Sex ratios of sea turtle hatchlings: direct and indirect estimates

by

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A thesis submitted in conformity with the requirements
for the degree of Doctor of Philosophy
Graduate Department of Zoology
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Abstract

To generate more data on sex ratios of sea turtle hatchlings, I developed a simple method of collecting daily mean sand temperatures on nesting beaches. Sand temperature can be used as an index of sex ratio. I then measured sand temperatures and sampled gonads of hatchlings of two sea turtle species on a nesting beach in Suriname during 1993. From histological analysis of the gonads, combined with information on the relative numbers of turtles nesting during the season, I generated overall estimates of sex ratio for green sea turtles (63.8% female) and for leatherback turtles (69.4% female). Relative numbers of male and female hatchlings varied throughout the season, and this corresponded to changes in sand temperatures and rainfall. From historical rainfall records and from past records of nesting frequency, I estimated overall sex ratios for another 13 seasons. Seasonal sex ratios fluctuated yearly.

A revised estimate of the pivotal temperature (that constant incubation temperature which produces equal numbers of each sex) of green turtles in Suriname was 29.4 °C; this was slightly higher than past estimates. The transitional range of temperature (TRT), which is the range of incubation temperatures over which both sexes are produced, spanned several degrees.

Metabolic warming was monitored in leatherback nests. It was found that eggs were about 0.8 °C warmer on average than the surrounding sand during the thermosensitive period of sexual differentiation. Because the leatherback has a narrow TRT, the influence of metabolic warming on sex ratio probably is restricted to a narrow range of incubation temperatures.

Lastly, a new method of estimating sex ratios was devised, based on the rate of development of eggs and prevailing incubation temperatures. In general, nests with shorter incubation times produce mostly females and nests with longer incubation times produce mostly males. This relationship could be useful for generating predictions of sex ratio from natural nests for which only the incubation duration is known. In addition, by comparing this relationship from laboratory and field studies, it was possible to quantify the time it takes loggerhead hatchlings to dig up from the nest once they have hatched.

Résumé

Afin d'obtenir plus d'informations sur la proportion de tortues mâles et femelles chez les tortues marines nouveau-nées, j'ai développé une méthode simple pour relever les températures quotidiennes moyennes du sable sur les plages où se trouvaient les nids. On peut se servir de la température du sable pour déterminer la proportion de tortues mâles et femelles. Au cours de l'année 1993, j'ai relevé les températures du sable et j'ai effectué des prélèvements sur les gonades des nouveau-nés de deux espèces de tortues marines sur une plage du Suriname. A partir de l'analyse histologique des gonades et d'informations sur les nombres relatifs de tortues nichant durant la saison, je suis parvenu à des estimations globales quant à la proportion de tortues mâles et femelles chez les tortues marines vertes (63,8% de tortues femelles) et chez les tortues luth (69,4% de tortues femelles). Les nombres relatifs des nouveau-nés mâles et femelles variaient pendant la saison, et cette variation correspondait à des changements dans la température du sable et les pluies. A partir de relevés historiques sur les précipitations et d'anciens relevés sur la fréquence des nichées, j'ai calculé les proportions globales des tortues mâles et femelles sur une période de 13 ans. Les proportions de chaque saison variaient d'une année à l'autre.

Une nouvelle estimation de la température pivot (c'est-à-dire la température d'incubation constante qui produit des nombres égaux de tortues mâles et femelles) pour les tortues vertes du Suriname était de 29,4 ºC, ce qui était légèrement plus élevé que des estimations précédentes. L'échelle des températures, à savoir l'échelle des températures d'incubation à l'intérieur de laquelle les tortues mâles et femelles sont produites, a augmenté de plusieurs degrés.

On a contrôlé le réchauffement métabolique dans les nids de la tortue luth. On a trouvé que les oeufs étaient en moyenne plus chauds de 0,8 ºC que la température du sable environnant, durant la période thermosensitive où a lieu la différentiation du sexe. Parce que la tortue luth a une échelle des températures réduite, l'influence du réchauffement

métabolique sur la proportion des mâles et des femelles ne porte probablement que sur une échelle réduite de températures d'incubation.

Enfin, on a inventé une nouvelle méthode pour estimer la proportion des mâles et des femelles, en se basant sur le taux de développement des oeufs et sur les températures d'incubation dominantes. En général, les nids qui ont des périodes d'incubation plus courtes produisent principalement des tortues femelles, et ceux qui ont des périodes d'incubation plus longues produisent principalement des tortues mâles. On pourrait se servir de ce lien pour estimer la proportion des mâles et des femelles dans les nids naturels pour lesquels seule la période d'incubation est connue. D'autre part, en comparant ce lien découvert en laboratoire et les études sur le terrain, il a été possible de quantifier le temps nécessaire aux nouveaux-nés pour sortir du nid après leur éclosion.

Proporción de sexos de crías de tortugas marinas: estimaciones directas e indirectas.

Una tésis presentada para obtener el grado de Doctorado de Filosofía
Departamento de Zoología, Universidad de Toronto

Resumen

Para generar mas información sobre las proporciones de sexos de las crías de tortugas marinas, desarrollé un método simple de registrar el promedio diario de la temperatura de la arena en las playas de anidación. La temperatura de la arena se puede usar como un índice de la proporción de sexos. En 1993, medí y registré temperaturas de arena, y tomé una muestra de gónadas de crías de dos especies de tortugas marinas que anidan en la misma playa en Surinám. El análisis histológico de las gónadas, junto con información sobre la frecuencia y número de tortugas que anidaron durante la temporada, se usaron para generar estimaciones globales de la proporción de sexos de la tortuga blanca (63,8% hembras) y la tortuga baula (69,4% hembras). La proporción de machos y hembras varió durante la temporada de anidación, y esto correspondió a cambios en la temperatura de la arena y en la precipitación fluvial. Registros históricos de precipitación e información sobre la frecuencia de anidación en años anteriores fueron usados para estimar la proporción de sexos de crías para 13 temporadas adicionales. La proporción de sexos de crías varió anualmente.

