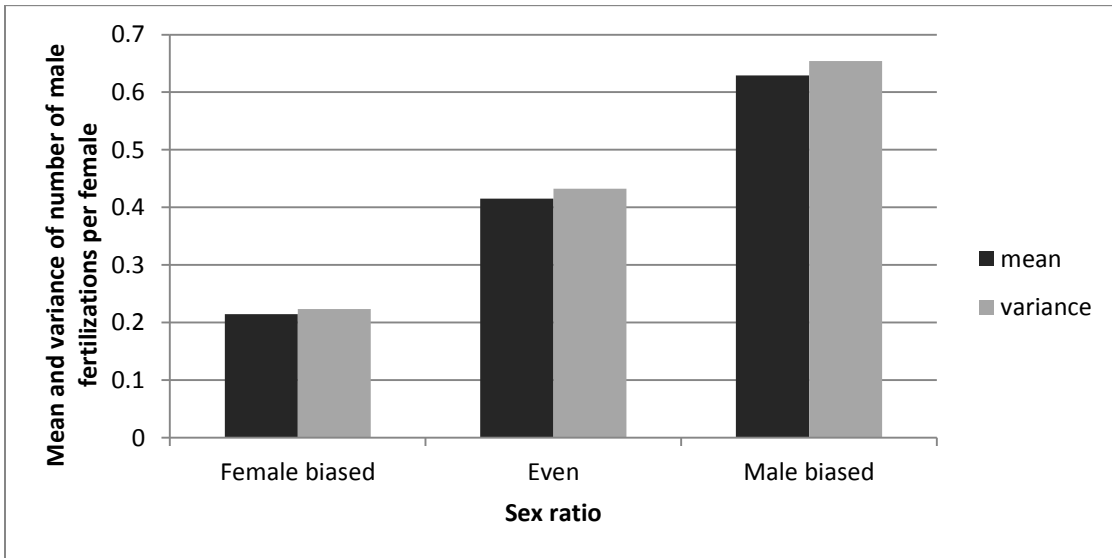
*d*

Figure 12 continued – Results of simulations varying sex ratio.

*e* Mean variation in male encounter rate in each iteration, *f* Mean mean and mean variance of number of male fertilizations per female in each iteration.



*e*

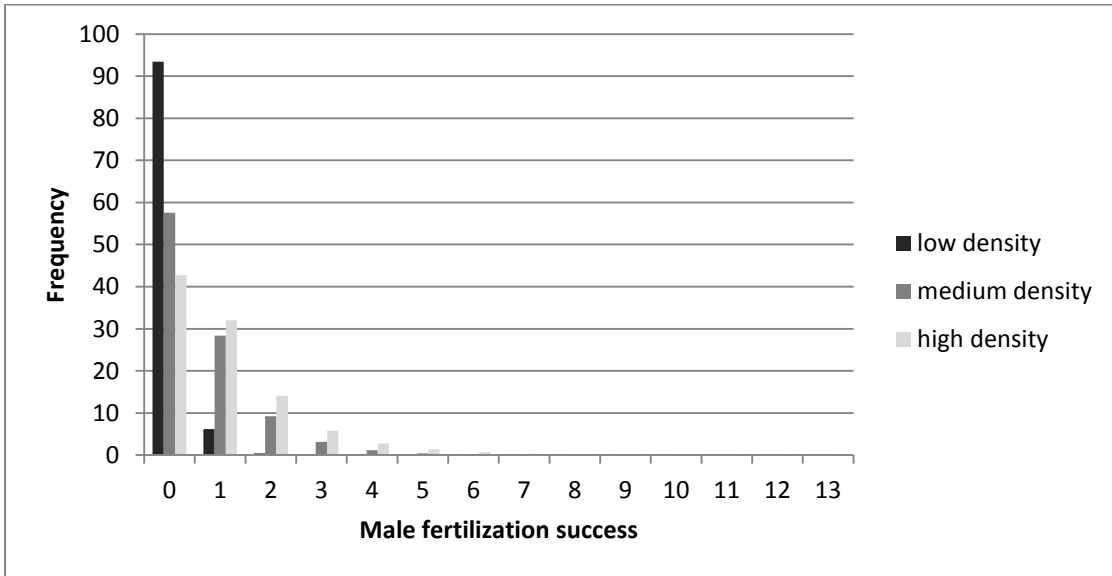


*f*

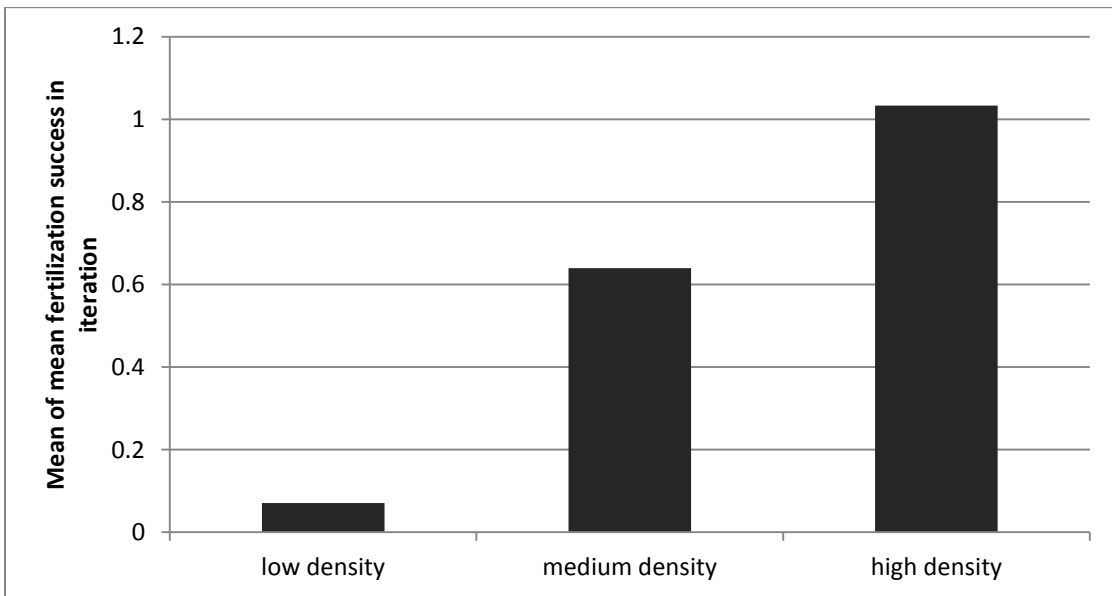


**Figure 13 – Results of simulations varying population density.**

a Mean frequencies of male fertilization success across 10000 iterations, b Mean of mean male fertilization success in each iteration.



