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TESTING FOR INDIRECT BENEFITS OF POLYANDRY IN THE FLORIDA GREEN TURTLE

by

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B.S. University of Florida, 2009

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ABSTRACT

Behavioral studies in the green turtle (*Chelonia mydas*) have indicated that promiscuous mating is commonplace. Though it has been shown that there is much variation in the rate of polyandry (females mating with multiple males), the drivers behind polyandry in this species are unknown. It has been speculated, but never demonstrated, that indirect benefits (fitness benefits resulting from offspring genetic diversity) play a role. However, previous tests of this hypothesis have limited scope of inference due to lack of environmental control. In this thesis, I attempted to study the indirect benefits of polyandry in Archie Carr National Wildlife Refuge (ACNWR) green turtles, limiting environmental variation by selecting nests over two week periods in a small subset of the ACNWR. Through the use of highly polymorphic microsatellite markers, I show that 85.7% of ACNWR green turtle females mate with multiple males, the highest rate yet reported for green turtles. I was successful in limiting environmental variation; however, I was unable to make comparisons among nests with one or multiple fathers because of a limited sample size of single father nests. Regardless, my thesis provides preliminary evidence (number of males per nest) that the density of males off Florida's beaches may be relatively high, which is expected to be a driver behind the evolution of polyandry and likely plays a large role both in this population and the prevalence of multiple paternity in green turtles as a whole.

ACKNOWLEDGMENTS

My Master's experience has truly taught me that no one can go it alone in science, and this is especially true in marine turtle research. My committee of Doc, John, and Eric have been unbelievably helpful and patient as I worked my way through the morass of completing my thesis. Doc especially has been an inspiration for me; he would say that his success has only come from a combination of pigheadedness and an ability to attract good young people, but everyone around him knows that it is his force of personality and hard work that transform those young people into what they become. One only needs to see the former UCF brigade at a turtle meeting to know that. John was always there for advice, and has become a good friend in his own right. Eric took my procrastinating field biologist self and transformed it into a procrastinating but competent molecular biologist in less than 2 months, and without his advice and direction I would have made it absolutely nowhere with my project.

Coming into UCF, I had one family; now I have three! I couldn't live without any of them; they truly are what kept me sane throughout this program (although sometimes they contributed to the insanity as well). My turtle family has been the most obvious contributor to my incredible experience in graduate school so far. There have been countless members of the UCF Marine Turtle Research Group that I count as friends, including all of the interns and other lab members that have gone through the grind of a nesting season with me. But the most important people in my life over the last three years have been my labmates, I would have been exposed to much less of the turtle world, and wouldn't have my closest friends. I've laughed with each of my fellow graduate students so much, and I couldn't be more thankful for that. Alli "Bill" Hays was always there to lift my spirits with one of her ridiculous stories, to make fun of my hair when getting off the ATV after a long day on the beach, and to say "Bye, Buddy, hope you find your dad!" whenever we parted. Andrew Sterner "taught me everything I know" (he thinks), but he also has been a steadfast friend and sounding board for some of my more colorful rants. Simo Ceriani, aka “The Barefoot Contessa”, has been there for me throughout to ask pointed questions that clarify my thoughts, to make “dirty” statements, and ask me what things mean. Constantly. Ryan Chabot, Emily Crews, Kendra Cope, and Fernanda Gusmão, I group you together because you guys are my little brother and sisters, and like any little siblings, I care for you more than you know. Céline Mollet-St. Benoît is the newest addition to my growing list of turtle family members (as Kendra would say, she is my G-Lil) (barf), who has been the patient and helpful presence I needed when lab work and life seemed to be crumbling around me as I crawled towards the finish line. Lastly, and most influentially, Cheryl Sanchez has influenced my entire outlook on life; her cheerful presence and determination to do good has made me a much better person, scientist, and conservationist.

My BGSA family has been there for me whenever I needed them, both personally and professionally. I couldn’t have done anything without their help, encouragement, and friendship. Ian Kutch, Matt Tye, Steffan Pierre, Joe Waddell, and Tyler Raszick are my brothers. Whether sitting around the D&D table, drinking at a bar making up acronyms (PF Chang’s, anyone?), or just hanging out around the department, they have been the best of friends. Julia Behler has been there for me literally whenever I needed her, including at times most inconvenient for her. I could spend 80 pages writing about each of the rest of my BGSA family individually, but I will limit it to a list of the rest of my BGSA family, each of whom is more loved than they realize: Molly Grace, Christina Toms, Hester Dingle, Madison Hall, Josh Solomon, Jason Strickland, Greg Territo, Lina Sánchez, Vicki Villanova, Danny and Laura Goodding, Monette Schowerer, Jessica Hearns, Allyson Fenwick, Alexis Maldonado, Stephanie Garvis, Rachel Odom, Jenny Manis, Sam Yuan, Karlie Carman, Christen Steele, Ji Min Noh, Angela Tringali, Caitlyn Debevec, Derek Woller, Ricardo Mariño Pérez, Steve Gotham, Kirsten Martin, and Danae

Perry. My BGSA family will continue to grow as I spend more time at UCF, and nothing could make me happier.

Last and certainly not least, my actual family has shaped and influenced me, as every family does. I love them so much. My dad, even though he passed away during my time in graduate school, has been the one person I've thought about every day since. My dad always cared about every person near him, even if he had no idea who they were, and I hope to live up to that example. My mom has always been the person to push me to do better, to talk me out of my crazier ideas, to listen and comfort and explain and well, to just BE THERE. My brother instilled in me my love of all things nerdy, my love of both big toys and small ones, and my desire to question everything around me. My extended family as well has been incredible. Every year on Thanksgiving, all of us get together, and the overwhelming sense of home makes me incredibly happy. My Aunt Agnes deserves special mention, only if because without her influence, I would never have gotten on to this crazy sea turtle rollercoaster to begin with.

I love every member of my three families, and I hope they know that.

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CHAPTER ONE: INTRODUCTION

Understanding behavior and its relationship to an individual's fitness has been a central theme of ecological research since Darwin. The inherent plasticity of behavior in response to environmental factors often precludes analysis of a single cause as the "reason" for its evolution. Despite this complexity, a superficial grasp of the mechanisms driving behaviors is needed to both understand how such behaviors evolve and, in the case of species of conservation concern, how to apply this knowledge in species management.

The complexity of behavior is perhaps most apparent in mating strategies. Often males and females have different approaches for resource usage that can interact to produce drastically different mating strategies (Emlen and Oring 1977). Such differences in mating strategy are often based on questions of "fitness capital" (*sensu* Williams 1966). Fitness capital, in this context, is not limited to how much energy is spent in the actual conduct of mating. It also includes parental investment in the offspring and increased predation risk as a result of mating [for a review of possible factors, see Daly (1978)]. Difference in "investment" can lead to differing strategies. For example, an environment in which males invest less per reproductive event than females can lead to a polygynous system (Emlen and Oring 1977), where males mate with multiple females.

In many species, the opposite scenario is true: females invest more per reproductive event than males. Females generally invest heavily to feed and raise offspring, while males often simply mate with the female and provide no other resources. Consequently, in such systems female fitness is driven by energetic limits on offspring production while male fitness is driven by number of successful mating events (Bateman 1948), leading to a conflict between the male and female over mating. All else being equal, as long as a female is provided with sufficient sperm to fertilize all of her eggs, her fitness has been maximized.

For a female to incur the cost of mating with an additional male, then this action should provide a benefit that offsets the associated cost (Bateman 1948). Some benefits of polyandry are immediately apparent through behavioral observation; such benefits, termed direct benefits, can include increased parental care (Ihara 2002), nuptial gifts (Fedorka and Mousseau 2002), and protection from predation (González 1999). In the absence of direct benefits, indirect benefits, which include increased offspring genetic diversity, bet-hedging through phenotypic diversity, inbreeding avoidance, and fertility assurance [for review, see Jennions and Petrie (2000)], are hypothesized to play a role in driving the evolution of polyandry.

However, other factors must be considered before making a blanket statement about the mating system. Individual variation in habitat quality and food availability can play a role in reproductive investment, affecting both the number of mating events (Hunt et al. 2005, Hebets et al. 2008) and the variable of interest in most studies of indirect benefits, offspring fitness (McGinley 1987). In addition, absolute mate quality can affect the success of offspring, and maternal reproductive investment can change among different males (Cunningham and Russell 2000, Maklakov and Lubin 2004). Finally, population-level factors such as operational sex ratio (Emlen and Oring 1977) and population density (Kokko and Rankin 2006) can have large effects on mating strategies through changes in the cost of finding a mate. These factors must be considered before undertaking any study examining the indirect benefits of polyandry.

