ESTIMATING INCIDENCE OF MULTIPLE PATERNITY OF KEMP'S RIDLEY SEA TURTLES ON SOUTH PADRE ISLAND, TEXAS

by

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Introduction:

Genetic estimates of paternity can offer insight into the breeding patterns of animals like sea turtles which are difficult to observe in the wild and can have potentially important conservation implications (Jensen et al. 2006, Uller and Olsson 2008). For instance, polyandry can increase heterozygosity (Foerster et al. 2003), genetic variation (Jennions and Petrie 2000) and effective population size (Sugg and Chesser 1994, Pearse and Anderson 2009) relative to strict monogamy. These same genetic data can also be used to estimate the number of effective breeders in a population (Wang 2009) and over time can be used to monitor population stability or detect population declines.

Multiple paternity (MP), defined as multiple sires per clutch, is common in sea turtles (Fitzsimmons 1998, Bollmer et al. 1999, Moore and Ball 2002, Ireland et al. 2003, Jensen et al. 2006, Zbinden et al. 2007). Genetic parentage studies of sea turtles estimate the incidence of MP to range from 0 to ~100% of clutches with an average of approximately 48% MP (e.g. Fitzsimmons 1998, Bollmer et al. 1999, Moore and Ball 2002, Ireland et al. 2003, Jensen et al. 2006, Zbinden et al. 2007). For species such as sea turtles, in which males do not provide energetically expensive resources to females, such as defense of territories or care of young, the fitness of males is predicted to increase with the number of matings they can obtain (Fox and Rauter 2003). The benefits of multiple mates to females in these types of species are less clear, but can potentially include increased fertilization success or increased offspring survival (Jennions and Petrie 2000). To date, there is no evidence that female sea turtles obtain these types of benefits from mating with multiple males. Alternatively, females may simply mate with multiple males to reduce harassment and risk of injury (Lee and Hays 2004, Uller and Olsson 2008). A positive relationship exists between the incidence of MP and the estimated

population size of rookeries, especially for the genus *Lepidochelys* (Ireland et al. 2003, Jensen et al. 2006). Jensen et al. (2006) hypothesized that large aggregations of females, such as those that congregate offshore before arribada (mass nesting) events, result in a "mating frenzy" and subsequently higher levels of MP. In a study of the olive ridley, Jensen et al. (2006) found that 30% of nests at a solitary nesting rookery exhibited MP, whereas 92% of nests at an arribada nesting rookery exhibited MP.

In 1947 Andres Herrera was witness to a Kemp's ridley (*Lepidochelys kempii*) arribada in Mexico, which he caught on film. Today this single image of approximately 40,000 female turtles on the beach is the only estimate for the original Kemp's ridley population size (Hildebrand 1963). Forty years later, in 1987, fewer than 600 females nested in a single season, a substantial decrease attributable to decades of severe poaching of both female turtles and eggs (Manzella et al. 1988). Additionally, the Kemp's ridley is restricted to the Gulf of Mexico and has historically nested only between Mustang Island, Texas and Veracruz, Mexico (approximately 1,300 km), with highest nesting density in Rancho Nuevo, Mexico (www.nps.gov/pais). Declining numbers combined with their restricted habitat have made the Kemp's ridley the most endangered of all sea turtle species (www.iucnredlist.org).

Between 1978 and 1986, Mexico and the United States launched a program to increase productivity at a secondary nesting site for the Kemp's ridley in the Padre Island National Seashore of Texas (Manzella et al. 1988). Because these animals reach sexual maturity between 7 and 15 years of age, it was not until 1996 when the first females from the preliminary stages of this experimental program returned to Texas to nest (Shaver and Caillouet 1998, Turtle Expert Working Group 1998). As of 2009, an estimated 6,666 - 8,000 females (based on average

of 2.5 - 3 nests per female per nesting season) nested along the coastline of Mexico and Texas. Moreover, a record 197 nests were found on the Texas Coastline, 80.7% of which occurred on beaches between Boca Chica Beach and Padre Island (approximately 275 km) (www.nps.gov/pais).

Despite extensive protection of the Kemp's ridley sea turtle, little is known about the genetic mating system or the effective number of male breeders of this species, especially on Texas nesting beaches. The single study of MP in the Kemp's ridley, carried out in the high density Rancho Nuevo rookery, estimated that 58% of nests were multiply sired, despite restrictive sampling limitations which only allowed sampling of dead hatchlings (Kichler et al. 1999). The authors suggest, however, that true MP levels might be as high as 100%, but would only be seen with additional samples, a postulate also suggested by Neff et al. (2002). A reanalysis of Kichler's et al. (1999) data using a Bayesian model and a sibship method (Neff et al. 2002, Wang 2004) suggest that 81% of nests exhibited MP.