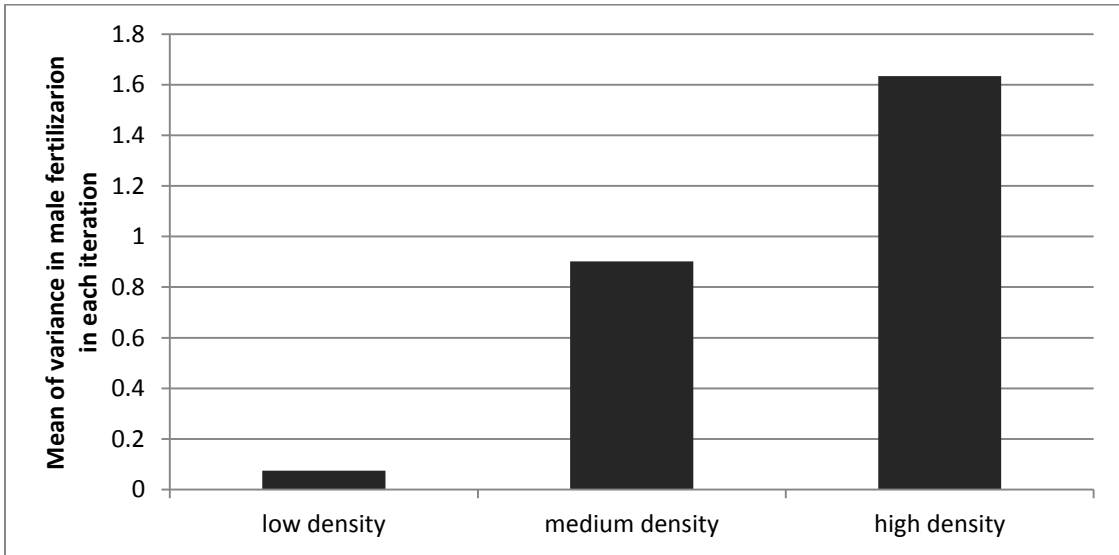
*a*



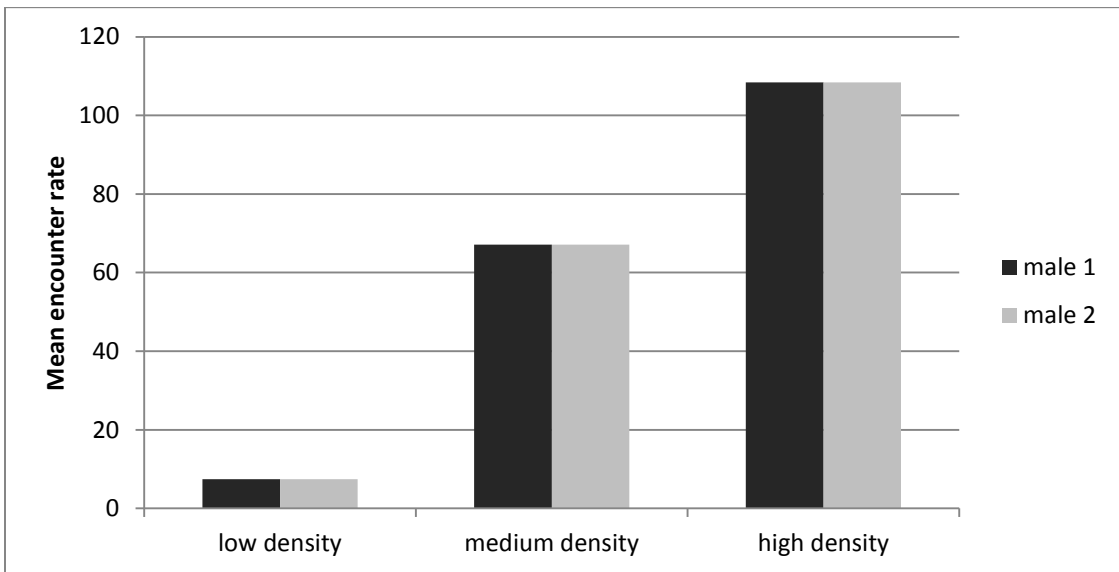
*b*

Figure 13 continued – Results of simulations varying population density.

*c* Mean of variation in male fertilization success in each iteration, *d* Mean of the mean male encounter rate in each iteration.



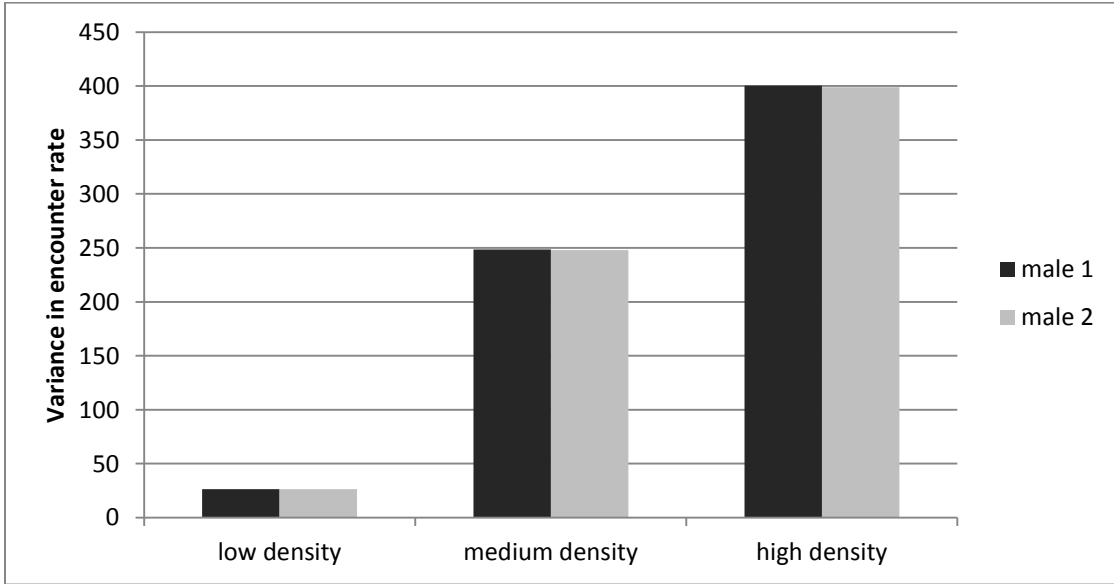
*c*



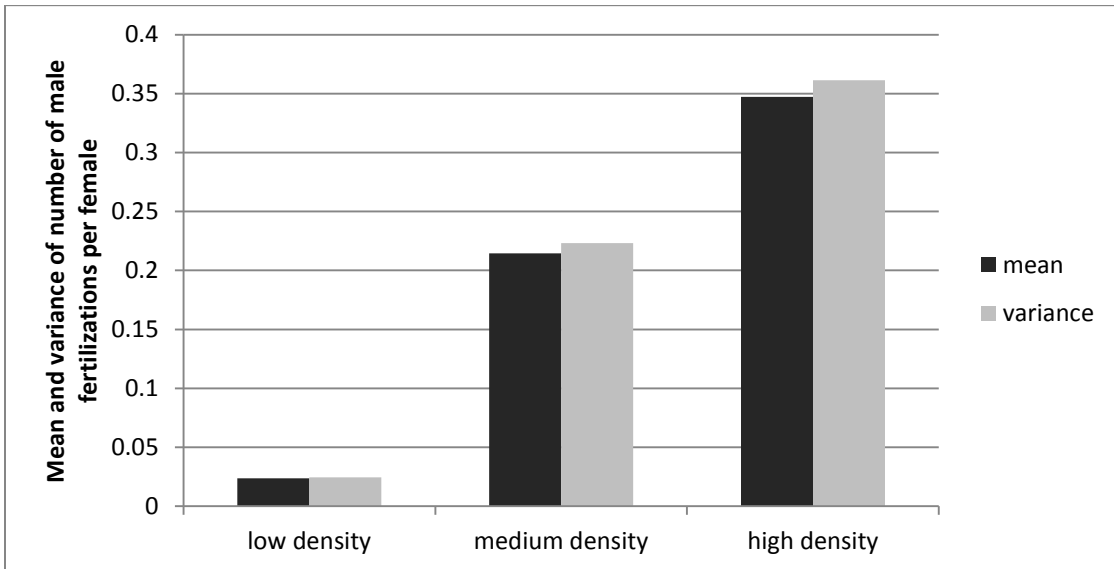
*d*

Figure 13 continued – Results of simulations varying population density.

*e* Mean variation in male encounter rate in each iteration, *f* Mean mean and mean variance of number of male fertilizations per female in each iteration.