Although interesting as theoretical questions, the study of mating strategies in a population or species can also be of great benefit to conservation. Study of the island fox (*Urocyon littoralis*) showed fox population density and mating system affect demographic parameters, and concluded that removal of a top predator was necessary for population survival (Angulo et al. 2007). Additionally, a study of the flatback turtle (*Natator depressus*) mating system (Theissinger et al. 2009) provided insight into both current and long-term effective population sizes in this threatened species. Conservation and management of the green turtle (*Chelonia mydas*), listed as an endangered species by both the U.S. Endangered Species Act and IUCN Red List, could benefit similarly from enhanced understanding of its mating system. Mating is energetically costly for green turtle females, as they must carry the male for the duration of mating [up to 6 hours (Booth and Peters 1972)], and is also subject to the harassment of other males at this time. Females have demonstrated the ability to refute mating attempts by traveling in all-female groups, blocking the cloaca with the rear flippers, or even beaching themselves (Booth and Peters 1972).

Despite the high cost of mating, behavioral observations of mating green turtles indicate that they are polygynandrous, i.e., both males and females mate with multiple members of the opposite sex. However, molecular analyses suggest that the level of polyandry is more variable (Table 1). It is unknown whether polyandry provides indirect benefits to the female; Lee and Hays (2004) attempted to compare the hatching success of monandrous and polyandrous clutches but found no difference. However, this study did not control for environmental factors such as temperature and substrate which can have a large effect on hatching success (Mortimer 1990, Maloney et al. 1990, Ackerman 1997). More recently, Wright et al. (2013) again compared the hatching successes of polyandrous and monandrous clutches; again environmental factors such as lay date (and by proxy, nest temperature) and substrate were not controlled for *a priori.* Both of these studies attempted to control for environmental variation analytically; however, with the small sample size (18 nests on two different beaches) of Lee and Hays (2004) and the large temporal and spatial variation of Wright et al. (2013), it is unclear if the results of these studies can be used as a general statement on the indirect benefits of polyandry in the green turtle.

Table 1: Previous green turtle paternity studies.

Location	**Breeding population size (All ref. cited by Jensen et al. 2006)**	**Multiple paternity (Number of nests sampled with multiple fathers)**	**Source**
Alagadi beach, Northern Cyprus	Unknown	24% (22 of 78)	Wright et al. (2013)
Southern Great Barrier Reef, Australia	≈5500 (N. Fitzsimmons pers. comm.)	9% (2 of 18)	Fitzsimmons (1998)
Ascension Island	≈8000 (Broderick et al. 2002)	61% (11 of 18)	Lee and Hays (2004)
Tortuguero, Costa Rica	≈16000 (Harrison and Troëng 2004)	50% (5 of 10)	Peare and Parker (1996)

For the critically endangered green turtle, knowledge of the mating system also can provide management utility in the face of climate change. Green turtles, like all species of marine turtle, exhibit temperature-dependent sex determination. In green turtles, temperature is related to offspring sex ratio in a sigmoidal fashion, with higher temperatures producing a higher proportion of females (Spotila et al. 1987, Standora and Spotila 1985). In most green turtle populations, offspring sex ratios are highly female-biased (Godfrey et al. 1995, Booth and Freeman 2006). However, it is unknown what functional effect, if any, this has on the population as a whole. More frequent remigrations by males to breed and movement among nesting beaches has been suggested as a potential buffer against the effects of climate change in northern Cyprus (Wright et al. 2012), but whether the mating patterns in Cyprus are a reflection of green turtles everywhere is unknown.

Understanding how the mating system works in other populations and what drives it could inform management decisions being considered on many nesting beaches about whether to manipulate nest temperatures *in situ* or relocate them to hatcheries. A 1:1 female:male ratio is often cited as the goal, but it is unknown whether this is needed or even desirable. My goals for this thesis were two-fold. First, I aimed to get a general understanding of the mating system in the Florida population of green turtles. Second, I aimed to understand how indirect benefits and demographic factors might play a role in the maintenance of polyandry.

CHAPTER TWO: METHODS

Study Site and Nest Selection

The Florida green turtle population is the fastest growing nesting population in the world, with an annual growth rate of 13.9% year (Chaloupka et al. 2008). Over the last five years, an average of approximately 6800 green turtle nests were laid on Florida beaches between May 15 and August 31 (INBS 2013). Of these nests, an average of 41% was laid within the boundaries of the ACNWR, making it an ideal site to study both the mating system of the population and indirect benefits of polyandry.

In order to effectively evaluate the effect of paternity on hatching success, the influence of other variables on hatching success needed to be limited. The high nesting densities within the boundaries of the Archie Carr National Wildlife Refuge (ACNWR, 262 nests per km in 2011) allow for *a priori* consideration of environmental factors when setting up an observational study of the indirect benefits of polyandry. As temperature and other meteorological factors are known to have a large effect on hatching success, I limited nest marking to a continuous two-week period in late June and early July, near the peak of the green turtle nesting season. To control for substrate factors, I limited nests laid to a small, undeveloped area of the nesting beach.

In 2011, this area consisted of one kilometer of nesting beach between University of Central Florida (UCF) kilometer markers 9 and 10. Due to lower nesting densities in 2012, this area was expanded to include all undeveloped areas between UCF kilometer 8.3 and 10.3. Historically, this area was the highest density natural area on the ACNWR. I excluded nests laid in substrate that was altered by dune restoration, e.g., in front of Juan Ponce de Leon Landing County Park. I marked all nests in suitable areas until a total of 20 was reached in each season.

Nesting Female Sampling and Reproductive Parameters

Each female encountered for this study had a standard set of morphometric measurements taken to examine their relationship with clutch size, egg mass, and paternity of the nest. Each individual was biopsied using a 4mm biopsy punch after nest deposition was completed. All sampled individuals were tagged using both Inconel and subcutaneous PIT (passive integrative transponder) tags to avoid pseudoreplication. Each sampled nest location was recorded using stakes in the dune and GPS coordinates. Additionally, measurements were taken from the clutch to the dune line (generally vegetation line where the slope of the beach begins to increase more rapidly) and the most recent high tide line (indicated by smoothed over sand and a small line of sediment).Clutch sizes were obtained by excavating the nest after the female returned to the ocean, and egg mass was measured for a random sample of 25 eggs from each clutch (in 2012 only).

Nest Monitoring and Hatchling Sampling

A restraining cage was placed over each sampled nest after 45 days of incubation in order to detain any emergent hatchlings. Upon emergence, 24 random hatchlings from each nest were biopsied using a 2mm biopsy punch. Samples were stored in 100% ethanol. In the event that fewer than 24 hatchlings were available to sample, dead hatchlings and unhatched fetuses in good condition were sampled as a substitute. After emergence, nests were excavated to determine the hatching success (number of hatched eggs/total eggs).

DNA Extraction, Amplification, and Analysis

Template DNA was extracted from samples using a phenol-chloroform procedure followed by precipitation with 100% ethanol. Following extraction, I attempted to amplify template DNA at four microsatellite loci: CHMY02, CHMY04, CHMY15, and CM84. (Fitzsimmons et al. 1995, Shamblin et al. 2012)(GenBank accession numbers JQ728653, JQ728654, JQ728659) Loci CHMY02, CHMY04, and

CHMY15 were amplified using the following touchdown PCR cycling parameters: an initial denaturation step of 95°C for 5 minutes followed by 30 cycles of 95°C for 30 seconds, annealing temperatures starting at 55°C and decreasing by 0.5°C each cycle, 72°C for 1 minute and then an additional 35 cycles of 95°C for 30 seconds, 43˚C for 1 minute, and 72°C for 1 minute, and a final extension step of 72°C for 10 minutes . Locus CM84 was amplified using an initial denaturation step of 95°C for 5 minutes followed by 37 cycles of 95°C for 30 seconds, annealing temperature of 43˚C for 30 seconds, and 72°C for 1 minute, with a final extension step of 72°C for 10 minutes . PCR products were sent to the University of Arizona Genetics Core for fragment analysis, and these results were analyzed in the program GeneMarker (Softgenetics LLC).

Paternity Determination

To determine whether a nest was monandrous or polyandrous, I used the methods described by Fitzsimmons (1998). This method was later termed the Minimum Method by Myers and Zamudio (2004), and I will refer to it as such. This procedure entails first identifying the female's alleles in each hatchling and eliminating them from consideration. If, after the female's alleles are removed, there were more than two alleles at more than one locus within a nest of hatchlings, the nest was determined to be polyandrous. If for some reason female allele data were not available, then I used five alleles at a locus as my criterion. This conservative procedure allows for the presence of null alleles and errors in genotyping. The probability of detecting multiple paternity using this method was assessed for each nest using the PrDM software (Neff and Pitcher 2002).