In this study, I used microsatellite markers to estimate the proportion of Kemp's ridley nests on South Padre Island, Texas (SPI) that were sired by multiple males. If the incidence of MP is positively related to nesting female density, as suggested by previous studies, then MP should be lower on SPI (~19-20 females) than in the much larger nesting population of Rancho Nuevo, Mexico (4,333 - 5,200 females). I also estimated the effective number of breeders currently contributing to nests on SPI.

Methods:

DNA sampling:

During the 2010 summer nesting season samples were collected from the 34-mile area of beach on SPI and the 7.5-mile area of Boca Chica Beach south of SPI, which are regularly patrolled by trained interns and volunteers from the non-profit organization Sea Turtle, Inc. (STI). In accordance with STI permits and protocols, all sea turtle nests laid on these beaches are relocated to a hatchery where they can be closely monitored until hatching.

All husbandry and conservation activities at STI are carried out in compliance with federal permits issued by the US Fish and Wildlife Service. Following the limitations of this federal permit issued to STI and the subsequent amendment, which extends coverage to seasonal researchers, I collected dermal samples from dead hatchings, dead fetuses and dead embryos. This sampling procedure is very similar to that of Kichler et al. (1999) who found high levels of MP, even with very limited sampling ranging from 2 to 14 samples per clutch.

Following hatching, all remaining nest contents, including egg shells, dead hatchlings and unhatched eggs, were bagged and prepared for transport to the Padre Island National Seashore (PINS) facility on Padre Island, Texas. At the PINS facility, sampling of dead hatchings, dead fetuses and dead embryos was carried out under the supervision and with the assistance of the PINS staff. A portion of flipper tissue was removed from dead hatchlings and fetuses and placed into vials containing 20% DMSO/6M NaCl and stored at room temperature until DNA extraction. In some instances, the embryo was so small that the entire embryo was collected as a tissue sample and was stored in DMSO. Additionally, Robin Tillitt of the USGS supplied 72 chorioallantoic membranes (CAMs), which were sorted from the nest contents or shifted from sand at the hatchery. Each CAM was placed in a vial, also containing 20% DMSO/6M NaCl.

DNA Extraction:

Tissue samples from 154 Kemp's ridley sea turtle hatchlings, embryos and CAMs were used in this study. Tissue from flippers and CAMs were placed in 300 µL lysis buffer (75 mM NaCl, 25 mM EDTA, 1%SDS) with 15 µL Proteinase K (20 mg/ml), then incubated at 55°C overnight. To further breakdown the highly keratinized flipper tissue each sample of this type was ground using a drill and a pestle attachment prior to incubation. One and a half volumes of 7.5 M ammonium acetate was then added to precipitate proteins, which were pelleted by centrifugation for 15 minutes. I then added 0.7 volume isopropanol to the supernatant to precipitate the DNA and centrifuged the sample for 15 minutes to pellet the DNA. The DNA pellet was washed with 70% ethanol and then allowed to dry before resuspending in 100 µL 10 mM Tris-HCl pH 8.5.

Samples were genotyped at 14 microsatellite loci developed for Kemp's and olive ridleys, green (*Chelonia mydas*), loggerhead (*Caretta caretta*), and hawksbill sea turtles (*Eretmochelys imbricata*; Kichler et al. 1999, FitzSimmons et al. 1995, Jensen et al. 2006, Shamblin et al. 2007, Shamblin et al. 2008; Table 1). Twelve loci were amplified in three multiplex sets and two loci (Cc2H12 and Cc5C08) were amplified singly. Polymerase chain reactions (PCR) (10 µL) contained 1 µL DNA, 0.20 µM of each primer, 5 µL Qiagen Multiplex PCR Master Mix containing HotStar Taq DNA polymerase, dNTPS and Multiplex PCR buffer with 3 mM $MgCl_2$ pH 8.7, and 3 µL deionized water**.** Reactions were cycled in an ABI 2720 thermalcycler with an initial 15 minute denaturation period (94^{o}C), followed by 30 cycles of 30 seconds of denaturation (94 ^{o}C), 1.5 minutes of annealing (55 ^{o}C/60 ^{o}C) and one minute of

elongation (72 ^{o}C). Reactions ended with a 30 minute elongation period (60 ^{o}C). The resulting multiplexes were diluted 40x with deionized water for flipper samples, 15x for embryo samples and 8x for CAM samples. For genotype analysis, 0.5 µL of diluted PCR product from each sample was loaded into 10 µL HIDI formamide with 0.1 µL LIZ-500 size standard (Applied Biosystems USA) and electrophoresed on an ABI3130 Genetic Analyzer (Applied Biosystems USA). Genotypes were scored using Genemapper v4.0 (Applied Biosystems USA).

Table 1. Characterization of microsatellite loci utilized in Kemps ridley sea turtles (N = 154 individuals).