Se revisó la estimación de la temperatura umbral (la temperatura constante que produce 50% hembras y 50% machos) de la tortuga blanca de Surinám. La nueva estimación es 29,4°C y es un poco más alto que las estimaciones anteriores. El Rango Transitorio de Temperatura (RTT), que es el rango de temperaturas de incubación en que crías de ambos sexos son producidos, cubre varios grados centígrados.

El calor metabólico se midió en nidos de la tortuga baula. Se encontró que los huevos en estos nidos estaban un promedio de 0,8°C más cálidos que la arena adyacente durante el periodo termosensitivo de diferenciación sexual. Debido al estrecho RTT en la

tortuga baula, la influencia del calor metabólico sobre la proporción de sexos es seguramente limitada a un estrecho rango de temperaturas de incubación.

Finalmente, un nuevo método para estimar las proporciones de sexos fue originado basado en la rata de desarrollo embriónico de los huevos y la temperatura de incubación prevaleciente. En general, nidos con duraciones de incubación cortas producen más que todo hembras, y nidos con duraciones de incubación largas producen mayormente machos. Esta relación puede ser útil para generar estimaciones de proporción de sexos de nidos in situ cuando solamente se sabe la duración de incubación. Además, comparando esta relación usando datos de trabajo de laboratorio con estudios de campo, fue posible calcular el tiempo que les toma a las crías de la tortuga caguama en salir del nido.

Acknowledgements

Although I am listed as the sole author of this thesis, I know that my graduate career has been dependent upon the help, guidance, suggestions, criticisms, advice, and general camaraderie of scores of people. Without them, this thesis would not exist.

My supervisor, Nicholas Mrosovsky, was kind enough to take me on as a student, despite my lack of experience, and he invested much time and effort in my academic career. Although I am grateful for all the things he has done for me (including occasionally letting me beat him in squash), I am most indebted to him for the strong training he has provided in all areas of research, from fieldwork to paperwork.

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Table of Contents

Preface

I have selected the research paper format for this thesis. Six papers (Chapters 2-7) follow a general introduction (Chapter 1). These are followed by a general discussion (Chapter 8) and 3 appendices containing reports and papers relating to the application of sex ratio data to conservation. Three of these papers have been published in refereed journals, 2 have been submitted for review, and 1 has been accepted for publication in a manual of sea turtle conservation. Permission to reproduce the published papers was obtained from different journals:

Chapter 2 is reprinted from **Copeia, 1993 (3), Godfrey, M.H., and Mrosovsky, N., Simple method of estimating mean incubation temperatures on sea turtle beaches, pp. 808-811**, ©1994, with kind permission of M.E. Douglas, Managing Editor, Copeia, Department of Zoology, Arizona State University, Tempe, Arizona, 85287-1501, USA.

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Chapter 5 has been submitted for review for publication in the **Journal of Herpetology.**

Chapter 7 has been submitted for review for publication in **Chelonian Conservation and Biology**.

Appendix 1 will appear in the **Manual of Techniques for Sea Turtle Research and Conservation,** to be published by the **Marine Turtle Specialist Group, World Conservation Union (IUCN)**.

Appendix 3 is reprinted from **Biological Conservation, 74, Godfrey, M.H., and Barreto, R., Beach vegetation and seafinding orientation of turtle hatchlings, pp. 29-32**, ©1995, with kind permission from Elsevier Science Ltd., The Boulevard, Langford Lane, Kildington, OX5 1GB, UK.

The papers reproduced here are identical to those published with a few exceptions, as follows: a) the abstract, acknowledgment and reference sections have been omitted; b) the figures have been numbered consecutively throughout the work; c) the reference format has been modified where necessary for consistency, and all literature cited is found at the end of the thesis; d) a few minor additions have been made to Chapter 3.

Appendix 2 is a report (unpublished) submitted by M. H. Godfrey and R. Barreto to the Stichting Natuurbehoud Suriname (STINASU) following the 1993 field season at Matapica Beach, Suriname.

The relative contribution of authors is as follows. For papers included in this thesis, the original ideas for the work were generated through interactions in meetings and discussions with individuals within and outside of the laboratory. The research projects were designed by me but critically evaluated by members of the department and elsewhere. The papers were written by me but received editing and comments from Dr. N. Mrosovsky, co-authors, and others in and out of the laboratory.

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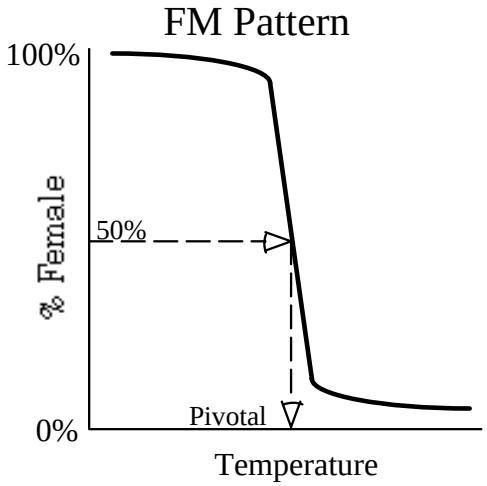
Chapter 1: Introduction

The direction of sexual differentiation in sea turtles, as in many other reptiles, is influenced by ambient temperatures during embryonic development (Raynaud and Pieau, 1985; Janzen and Paukstis, 1991b; Ewert and Nelson, 1991). More precisely, at relatively cool incubation temperatures, the hatchlings produced are all or mostly all male, at relatively warm incubation temperatures, the hatchlings produced all or mostly all female. The constant incubation temperature that results in a 1:1 ratio of male: female hatchlings is called the pivotal temperature; those temperatures which produce some of each sex fall into what is called the transitional range of temperature for sexual differentiation (for definitions, see Mrosovsky and Pieau, 1991). All crocodilians, many turtles, and some lizards display temperature-dependent sex determination, although the precise patterns vary (Fig 1.1).