Preliminary parentage inferences beyond one or multiple fathers were carried out in two manners. I used the maximum likelihood sibship clustering program COLONY v2.0 (Wang 2004, Wang and Santure 2009) using the "Medium" run length and 10 runs per nest. This program estimates paternal genotypes from the known offspring and maternal genotypes, and uses these estimates to determine the number of fathers in the clutch. However, COLONY ideally needs at least 6-8 microsatellite loci to effectively conduct this type of analysis. Secondly, in a similar manner to using the Minimum Method to determine whether there one or multiple fathers in a nest, I estimated the number of fathers by counting the number of paternal alleles at each locus. 3-4 alleles corresponded to two fathers, 5-6 corresponding to alleles to three, and so on. This method (which I will refer to as the "Brute Force" method) is a minimum estimate of the number of fathers in a clutch, as identical alleles at a locus among fathers would be unrecognizable as being from distinct individuals.

Statistical Analyses

In order to evaluate how effective my strategy of limiting nests to a small temporal and spatial scale, I analyzed the effect of all environmental variables on hatching success. Hatching success, a proportional variable, was arcsine transformed to better conform to the normality and equal variance assumptions of parametric tests. These variables were: relative lay date (day relative to the first study nest in each season), distance to dune divided by total beach length (a measure of how far up the beach the nesting female crawled before depositing her eggs), and year. I conducted individual linear regressions of hatching success and these variables, and a forward stepwise regression including all these variables and their interactions (p-value<0.05 to enter the model, with more highly significant p-values included in the model first), to evaluate these effects.

To evaluate the effect of nesting female and egg characteristics on hatching success, I conducted individual linear regressions and a stepwise forward regression (as before) of the following variables: over-the-curve length, clutch size, and egg mass. To understand better what could affect maternal investment, I analyzed the relationships between over-the-curve length, clutch size, and the preliminary number of fathers estimated by COLONY v2.0 (referred to as "number of fathers" hereafter) with egg mass.

Finally, I conducted two analyses to determine how indirect benefits could play a role in the evolution of polyandry in the green turtle. First, I conducted linear regressions of the number of fathers (from both the Brute Force and COLONY methods) and hatching success of nests. In addition, I used all significant variables covariates from previous analyses in a multiple regression models with number of fathers (both methods) as an additional factor. All analyses were conducted in the statistical software package JMP v.11 (SAS Institute Inc. 2013)

CHAPTER THREE: RESULTS

Figure 1. Locations of study nests (2011=Blue, 2012=Purple).

Reproductive Parameters and Nest Marking

Over the two year study period, I marked 40 nests for this study. The distribution of nests within my study area is displayed in Figure 1. Average over-the-curve length (OCL) of the females that laid these nests was 107.2 cm with a standard deviation of 5.8 cm. Average clutch size was 130 eggs with a standard deviation of 27.9. For nests marked in 2012, the average mass of an egg was 49.97 grams, but egg mass varied among clutches (F-Ratio=193.01, $p<0.0001$). Average egg mass was unrelated to clutch size and all morphometric parameters (head width, $p=0.07$; all others, $p>0.27$).

Hatchling Sampling and Hatching Success Determination

Of the 40 nests marked for this study, I was able to obtain hatchlings from a subset of 34. I was unable to locate the stakes for two nests; one nest emerged as Hurricane Irene passed by Florida; one nest emerged as Hurricane Isaac passed by Florida; one nest emerged outside of the restraining cage, one nest was depredated by raccoons; and one nest had another green turtle clutch deposited directly over it, making it impossible to determine both whether hatchlings were from the correct nest and hatching success of the study nest.

Of the remaining 34 nests, one nest (12/133) was significantly affected by plant roots (46 eggs infested) and therefore was not included in hatching success analyses; however, I was still able to obtain hatchling samples from this nest and included it in paternity analyses. Average hatching success was 66.7% with a standard deviation of 24.2% for the remaining 33 nests. For five of these nests, I was unable to obtain 24 hatchling or fetus samples: 11/161 (18 samples), 11/167 (22), 11/168 (15), 11/169 (15), 12/153 (6).

Amplification and Genotyping

Difficulty in PCR amplification resulted from a variety of factors, most prominently low yield from phenol-chloroform extraction (<5 ng template DNA/microliter) of 2012 hatchling samples. As a result of this difficulty, I was unable to generate genotypic data for five nests. For the remaining 28 nests, I had varying degrees of success in getting hatchling template DNA to amplify, but enough data were generated for all nests to discern whether they were monandrous or polyandrous by the Minimum Method alone (Table 2).

For the nests that I obtained genetic data, 24 (85.7%) were polyandrous (Table 2). All nests that I determined to be monandrous had probabilities of detecting multiple paternity above 0.95 (mean=0.977, 0.967-0.986). Probability of detecting multiple paternity was much more strongly related to the number of hatchling samples I genotyped than the number of loci I could get to amplify in a given nest (Figure 2). Using COLONY v2.0 to preliminarily analyze the polyandrous nests, I determined that 2-7 fathers were present in a given nest (mean= 4.39 fathers) (Table 2). By the Brute Force method, I determined that 1-4 fathers were present in a nest (mean=2.21 fathers) (Table 2).

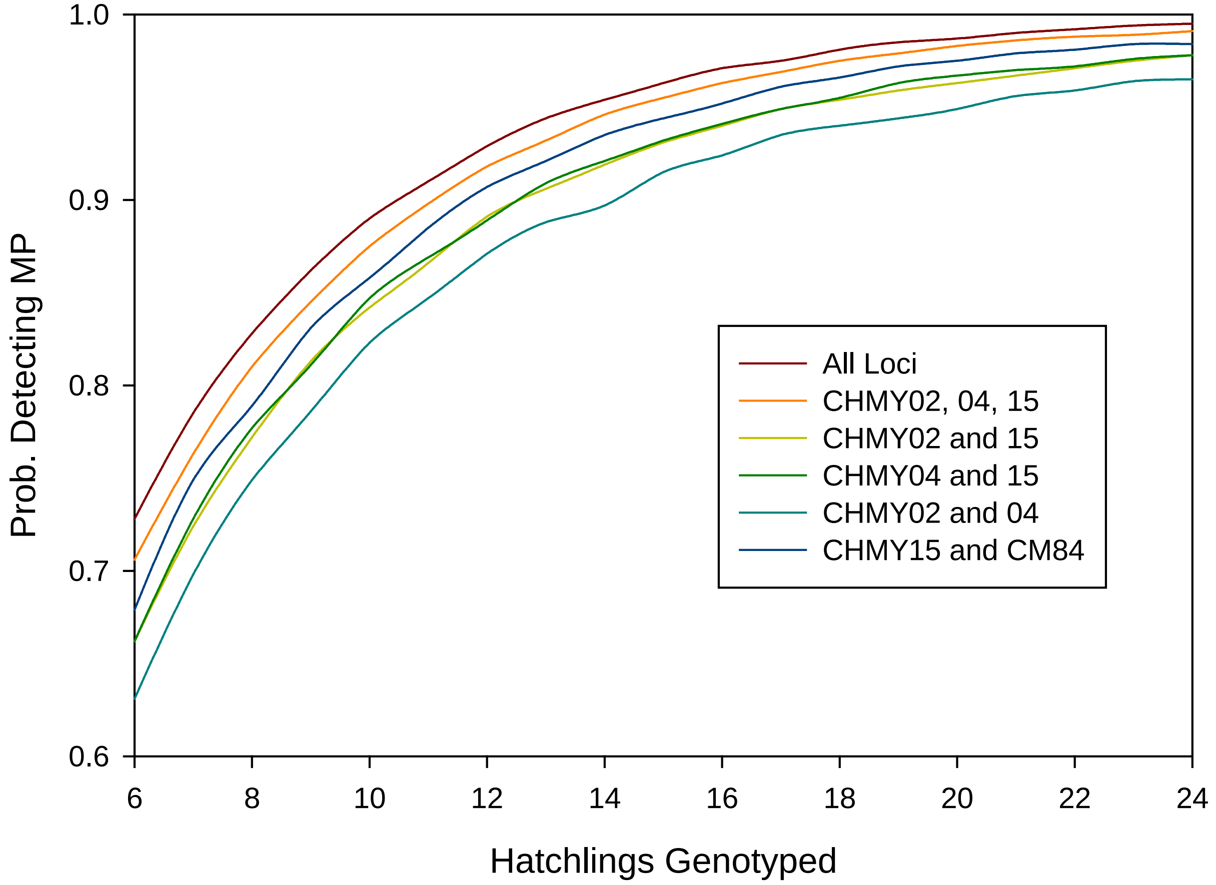

Figure 2. Relationship between number of hatchlings genotyped and probability of detecting multiple paternity with different combinations of loci. These values are conservative, as this scenario assumes only two fathers and an 80%-20% paternity skew.