Locus	Size Range (bp)	N_a	H_o	H_e	F_{IS}	Species loci originally developed for	Reference
Klk316	107-138	8	0.864	0.758	-0.143	Kemp's ridley (*Lepidochelys kempii*)	Kichler et al. 1999
Or-1	151-197	8	0.682	0.611	-0.120	olive ridley (*Lepidochelys olivacea*)	Jensen et al. 2006
Or-2	153-173	6	0.487	0.546	0.104	*Lo*	Jensen et al. 2006
Cm84	314-344	9	0.864	0.790	-0.097	green (*Chelonia mydas*)	Jensen et al. 2006
Ei8	179-194	6	0.792	0.722	-0.101	hawksbill (*Eretmochelys imbricata*)	Jensen et al. 2006
Cc7C04	201-250	11	0.968	0.870	-0.116	loggerhead (*Caretta caretta*)	Shamblin et al. 2007
Cc1G02	264-320	14	0.896	0.901	0.002	*Cc*	Shamblin et al. 2007
Cc5C08	297-358	14	0.890	0.905	0.014	*Cc*	Shamblin et al. 2007
Cc1F01	282-290	2	0.617	0.494	-0.253	*Cc*	Shamblin et al. 2007
Cc5H07	210-268	13	0.864	0.881	0.016	*Cc*	Shamblin et al. 2007
Cc7G11	259-279	6	0.506	0.565	0.101	*Cc*	Shamblin et al. 2007
Cc7D04	329-406	18	0.838	0.881	0.046	*Cc*	Shamblin et al. 2009
Cc2G10	266-327	17	0.974	0.914	-0.070	*Cc*	Shamblin et al. 2007
Cc2H12	323-386	15	0.981	0.873	-0.126	*Cc*	Shamblin et al. 2007

Na – number of alleles, H_O – observed heterozygosity, H_E – expected heterozygosity, F_{IS} – inbreeding coefficient

Table 2. Characterization of microsatellite loci for a single sample per female/nest in Kemp's ridley sea turtles (N = 24 individuals).

Locus	N_a	H_o	H_e	F_{IS}
Klk316	8	0.833	0.803	-0.060
Or-1	6	0.792	0.695	-0.163
Or-2	4	0.417	0.448	0.050
Cm84	6	0.833	0.790	-0.077
Ei8	5	0.708	0.733	0.013
Cc7C04	9	0.958	0.882	-0.110
Cc1G02	13	0.917	0.926	-0.011
Cc5C08	14	0.917	0.929	-0.008
Cc1F01	2	0.625	0.510	-0.252
Cc5H07	13	0.958	0.916	-0.069
Cc7G11	6	0.625	0.638	0.000
Cc7D04	15	0.875	0.917	0.025
Cc2G10	17	0.958	0.937	-0.044
Cc2H12	13	0.958	0.905	-0.081

Na – number of alleles, H_O – observed heterozygosity,

H_E – expected heterozygosity, F_{IS} – inbreeding coefficient

Genetic diversity:

I tested microsatellite loci for deviation from Hardy-Weinberg equilibrium (HWE) and genotypic linkage equilibrium using Genepop v 4.0 (Rousset 2008). For these analyses I utilized one individual from each nest (N = 24 individuals; Table 2). I used the program GenAlEx 6.3 to calculate the number of alleles, observed heterozygosity (H_O), and expected heterozygosity (H_E) for each microsatellite locus (Peakall and Smouse 2006).

Sibship and MP:

I first used a simple allele counting method to determine the level of MP in clutches that contained three or more samples. If a nest had more than four alleles at a single locus it was determined to be sired by more than one male.

I then used the computer program COLONY 2.0 to cluster samples into full sibling groups (Wang 2004, Wang 2009, Wang and Santure 2009). COLONY randomly compares each individual genotype and determines the likelihood of that relationship. This process is repeated over many iterations, ultimately grouping offspring into full sibship clusters. This program was designed to handle input from polygamous species even without known parental genotypes (Wang 2009). I used all 154 genotyped tissue samples to estimate full sibship clusters using the full likelihood method and a 0.01 or 0.05 error rate for five short and five long runs. I also used COLONY to estimate the effective number of breeders (N_b) using the sibship assignment method given in Wang (2009).

Results

Kemp's ridley females laid a total of 32 nests on SPI and Boca Chica beach during the 2010 summer nesting season. Ten females were identified while nesting and three of these were known to nest twice during the season. I was only able to collect samples from both nests that were confirmed to have been laid by a single female in one of these instances. The average season hatch rate was 91%; however, not all unhatched eggs were fertilized, which limited sampling. A total of 167 samples (< 6% of eggs laid on the beach) were collected for this study from 25 nests belonging to a minimum of 19 or a maximum of 24 nesting females. Of these samples 154 successfully amplified and were included in the paternity analysis. The number of samples successfully amplified from each nest ranged from 1 to 42 and had a median value of three (Fig. 1).