Temperature-dependent sexual differentiation (TSD) is usually contrasted with genotypic sexual differentiation (GSD), which is the common sex determining mechanism for most vertebrates (Mittwoch, 1996). With GSD sex is determined at fertilization. For example, in mammals, presence of the paternally-inherited Y chromosome causes the fertilized egg to differentiate into a male; on the other hand, if the egg is fertilized by a spermatozoon with an X chromosome, then it will differentiate into a female. This is in contrast to TSD, which is characterized by a temperature sensitive critical period of sexual differentiation during development (after fertilization). In sea turtle species studied so far, the thermosensitive period for TSD is roughly the middle third of incubation (Yntema and Mrosovsky, 1982; Maxwell, 1987; Desvages et al., 1993).

A straightforward dichotomy, however, between GSD and TSD is probably too simple a story. Although Bull (1983) hypothesized that evolutionary

Figure 1.1. Patterns of sex determination in reptiles. Shown are the different relationships between egg incubation temperature and offspring sex ratio (expressed as % female). Note that in the case of GSD (genetic sex determination), sex ratio remains unchanged at different incubation temperatures. For the other patterns, the pivotal temperatures (that constant incubation temperature which results in 50% female offspring) are indicated. Adapted from Bull (1980). Pattern abbreviations are as follows: FM = female-male; MF = male-female; FMF = female-male-female.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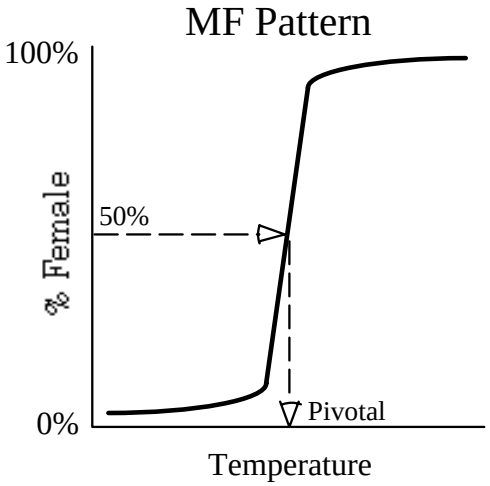
100%
% Female
50%
Pivotal
0%
Temperature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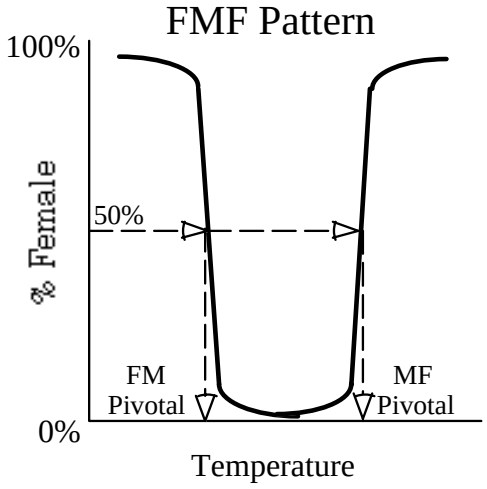
FMF Pattern
100%
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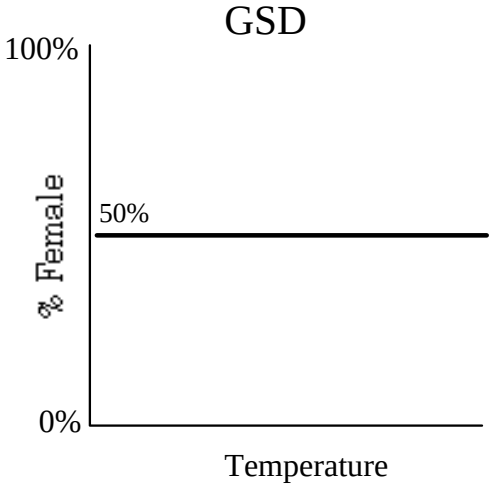

GSD
100%
% Female
50%
0%
Temperature

transitions from GSD to TSD involve the loss of heteromorphic sex chromosomes and vice versa, there seem to be at least some species in which both conditions exist. In an elegant set of experiments, Dournon et al. (1990) have shown that in the salamander *Pleurodeles waltl*, TSD appears to be superimposed onto GSD. *P. waltl* females have heterogametic sex chromosomes (ZW). When their eggs are incubated at pivotal temperatures, phenotypic sex is correlated with genotypic sex, and the sex ratio is 1:1. However, at higher incubation temperatures, all the offspring are male, regardless of genotype (i.e. half of the phenotypic males are sex-reversed ZW genotypic females). When these sex reversed ZW males are mated with ZW females, and the resultant eggs are incubated at pivotal temperature, 25% of the offspring are phenotypic males (and have ZZ genotypes), while 75% are phenotypic females (with either ZW or WW genotypes). If the WW females are crossed with ZZ males, and the eggs are incubated at pivotal, all the offspring will be phenotypic females (with a ZW genotype). Note that in all the above cases, if the incubation temperatures had been above pivotal, then all the offspring would be phenotypic males. The interpretation of these data is that in *P. waltl*, a GSD system is expressed when eggs are incubated at pivotal temperatures. At higher temperatures, the GSD system is over-ridden by TSD.

In *Emys obicularis*, the European pond turtle, heteromorphic sex chromosomes have not been found, and this species displays TSD. Despite the lack of heteromorphic sex chromosomes, sexual genotype is thought to be revealed by serologically defined H-Y antigen (H-Y) in non-gonadal tissues: males are H-Y negative, females are H-Y positive (Zaborski et al., 1988). When *E. obicularis* eggs are incubated at pivotal temperature, there is a high concordance between sexual H-Y genotype and phenotype (Zaborski et al., 1988). Hatchlings from eggs incubated at temperatures widely different from pivotal show much weaker correlations between H-Y genotype and sexual phenotype.