Table 2. Summary of genotyping success and paternity results. PrDM varies slightly dependent on which loci were used for genotyping.

Nest Number (Year/Number)	Polyandrous (Yes/No)	Number of Fathers (COLONY)	Number of Fathers ("Brute Force")	Hatchlings Genotyped	Loci	PrDM
11/150	Yes	4	3	23	2	0.975
11/151	No	1	1	21	3	0.986
11/152	Yes	7	3	23	2	0.975
11/153	Yes	5	3	22	2	0.981
11/154	No	1	1	24	2	0.978
11/155	Yes	7	3	23	2	0.984
11/156	Yes	6	2	24	3	0.991
11/158	Yes	6	3	22	3	0.988
11/160	Yes	6	4	24	4	0.995
11/161	Yes	3	2	15	2	0.931
11/162	Yes	5	2	22	2	0.972
11/163	Yes	4	2	22	3	0.988
11/165	Yes	7	3	23	3	0.989
11/167	Yes	4	2	22	3	0.988
11/168	Yes	3	2	15	2	0.931
11/169	Yes	4	2	13	2	0.906
12/110	Yes	4	2	22	3	0.988
12/111	Yes	5	2	12	3	0.918
12/117	Yes	3	2	14	2	0.897
12/126	Yes	6	3	16	2	0.924
12/127	Yes	4	2	22	2	0.971
12/133	Yes	4	3	13	2	0.906
12/137	Yes	5	2	21	2	0.967
12/138	No	1	1	24	2	0.978
12/139	No	1	1	21	2	0.967
12/140	Yes	5	2	24	2	0.978
12/141	Yes	5	2	24	2	0.978
12/142	Yes	7	2	24	2	0.978

Effect of Paternity and Environmental Factors on Hatching Success

Year was the only environmental or maternal variable to have a statistically significant effect both individually and when all environmental variables and their interactions were evaluated within a stepwise regression framework (F= 9.01, p=0.005).Therefore, in further analyses, I only included year as a blocking factor. Statistical significance of environmental and maternal effects is summarized in Table 3.

Table 3. Relationship between environmental and maternal effects and hatching success. (Egg mass data taken only for 2012 nests.)

Effect	**N**	**R^2**	**F-Ratio**	**p-value**
Environmental Factors				
Year	33	0.24	9.59	0.004
Distance to High Tide/Beach Width	33	0.071	2.36	0.135
Relative Lay Date	33	9e-6	0.0003	0.987
Maternal Factors				
Over-the-curve length	33	0.012	0.40	0.532
Clutch Size	33	0.014	0.44	0.510
Egg Mass	17	0.082	1.34	0.265

The high proportion of multiple paternity nests precluded a comparison of hatching success among monandrous and polyandrous nests. I conducted multiple regressions with year as a blocking factor and number of fathers as the variable of interest. In both analyses, the year effect remained significant. For the COLONY estimates, number of fathers was not significantly related to hatching success (t= 1.14, p=0.267; Figure 3) over both years. Within 2011, COLONY number of fathers had a marginally significant effect (F=2.97, p=0.105). For the Brute Force estimates, number of fathers was again not significantly related to hatching success (t=0.020, p=0.888) (Figure 4).

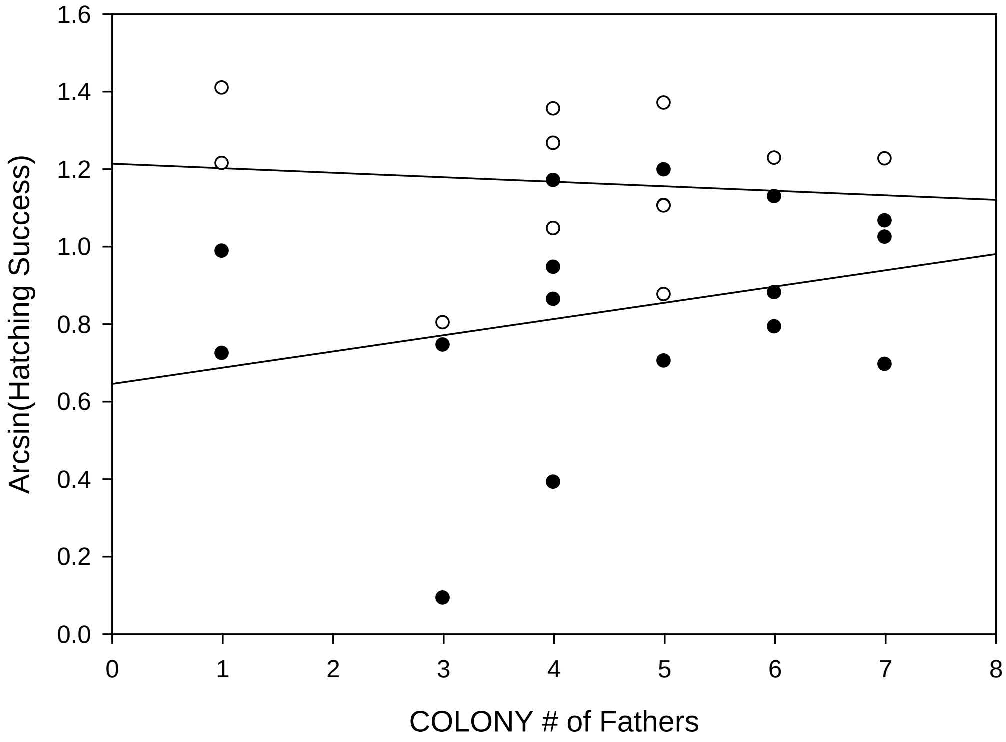

Figure 3. Regressions within years (2011=filled circles, 2012=unfilled circles) of COLONY results for number of fathers and hatching success. Neither number of fathers nor its interaction with year had a significant effect on hatching success.

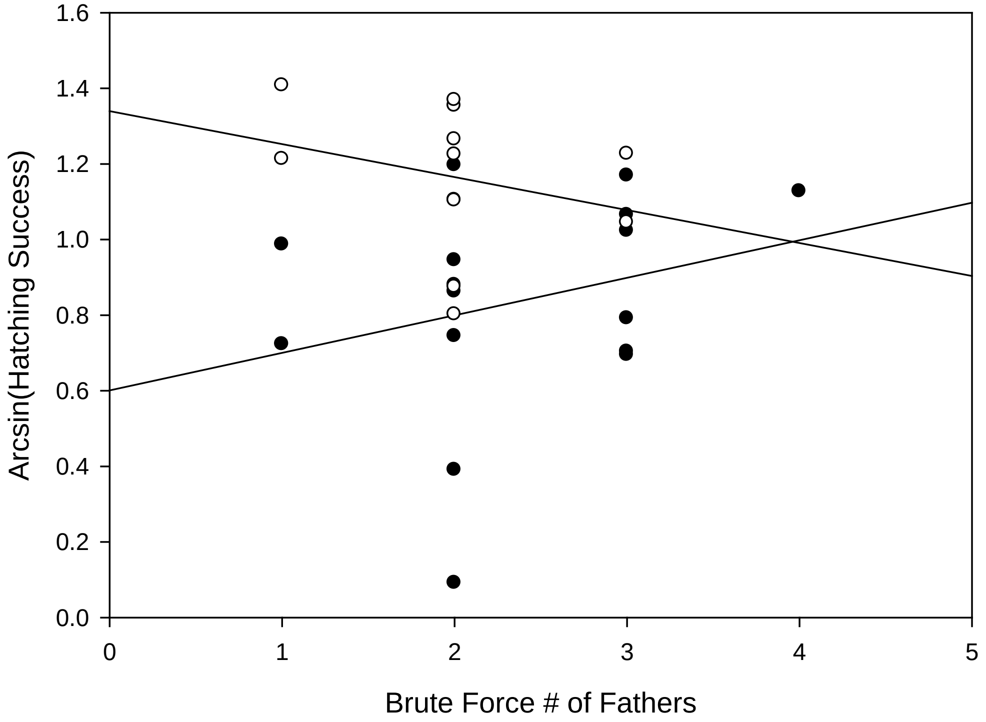

Figure 4. Regressions within years (2011=filled circles, 2012=unfilled circles) of Brute Force results for number of fathers and hatching success. Neither number of fathers nor its interaction with year had a significant effect on hatching success.

CHAPTER FOUR: DISCUSSION

I was effective at limiting environmental variation where possible: no environmental factor aside from year was significant, and a year effect was unavoidable given my other constraints on turtle selection. A year effect is also unsurprising, given the differences in air temperature during the incubation periods of my study nests (2011 average high: 32.78 C˚, 2012 average high: 31.67 C˚; F(1,119)=12.811, $p<0.001$). Although a seemingly small difference, the 2011 average is near previously reported limits for successful development on some nesting beaches, and this likely played a large role in the difference I saw in hatching success among years.