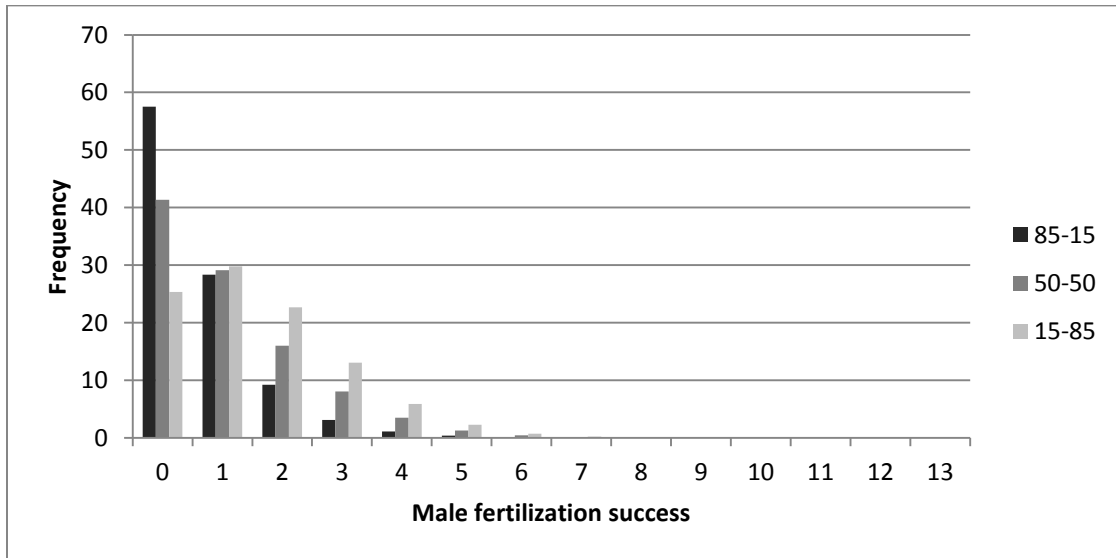
*e*



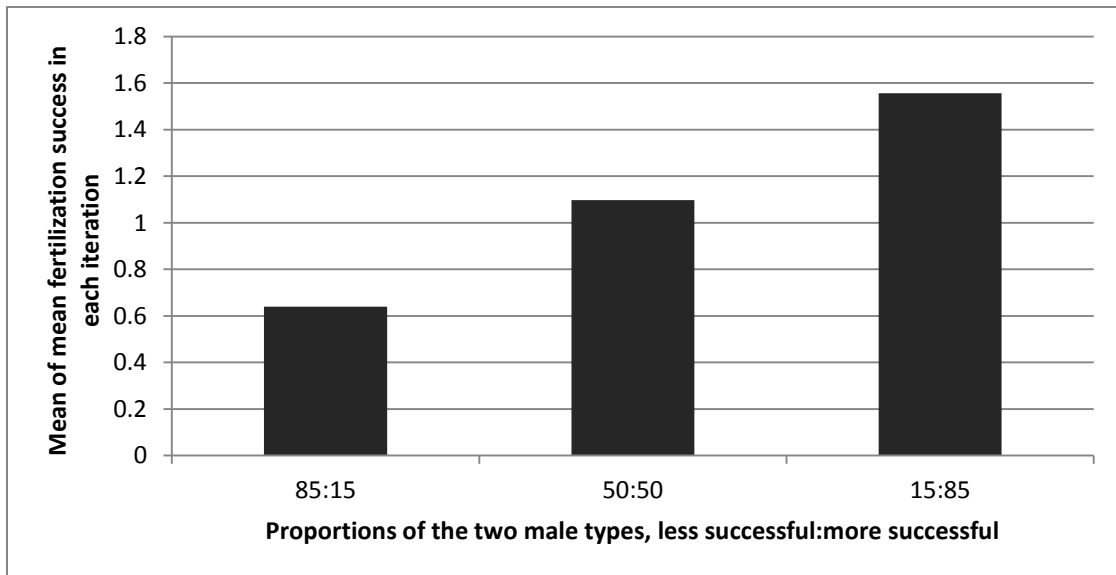
*f*

**Figure 14 – Results of simulations varying proportions of the two male types.**

a Mean frequencies of male fertilization success across 10000 iterations, b Mean of mean male fertilization success in each iteration.



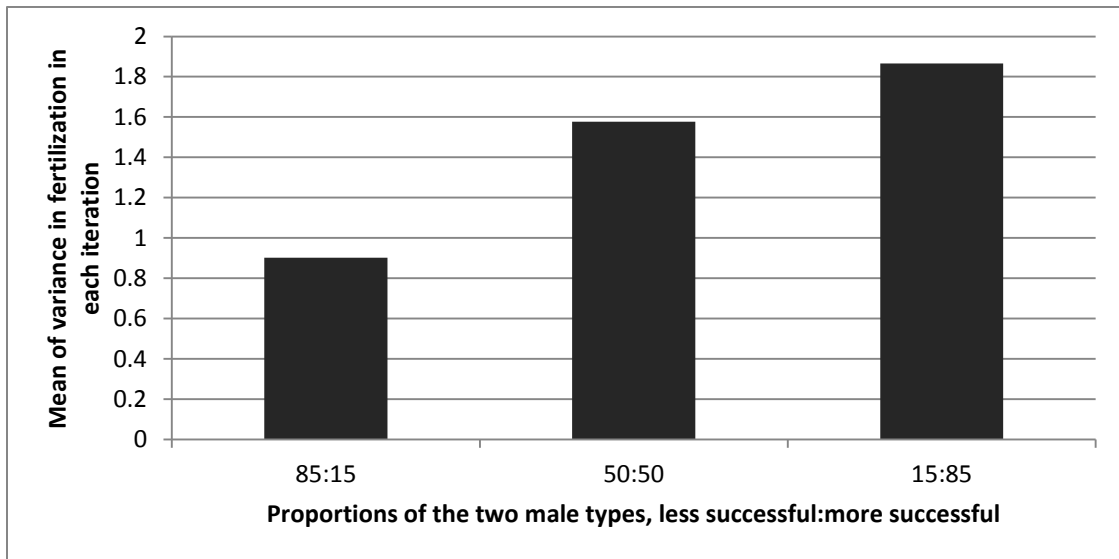
a



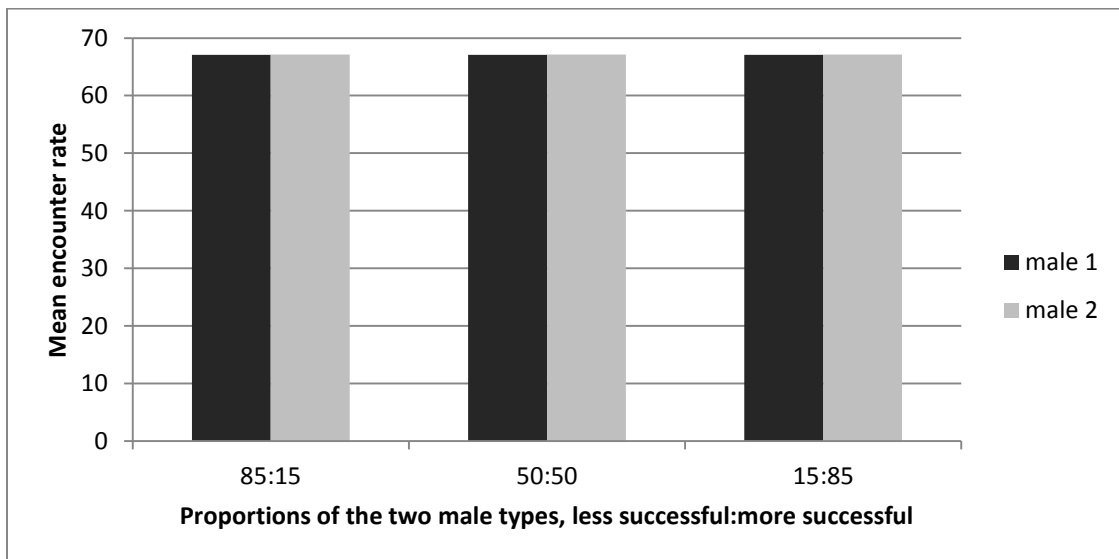
b

**Figure 14 continued – Results of simulations varying proportions of the two male types.**

***c*** Mean of variation in male fertilization success in each iteration, ***d*** Mean of the mean male encounter rate in each iteration.



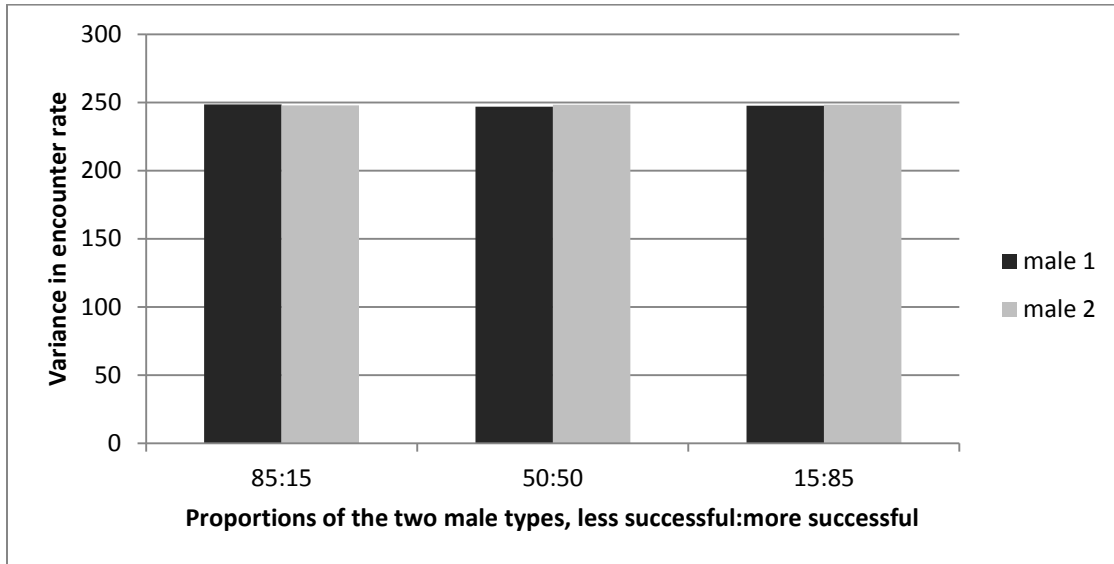
*c*



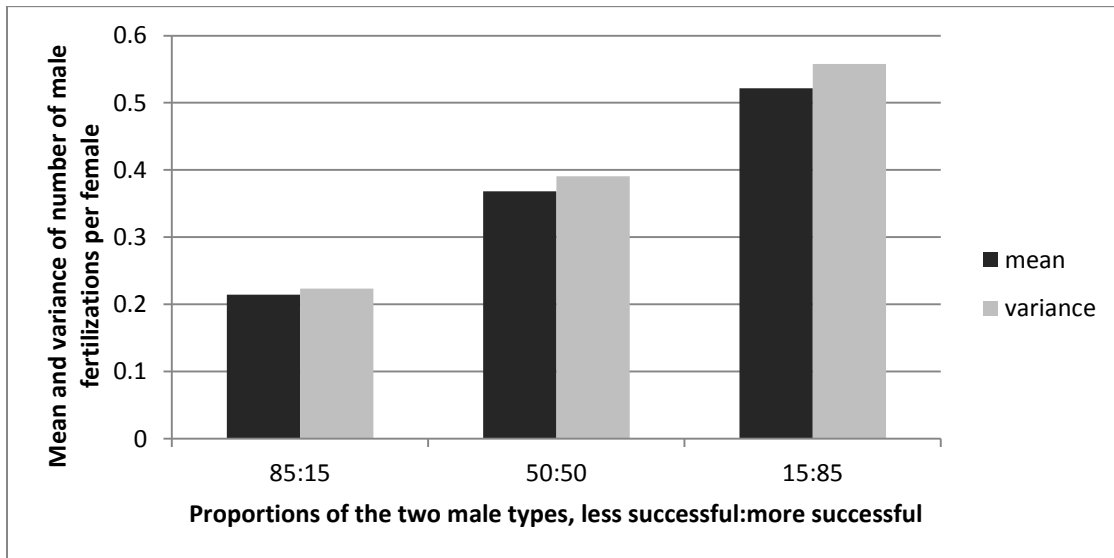
*d*

**Figure 14 continued – Results of simulations varying proportions of the two male types.**

*e* Mean variation in male encounter rate in each iteration, *f* Mean mean and mean variance of number of male fertilizations per female in each iteration.