It is often assumed that in natural conditions, nest temperatures often are close to pivotal. Consistent with this idea, when *E. obicularis* adults in a natural population in France were sampled, it was found that in most adults phenotype and H-Y genotype agreed, while

the remaining were considered to be sex-reversed individuals (Servan et al., 1988). This is to be expected if TSD is superimposed on GSD, as variation in weather patterns during incubation is likely to cause egg temperatures to deviate occasionally from pivotal.

Other data suggest that there are genetic components to TSD. *Staurotypus salvinii*, the Central American musk turtle, has visible heteromorphic sex chromosomes; and yet, at least some individual clutches appear to display TSD (Ewert and Nelson, 1991). Also, there is variation in the pivotal temperatures of clutches from different individuals of some species of turtle (Bull et al., 1982a; Mrosovsky, 1988; Janzen, 1992; Bobyn and Brooks, 1994). This suggests that at least one aspect of TSD, the pivotal temperature, is heritable and thus involves a genetic component.

The question of the underlying physiological mechanisms of TSD still remains largely unanswered (cf. Spotila et al., 1994), although some pieces of the puzzle have been slotted into place. For instance, in turtles, it is likely that steroid hormone levels are the specific factors that control whether the gonad differentiates into a testis or ovary. When European pond turtle eggs are incubated at 30 °C (all females produced), there is a high level of estrogen found in the gonads during gonadal differentiation; at 25 °C (all males produced), the relative level of estrogen in the gonad is low (Pieau et al., 1994a). In addition, during the thermosensitive period of sexual differentiation of turtle eggs, when estrogen is applied topically to eggs being incubated at male-producing temperatures, the gonads will differentiate into ovaries (Pieau et al., 1994a; Wibbels et al., 1994). Antiestrogens injected into eggs developing at female-producing temperatures produce partial masculinisation of the gonads (Pieau et al., 1994a). Introduction of testosterone into eggs incubating at male producing temperatures will result in differentiation of the gonads into ovaries (Wibbels et al., 1994), which can be explained by the aromatisation of testosterone to estrogen. Such data point to a crucial role for aromatase, the enzyme that converts testosterone to estrogen, in gonadal differentiation. Aromatase activity in the gonads of turtles with TSD is high at female producing temperatures, and low at male producing ones (Desvages and Pieau, 1992;

Desvages et al., 1993). Pieau et al. (1994b) have put forward a model based on temperature regulation of the expression of the aromatase gene. Relative levels of aromatase will determine whether or not the gonads will differentiate into testes or ovaries. Research into the nature of the regulatory elements (such as promoter regions) of aromatase gene is the focus of current research (Pieau, 1996)

Clarification of the physiology of TSD would only reveal half of the solution to this intriguing puzzle. What still remains is the question of why TSD exists, and what is its evolutionary significance. One general answer is that TSD allows offspring sex ratio to vary (Korpelainen, 1990). Fisher (1930) predicted that, given equal cost of either males or females, the offspring sex ratio should be in a 1:1 ratio at the time when parental investment ends. This can be understood by considering what happens when the sex ratio is not 1:1. If there are fewer of one sex, then these offspring will have a higher reproductive success. This in turn would favour production of more of the rarer sex, until the ratio balances out at 1:1 again.

There are several assumptions underlying Fisherian theory, including random mating in the population and biparentalism (two parents for each offspring) (for review, see Bull and Charnov, 1988). The violation of one could lead to an unequal offspring sex ratio. In the case of sea turtles, it is difficult to discern if the Fisherian assumptions are met, given the lack of basic information on population structure. For instance, it is not known whether or not most sea turtle populations are in equilibrium, which is another Fisherian assumption (for further discussion, see Mrosovsky, 1994).

Since Fisher's (1930) proposals, there have been suggestions that in certain situations one would expect offspring sex ratios to be different than 1:1. For example, if there is local mate competition between related males, then offspring sex ratios should be biased in favour of females (Bull and Charnov, 1988). In the case of certain invertebrates that may inhabit the same small patch for their entire lifetime, as do their offspring, one would expect competition for mates among male siblings. In such a scenario, it would be better for the parents to

produce one son and many daughters, since the one son could inseminate all the daughters thereby produce far more offspring than if equal numbers of each sex had been produced. Indeed, skewed offspring sex ratios have been found in such populations of mites (Amano and Chant, 1978). However, this situation depends on a high level of inbreeding: if there is even a low amount of admixture of offspring from different parents into the breeding population, the offspring sex ratios are pushed quickly back to 1:1 (Bull and Charnov, 1988).

Another suggested scenario for biased offspring sex ratios was first formulated by Trivers and Willard (1973) and reformulated by Charnov and Bull (1977) for species with ESD (environmental sex determination, of which TSD is a subset). Briefly, the Charnov-Bull model states that given two different environments, with one being more advantageous to one sex than the other (and vice versa), and given that the parents and offspring have no choice about which environment the offspring will enter, then ESD will be favoured over GSD. For instance, if in a more acidic environment it is better to be female (perhaps because lower pH fosters greater growth and hence greater fecundity), then those offspring that find themselves in a more acidic environment would be better off as females. On the other hand, in less acidic environments, which perhaps would impede growth, males might be favoured because they could still mate despite their smaller size (and decreased fecundity). In this hypothetical situation, large females have greater fitness (as measured by fecundity) than large males, while small males have greater fitness than small females. ESD, as characterized by females produced in acidic environments and males produced in basic environments, would be better than GSD, which would cause some of each sex to be produced in each environment.