Despite my success, the sheer prevalence of multiple paternity nests prevented a comparison of hatching success among single and multiple paternity nests. The proportion of green turtle nests on the ACNWR with multiple paternity is the highest yet recorded in marine turtles, only rivaled by arribada-nesting olive ridleys in Ostional, Costa Rica (Jensen et al. 2006). Although I attempted to understand how the number of fathers is correlated with hatching success, it should be emphasized that these analyses are preliminary and need more genetic data to be truly informative. For COLONY v2.0 to provide accurate estimates of how many fathers are present within a group of hatchlings, 6-8 microsatellite loci should be used (Wang 2004). In previous studies in the green turtle, the maximum number of fathers found in a nest was five (Lee and Hays 2004); COLONY v2.0 determined that there were five or more fathers in 15 of my 28 nests. This likely highlights the unreliability of my current dataset for this type of analysis, but it also holds promise for a more complete understanding of female mating behavior in the future. A distinct possibility is that the number of fathers in some nests with >5 fathers will drop. In many of these nests, paternity was highly skewed toward 1-3 fathers, and remaining fathers represented by much smaller clusters (often only one hatchling). While skewed paternity is expected, these smaller clusters were often not as well supported by the genetic data, with high probabilities of sharing a paternal genotype with another from the same clutch. Because COLONY only reports the number of fathers that maximizes the probability of generating the data, that is the result I chose to use; however it is clear from the COLONY results that competing hypotheses with less fathers are nearly as likely. With increased genetic information, I expect many of these situations to resolve themselves into smaller numbers of fathers. The Brute Force estimate of 2.21 fathers per nest is likely a minimum bound for these decreases.

Generating more genetic data will also help to identify individual male genotypes and generate an operational sex ratio (ratio of breeding males to females at a given time, OSR hereafter) for this breeding population. I hypothesize that the OSR will be more highly male-biased than other green turtle nesting populations for two reasons. First, my data show an unprecedented rate of multiple mating by females in this population. This could be due to higher densities of males related to increased population density as a whole (Jensen et al. 2006) limiting females' ability to avoid mating with multiple males, increasing the cost of avoiding mating to prohibitive levels. In Florida, however, high population density seems an unlikely cause given the small population size and our knowledge of how male green turtles move among nesting beaches (Wright et al. 2012, D. Bagley unpublished data). More likely is that the OSR is more biased towards males relative to other populations. Hypothetically, a relatively male-biased OSR is likely in Florida because it is at the northern extent of green turtle nesting worldwide. The resulting lower nest temperatures in this population should produce a higher percentage of male offspring through temperature-dependent sex determination. If there is no sex bias in dispersal or survival, Florida should exhibit a more male-biased adult population as well.

My study both corroborates and refutes statements in the literature about the green turtle mating system. It has been implicitly assumed that mating is costly to green turtle females, and that this cost must either be so extreme as to make mating beneficial in comparison (Lee and Hays 2004) or there must be an indirect benefit to multiple mating. My study is inconclusive on the second point; however, the preliminary indicators of high male densities and their correlation with high proportions of multiple paternity in this study and others (Jensen et al. 2006, Lee and Hays 2004) suggest that avoiding mating is a relevant factor in the evolution of polyandry in green turtles.

In contrast, my results call into question the conclusions of Booth and Peters (1972). Their study strongly suggested that females have a large degree of control over whether they mate with a given male, and this study has been cited repeatedly as evidence that females are choosy. However, the high prevalence of multiple mating that I saw is unlikely to be present in a structured system like Booth and Peters (1972) describe. This could be a result of the habitat, as Booth and Peters describe specific, shallow areas in their lagoon study site (Fairfax Island in Australia) where females would congregate to avoid mating with males. Similar areas are not present off of Florida's east coast.

It is apparent from my study of the mating system that the Florida population (which nests disproportionately within the ACNWR) is unusual. Florida's green turtle population has exploded in the last 30 years, and is the fastest growing population in the world (Chaloupka et al. 2008); perhaps the unusually high prevalence of polyandry is playing a role. Although I was unable to make conclusions regarding the effects of polyandry on hatching success, it could be that this is just not the right measure of fitness. Using other measures, such as hatchling crawl speed or one-year survival, might reveal the benefits of increased genetic diversity resulting from polyandry. Fitness proxies from other life stages would be very difficult to study; hatching success is commonly used as a proxy success precisely because it is easy to measure. But the association of a mating system typically associated with more dense populations and the rapid growth of the Florida population is hard to ignore, and perhaps calls for action to preserve high male densities off the nesting beach.

If Florida has a relatively high male to female ratio, this ratio is almost certain to change as the climate warms. Changes of less than a degree could swing nests from male-biased offspring sex ratios to female-biased sex ratios (Spotila et al. 1987, Standora and Spotila 1985). Recent research (M. Schwoerer unpublished data) has indicated that nest shading can have a significant impact on nest and sand temperatures on a Florida nesting beach, and this strategy has been tested and implemented on other marine turtle nesting beaches (Patino-Martinez et al. 2012, Jourdan and Fuentes 2013). It may be appropriate to explore this strategy in Florida as well, given the results of my study of the mating system, a recent study that showed a persisting female bias in Indian River Lagoon juveniles (Sanchez unpublished thesis) and previous knowledge of how the mating system is affected by the availability of males (Emlen and Oring 1977, Jensen et al. 2006). More study of male behavior and how offspring sex ratios translate to the adult population is needed to be certain of the necessity of these actions.

In conclusion, I conducted a focused evaluation of the mating system in the green turtles nesting on the ACNWR. I found that this population has unprecedented rates of multiple paternity (85.7% of nests), but I was unable to evaluate the evolutionary relevance of the indirect benefits of polyandry as a result of this extremely high prevalence. My results, and other preliminary analyses, suggest that there is a relatively high male to female OSR in the Florida breeding population, and that the effects of this ratio drive the mating system in green turtles.

APPENDIX: GENETIC RESULTS FOR ALL NESTING FEMALES AND THEIR HATCHLINGS

In this appendix, I tabulate all of the microsatellite allele data that I was able to generate. They are sorted by nest, with the associated nesting female included at the top of the table. A non-zero number is the base pair size of the allele for that individual. Two zeroes indicate that I did not have any data for that individual at that locus. A non-zero number for one allele at locus and a zero for the other indicates I only saw one peak in GeneMarker that was the mother's allele and no others and to be conservative I included a 0 for the other allele.

11/150 Hatchlings	1010	1010	1014	1014
NF	0	0	240	276
1	252	276	240	244
2	252	276	240	276
3	252	276	0	0
4	252	276	0	0
5	240	252	0	0
6	0	0	0	0
7	240	252	240	268
8	248	272	240	276
9	252	276	268	276
10	252	276	240	276
11	252	276	240	276
12	240	252	240	276
13	240	252	0	0
14	240	252	240	276
15	236	252	0	0
16	240	252	0	0
17	240	252	268	276

18	252	276	278	276
19	240	252	240	244
20	240	252	240	244
21	240	252	0	0
22	252	276	0	0
23	252	276	240	276

11/151 Hatchlings	CHMY02	CHMY02	CHMY04	CHMY04	CHMY15	CHMY16
NF	238	266	168	184	252	268
1	258	266	176	184	260	268
2	238	258	168	176	252	260
3	0	0	168	176	252	260
4	0	0	176	184	260	268
5	0	0	168	176	252	260
6	0	0	176	184	260	268
7	0	0	176	184	252	260
8	0	0	168	176	252	260
9	238	258	0	0	252	260
10	238	258	168	176	252	260
11	0	0	168	176	252	260
12	258	266	0	0	252	260
13	0	0	0	0	252	260
14	0	0	0	0	252	260
15	0	0	176	184	260	268

16	0	0	176	184	260	268
17	238	246	0	0	252	260
18	238	258	168	176	252	260
19	0	0	168	176	252	260
20	0	0	0	0	260	268
21	0	0	0	0	252	260

11/152 Hatchlings	CHMY02	CHMY02	CHMY04	CHMY04
NF	242	246	252	252
1	242	246	244	252
2	246	250	248	252
3	246	246	252	272
4	242	246	244	252
5	242	246	248	252
6	246	250	248	252
7	242	246	252	272
8	242	246	244	252
9	242	246	244	252
10	242	246	252	264
11	242	246	244	252
12	246	254	248	252
13	242	254	248	252
14	246	258	252	272
15	0	0	252	264

16	0	0	248	252
17	238	242	244	252
18	0	0	252	264
19	242	246	244	252
20	246	246	252	272
21	246	246	252	272
22	0	0	248	252
23	242	246	244	252