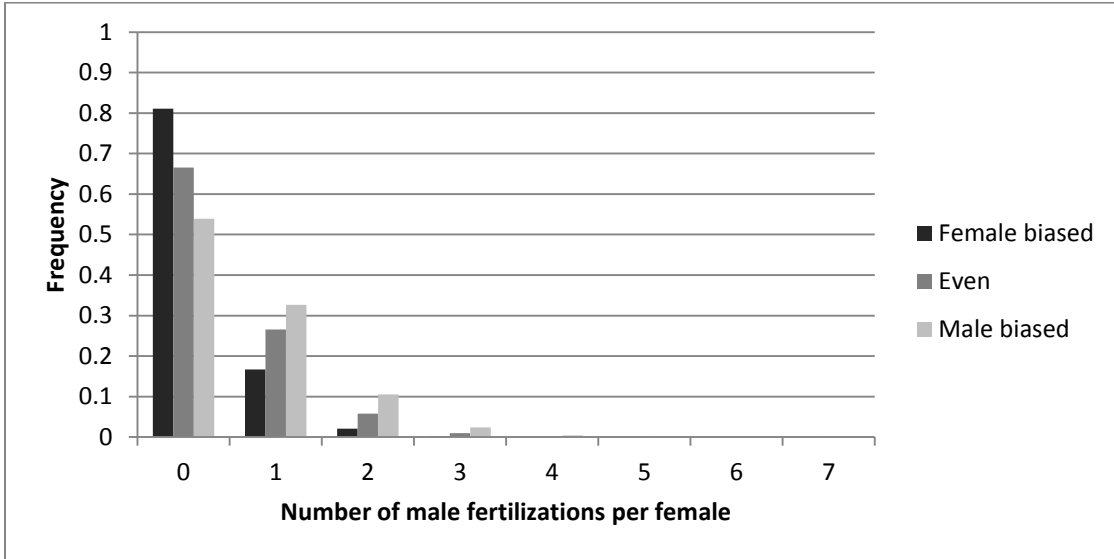
*e*



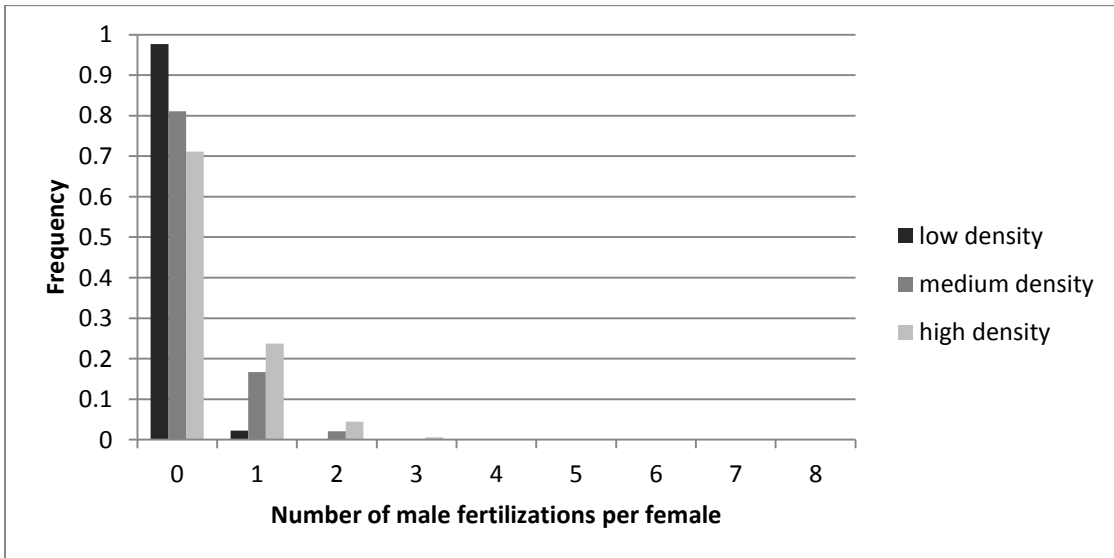
*f*

**Figure 15 – Mean number of male fertilizations per female across 10000 iterations.**

a Sex ratio varied, b Population density varied.



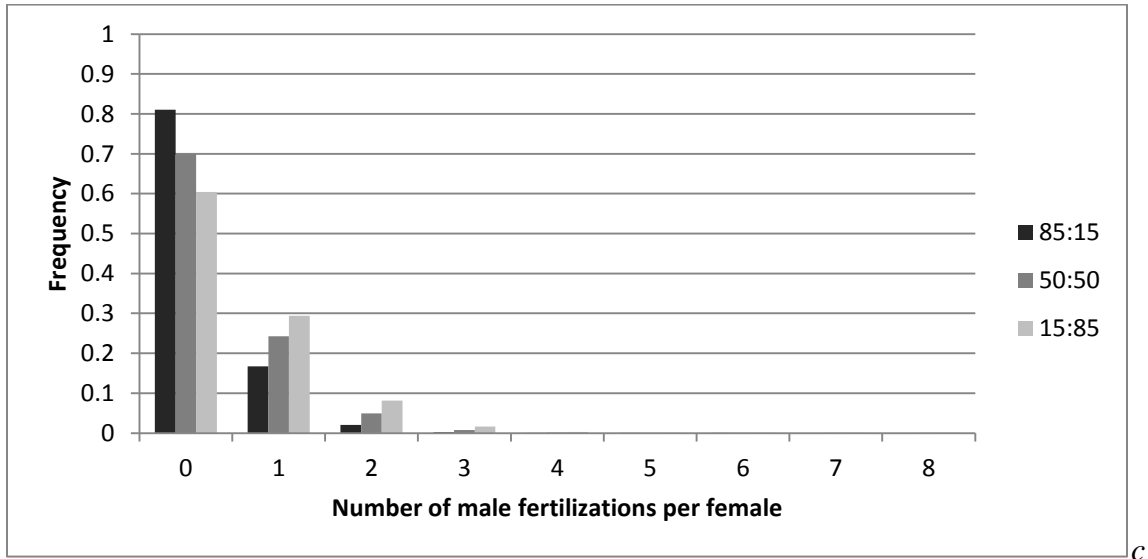
*a*



*b*

**Figure 15 continued – Mean number of male fertilizations per female across 10000 iterations.**

**c Proportions of the two male types varied.**





## *Conclusions*

### **Poisson modelling**

The statistically significant fit of the two-male-type modified Poisson model to the observed male fertilization success data suggests that there is, indeed, variation amongst male painted turtles in relation to their fertilization success. However, these results do not elucidate the possible mechanism of discrimination: whether the power of discrimination is held by the females of the species or by the males, whether the criteria are consistent from one individual to another or whether the criteria remain consistent from one mating season to the next. Regardless, the distribution of male fertilization successes observed in chapter 1 may be generated by the simple assumptions of random encounter and two categories of male fertilization success.

While the two-male type modified Poisson distribution is a statistical fit to the observed data and indeed fits the left-hand portion of the observed data very well, it underestimates the number of males that contributed sperm to three or more female's clutches. Stochastic variation from year to year in male fertilization success may be sufficient to explain such a deviation from the expected distribution. The agent-based simulations of the basic model and the two-male-type model support this contention to a point. The two-male-type model has a higher probability than the basic model of generating the extreme values in the right-hand-tail in the observed male fertilization success distribution. Neither distribution, however, generated a probability that was close to a 5% level of significance, suggesting that more research is required to uncover what additional factors might influence male fertilization success in painted turtles. One possibility is that males are divided into more success categories. Although I could not test this possibility statistically, I created a distribution based on the Poisson model that categorized males into three types. Using the least squares method, I fit a predicted distribution to the observed distribution. The distribution that minimized the sums of squares was based one category of males comprising 50% of the population with a low success rate (0.0062), a second category of males comprising 45% of the population with a moderate success rate (0.009) and a third category of males comprising 5% of the population with a high success rate (0.028). After 10000 mating season simulations with

the three-male-type model, the probability of generating values as or more extreme as those in the right-hand tail of the observed distribution was 0.000007 over two years (242/10000 in 2004, 3/10000 in 2005). This is far lower than the probability for the two-male-type model (0.002868), thus suggesting that the addition of more male categories may not be necessary or sufficient to explain the observed distribution of male fertilization success.