In terms of reptiles and TSD, it has been difficult to pinpoint the fitness correlate to TSD. It has been suggested that warmer incubation temperatures foster greater adult size, and this would benefit one sex differentially in certain situations: in crocodilians, it is more advantageous to be a large adult male because of intermale competition for mates; for turtles, it is more advantageous to be female, because larger females have greater reproductive

success than larger males (Head et al., 1987). A study comparing different taxa found no correlation between adult sexual dimorphism and presence/absence of TSD in turtles and lizards (Janzen and Paukstis 1991a), but it may be the case that too few data are available for this type of study, as less than 10% of all reptile species have been investigated for mode of sexual differentiation (Ewert et al., 1994).

Differential fitness between the sexes might be detected before adulthood in species with TSD. Indeed, in the case of the American alligator (*Alligator mississippinesis*), which displays a FMF pattern of TSD (see Fig 1), female hatchlings produced at cool temperatures have significantly more residual yolk in their abdomens than males produced at median temperatures (Ferguson and Joanen, 1983). However, this increased yolk does not appear to cause fitness differences among hatchlings: when alligator hatchlings from different incubation temperatures are reared at constant temperatures for 18 months, those hatchlings produced near the FM pivotal temperature (see Fig. 1.1) are significantly larger and heavier than hatchlings from incubation temperatures that produce only one sex (Joanen et al., 1987). Therefore, there is little evidence that size is indicative of differential fitness of the sexes.

For turtle hatchlings, although there appear to be trends in some data to support the hypothesis that contrasting thermal regimes during incubation affect the fitness of the sexes differentially, the results are not very robust. Often, the differences in fitness due to genetic (clutch) effects are greater than those due to incubation temperatures (e.g. Bobyn and Brooks, 1994), or there were no significant differences in fitness due to different incubation temperatures (e.g. Janzen, 1996). Another puzzling point is that it has been found that in response to different incubation temperatures, body size and performance were affected differentially in male and female hatchlings and juveniles of pine snakes (*Pituophis melanoleucus*) and Australian skinks (*Bassiana duperreyi*) (Burger, 1989; Shine et al., 1995). Yet these two species do not display TSD! If differential fitness of males and females in different environments is postulated as the adaptive value of TSD, then one might expect that TSD would have evolved in these species.

In the case of sea turtles, it is difficult to understand why slight changes in sand temperatures in a 3 week or so period during incubation on a nesting beach should alter the fitness of males and females differentially. For example, leatherback sea turtles (*Dermochelys coriacea*) are long distance migrants, and live in many different environments, and yet a difference of 1 °C during incubation can cause an entire clutch of eggs to differentiate into 100% males or females. Despite their challenging nature, more studies are needed to help evaluate this hypothesis of differential fitness of TSD.

Of course, it could be the case that there is no adaptive advantage of TSD for sea turtles, and TSD simply works adequately for these organisms (Mrosovsky, 1980). But, in general, it is assumed that TSD exists in order to allow these animals to alter their offspring sex ratio (Korpelainen, 1990). Rather than concentrate on <u>why</u> this should be so, I will concentrate on <u>whether</u> this is so. Although TSD in sea turtles was first described in 1979 (Yntema and Mrosovsky, 1979), few data on offspring sex ratio exist. Even with TSD, it could be the case that sea turtles have 1:1 offspring sex ratios in general.

Indeed, some studies have suggested that sea turtle hatchling sex ratios are close to 1:1 (see Table 1.1). It should be noted that in most cases, the studies were limited to one or part of one season, the sampling may not have been random, and/or the estimates did not take into account variation in numbers of hatchlings produced during the nesting season (see column 5 in table 1.1). The most detailed study to date is on loggerheads (*Caretta caretta*) in Florida (Mrosovsky and Provancha, 1992). Data from five consecutive years show that the overall hatchling sex ratios in Florida are heavily biased towards females (roughly 90% female), although there was some year to year variation. In several additional years, it has been estimated that hatchling sex ratios are also skewed in favour of females (J. Provancha, personal communication).

Thus, some studies suggest relatively balanced sex ratios in sea turtle hatchlings, while others suggest highly skewed sex ratios. Clearly, more study is needed. It is important that the studies be long term, both within and across seasons (see Appendix 1). Often, the

nesting season of one population is several months long, during which hatchling sex ratio and relative numbers of nests laid change. An accurate estimate of seasonal sex ratio must take all these factors into account (see Appendix 1). Also most adult female sea turtles nest not only several times in one season, but in multiple seasons with intervals of 1-4 years

Table 1.1. Sex ratios of sea turtle hatchlings reported for different nesting beaches.

Species	Location	Year	Sex ratio % female	Comments Code	Reference
Caretta caretta	SC and GA, USA	1979, 80, 82	48.2%	A, E	Mrosovsky et al., 1984b
	Florida, USA	1987-89	~90%		Mrosovsky and Provancha, 1992
	Tongaland, South Africa	198?	~50%	A, D	Maxwell et al., 1988
	Mon Repos, Australia	1980-81	>50%	A, D	Limpus et al., 1983
	Heron I., Australia	1980-81	<40%	A, D	Limpus et al., 1983
Chelonia mydas	Tortuguero, Costa Rica	1988	40%	A	Horikoshi, 1992
	Tortuguero, Costa Rica	1980	67%	A, B	Spotila et al., 1987
	Suriname	1981	67.8%	A, B	Mrosovsky et al., 1984a
	Suriname	1982	55.2%		Mrosovsky et al., 1984a
	Heron Isl., Australia	1981-82	>50%	A, B	Limpus et al., 1983
Dermochelys coriacea	Pacific Mexico	1983/84	51.7%	C	Benabib, 1984
	French Guiana	1981, 83-85	ca. 50%	B, D	Rimblot-Baly, 1987

Table 1.1 continued

Species	Location	Year	Sex ratio (% female)	Comments Code	Reference
Dermochelys coriacea	Suriname	1982	60.5%	A	Mrosovsky et al, 1984a
	Malaysia	1986	100%	A, E	Chan and Liew, 1995
	Pacific Costa Rica	1994/ 95	93.5%	A, B	Binkley, 1996
Eretmochelys imbricata	Antigua, Caribbean	1989-90	not highly female-biased	B, F	Mrosovsky et al., 1992
	Milman Isl., Australia	1991	>50% female	A, B, E, F	Loop et al., 1995
	Buck Isl., USVI	1995?	>90% female	B, G	Wibbels and Hillis, 1996