11/153 Hatchlings	CHMY02	CHMY02	CM84	CM84
NF	239	267	341	363
1	267	279	341	353
2	239	251	341	353
3	263	267	353	363
4	263	267	341	353
5	263	267	343	363
6	267	271	341	363
7	239	251	341	351
8	239	271	343	363
9	239	251	361	363
10	239	279	341	353
11	251	267	353	363
12	267	271	341	343
13	267	279	353	363
14	263	267	343	363
15	267	279	351	363

16	239	267	341	343
17	239	279	0	0
18	239	279	341	353
19	239	251	361	363
20	239	251	0	0
21	251	267	341	353
22	251	267	351	363

11/154 Hatchlings	CHMY02	CHMY02	CHMY15	CHMY15
NF	238	254	240	252
1	254	254	252	268
2	254	254	252	256
3	238	254	252	268
4	238	254	252	256
5	254	254	252	268
6	254	258	240	268
7	0	0	240	256
8	254	258	240	268
9	238	254	252	268
10	254	254	240	268
11	238	254	240	256
12	254	258	240	268
13	238	254	252	256
14	254	258	240	268

15	0	0	240	256
16	238	258	252	268
17	254	254	240	256
18	254	254	240	256
19	238	254	240	256
20	254	254	240	268
21	238	258	252	256
22	238	254	252	256
23	238	258	0	0
24	254	254	252	256

11/160 Hatchlings	CHMY02	CHMY02	CHMY04	CHMY04	CHMY15	CHMY15	CM84	CM84
NF	0	0	160	168	244	252	345	351
1	242	246	168	168	248	252	351	351
2	238	242	168	176	252	260	345	361
3	242	246	160	168	244	252	345	361
4	242	246	160	168	244	252	345	361
5	242	246	168	168	252	252	351	357
6	0	0	160	168	244	252	345	361
7	242	262	168	168	248	252	345	357
8	246	281	168	176	252	260	351	361
9	242	285	160	168	244	252	351	361
10	242	246	160	168	244	252	341	345
11	242	250	168	188	252	272	345	361

12	246	285	160	168	244	252	351	351
13	238	242	160	168	244	252	341	351
14	242	250	160	188	244	272	345	345
15	246	285	160	168	244	252	341	351
16	242	285	160	168	244	252	351	361
17	246	285	168	168	248	252	345	351
18	238	242	168	176	252	260	345	351
19	242	246	168	168	248	252	345	357
20	242	262	160	168	244	252	341	351
21	242	262	168	168	248	252	341	351
22	242	246	0	0	252	252	351	351
23	242	246	168	168	252	252	351	361
24	242	246	168	168	248	252	351	351

11/155 Hatchlings	CHMY15	CHMY15	CM84	CM84
NF	256	268	341	363
1	0	0	341	345
2	252	256	341	345
3	268	272	341	343
4	268	272	341	353
5	0	0	341	353
6	252	268	345	363
7	252	256	341	363
8	256	272	361	363

9	256	272	353	363
10	268	268	363	363
11	268	276	345	363
12	268	272	361	363
13	256	268	361	363
14	268	272	353	363
15	252	276	341	345
16	268	272	361	363
17	268	276	0	0
18	256	276	361	363
19	268	276	353	363
20	252	256	345	363
21	0	0	341	345
22	252	268	341	345
23	256	276	341	343

11/156 Hatchlings	CHMY02	CHMY02	CHMY04	CHMY04	CHMY15	CHM15
NF	254	254	168	172	256	272
1	234	254	168	172	252	272

2	254	290	168	172	252	256
3	0	0	168	172	252	272
4	0	0	168	172	248	256
5	0	0	168	172	256	0
6	254	282	160	172	244	256
7	254	282	160	172	252	256
8	234	254	168	188	0	0
9	254	282	168	172	252	272
10	234	254	168	188	252	272
11	0	0	160	172	0	0
12	234	254	160	168	0	0
13	254	282	160	172	0	0
14	0	0	160	172	0	0
15	254	282	0	0	244	272
16	234	254	168	188	252	272
17	254	282	168	172	252	256
18	234	254	168	172	0	0
19	0	0	160	172	0	0
20	254	282	168	188	0	0
21	254	282	168	172	0	0
22	234	254	168	172	0	0
23	234	254	168	188	0	0
24	234	254	168	172	0	0

11/158 Hatchlings	CHMY02	CHMY02	CHMY04	CHMY04	CHMY15	CHMY15

NF	258	282	156	184	240	268
1	242	282	180	184	268	284
2	246	258	156	156	240	240
3	246	258	156	188	240	240
4	246	258	156	156	0	0
5	0	0	156	156	0	0
6	242	282	184	204	0	0
7	242	258	184	204	0	0
8	246	258	156	184	0	0
9	0	0	156	156	0	0
10	242	258	156	168	0	0
11	246	282	0	0	0	0
12	242	258	156	156	240	240
13	242	258	184	204	0	0
14	242	258	0	0	0	0
15	242	282	0	0	268	288
16	242	258	156	204	240	288
17	242	258	156	204	240	0
18	242	282	156	204	240	284
19	0	0	176	184	0	0
20	242	282	184	204	0	0
21	242	282	0	0	0	0
22	242	258	0	0	0	0

11/161 Hatchlings	1010	1010	1014	1014
NF	258	262	240	264
1	242	258	248	264
2	242	258	0	0
3	242	262	240	268
4	242	258	240	248
5	242	262	240	248
6	254	262	240	248
7	242	258	264	268
8	242	262	264	268
9	254	262	248	264
10	242	262	264	268
11	242	258	0	0
12	242	258	0	0
13	242	262	240	252
14	238	262	0	0
15	242	262	264	268

11/162 Hatchlings	CHMY02	CHMY02	CHMY04	CHMY04
NF	238	246	180	184
1	238	254	176	184
2	238	258	180	184
3	238	254	176	180

4	246	274	156	180
5	238	0	176	180
6	238	254	176	180
7	238	254	176	180
8	238	238	176	184
9	238	254	176	180
10	238	254	176	184
11	238	274	180	184
12	246	254	176	180
13	246	274	180	184
14	238	274	156	184
15	246	258	176	180
16	238	254	176	180
17	238	254	176	180
18	238	258	184	184
19	246	258	184	184
20	246	258	0	0
21	238	0	156	184

11/163 Hatchlings	CHMY02	CHMY02	CHMY04	CHMY04	CHMY15	CHMY15
NF	234	254	160	176	244	268
1	234	254	160	176	244	260
2	0	0	168	176	0	0
3	234	234	160	176	244	260

4	234	234	160	184	244	268
5	234	246	0	0	0	0
6	234	254	176	192	0	0
7	234	246	160	184	244	268
8	234	234	160	176	244	260
9	246	254	176	192	244	260
10	246	254	176	192	0	0
11	234	234	0	0	268	276
12	246	254	176	192	0	0
13	246	254	0	0	0	0
14	234	234	160	184	244	268
15	234	254	160	176	244	260
16	234	254	160	176	244	260
17	246	254	176	192	0	0
18	234	254	176	192	0	0
19	246	254	160	176	244	260
20	234	234	0	0	244	268
21	234	246	0	0	268	276
22	234	254	176	192	0	0

11/165 Hatchlings	CHMY02	CHMY02	CHMY04	CHMY04	CHMY15	CHMY15
NF	246	266	156	168	240	252
1	258	266	156	184	240	268
2	246	258	156	168	240	252

3	258	266	156	184	0	0
4	238	0	156	156	0	0
5	246	258	0	0	252	268
6	0	0	156	168	0	0
7	238	246	156	168	240	252
8	266	278	156	168	0	0
9	266	278	168	184	0	0
10	246	258	168	184	0	0
11	234	266	156	168	0	0
12	234	246	164	168	0	0
13	246	258	168	184	252	268
14	246	278	156	184	0	0
15	246	278	168	184	252	268
16	246	278	156	168	0	0
17	266	266	156	168	240	252
18	0	0	168	184	0	0
19	238	266	156	168	0	0
20	238	246	168	184	0	0
21	238	266	156	168	240	252
22	258	266	156	184	0	0
23	238	246	156	156	240	240

11/167 Hatchlings	CHMY02	CHMY02	CHMY04	CHMY04	CHMY15	CHMY15
NF	250	282	160	188	244	272

1	234	250	160	168	244	252
2	242	282	160	160	240	244
3	234	282	160	0	244	252
4	242	282	160	176	240	244
5	242	282	156	160	240	244
6	234	250	0	0	252	272
7	242	250	156	188	240	272
8	238	282	184	188	268	272
9	234	250	168	188	252	272
10	242	282	160	168	244	252
11	238	282	160	184	244	268
12	242	250	156	188	240	272
13	242	250	156	160	240	244
14	234	282	160	168	244	252
15	234	282	160	168	244	252
16	242	250	156	188	240	272
17	234	282	160	168	244	252
18	234	282	156	188	240	272
19	234	282	168	188	252	272
20	242	250	156	188	240	272
21	242	282	156	160	240	244
22	238	250	0	0	252	272