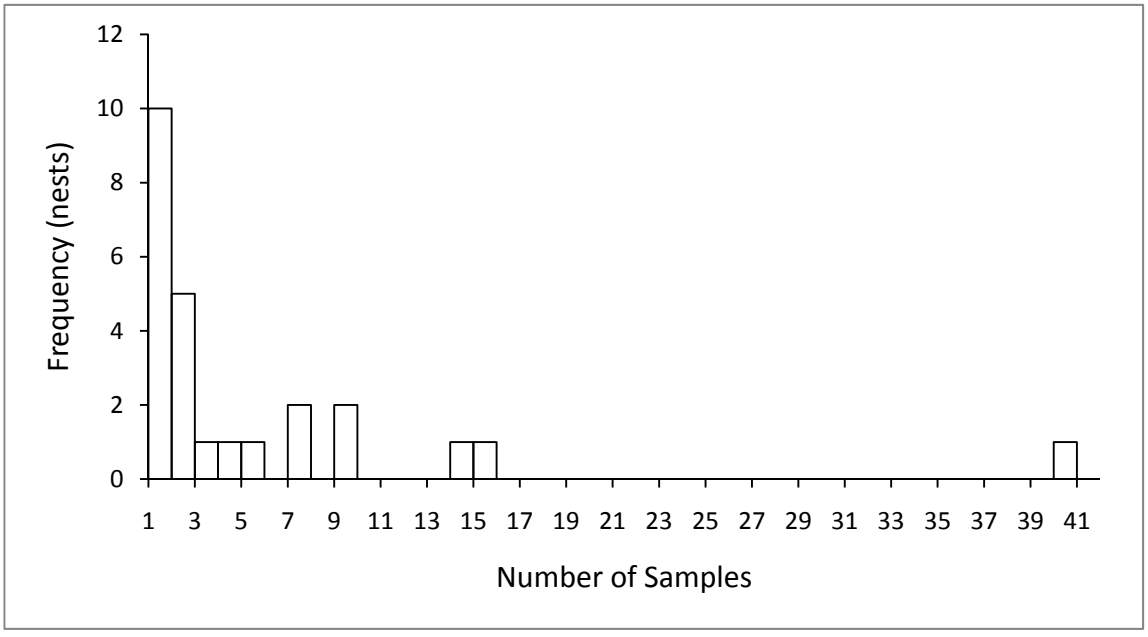

Figure 1. Frequency of number of Kemp's ridley sea turtle tissue samples by nest. Median value three samples.

All loci were highly polymorphic with 2 - 18 alleles, and observed heterozygosity ranged from 0.487 to 0.981 (Table 1). All loci were in Hardy-Weinberg and genotypic linkage equilibrium ($P > 0.05$ in all cases). Re-extraction of 40 tissue samples (26% of total) gave identical genotypes to the original DNA extractions and so the genotyping error rate was well below 0.01%.

MP:

Using the allele counting method it was only possible to determine levels of MP in the 15 nests from which three or more tissue samples had been collected (Kichler et al. 1999). Of these nests, eight had loci containing more than four alleles. Nests with more than four alleles at a locus had on average five loci with more than four alleles (range 1 – 10). These data suggest that eight (53%) nests had been multiply sired (Appendix 1).

COLONY:

Colony grouped the 154 samples into 29 full sibling groups or clusters, in which all hatchlings shared both parents (probabilities ranged from 0.70 to 1.0 across runs). These full sibling clusters contained between 1 and 37 individuals. These groupings were identical across runs of varying lengths and error rates with the exception of a single individual from nest 9 that was sometimes put into its own cluster. Fourteen nests had a single sibship cluster, nine had two clusters, and two had three clusters. There was a positive correlation between sample size of a nest and the number of sibship clusters ($y = 0.047x + 1.23$, $R^2 = 0.37$, $F_{24} = 13.44$, $P = 0.001$). Initially, nest 1 appeared to be an outlier with the largest number of tissue samples ($N = 42$). However, this positive relationship remained after removing nest 1 from the data set ($y = 0.061x + 1.17$, $R^2 = 0.22$, $F_{23} = 5.57$, $P = 0.028$) (Fig. 2). Most sibships (23 of 29) occurred within a

clutch, whereas six were comprised of individuals from two different clutches. In one case, the same known female laid the two nests. In four instances the female of one or both nests was not identified; however, the internesting period averaged 28.3 days and so it is possible that the two nests could have been laid by the same female. In one case, a sibship was formed between two nests that were laid on the same day by two different females, each identified by flipper tags (Table 3).