Comments code:

A - Estimate based on small sample size

B - Only part of season was studied

C - Based on unreliable sexing method (see Mrosovsky and Benabib, 1990)

D - Changes in nesting frequency not taken into account

E - Estimate based on relocated nests

F - No *in situ* nests actually sampled

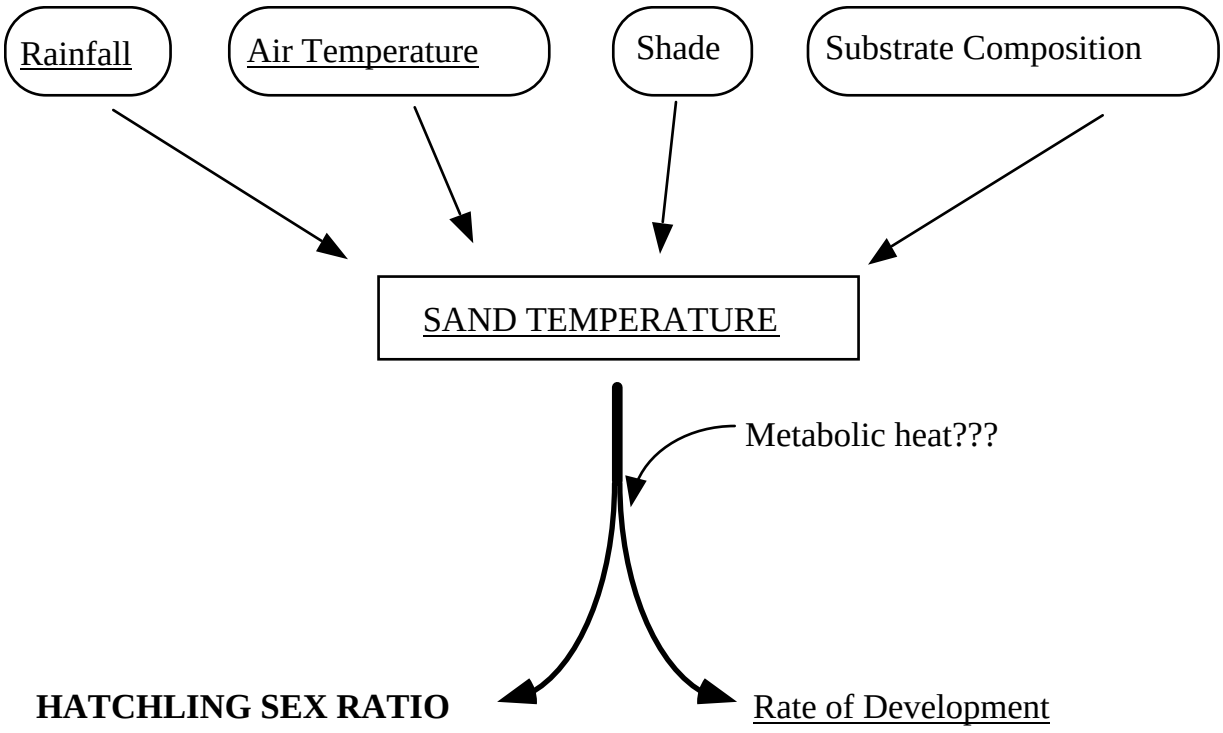
G - Only dead hatchlings investigated

between them (Ehrhart, 1982). Therefore, in a nesting population, only a portion of females of reproductive age will lay eggs in a particular season. A fair estimate of overall population hatchling sex ratio should be based on several years. In addition, given that nesting seasons are usually several months long, and given the variation in weather (and hence sand temperature) both during and among seasons, one might expect there to be variation in sex ratios during different years. It could be the case that overall sex ratios from several different years is close to 1:1, despite biased sex ratios in individual years (cf. Janzen, 1994b).

The challenge in such studies is to devise a method to predict sex ratios for a number of years. One of the logistic difficulties with such studies is the sexing of sea turtle hatchlings. At emergence, sea turtle hatchlings lack distinct morphological differences between the sexes. The method of sexing has been limited to investigation of the gonad, either by gross morphology or histological examination (Yntema and Mrosovsky, 1980; Rimblot-Baly et al., 1987; van der Heiden et al., 1985). Although gross morphology can be used in limited cases, overall the most reliable method at the moment is histological examination (Whitmore et al., 1985; Rimblot-Baly et al., 1987). The main drawbacks to the histology method include its labour intensive nature and that the sampled hatchlings are killed (but see Appendix 2 for details on mitigation). A new method of sexing hatchlings, by measuring ratios of steroids in the allantoic fluid left in the egg shell after hatching (Gross et al., 1995) is promising, but remains to be validated in different species and in different incubation conditions. In addition, the logistic problem of sampling the allantoic fluid before hatching, and subsequent cross contamination of fluid from different hatched eggs within a nest, makes it unlikely for this method to be useful in extended field studies.

Histological examination is laborious and the collection of samples during a season is time-consuming. In addition, the use of these methods alone will only

Figure 1.2. Possible environmental correlates that might be used as indices of sex ratios of turtle hatchling. Those underlined are measures which have been used (either in this thesis or in published studies).


Rainfall
Air Temperature
Shade
Substrate Composition
SAND TEMPERATURE
Metabolic heat???
HATCHLING SEX RATIO
Rate of Development

generate sex ratio data from the season the samples were studied. For long-term studies, it would be advantageous to collect data on some easily-measured variable that is correlated with sex ratio, and use this information to predict hatchling sex ratios (Fig. 1.2). The most obvious variable is sand temperature. Once collected, sand temperature data could be used to predict hatchling sex ratios, which could then be combined with relative nesting data to generate a seasonal hatchling sex ratio estimate. Although sand temperature information has been collected on nesting beaches for several years, most methods traditionally used were laborious, the equipment expensive, or the techniques flawed (e.g. not allowing time for equilibration of sand after burying temperature equipment on a beach, before recording sand temperature data [e.g. Hays and Speakman, 1993]). For these reasons, it would be beneficial to design a simple new method of collecting nesting beach sand temperatures that would both be less laborious and expensive.