A second factor that may contribute to the lack of fit with the right-hand tail of the observed distribution is that both the basic Poisson and the two-male-type modified Poisson models underestimate the number of females that reproduce in a year. The model did not take into account the use of stored sperm by females to fertilize clutches in multiple seasons (Pearse and Avise, 2001). Females that do not use sperm acquired from mating in the current year may yet produce offspring fathered by males from a previous year's mating. Estimates of use of stored sperm in female painted turtles range from 22.7% across years to 100% within years (Pearse *et al.*, 2001) and 16% across years to 24.9% within years (this study, chapter 1). If fertilization success as a result of stored sperm from a previous season is added to fertilization success as a result of mating in the current season, then more males may achieve higher fertilization success and this may result in a better fit to the right-hand tail of the observed distribution. Again, a lack of degrees of freedom precluded testing such a model for goodness of fit.

A third option is that males may not be divided according to factors that fit into discrete categories or that are consistent from year to year. If females use major histocompatibility complex complementarity to determine which male's sperm they use to fertilize their clutches, then theoretically, there could be as many male success categories as there are females (Milinski, 2006). Females may use multiple cues to discriminate amongst male types (Andersson, 1994, van Doorn and Weissing, 2004), thus increasing the number of male type categories and increasing the variance within the population of male fertilization success rates, particularly if there is a specific weighting associated with each cue. There may be discrimination by females and by males concurrently, for example, males may employ different courtship strategies, according to their age or size (red-eared slider turtle, *Trachemys scripta*, Thomas, 2002) or there may

be sperm competition in addition to any female choice that occurs (Andersson, 1994). Criteria may change from year to year as a result of changes in sex ratio, density or proportions of male types encountered (as in mealworms, *Tenebrio molitor*, Fairbairn and Wilby, 2001). If females mate with multiple males and then cryptically “choose” which sperm to use after comparing all of the current year’s mates, then yearly variation in pond size, turtle density, sex ratio and male type proportion will all influence the number of males a female encounters and thus from what pool of males she may choose.

Simulations varying density support this last contention. Increasing the number of turtles increases the number of encounters between male and female turtles, thus potentially allowing females to discriminate among potential mates to a greater degree.

### **Sex ratio**

Literature on the effects of sex ratio on sexual selection predicts that a male-biased sex ratio will lead to an increase in the variance in male fertilization success as a result of females being choosier when selecting their mates (Andersson, 1994; Kvarnemo and Ahnesjo, 2002). Females have more males to compare against one another and thus will wait for (or cryptically select) a mate that is the most fit (Andersson, 1994; Kvarnemo and Ahnesjo, 2002). This is predicted to increase both the mean and the variance of male fertilization success as the most-successful males in a male-biased population will enjoy greater reproductive success than the most-successful males in a female-biased population, thus pulling the distribution to the right. However, the sex ratio simulations show a greater variance in male reproductive success in the female-biased population and lesser variance in male reproductive success in a male-biased population. Variance in male reproductive success in all simulations is strongly tied to differences in encounter rate among simulations. In the female-biased scenario, male painted turtles encounter many more females over the mating season than do males in a female-biased population and thus have the opportunity to contribute sperm to a the clutches of a greater number of females, thus increasing both the mean and the variance of male fertilization success. Further research should include simulations of the effect of increased female choosiness on male reproductive success. Subsequently, the results of the choosiness simulations should be compared both to the results presented here and to

reproductive success data from populations of painted turtle with varying sex ratios to determine if the mechanism of increased choosiness best explains variation in male reproductive success or if this variation may be explained simply by changes in encounter rate.

### **Density**

If there are more turtles within a specific area, then each male will both encounter more turtles overall and also more female turtles, thus resulting in an increase in both the mean and the variance of male fertilization success. However, this result may not accurately reflect the dynamics of *C. picta* interactions. The model does not specify how many turtles can share a plot in the model space, so if multiple turtles all end up on the same plot in the same unit of time, then each of those that are male will have specific probability of fertilizing the eggs of each of those turtles that are female. Although there is little research on competition amongst painted turtles for space within a pond or amongst males for access to females, painted turtles respond aggressively to one another when competing for a basking site (Lovich, 1988). However, in my artificial ponds experiment (chapter 2), there was no difference in rates of courtship behaviour between 2005 trials with 4 males in each trial, and 2006 trials with 2 males in each trial. At these low densities, male turtles did not interfere with each other's courtship attempts, nonetheless, future models may benefit from additional parameters that reflect the potential for males to disturb the courtship efforts of other males.

### **Proportions of male type**

With a greater proportion of the more-successful male type, females are more likely to meet a more-successful male than a less-successful male. Since the female has a higher probability of accepting the sperm contribution from a more-successful male, more males will fertilize the offspring of more females, thus increasing the mean and the variance of male fertilization success. There are currently little data on differences in mean and variation of male phenotypes among populations of painted turtle, since there are few long-term morphometric studies of the species. Indeed, any investigation of the relationship between male phenotype, male fertilization success and variation of these

among populations first requires a much greater body of research on whether male phenotype is related to fertilization success, which male traits are important and whether such traits can be neatly categorized as in this simulation. Regardless, there are many hypothetical scenarios in which differences in ecology, demographics or latitude of *C. picta* populations will affect the proportions of males of differing phenotypes, such as differential predation risk, recruitment rates, or age and/or size at maturity.

### **Female reproduction**

All three scenarios predict small proportions of females with a greater number of fertilizations than have been observed in any natural populations. In the populations with published data, females produce a maximum of two clutches in a season at northern latitudes (e.g. Algonquin Park, Samson, 2003) and a maximum of two males have been found to contribute to a single clutch (Pearse *et al.*, 2001, McGuire *et al.*, in press; this research, chapter 1). Hence, females should use sperm contributions from a maximum of four fertilizations in a single season. The predicted rates of females using sperm from more than four males in a season are low enough as to not be inconsistent with published data; females using sperm from more than four males in a season may not have been sampled, or the contributions from more than four males may not have been detected or may have been discarded as genotyping error.

The distribution of male fertilization success observed in this research may be generated using a simple model of random encounter rates and two male types with different probabilities of fertilization success. Changes to the sex ratio, density and proportions of male types all influence the mean and variance of male fertilization success. Future research should include tests of this simple model to determine if the predictions of the model simulations are supported by data from natural populations of painted turtle.

## ***GENERAL CONCLUSIONS***

In this thesis, I proposed to investigate the effect of sex ratio on male reproductive success in painted turtles by addressing three main topics. First, I investigated male reproductive success and its relationship to male phenotype in a female-biased population of painted turtle. Second, I used *ex situ* behavioural observation to look at the mechanism driving differences in male reproductive success, specifically, female choice vs. variation in male behaviour. Third, I used agent-based modeling to investigate the effect of changes in population sex ratio, density and proportions of different male types on the mean and variance of male reproductive success. Herein I summarize the conclusions for each of these topics and then offer a synthetic analysis of the overall results.

### ***Male reproductive success and male phenotype***

Male painted turtles enjoy different rates of fertilization success depending on body size, but not on claw length. Small male body size may be maintained in the population as a result of discriminating mate choice by females. Female painted turtles may also choose males with longer claws; however, claw length is not likely currently under selection. Because there is little variation in claw length among males within the population, the trait is fixed and the trait distribution cannot be influenced by selection (Darwin, 1874). Males with high fertilization success (small males) did not produce offspring with greater hatch success or survival post-hatching. A previous study on the same population found evidence for a relationship between body size and hatch success, and also for the good genes theory of sexual selection (McTaggart, 2000). The lack of evidence in the current research could be a result of the quality of hatchling samples and difficulty in successfully genotyping many poor-quality samples, or differences in sample size between the two studies. Additionally, however, females in this female-biased population may not have the opportunity to exert active choice over their mates, and thus there may be no concomitant expectation of a fitness advantage to one male phenotype as compared to others. A lack of significant difference between body size of unsuccessful males and successful males (males who contribute sperm to one or more clutches) could

indicate that females in this population accept or reject copulation primarily on whether or not they have sufficient sperm to fertilize reproductive output for the season and only secondarily on the perceived quality of the males. More research is needed to determine if small males are in fact higher quality mates and also if females preferentially use the sperm of smaller males to fertilize their clutches in populations with various adult sex ratios.