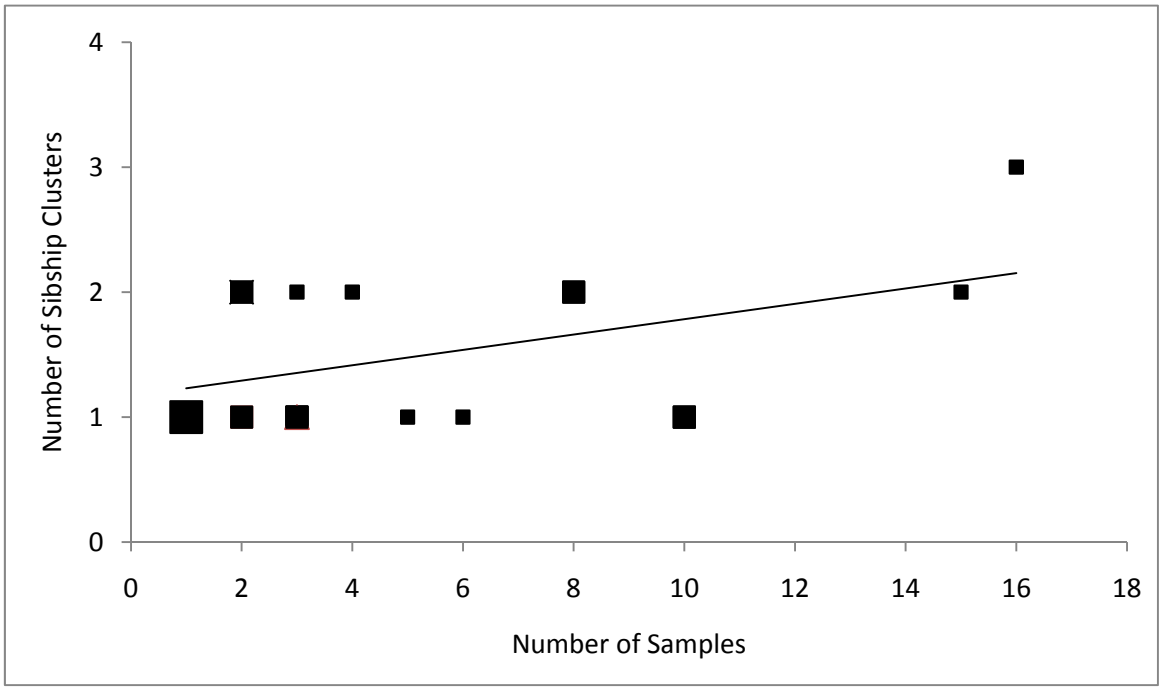

Figure 2. Number of Kemp's ridley sea turtle sibship clusters as a function of the number of tissue samples (N = 154) from a nest. Large squares represent four data points with the same value, medium sized squares represent two data points with the same value, and the smallest squares represent single data points. $y = 0.061x + 1.17$ and $R^2 = 0.22$.

Table 3. Kemp's ridley nests grouped by sibship cluster. Paired clutches may represent nests laid by the same female (see text). In nest columns, data given as nest id number (number of samples from that nest).

Sibship Cluster	Probability	First Nest	Second Nest	Comment
1	1	1 (7)	-	-
2	1	1 (28)	3 (10)	same day
3	0.99	1 (8)	-	-
4	1	2 (1)	-	-
5	1	2 (12)	29 (3)	same female
6	1	2 (3)	29 (1)	same female
7	1	5 (11)	-	-
8	1	6 (1)	-	-
9	1	7 (1)	-	-
10	1	9 (5)	24 (4)	33 days between nests
11	1	9 (10*)	24 (4)	33 days between nests
12	1	10 (1)	-	-
13	1	10 (1)	-	-
14	1	11 (5)	-	-
15	1	12 (1)	-	-
16	1	14 (6)	-	-
17	1	14 (2)	-	-
18	1	15 (6)	-	-
19	0.7854	16 (2)	-	-
20	1	16 (1)	-	-
21	0.7382	18 (1)	26 (1)	25 days between nests
22	0.945	18 (1)	26 (1)	25 days between nests
23	1	19 (3)	30 (1)	30 days between nests
24	1	20 (2)	27 (3)	25 days between nests
25	1	22 (2)	-	-
26	1	23 (3)	-	-
27	1	25 (1)	-	-
28	1	25 (1)	-	-
29	1	31 (3)	-	-

* In some runs of COLONY one of these hatchlings was placed alone in a separate sibship group.

Of the nests with two or more samples (N = 21), 11 had more than one sibship for a MP estimate of 52% of nests, which is close to the estimate obtained by the simple allele counting method. For nests with three or more samples, seven had more than one sibship cluster, giving a MP estimate of 46% of nests (Appendix 2). The effective number of breeders (N_b) ranged from 19 - 20 across separate runs of COLONY.

Discussion:

MP within a clutch appears to be common in Kemps ridley sea turtles. The 95% confidence intervals surrounding my estimate of 52% range from 32-72% and overlap with the confidence intervals ($p \pm 1.96 \sqrt{[p(1-p)/N]}$, where p is the proportion and N is the sample size) around the 81% estimate from the much larger Rancho Nuevo colony (66-96%), suggesting the level of MP is not related to nesting density in this species. The positive relationship between sample size from a nest and number of sibling clusters also suggests that with more complete sampling the estimate of MP on SPI would be even higher than currently estimated. A similar relationship was found for Kichler et al's. (1999) data (Neff 2002).