The use of sand temperature as an indicator of sex ratio also requires information on the pivotal temperature of a particular population of sea turtle species, unless sand temperature data have been directly correlated to sex ratios of previously sampled turtle clutches from that area. Metabolic heat generated by developing eggs within a clutch increases the temperature of the nest, relative to the sand (e.g. Morreale et al., 1982; Maxwell et al., 1988). It might be necessary to add a correction factor to sand temperatures in order to predict the true sex ratio of a clutch of eggs incubating in that sand, particularly if the sand temperature is close to pivotal temperature. Such metabolic warming has never been quantified using a large number of nests.

Sand temperatures are not the only type of environmental variables which have been used to predict sex ratios of reptiles. Air temperatures, relative amount of vegetation cover, and beach sand albedo are known to be correlated with changes in sand temperatures at nest depth and/or turtle hatchling sex ratios (Limpus et al., 1983; Janzen, 1994a,b; Hays et al., 1995). Other ecological variables also might be correlated to nest temperatures and therefore hatchling sex ratios. If data on such variables were collected in the past, one might be able to

reconstruct historical sex ratio estimates, thereby extending and enhancing overall sex ratio information for a population.

The main emphasis of this thesis is on determining overall sea turtle hatchling sex ratios. Because of the methodological problems outlined above, it is necessary to develop different procedures to aid in estimating sex ratios. Therefore, the structure of this thesis is as follows:

Chapter 2: Outline a new technique of collecting sand temperature data on nesting beaches.

Chapter 3: Directly estimate sex ratios of two species of sea turtles on a single nesting beach in Suriname, and attempt to estimate past sex ratios from historical records of environmental variables.

Chapter 4: Attempt to accurately describe the pivotal temperature of the Suriname green turtle population.

Chapter 5: Quantify the metabolic heat generated by developing sea turtle eggs, in order to assess its potential impact on hatchling sex ratios.

Chapter 6: Outline a method of indirectly estimating sex ratios of sea turtle hatchlings from the rates of development of the eggs.

Chapter 7: Estimate the time it takes for hatchling sea turtles to emerge from the nest after pipping from the eggs (which is useful in the method outlined in Chapter 6).

The appendices contain information pertaining to the application of sex ratio studies to sea turtle conservation. Although it is the theoretical problem of the ecological and evolutionary significance of TSD in sea turtles this thesis addresses, it is hoped that this information also will be of practical use in the design and implementation of successful management plans for these “noblest of God’s creatures” (Gaffney and Meylan, 1988).

Chapter 2:
A Simple Method of Estimating Mean Incubation Temperatures on Sea Turtle Beaches

Introduction

For studies of sex ratios in reptiles, it is important to know about incubation temperatures. Soil temperature at nest depth varies over the course of 24 hours. This is true even for marine turtles whose eggs are deposited well below the surface. Therefore, to obtain a mean daily temperature, frequent readings are required. A datalogger is one way to provide these, but there are some associated disadvantages. Dependence on a single piece of equipment may be a drawback in isolated places. In more accessible places there may be a risk of theft. Dataloggers are expensive and may not be suitable where recording sites are far apart, because long leads are then required to connect the thermosensors to the recorder. A way to avoid relying on a datalogger to obtain a daily mean temperature is simply to take measurements manually every few hours. However, such a labor intensive procedure can seldom be sustained for long periods.

We have developed a compromise procedure that includes sufficient automation to avoid having to take readings frequently, but is cheaper than a datalogger, and also enables monitoring of widely scattered sites. This consists of a module that memorizes the maximum and minimum temperatures since it was last cleared. For beach temperatures at sea turtle nest depth, the mean of the maximum and the minimum temperatures over a 24 hour span is very close to the mean based on frequent readings over the same period.

Materials and Methods

The maximum minimum memorizer consists of a commercially available thermistor with a digital readout (Radio Shack #277-302, $Can 22.42 in 1992; also #630-1020 can be used) powered by an alkaline AA battery. The module is encased in clear acrylic (Plexiglas) with water-tight seals, and its displays can be activated via magnetic reed switches without opening its case (Plate 2.1). The type of magnetic switch is not critical but those found in security devices for doors and windows are generally suitable. A wiring diagram is provided in Fig. 2.1. The display shows the current temperature. The maximum temperature since the time when the module was cleared is obtained by first activating reed switch A and then reed switch B. The minimum temperature since the time when the module was cleared is obtained by first activating reed switch A and then reed switch C. To clear the memory, a magnet is held over reed switch A while simultaneously activating reed switch B with another magnet, and then repeating the process with reed switch C. To avoid unwanted simultaneous activation of reed switches, these should not be mounted too close to each other within the Plexiglas case.

Calibration: different probes do not give exactly the same readings and each must be calibrated separately against a good quality mercury thermometer (accuracy 0.1 °C). Periodic calibration is advised to detect the occasional faulty or erratic system. Although the relationship between readings from the memorizer module and a standard mercury thermometer is close to linear from 23 - 36 °C, slightly different corrections must be made at different temperatures (see Fig. 2.2, for example). We did not detect any change in the readings obtained from a thermistor probe kept at constant temperature when the module and its housing were left in icy water or in front of a warm air blower.

Plate 2.1. Maximum-minimum memorizer in its casing. Top shows digital display at front. Bottom shows reed switches (arrows) and battery at back. Scale bar = 1.5 cm. The probe can be uncoiled and buried about 1 m away from the casing.