11/168 Hatchlings	CHMY02	CHMY02	CHMY15	CHMY15
NF	254	266	240	268
1	262	266	244	268
2	254	258	244	268
3	254	266	240	244
4	262	266	240	244
5	258	266	244	268
6	254	258	0	0
7	254	266	244	268
8	254	258	240	244
9	254	258	268	272
10	258	266	268	272
11	254	258	268	272
12	262	266	268	272
13	262	266	244	268
14	254	258	268	272
15	254	266	244	268

11/169 Hatchlings	CHMY02	CHMY02	CHMY15	CHMY15
NF	234	250	252	256
1	242	250	0	0
2	250	282	244	256
4	234	242	248	252

5	234	242	248	252
6	250	274	252	252
7	250	254	252	256
8	242	250	248	252
10	250	254	252	256
11	234	254	252	256
12	242	250	248	252
13	250	254	252	256
14	242	250	252	256
15	250	274	252	256

2012 Nests

12/110 Hatchlings	CHMY02	CHMY02	CHMY04	CHMY04	CHMY15	CHMY15
NF	246	258	160	168	244	252
1	254	258	164	168	0	0
2	0	258	160	168	244	252
3	258	254	160	168	244	252
4	0	0	160	168	244	252
5	246	282	156	160	240	244
6	254	258	168	160	252	240
7	246	258	160	168	244	252
8	254	258	160	168	244	252
9	246	282	160	168	244	252

10	254	258	164	168	252	252
11	246	258	160	168	244	252
12	246	258	164	168	252	252
13	254	258	160	168	244	252
14	246	282	0	0	0	0
15	246	282	160	168	244	252
16	246	258	156	160	240	244
17	258	0	168	168	252	252
18	246	258	168	0	252	0
19	246	282	160	168	244	252
20	246	258	160	168	244	252
21	246	258	160	168	244	252
22	246	282	156	160	244	244

12/111 Hatchlings	CHMY02	CHMY02	CHMY04	CHMY04	CHMY15	CHMY15
NF	0	0	156	156	240	240
1	0	0	0	0	240	244
2	254	274	0	0	0	0
3	0	0	0	0	240	280
4	250	266	156	176	240	260
5	250	266	156	176	240	260
6	0	0	156	176	240	260
7	250	254	0	0	240	268
8	0	0	156	160	240	244

9	254	274	156	184	240	268
10	0	0	0	0	240	268
11	0	0	0	0	240	240
12	238	274	156	156	240	0

12/117 Hatchlings	CHMY04	CHMY04	CHMY15	CHMY15
NF	172	184	256	268
1	0	0	256	268
2	168	172	252	256
3	172	184	256	268
4	168	172	252	256
5	168	172	0	0
6	172	184	256	268
7	0	184	264	268
8	168	172	252	256
9	168	172	256	256
10	172	184	256	268
11	168	184	0	0
12	172	184	256	268
13	180	184	264	268
14	172	184	256	268

12/126 Hatchlings	CHMY04	CHMY04	CHMY15	CHMY15
NF	176	184	260	268
1	0	0	260	264
2	156	176	240	260
3	156	184	240	268
4	184	192	268	276
5	156	184	240	268
6	176	192	260	276
7	156	176	240	260
8	0	0	256	260
9	156	176	0	0
10	0	0	240	268
11	156	176	240	260
12	176	176	260	256
13	176	192	260	276
14	176	0	260	260
15	168	184	252	268
16	156	184	240	268

12/127 Hatchlings	CHMY02	CHMY02	CHMY04	CHMY04
NF	0	0	252	268
1	270	0	248	268
2	0	0	256	268
3	262	0	252	268
4	0	0	252	256
5	0	0	256	268
6	0	0	256	268
8	0	0	268	268
9	254	270	244	252
10	254	262	244	268
11	242	270	248	268
12	0	270	248	252
13	254	270	244	252
14	0	0	244	252
15	254	262	244	268
16	254	0	0	0
17	254	262	244	252
19	254	270	0	0
20	254	262	244	252
21	254	270	244	268
22	0	270	248	252
23	254	0	244	252
24	254	262	244	252

12/133 Hatchlings	CHMY02	CHMY02	CHMY04	CHMY04
NF	0	0	240	256
1	254	270	256	268
2	254	270	256	268
3	270	282	256	268
4	270	282	256	268
5	262	270	0	0
6	254	270	256	268
7	254	270	256	268
8	0	0	268	240
9	254	270	256	268
10	258	274	0	0
11	254	270	0	0
12	0	0	256	268
13	250	270	0	0

12/137 Hatchlings	CHMY02	CHMY02	CHMY15	CHMY15
NF	246	266	256	268
1	254	266	256	268
2	246	250	0	0
3	242	246	0	0

4	242	266	0	0
5	242	246	0	0
6	250	266	0	0
7	246	250	268	0
8	246	250	0	0
9	242	246	252	256
10	250	266	248	256
11	0	0	256	268
12	246	250	268	0
13	242	246	268	0
14	246	254	252	268
15	246	250	252	268
16	242	266	0	0
17	242	266	252	256
18	246	250	252	268
19	250	266	252	256
20	246	250	268	0
21	250	266	252	268

12/138 Hatchlings	CHMY02	CHMY02	CHMY15	CHMY15
NF	242	250	240	244
1	242	266	240	244
2	250	266	244	252
3	242	266	244	252

4	242	266	0	0
5	250	266	244	252
6	250	266	240	244
7	242	266	0	0
8	242	266	0	0
9	250	266	240	244
10	242	266	240	244
11	242	266	244	252
12	250	266	244	252
13	250	266	240	244
14	242	266	244	252
15	242	266	244	252
16	242	266	240	244
17	242	266	244	252
18	242	266	0	0
19	242	266	0	0
20	242	266	244	252
21	242	266	0	0
22	250	266	244	252
23	242	266	244	252
24	250	266	240	244

12/139 Hatchlings	CHMY02	CHMY02	CHMY15	CHMY15
NF	242	246	256	260

1	242	262	260	268
2	242	262	260	268
3	242	0	240	256
4	242	262	260	268
5	246	262	256	268
6	246	262	256	268
7	242	246	240	260
8	242	246	240	260
9	246	262	260	268
10	246	262	260	268
11	242	262	240	256
12	242	262	256	268
13	246	262	260	268
14	242	246	240	260
15	246	262	260	268
16	242	246	240	260
17	242	246	240	260
18	242	246	240	256
19	246	262	256	268
20	242	246	260	268
21	242	246	240	260

12/140 Hatchlings	CHMY02	CHMY02	CHMY15	CHMY15
NF	242	258	268	268

1	0	0	256	268
2	0	0	252	268
3	254	258	252	268
4	242	254	268	268
5	258	270	268	268
6	254	258	252	268
7	0	0	252	268
8	254	258	268	272
9	250	258	256	268
10	0	0	256	268
11	242	254	252	268
12	254	258	252	268
13	242	270	268	268
14	242	254	252	268
15	242	250	268	272
16	242	254	256	268
17	258	270	256	268
18	0	0	252	268
19	242	270	256	268
20	242	254	268	268
21	258	270	268	268
22	254	258	0	0
23	258	270	268	272
24	242	274	256	268

12/141 Hatchlings	CHMY02	CHMY02	CHMY15	CHMY15
NF	242	250	256	268
1	242	254	268	272
2	250	274	264	268
3	242	274	264	268
4	242	274	264	268
5	242	274	256	268
6	250	254	268	272
7	242	254	256	272
8	242	0	260	268
9	242	274	256	268
10	242	274	256	268
11	250	274	264	268
12	250	254	256	268
13	242	274	264	268
14	250	274	268	268
15	242	254	256	272
16	242	246	260	268
17	250	254	268	272
18	250	274	268	272
19	242	254	256	272
20	250	274	256	268
21	242	274	264	268
22	242	254	256	272
23	242	254	256	272
24	242	246	0	0

12/142 Hatchlings	CHMY02	CHMY02	CHMY15	CHMY15
NF	234	254	252	272
1	0	0	252	252
2	250	254	240	272
3	250	254	240	252
4	254	282	252	272
5	234	250	240	252
6	250	254	0	0
7	234	254	252	272
8	234	282	252	272
9	0	0	252	252
10	242	254	252	252
11	254	282	0	0
12	234	250	240	252
13	234	282	252	272
14	250	254	240	252
15	234	250	240	252
16	234	282	252	252
17	254	282	252	252
18	234	282	252	252
19	234	282	240	252

20	234	250	252	252
21	250	254	240	272
22	254	282	252	272
23	254	282	240	252
24	234	250	252	252

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LIST OF REFERENCES

Ackerman RA. 1997. The nest environment and the embryonic development of sea turtles, p. 83–106. *In:* The biology of sea turtles. P. L. Lutz and JA Musick (eds.). CRC Press, Boca Raton, FL.