To reconsider the Jensen et al. (2006) female density hypothesis I added the MP estimate from this study (52%) and the reanalyzed Kichler et al. (1999) estimate (81%) for Kemp's ridley to those from their previous analysis, after removing Kichler's earlier estimate of 52%. With these changes there is still a positive, but weaker relationship that is not significant between population size and percent MP ($R^2 = 0.09$, $F_9 = 0.80$, $P = 0.40$) (Fig. 3). Both of the Kemp's ridley estimates fall above the regression line, suggesting that the sibship method in COLONY may detect relatively higher levels of MP or that the Kemp's ridley mating system may result in relatively higher MP than olive ridleys, green, loggerhead and leatherback sea turtles.

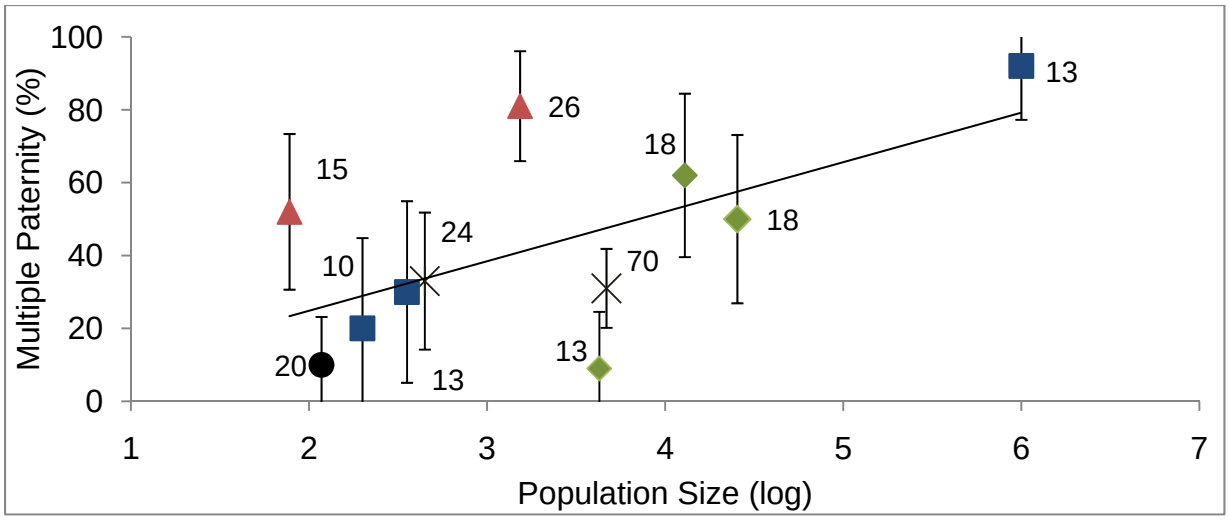

Figure 3. Effect of population size on estimates multiple paternity in five species of sea turtles. Reanalysis of data from Jensen et al. 2006, including data from Kichler et al. (1999) and Wang (2004) and the estimate from this study. Error bars represent 95% confidence intervals ($p \pm 1.96 \sqrt{[p(1-p)/N]}$). Numbers near each data point represent the total number of nests sampled and analyzed from that study. Legend: triangles (Kemp's ridley), circles (leatherback), squares (olive ridley), diamond (green) and X (loggerhead). $y = 7.90x + 13.70$ and $R^2 = 0.09$.

Studies using satellite transmitters to monitor migratory behavior of Kemp's ridley sea turtles during and after the April – July nesting season reveal that females generally move North-East through the Gulf of Mexico, remaining in the shallow waters near the coastline at the end of the nesting season (Shaver and Rubio 2007). The satellite tracking of 11 Kemp's ridley males off of the coast of Mexico, however, suggested most (10 of 11) remained near the nesting beach year-round. The authors propose that food is plentiful enough to support this male population throughout the year and it may expose them to a higher number of mates (Shaver et al. 2005). This is a very different pattern than what is seen in other sea turtle species, where males make cross-oceanic migrations between breeding and feeding areas (Hays et al. 2001, James et al. 2005). It is possible that by minimizing their migratory effort, male Kemp's ridley sea turtles have been able to increase mating effort, thereby increasing levels of multiple paternity.