### ***Female choice vs. variation in male courtship behaviour***

In artificial ponds, large males courted more vigorously than small males, suggesting that the relationship observed in chapter 1 whereby smaller males have higher fertilization success is unlikely to be a result of increased courtship activity levels by smaller males. Although there was no direct evidence of female choice (from artificial ponds or y-mazes), that smaller males enjoy greater fertilization success even though larger males court more vigorously may be viewed as tangential support of female choice. In the slider turtle (*Trachemys scripta*), a closely-related species with similar courtship behaviour, mating behaviour and sexual dimorphism to *C. picta*, small males court by displaying with their elongated foreclaws, whereas large males bite females, possibly as a coercive strategy (Thomas, 2002). Similarly, large male painted turtles may be employing an alternative mating strategy by courting persistently to increase their fertilization success and thus partially counteract the effects of female choice on their fitness. Many of the Algonquin painted turtles have been captured with small round wounds on their heads and necks, and females are much more likely to have injuries than are males (R.J. Brooks, unpub. data), possibly indicating that these injuries are the result of male painted turtle aggression towards females. The existence of a male mating tactic that may override female choice in pond turtles is possible evidence of sexual conflict, an understudied field in all turtle species. There is much interesting work to be done on the influence of sexual conflict on male and female reproductive success in pond turtles.

### *Male fertilization success, population sex ratio and population density*

The goodness of fit for the two-male-type modified Poisson model provides further support that patterns of male fertilization success in this female-biased population can be explained by the existence of two types of male each with its own fertilization success rate. However, the two-male-type model does not sufficiently account for the number of highly successful males in the observed data. Inclusion of more parameters may produce a better fit to the right-hand tail of the distribution, but a lack of degrees of freedom in the current study precludes any further goodness-of-fit tests. Additionally, more information regarding what factors influence male fertilization success must be gleaned from natural populations of painted turtle or closely-related pond turtles to inform what parameters should be considered. The reaction of the two-male-type modified Poisson model to changes in sex ratio, turtle density and proportions of male types is predictable, however sex ratio simulations produced the opposite effect on variance in male reproductive success than that predicted by sex ratio theory (Emlen and Oring, 1977). More males (a male-biased sex ratio) resulted in a decrease in both the mean and the variance of male fertilization success. More turtles (increased turtle density) and a greater proportion of more-successful males resulted in an increase in the mean and variance of male fertilization success. A greater number of males (male-biased population) decreases the likelihood that a male will encounter a female in any unit of time because the model kept the number of males constant and varied the number of females. This limits the number of opportunities (or rolls-of-the-dice) for each individual male. A greater number of turtles in the population increases the likelihood that a male will encounter a female in any unit of time and thus increases the number of accepted mates. A greater proportion of more-successful males increases the probability that a female will use the sperm contribution of any male encountered in a given unit of time. Highly simplistic models such as this do not address what factors influence the decision of a female to either accept copulation or to use a male's sperm. Ecological and behavioural data are required to parse the fertilization probability into component parts and thus allow the development of more complex models that compare the relative importance of different factors. Female reproductive rates are underestimated in all models and thus future models should address the use of stored sperm both within and



between seasons, which requires better estimates of intra- and interseasonal stored sperm use. Currently, researchers either assume use of stored sperm when clutches in consecutive seasons are fertilized by the same male, when mating with the same male in consecutive years is unlikely, e.g. in a male-biased population (Pearse *et al.*, 2001; McGuire *et al.*, in press) or do not assume the use of stored sperm when remating with the same male is more likely from one season to the next, e.g. in a female-biased population (this study). *Ex situ* studies in which female turtles are allowed to mate with specific males and then are isolated from males between nesting seasons or where captive females are allowed access to specific males and then observed throughout the mating season for copulation are possible methods for determining the rates of copulation versus fertilization and for determining the mechanism of sperm storage and subsequent selection.

#### ***Sex ratio, sexual selection and male reproductive success***

In this female-biased population of painted turtles, differences in male fertilization success may be accounted for by the presence of two male types each with its own probability of fertilization success. Further, these two groups may be characterized by body size, with smaller males enjoying greater fertilization success than larger males, but there appear to be no fitness advantages accrued by females that use the sperm from smaller males to fertilize their clutches. Additionally, the current research does not demonstrate that differences in male fertilization success may be attributed to female choice (either overt or cryptic), that the same males enjoy high fertilization success in multiple years, that the same criteria is applied to all males (if female choice is the mechanism), or that any fertilization success advantages are passed on from the parent generation to the offspring. More research is needed in the Algonquin population and other populations of painted turtle to investigate female mate choice and any associated benefits.

The recent increase in the number of published genetic markers for freshwater turtles will facilitate an increase in the number of paternity analyses on this taxon. When paired with estimates of sex ratio and population density, the predictions of the agent-

based model may be tested. If change in variance of male reproductive success is mainly due to increased encounter rate between the sexes, then populations with male-biased adult sex ratios should report a greater mean and variance of male fertilization success as compared to female-biased populations. If the change is due to increased choosiness of female turtles, then populations with male-biased sex ratios should report a lower mean and variance of male reproductive success as compared to female-biased populations. The addition of a parameter reflecting stored sperm use, which is higher in male-biased populations (McGuire *et al.*, in press) may help to discriminate further between these two options, as some males will likely achieve very high fertilization success through preferential use of their stored sperm by females. A greater body of literature regarding reproductive success, sperm storage and multiple paternity in freshwater turtle populations with a range of sex ratios will provide invaluable evidence with which to test the assumptions of the agent-based model.

Although the current research indicates that there is variation in male reproductive success in painted turtles, that reproductive success is related to a sexually dimorphic trait (body size) and that the mean and variance of male reproductive success may be influenced by the adult sex ratio of a population, there remains a lack of evidence that sexual selection, specifically mate choice, is required to explain these patterns. The pattern of large female size dimorphism in emydid turtles is likely the ancestral form (Stephens and Weins, 2009) and maintenance of sexual dimorphism in pond turtles may be explained by natural selection alone. In general, small pond turtles may be at an advantage because they have lower energy requirements and heat up faster (Carrière *et al.*, 2008; but see Lefevre and Brooks, 1995) and have greater ease of movement within the pond (McTaggart, 2000; Morreale *et al.*, 1984). Large females may be at an advantage because of greater reproductive output in a season (Congdon and Gibbons, 1985). That smaller males enjoy greater reproductive success could be due to the size-based maturity and indeterminate growth in painted turtles. Smaller males are generally younger males (Ernst and Lovich, 2009) and sperm quality may degrade with age (as in beetles, *Tenebrio molitor*, Carazo *et al.*, 2011; feral fowl, *Gallus gallus domesticus*, Dean

*et al.*, 2010; black-footed ferret, *Mustela nigripes*, Wolf *et al.*, 2000) giving an advantage to the sperm of smaller/younger males when a female has the contributions of multiple males stored in her oviduct. Thus, the relationship between sex ratio and male fertilization success demonstrated by the agent-based model may be a result of differential fertilization probabilities of younger and older males (based on sperm quality) and an increase in overall encounter rates in populations with male-biased sex ratios. Berry and Shine (1980) found that all highly aquatic pond turtles had similar patterns of sexual dimorphism (females larger than males), similar courtship (display by males) and a lack of coercive copulation (but see Thomas, 2002). While the assumption is that these factors together indicate a system of female choice, no such assumption is necessary. Sexual dimorphism can be explained by natural selection favouring small size in pond turtles along with natural selection on larger body size in females to increase reproductive output (as discussed above), courtship displays may be required for courting males to be detected by females in the often murky pond environment and a lack of coercive copulation may be simply that small males are unable to coerce females and given the opportunity (i.e. larger body size) males will employ coercion to gain copulations (e.g. biting, R. J. Brooks, unpublished data; Thomas, 2002). While future research may demonstrate that the predictions of the simple agent-based model do not correspond with the change in mean and variance of male reproductive success observed in populations of different sex ratios and that intersexual selection is, indeed, required to maintain the observed sexual size dimorphism in pond turtles, the simple model of increased encounter rate proposed here should not be discounted.

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**APPENDIX 1 FATE OF EGGS BY CLUTCH**

Clutch ID	Eggs laid	Dead in				De- predated	Un- accounted	Eggs discarded	Eggs in paternity	Eggs with		Proportion successful paternity
		Live hatchling	Dead hatchling	egg/partly developed	Infertile/ no tissue					paternity output	Eggs with father's ID	
0032 1 04	9	0	2	6	1	0	0	6	3	2	1	0.11
0042 1 04	7	1	3	1	0	2	0	3	4	2	2	0.29
0054 1 04	7	0	0	7	0	0	0	4	3	3	3	0.43
0054 2 04	6	0	1	4	0	0	1	2	3	3	3	0.50
0072 1 04	8	0	3	3	1	0	1	2	5	5	4	0.50
0081 1 04	9	0	1	5	1	1	1	7	1	1	1	0.11
0104 1 04	8	0	0	3	4	0	1	6	1	1	1	0.13
0314 1 04	7	0	5	1	1	0	0	3	4	4	4	0.57
0322 1 04	6	0	1	5	0	0	0	5	1	1	1	0.17
0322 2 04	5	1	0	3	1	0	0	1	4	4	4	0.80
0363 1 04	7	1	2	2	2	0	0	2	5	5	4	0.57
0363 2 04	5	4	1	0	0	0	0	0	5	5	4	0.80
0375 1 04	8	0	4	4	0	0	0	4	4	4	4	0.50
0375 2 04	9	5	2	2	0	0	0	9	0	0	0	0.00
0632 1 04	8	1	1	3	0	2	1	7	0	0	0	0.00
1228 1 04	7	1	4	2	0	0	0	5	1	1	1	0.14
1233 1 04	5	0	0	5	0	0	0	5	0	0	0	0.00
1286 1 04	8	0	0	6	2	0	0	8	0	0	0	0.00
1292 1 04	6	0	0	5	0	0	1	5	0	0	0	0.00
1292 2 04	6	2	0	3	1	0	0	3	3	2	2	0.33
1293 1 04	6	0	0	6	0	0	0	6	0	0	0	0.00