Angulo E, GW Roemer, L Berec, J Gascoigne, F Courchamp. 2007. Double Allee effects and extinction in the island fox. *Conservation Biology*, 21: 1082-1091.

Bagley DA. 2003. Characterizing juvenile green turtles (*Chelonia mydas*) from three east central Florida developmental habitats. Master's Thesis, University of Central Florida Department of Biology.

Bateman, AJ. 1948. Intra-sexual selection in *Drosophila*. *Heredity*, 2: 349–368.

Booth J, JA Peters. 1972. Behavioural studies on the green turtle (*Chelonia mydas*) in the sea. *Animal Behaviour* 20: 808-12.

Chaloupka M, CJ Limpus. 2001. Trends in the abundance of sea turtles resident in southern Great Barrier Reef waters. *Biological Conservation*, 102: 235-249.

Chaloupka M, KA Bjorndal, GH Balazs, AB Bolten, LM Ehrhart, CJ Limpus, H Suganuma, S Troëng, M Yamaguchi. 2007. Encouraging outlook for recovery of a once severely exploited marine megaherbivore. *Global Ecology and Biogeography*, 17: 297-304.

Cunningham, EJ and AF Russell. 2000. Egg investment is influenced by male attractiveness in the mallard. *Nature* 404: 74-77.

Daly M. 1978. The Cost of Mating. *The American Naturalist* 112: 771-774.

Emlen ST, LW Oring. 1977. Ecology, sexual selection, and the evolution of mating systems. *Science*, 197: 215–223.

Fedorka K, T Mousseau. 2002. Material and genetic benefits of female multiple mating and polyandry. *Animal Behaviour* 64: 361-367.

Foley AM, KE Singel, PH Dutton, TM Summers, AE Redlow, J Lessman. 2007. Characteristics of a green turtle (*Chelonia mydas*) assemblage in northwestern Florida determined during a hypothermic stunning event. *Gulf of Mexico Science*, 25: 131-143.

Fitzsimmons NN, C Moritz, and SS Moore. 1995. Conservation and dynamics of microsatellite loci over 300 million years of marine turtle evolution. *Molecular Biology and Evolution*, 3: 432-440.

Fitzsimmons NN, C Moritz, L Pope, CJ Limpus. 1997. Geographic structure of mitochondrial and nuclear gene poly-morphisms in Australian green turtle populations and male- biased gene flow. *Genetics*, 147: 1843–1854.

Fitzsimmons, NN. 1998. Single paternity of clutches and sperm storage in the promiscuous green turtle (*Chelonia mydas*). *Molecular Ecology* 7: 575-84.

Godfrey MH, R Baretto, N Mrosovsky. 1996. Estimating past and present sex ratios of sea turtles in Suriname. *Canadian Journal Zoology*, 74: 267-277

González A, C Rossini, M Eisner, and T Eisner. 1999. Sexually transmitted chemical defense in a moth (*Utetheisa ornatrix*). *Proceedings of the National Academy of Sciences of the United States of America* 96: 5570-4.

Hamann M, CJ Limpus, DW Owens. 2003. Reproductive cycles of males and females. In: Lutz PL, JA Musick, J Wyneken (editors) Biology of sea turtles, Vol 2. CRC Press, Boca Raton, FL, p 275–306.

Harris WE, T Uller. 2009. Reproductive investment when mate quality varies: differential allocation versus reproductive compensation. *Phil. Trans. R. Soc. B,* 364: 1039-1048.

Hays GC, JR Speakman. 1991. Reproductive investment and optimum clutch size of loggerhead sea turtles (*Caretta caretta*). *Journal of Animal Ecology,* 60: 455-462.

Ihara Y. 2002. A model for evolution of male parental care and female multiple mating. *The American Naturalist* 160: 235-44.

Jennions MD, and M Petrie. 2000. Why do females mate multiply? A review of the genetic benefits. *Biological reviews of the Cambridge Philosophical Society* 75: 21-64.

Jensen MP, FA Abreu-Grobois, J Frydenberg and V Loeschcke. 2006. Microsatellites provide insight into contrasting mating patterns in arribada vs. non-arribada olive ridley sea turtle rookeries. *Molecular Ecology*, 15: 2567–2575.

Jourdan J, MMPB Fuentes. 2013. Effectiveness of strategies at reducing sand temperature to mitigate potential impacts from changes in environmental temperature on sea turtle reproductive output. *Mitigation and Adaptation Strategies for Global Change*, 1-13.

Kokko H, DJ Rankin. 2006. Lonely hearts or sex in the city? Density-dependent effects in mating systems. *Phil. Trans. R. Soc. B.,* 361: 319-334.

Lee PLM, GC Hays. 2004. Polyandry in a marine turtle: females make the best of a bad job. *Proceedings of the National Academy of Sciences*, USA, 101: 6530–6535.

Limpus CJ. 1993. The green turtle, Chelonia mydas, in Queensland: breeding males in the southern Great Barrier Reef. *Wildlife Research* 20: 513.

Makalov AA,Y Lubin. 2004. Sexual conflict over mating in a spider: increased fecundity does not compensate for the costs of polyandry. *Evolution*, 58: 1135-1140.

Maloney JE, C Darian-Smith, Y Takahashi, and CJ Limpus. 1990. The environment for development of the embryonic loggerhead turtle (*Caretta caretta*) in Queensland. *Copeia*, no. 2: 378-387.

McGinley MA, DH Temme, Geber MA. 2011. Parental investment in offspring in variable Environments: theoretical and empirical considerations. *Environments*, 130: 370-398.

Moore MK, RM Ball Jr. 2002. Multiple paternity in loggerhead turtle (*Caretta caretta*) nests on Melbourne Beach, Florida: a microsatellite analysis. *Molecular Ecology* 11: 281-288.

Mortimer JA. 1990. The influence of beach sand characteristics on the nesting behavior and clutch survival of green turtles (*Chelonia mydas*). *Copeia*, no.3: 802.

Myers EM, KR Zamudio. 2004. Multiple paternity in an aggregate breeding amphibian: the effect of reproductive skew on estimates of male reproductive success. *Molecular Ecology*, 13: 1951-1963.

Neff BD, Pitcher TE (2002) Assessing the statistical power of genetic analyses to detect multiple mating in fishes. *Journal of Fish Biology*, 61:739–750.

Patino-Martinez J, A Marco, L Quiñones, L Hawkes. 2012. A potential tool to mitigate the impacts of climate change to the Caribbean leatherback sea turtle. *Global Change Biology*, 18:401-411.

Peare T, PG Parker. 1996. Local genetic structure within two rookeries of *Chelonia mydas* (the green turtle). Heredity, 77: 619– 628.

Sanchez CL. 2013. Sex ratios of juvenile green turtles (*Chelonia mydas*) in three developmental habitats along the east coast of Florida. Master's thesis, University of Central Florida.

SAS Institute Incorporated. 2013. JMP Version 11. Cary, NC.

Spotila JR, EA Standora, SJ Morreale, GJ Ruiz. 1987. Temperature dependent sex determination in the green turtle (*Chelonia mydas*): effects on the sex ratio on a natural nesting beach. *Herpetologica*, 43: 74-81.

Standora EA, JR Spotila. 1985. Temperature dependent sex determination in sea turtles. *Copeia* 3: 711-722.

Theissinger K, NN Fitzsimmons, CJ Limpus, CJ Parmeneter, AD Phillott. 2009. Mating system, multiple paternity and effective population size in the endemic flatback turtle (*Natator depressus*) in Australia. *Conservation Genetics*: 10- 329-346.

Wang J. 2004. Sibship reconstruction from genetic data with typing errors. *Genetics*, 166:1963-1979.

Williams GC. 1966. Natural selection, the costs of reproduction, and a refinement of Lack's principle. *The American Naturalist*, 100: 687-690.

Wright LI, KL Stokes, WJ Fuller, BJ Godley, A McGowan, R Snape, T Tregenza, AC Broderick. 2012. Turtle mating patterns buffer against disruptive effects of climate change. *Proceedings of the Royal Society: Biological Sciences*, 279: 2122-2127.

Wright LI, WJ Fuller, BJ Godley, A McGowan, T Tregenza, AC Broderick. 2013. No benefits of polyandry to female green turtles. *Behavioral Ecology*, 24: 1022-1029.