Studies of MP often suffer from small sample sizes, which can make it difficult to accurately determine the incidence of MP across clutches (Kichler et al. 1999, Uller and Olsson 2008). In this study, for example, I would have needed to sample 60 clutches, more than currently occur on SPI, to obtain confidence intervals that did not overlap with the Rancho Nuevo estimate, as determined by manipulation and modeling of confidence intervals. The addition of 95% confidence intervals around the point estimates included in the Jensen et al. (2006) study reveals fairly broad overlap between many of the estimates (Fig. 3). Reanalysis of the Jensen et al. (2006) data reveals that the 92% MP data point is an outlier that has high leverage (Cooks D = 1.05). Removing this data point results in a non-significant relationship between percent MP and population size ($P = 0.40$). Nevertheless, the large confidence intervals for many of the points may obscure a significant relationship. To further investigate the "density hypothesis" additional studies with sufficient sample sizes are needed to accurately estimate percent MP and a standardized method of parentage analysis (i.e., COLONY) should be used.

COLONY clustered 12 nests into six sibships. The same female, identified by flipper tags, laid two of the clutches. An additional four of these pairings can likely also be explained by the same nesting female, since Kemp's ridley females lay on average 2.5 - 3 clutches per year and sea turtles exhibit internesting periods ranging from 9 to 14 days (www.nps.gov/pais, Rostal 2007). One sibship cluster was formed between two nests that were laid on the same day by two separately identified females. To verify these results, I reamplified a random selection of 16 tissue samples from nest 1 and nest 3. This resulted in identical genotypes, suggesting these results were not due to genotyping errors. One possible explanation is that the two nesting

females were themselves closely related and possibly mated with the same male and therefore COLONY was unable to separate these offspring. Another more likely explanation is that these offspring simply shared many common alleles by chance and so were put into the same sibship.

The number of effective breeders at the SPI nesting site is relatively low with an estimated 20 individuals, yet there is no genetic evidence this nesting colony has experienced a genetic bottleneck. Heterozygosity and allelic richness of offspring is high and similar to the level of genetic diversity in the Rancho Nuevo nesting colony and olive ridleys (Kichler et al. 1999, Jensen et al. 2006). Although Kemp's ridley females are philopatric, the SPI colony is probably connected by male mediated gene flow from larger colonies in Mexico. One of the 11 males that were satellite tracked off the coast of Mexico moved up into Texas waters suggesting that male movement across these distances may be relatively frequent (Shaver et al. 2005). Male dispersal, a high incidence of multiple paternity, and the long-life span of Kemp's ridleys probably act in concert to maintain high levels of genetic diversity even though this species has experienced a historical decline.

Kemp's ridleys exhibit unique biological and migratory patterns compared to other species of sea turtles. However, lessons from their breeding biology can be applied to conservation of other declining populations. In the case of this endangered species MP increased heterozygosity and genetic variation, preventing the effects of a bottleneck even in light of drastic population declines. MP is a prevalent phenomenon in nature and increased study of this aspect of breeding biology may provide insight into genetic conservation of many of animals.

Appendix 1. The number of alleles for each microsatellite locus separated by individual Kemp's ridley sea turtle nests. Loci with more than four alleles indicate that more than one male sired the clutch.

Nest	Number of Samples	Cm84	Ei8	Klk316	Or-1	Or-2	Cc7C04	Cc1G02
1	**41**	6**	5**	3	3	3	6**	7**
2	**16**	5**	2	4	3	2	3	5**
3	**10**	4	3	2	2	2	4	4
5	**10**	2	2	3	2	3	5**	3
6	**1**	2	2	2	2	1	1	2
7	**1**	1	2	1	2	1	2	2
9	**15**	5**	2	4	4	2	6**	3
10	**2**	2	2	3	1	2	3	2
11	**5**	3	3	3	2	3	4	3
12	**1**	2	2	2	2	1	2	2
14	**8**	4	4	4	3	3	5**	5**
15	**6**	3	3	4	4	2	4	4
16	**3**	4	3	4	3	1	4	4
18	**2**	2	2	3	3	2	4	3
19	**3**	2	2	3	2	3	3	3
20	**2**	3	4	2	2	2	2	3
22	**2**	3	2	2	2	2	2	3
23	**3**	2	3	2	2	1	3	4
24	**8**	5**	2	4	4	3	5**	3
25	**2**	3	2	1	2	2	2	3
26	**2**	3	4	2	3	2	3	3
27	**3**	3	2	2	3	2	2	3
29	**4**	4	2	4	2	2	3	5**
30	**1**	2	1	2	1	2	2	2
31	**3**	2	3	2	2	2	2	4

nest with more than four alleles at one or more loci, representing multiple paternity

**locus with more than four alleles, representing multiple paternity

Appendix 1 continued:

Nest	Number of Samples	Cc5C08	Cc1F01	Cc5H07	Cc7G11	Cc7D04	Cc2G10	Cc2H12
1	**41**	7**	2	9**	4**	6**	5**	6**
2	**16**	4	2	5**	2	6**	5**	5**
3	**10**	3	2	3	3	2	4	4
5	**10**	4	2	4	2	2	3	4
6	**1**	2	1	2	2	2	2	2
7	**1**	2	1	1	1	1	2	2
9	**15**	6**	2	4	3	5**	5**	6**
10	**2**	3	2	2	3	3	3	3
11	**5**	4	2	3	2	4	4	4
12	**1**	2	1	2	2	2	2	2
14	**8**	4	2	5**	1	4	5**	5**
15	**6**	4	2	3	1	4	3	3
16	**3**	3	1	4	3	5**	3	3
18	**2**	3	2	3	4	3	3	4
19	**3**	3	2	3	3	3	4	3
20	**2**	2	1	3	2	4	4	3
22	**2**	3	2	2	1	2	2	3
23	**3**	4	2	3	2	2	3	3
24	**8**	6**	2	5**	3	6**	5**	5**
25	**2**	4	2	2	3	1	4	3
26	**2**	3	2	4	2	4	3	3
27	**3**	2	2	3	2	4	3	2
29	**4**	5**	2	3	2	4	6**	5**
30	**1**	1	2	2	2	2	2	2
31	**3**	3	2	3	2	3	4	3

nest with more than four alleles at one or more loci, representing multiple paternity

**locus with more than four alleles, representing multiple paternity

Appendix 2. COLONY maximum likelihood of sibships in Kemp's ridley sea turtles, with an error rate of 0.01.

Full Sibship Cluster									
1	N01_01	N01_02	N01_04	N01_12	N01_14	N01_32	N01_42		
2	N01_03	N01_05	N01_06	N01_07	N01_13	N01_16	N01_17	N01_18	N01_20
	N01_21	N01_22	N01_23	N01_24	N01_25	N01_26	N01_27	N01_28	N01_29
	N01_30	N01_31	N01_33	N01_35	N01_36	N01_37	N01_38	N01_39	N01_40
	N01_41	N03_60	N03_61	N03_62	N03_63	N03_64	N03_65	N03_66	N03_67
	N03_68	N03_69	N03_70						
3	N01_08	N01_09	N01_10	N01_11	N01_15	N01_19	N01_34	N01_43	
4	N02_44								
5	N02_45	N02_46	N02_48	N02_50	N02_51	N02_52	N02_53	N02_54	
	N02_55	N02_56	N02_57	N02_58	N29_152	N29_153	N29_154		
6	N02_47	N02_49	N02_59	N29_151					
7	N05_71	N05_72	N05_73	N05_74	N05_75	N05_76	N05_77	N05_78	
	N05_79	N05_80	N05_81						
8	N06_82								
9	N07_83								
10	N09_84	N09_85	N09_87	N09_88	N09_93	N24_137	N24_139	N24_140	N24_143
11	N09_86	N09_89*	N09_90	N09_91	N09_92	N09_94	N09_95	N09_96	N09_97
	N09_98	N24_136	N24_138	N24_141	N24_142				
12	N10_99								
13	N10_100								
14	N11_101	N11_102	N11_103	N11_104	N11_105				
15	N12_106								
16	N14_107	N14_108	N14_111	N14_112	N14_113	N14_114			
17	N14_109	N14_110							
18	N15_115	N15_116	N15_117	N15_118	N15_119	N15_120			
19	N16_121	N16_123							
20	N16_122								
21	N18_124	N26_147							
22	N18_125	N26_146							
23	N19_126	N19_127	N19_128	N30_H01					
24	N20_129	N20_130	N27_148	N27_149	N27_150				
25	N22_131	N22_132							
26	N23_133	N23_134	N23_135						
27	N25_144								
28	N25_145								
29	N31_156	N31_157	N31_158						

*In some runs of COLONY this sample was placed alone into its own sibship cluster resulting in 30 total sibship clusters.

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VITA

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ABSTRACT

ESTIMATING INCIDENCE OF MULTIPLE PATERNITY OF
KEMP'S RIDLEY SEA TURTLES ON SOUTH PADRE ISLAND, TEXAS

by

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Little is known about the genetic mating system of the Kemp's ridley sea turtle, *Lepidochelys kempii*. Jensen et al. (2006) found a positive relationship between the incidence of multiple paternity (MP) and the estimated population size of rookeries. In the high density Kemp's ridley Rancho Nuevo rookery, Kichler et al. (1999) estimated 81% of nests were multiply sired (Wang 2004). If MP is positively related to nesting female density, then MP in Kemp's ridley should be lower on a low-density nesting beach on South Padre Island, Texas (SPI) than in a Mexico's much larger nesting population. We genotyped 154 hatchling tissue samples from nests on SPI at 14 microsatellite loci. Results using full sibship reconstruction as implemented in COLONY indicated that 52% of nests with two or more samples (11 of 21) exhibited MP. This suggests that this species does not follow the female density trend proposed by Jensen et al. (2006) and maintains high levels of MP and genetic variation across its nesting range.