Clutch ID	Eggs laid	Live		Dead in		Infertile/ no tissue	De- predated	Un- accounted	Eggs discarded	Eggs in paternity	Eggs with		Proportion successful paternity
		hatchling	hatchling	egg/partly developed	hatchling						paternity	output	
A05 1 04	4	0	1	2	1	0	0	4	0	0	0	0	0.00
A20 2 04	6	2	3	1	0	0	0	2	4	4	4	3	0.50
A23 1 04	8	0	2	4	0	1	1	5	3	3	3	0	0.00
A23 2 04	6	1	3	0	0	2	0	2	4	4	4	4	0.67
A33 1 04	6	1	0	3	1	0	1	2	3	3	3	3	0.50
B09 1 04	6	0	4	1	0	1	0	2	4	4	4	4	0.67
X25 2 04	7	6	0	1	0	0	0	4	1	1	1	1	0.14
Z01 1 04	7	0	4	2	1	0	0	6	1	1	0	0	0.00
Z01 2 04	7	3	2	2	0	0	0	2	5	5	5	2	0.29
Z15 1 04	10	0	4	6	0	0	0	7	3	3	3	3	0.30
0032 1 05	10	4	1	0	3	0	2	3	7	7	7	4	0.40
0034 1 05	10	3	7	0	0	0	0	2	8	8	8	8	0.80
0054 1 05	6	4	0	0	1	0	1	1	5	5	5	4	0.67
0054 2 05	6	4	0	0	2	0	0	2	4	4	4	4	0.67
0060 1 05	8	2	4	0	0	0	2	2	6	6	6	6	0.75
0063 1 05	7	7	0	0	0	0	0	0	7	7	7	7	1.00
0072 1 05	7	0	6	1	0	0	0	0	7	7	7	6	0.86
0081 1 05	9	4	0	1	0	0	4	5	8	8	8	6	0.67
0095 1 05	8	1	7	0	0	0	0	1	3	3	3	3	0.38
0104 1 05	5	4	1	0	0	0	0	0	5	5	5	4	0.80
0301 1 05	5	0	0	0	5	0	0	4	1	1	1	1	0.20
0322 1 05	6	0	0	0	2	4	0	4	2	2	2	2	0.33
0322 2 05	7	5	0	1	1	0	0	2	5	5	5	5	0.71
0341 1 05	6	6	0	0	0	0	0	0	6	5	5	5	0.83

Clutch ID	Eggs laid	Dead in		Eggs with	Eggs in	Eggs with	Eggs with	Eggs	Eggs in	Eggs with	Eggs with	Proportion
		Live hatchling	Dead hatchling									
0363 1 05	6	2	2	2	0	0	0	0	6	5	4	0.67
0375 1 05	10	4	0	1	0	0	5	5	5	5	5	0.50
0375 2 05	8	7	0	0	1	0	0	1	7	2	2	0.25
0632 1 05	8	0	8	0	0	0	0	0	8	7	3	0.38
0633 1 05	7	5	0	0	0	0	2	2	7	5	5	0.71
0633 2 05	7	3	3	0	0	0	1	2	5	5	5	0.71
1292 1 05	8	0	0	0	7	0	1	7	2	2	2	0.25
A05 1 05	3	2	0	0	0	0	1	1	2	2	2	0.67
A13 1 05	5	1	0	0	2	0	2	3	2	2	2	0.40
A20 1 05	8	0	0	0	3	0	5	8	5	5	5	0.63
A23 1 05	7	7	0	0	0	0	0	0	7	7	7	1.00
A33 1 05	5	5	0	0	0	0	0	3	5	5	5	1.00
B04 1 05	8	6	1	0	1	0	0	1	7	7	7	0.88
B11 1 05	7	3	0	0	0	0	4	4	3	3	3	0.43
B40 1 05	6	6	0	0	0	0	0	0	6	6	6	1.00
C08 1 05	6	2	2	0	1	0	1	2	4	4	4	0.67
C08 2 05	7	2	4	0	1	0	0	0	7	7	7	1.00
C10 1 05	8	0	4	4	0	0	0	7	0	0	0	0.00
C47 1 05	7	5	0	0	2	0	0	2	5	3	3	0.43
E03 1 05	6	0	6	0	0	0	0	0	6	6	6	1.00
E04 1 05	6	4	1	0	0	0	1	1	6	6	6	1.00
E05 1 05	7	3	0	0	0	0	4	4	5	5	5	0.71
F18 1 05	8	5	1	0	2	0	0	1	6	6	6	0.75
J33 1 05	6	5	0	0	1	0	0	2	4	3	3	0.50

Clutch ID	Eggs laid	Live		Dead in		Infertile/ no tissue	De- predated	Un- accounted	Eggs discarded	Eggs in paternity	Eggs with		Proportion successful paternity
		hatchling	hatchling	egg/partly developed	hatchling						paternity	output	
J33 2 05	6	0	0	0	1	5	0	6	0	0	0	0	0.00
J39 1 05	9	7	0	2	0	0	0	0	9	8	8	8	0.89
J50 1 05	7	3	1	0	0	0	3	3	4	4	4	4	0.57
K06 1 05	8	4	3	0	0	0	1	1	8	8	8	8	1.00
K06 2 05	5	2	2	0	0	0	1	1	4	4	4	4	0.80
N13 1 05	6	6	0	0	0	0	0	0	5	5	5	5	0.83
X18 1 05	7	7	0	0	0	0	0	0	7	3	2	2	0.29
Z01 1 05	6	0	0	0	1	0	5	5	5	4	4	4	0.67
Z15 1 05	10	0	0	0	6	0	4	10	0	0	0	0	0.00
Z15 2 05	6	1	5	0	0	0	0	0	6	6	6	6	1.00

**APPENDIX 2 TISSUE SAMPLE QUALITY BY CLUTCH**

Year	Mother ID	Clutch	No sample	Bones/scutes	Decomp	Part decomp	Dead donor	Live donor	Embryo
2004	0032	1	1	0	0	6	2	0	0
2004	0042	1	2	0	0	1	3	1	0
2004	0054	1	0	0	2	4	1	0	0
2004	0054	2	1	0	1	2	2	0	0
2004	0072	1	2	0	1	2	3	0	0
2004	0081	1	3	3	2	0	1	0	0
2004	0104	1	6	0	0	1	1	0	0
2004	0314	1	0	0	1	0	5	0	0
2004	0322	1	0	0	3	2	1	0	0
2004	0322	2	1	0	0	1	2	1	0
2004	0363	1	2	0	0	1	4	0	0
2004	0363	2	0	0	0	0	2	4	0
2004	0375	1	0	0	2	2	4	0	0
2004	0375	2	0	0	2	1	1	5	0
2004	0632	1	3	0	2	1	1	1	0
2004	1228	1	0	0	2	0	4	1	0
2004	1233	1	0	2	3	0	0	0	0
2004	1286	1	2	0	6	0	0	0	0
2004	1292	1	1	1	3	1	0	0	0
2004	1292	2	1	0	0	2	1	2	0
2004	1293	1	0	0	5	1	0	0	0
2004	A05	1	1	0	2	0	1	0	0
2004	A20	1	0	0	4	1	2	0	0
2004	A20	2	0	0	1	0	2	3	0
2004	A23	1	1	0	4	1	2	0	0
2004	A23	2	1	0	0	0	3	1	0
2004	A33	1	2	0	1	1	1	1	0
2004	B09	1	1	0	1	1	3	0	0

Year	Mother ID	Clutch	No sample	Bones/scutes	Decomp	Part decomp	Dead donor	Live donor	Embryo
2004	B09	2	0	0	0	0	3	4	0
2004	B11	1	1	0	2	1	2	0	0
2004	B21	1	1	0	3	0	6	0	0
2004	B21	2	3	2	1	0	1	0	0
2004	B40	1	0	0	3	2	3	0	0
2004	B40	2	1	0	0	1	4	1	0
2004	C16	1	1	1	2	0	3	2	0
2004	C29	1	1	0	3	1	2	0	0
2004	C40	1	0	0	2	0	7	0	0
2004	C40	2	0	0	1	2	1	2	0
2004	E04	1	0	0	5	0	2	0	0
2004	E04	2	0	0	1	0	1	1	0
2004	E05	1	1	0	0	1	1	3	0
2004	E05	2	1	0	2	0	0	4	0
2004	E16	1	2	0	1	2	1	0	0
2004	E16	2	0	0	0	3	2	1	0
2004	E45	1	1	0	3	0	3	0	0
2004	E50	1	1	0	6	0	1	2	0
2004	E50	2	0	0	1	0	3	2	0
2004	J17	1	0	0	4	0	4	1	0
2004	J41	1	0	0	6	0	2	0	0
2004	J41	2	0	0	1	0	2	5	0
2004	J50	1	0	2	1	0	4	1	0
2004	K06	1	1	0	0	2	3	0	0
2004	K06	2	5	0	0	0	1	0	0
2004	X18	1	1	0	6	0	1	0	0
2004	X18	2	2	0	0	0	2	2	0
2004	X25	1	1	0	1	0	3	2	0
2004	X25	2	0	0	0	1	0	6	0
2004	Z01	1	1	0	2	0	4	0	0



Year	Mother ID	Clutch	No sample	Bones/scutes	Decomp	Part decomp	Dead donor	Live donor	Embryo
2004	Z15	1	0	0	3	3	4	0	0
2005	0032	1	4	0	0	0	0	4	0
2005	0034	1	0	0	0	0	2	8	0
2005	0054	1	0	1	0	1	0	4	0
2005	0054	2	2	0	0	0	0	4	0
2005	0060	1	2	0	0	0	0	6	0
2005	0063	1	0	0	0	0	0	7	0
2005	0072	1	0	0	0	1	3	4	0
2005	0081	1	0	0	1	1	0	7	0
2005	0095	1	0	0	0	0	2	6	0
2005	0104	1	0	0	0	1	0	4	0
2005	0301	1	4	0	0	0	0	0	1
2005	0322	1	4	0	2	0	0	0	0
2005	0322	2	1	0	1	0	0	5	0
2005	0341	1	0	0	0	0	0	6	0
2005	0363	1	0	0	0	1	2	3	0
2005	0375	1	5	0	0	1	0	4	0
2005	0375	2	1	0	0	0	0	7	0
2005	0632	1	0	0	0	0	0	8	0
2005	0633	1	1	0	0	0	0	6	0
2005	0633	2	1	0	0	1	0	5	0
2005	1292	1	1	6	0	0	0	1	0
2005	A05	1	0	0	0	0	0	3	0
2005	A13	1	2	0	1	0	1	1	0
2005	A20	1	2	0	1	0	5	0	0
2005	A23	1	0	0	0	0	0	7	0
2005	A33	1	0	0	0	0	0	5	0
2005	B04	1	1	0	0	1	0	6	0
2005	B11	1	3	0	0	0	0	4	0
2005	B40	1	0	0	0	0	0	6	0

Year	Mother ID	Clutch	No sample	Bones/scutes	Decomp	Part decomp	Dead donor	Live donor	Embryo
2005	C08	1	2	0	0	0	1	3	0
2005	C08	2	0	0	0	0	2	4	1
2005	C10	1	0	8	0	0	0	0	0
2005	C47	1	2	0	0	0	0	5	0
2005	E03	1	0	0	0	0	2	4	0
2005	E04	1	0	0	0	0	2	4	0
2005	E05	1	2	0	0	0	1	4	0
2005	F18	1	1	0	0	0	1	5	1
2005	J33	1	0	1	0	0	0	5	0
2005	J33	2	5	1	0	0	0	0	0
2005	J39	1	0	0	2	0	0	7	0
2005	J50	1	1	0	0	0	2	4	0
2005	K06	1	0	0	0	0	1	7	0
2005	K06	2	1	0	0	0	0	5	0
2005	N13	1	0	0	0	0	0	6	0
2005	X18	1	0	0	0	0	0	6	1
2005	Z01	1	1	1	0	2	0	2	0
2005	Z15	1	0	5	0	0	1	4	0
2005	Z15	2	0	0	0	0	0	6	0

### *APPENDIX 3 NETLOGO CODE FOR BASIC MODEL*

```
breed [females female]           ; ; specifies two types of agent: female and male  
breed [males male]
```

```
to setup  
  clear-all  
  setup-males  
  setup-females  
end
```

```
males-own [success encounter]    ; ; male attributes are success and encounter  
females-own [encounterf]        ; ; female attribute is encounterf
```

```
to setup-males  
  create-males number-male       ; ; number-male is generated by a slider control  
  ask males [ setxy random-ycor random-xcor ]  
  ask males [ set shape "turtle" ]  
  ask males [ set color blue ]  
end
```

```
to setup-females  
  create-females number-female   ; ; number-female is generated by a slider control  
  ask females [ setxy random-ycor random-xcor ]  
  ask females [ set shape "turtle" ]  
  ask females [ set color pink ]  
end
```

```
to go  
  if ticks > 5000 [stop]         ; ; stop the run after 5000 time units  
  move-turtles  
  tick  
end
```

```
to move-turtles  
  move-males  
  move-females  
end
```

```
to move-females  
  ask females [ right random 360 forward 1 ]  
  meetfem  
end
```

```
to meetfem  
  ask females [
```

```

let meetf one-of males-here      ; ; are any other agents on the plot male?
if meetf != nobody
[if random 10000 < 81 [set encounterf (encounterf + 1)] ]
]                                ; ; if so and the random number is less than 81,
end                              ; ; award +1 encounterf

```

```

to move-males
  ask males [ right random 360 forward 1 ]
  court
end

```

```

to court
  ask males [
    let mate one-of females-here      ; ; are any other agents on the plot female?
    if mate != nobody
      [ if random 10000 < 81 [ set success (success + 1)] ]
    ]                                ; ; if so and random number is less than 81, award
  ask males [                          ; ; +1 success
    let meet one-of females-here      ; ; are any other agents on the plot female?
    if meet != nobody [set encounter (encounter + 1)]
  ]                                    ; ; if so, award +1 encounter
end

```

#### *APPENDIX 4 NETLOGO CODE FOR TWO-MALE-TYPE MODEL*

```
breed [females female]           ; ; specifies three types of agent: female, male1 and
male2
breed [males1 male1]             ; ; male1 is the less-successful male type
breed [males2 male2]             ; ; male2 is the more-successful male type

to setup
  clear-all
  setup-males1
  setup-males2
  setup-females
end

males1-own [success1 encounter1] ; ; male1 attributes are success1 and encounter1
males2-own [success2 encounter2] ; ; male2 attributes are success2 and encounter2
females-own [encounterf]         ; ; female attribute is encounterf

to setup-males1
  create-males1 number-male1      ; ; number-male1 is generated by a slider control
  ask males1 [ setxy random-xcor random-ycor ]
  ask males1 [ set shape "turtle" ]
  ask males1 [ set color blue ]
end

to setup-males2
  create-males2 number-male2      ; ; number-male2 is generated by a slider control
  ask males2 [ setxy random-xcor random-ycor ]
  ask males2 [ set shape "turtle" ]
  ask males2 [ set color green ]
end

to setup-females
  create-females number-female    ; ; number-female is generated by a slider control
  ask females [ setxy random-xcor random-ycor ]
  ask females [ set shape "turtle" ]
  ask females [ set color pink ]
end

to go
  if ticks > 5000 [stop]          ; ; stop the run after 5000 time units
  move-turtles
  tick
end

to move-turtles
```

```

move-males1
move-males2
move-females
end

to move-males1
  ask males1 [ right random 360 forward 1 ]
  court1
end

to move-males2
  ask males2 [ right random 360 forward 1 ]
  court2
end

to move-females
  ask females [ right random 360 forward 1 ]
  meetfem1
  meetfem2
end

to meetfem1
  ask females [
    let meetf1 one-of males1-here      ; ; are any other agents on the plot male1?
    if meetf1 != nobody
      [if random 10000 < 66 [set encounterf (encounterf + 1)] ]
    ]
    ; ; if so and the random number is less than 66,
end      ; ;award +1 encounterf

to meetfem2
  ask females [
    let meetf2 one-of males2-here      ; ; are any other agents on the plot male2?
    if meetf2 != nobody
      [if random 10000 < 261 [set encounterf (encounterf + 1)] ]
    ]
    ; ; if so and the random number is less than 261,
end      ; ; award +1 encounterf

to court1
  ask males1 [
    let mate one-of females-here      ; ; are any other agents on the plot female?
    if mate != nobody
      [ if random 10000 < 66 [ set success1 (success1 + 1)] ]
    ]
    ; ; if so and random number is less than 66, award
ask males1 [      ; ;+1 success1
  let meet one-of females-here      ; ; are any other agents on the plot female?
  if meet != nobody [set encounter1 (encounter1 + 1)]

```

```

] ; ; if so, award +1 encounter1
end

to court2
  ask males2 [
    let mate one-of females-here ; ; are any other agents on the plot female?
    if mate != nobody
      [ if random 10000 < 261 [ set success2 (success2 + 1)] ]
    ] ; ; if so and random number is less than 261, award
  ask males2 [ ; ; +1 success2
    let meet one-of females-here ; ; are any other agents on the plot female?
    if meet != nobody [set encounter2 (encounter2 + 1)]
  ] ; ; if so, award +1 encounter2
end

```