FOR PLATE 2.1, SEE FIGURE 1 IN: Godfrey, M. H. and Mrosovsky, N. (1994). Simple method of estimating mean incubation temperatures on sea turtle beaches. Copeia 1994: 808-811. Available here: http://members.seaturtle.org/godfreym/Godfrey_1994_Copeia.pdf

Figure 2.1. Wiring diagram. A, B, and C are positions of magnetic switches.

FOR FIGURE 2.1, SEE FIGURE 2 IN: Godfrey, M. H. and Mrosovsky, N. (1994). Simple method of estimating mean incubation temperatures on sea turtle beaches. Copeia 1994: 808-811. Available here: http://members.seaturtle.org/godfreym/Godfrey_1994_Copeia.pdf

Figure 2.2. Calibration curve (circles and dashed line) for an individual module against a good quality mercury thermometer. If no calibration corrections were required, the points would fit on the solid line.

FOR FIGURE 2.2, SEE FIGURE 3 IN: Godfrey, M. H. and Mrosovsky, N. (1994). Simple method of estimating mean incubation temperatures on sea turtle beaches. Copeia 1994: 808-811. Available here: http://members.seaturtle.org/godfreym/Godfrey_1994_Copeia.pdf

Results

Three sets of data were analysed to determine how well the mean of the maximum and minimum over 24 hours corresponds to the mean based on more frequent readings. First, data from a study of sand temperatures on a hawksbill turtle nesting beach in Antigua were analyzed (Mrosovsky et al., 1992). In that study, sand temperatures were read with a BAT 12 (Sensortek Inc.) digital thermometer every two hours from thermocouples previously buried 30 or 60 cm deep in the sand. Twenty separate round-the-clock recordings of this type were analysed. The average of the maximum and minimum values was slightly lower than the average of the round-the-clock readings. The mean difference between the two methods of determining daily average sand temperature was 0.08 °C (range: 0.02 - 0.22, n = 20). A typical example is shown in Fig. 2.3. Second, in another check using the maximum minimum memorizer modules themselves, the daily means of sand temperatures at 4 different 30 cm sites at St. Croix were assessed by the two methods: the values based on readings taken every two hours over a 24 hour period ranged from -0.04 less to +0.03 °C more than the means obtained by averaging the maximum and minimum values over the same 24 hour period. Third, sand temperatures were studied in Suriname at Matapica: this is a beach where both green turtles and leatherbacks nest. Temperatures were read from the maximum minimum memorizer modules themselves every 2 - 3 hour round-the-clock at 30 and at 60 cm depth on 6 different dates from April to August (n = 12 round-the-clock readings). The means obtained by averaging the round-the-clock readings ranged from -0.21 less to +0.21 °C more than those obtained by averaging the maximum and minimum values. The mean difference between the two methods was 0.03 °C, with the round-the-clock method giving the marginally higher value.

Figure 2.3. Squares and solid line show temperatures taken every 2 hours at a single site over a day. Dashed line shows the average temperature of all time points. Solid line shows average temperature as determined from the maximum and minimum temperatures.

FOR FIGURE 2.3, SEE FIGURE 4 IN: Godfrey, M. H. and Mrosovsky, N. (1994). Simple method of estimating mean incubation temperatures on sea turtle beaches. Copeia 1994: 808-811. Available here: http://members.seaturtle.org/godfreym/Godfrey_1994_Copeia.pdf

To check on the durability of the casing, 5 modules were buried in the sand on Buck Island, St. Croix, US Virgin Islands, for 2 months. No signs of moisture within the casing were detected. The casing also withstood 6 months of more or less continuous use in Suriname.

Discussion

The maximum minimum memorizer provides two temperatures from which an estimate of mean daily temperatures can be made. Errors in these estimates can be in the order of 0.1 - 0.2 °C (but on average are smaller). These errors are small, and are comparable to the accuracy of recording equipment used in other studies of sea turtle ecology. Moreover, the present method overcomes a number of problems with other methods. It provides flexibility in its choice of recording sites, and it is far less time consuming than round-the-clock measures. Some investigators have taken temperatures at a single time of day, one selected on the basis of previous round the clock readings to correspond to the daily mean (e.g. Mrosovsky and Provancha, 1989). However, the validity of that approach depends on relative consistency of weather conditions.

We consider that the maximum minimum memorizer is valuable for simplifying long-term studies of sea turtle beach temperatures. Provided that the average of the maximum and minimum is validated against other estimates of daily mean temperature, this approach may also be useful for monitoring of nesting sites of other reptiles.

Chapter 3:
Estimating past and present sex ratios of sea turtles in Suriname

Introduction

Temperature affects sexual differentiation in sea turtles, as it does in many other reptiles (see Raynaud and Pieau, 1985; Ewert and Nelson, 1991; Janzen and Paukstis, 1991b, for reviews). For sea turtles, the thermosensitive period for temperature dependent differentiation occurs in the middle third of incubation of the eggs (Yntema and Mrosovsky, 1980; cf. Desvages et al., 1993). Sea turtles, being iteroparous, have nesting seasons spanning several months. Weather on nesting beaches usually varies over this period, and the associated thermal differences result in seasonal changes in the sex ratio of hatchlings (Limpus et al., 1983; Mrosovsky et al., 1984a, b; Benabib, 1984; Rimblot-Baly et al., 1987; Maxwell et al., 1988).

Such seasonal variation in sex production was monitored in 1982, for green (*Chelonia mydas*) and leatherback (*Dermochelys coriacea*) turtles nesting in Suriname (Mrosovsky et al., 1984a). When these data were combined with information about the numbers of turtles nesting in different months, it was possible to estimate the overall sex ratio. For both species in 1982, the overall sex ratio was calculated to be relatively close to 1:1.

In assessing these figures, the possibility that 1982 was an atypical year should be kept in mind. This is a general problem with single season studies of natural sex ratios in reptile populations (Mrosovsky, 1994). In the case of the 1982 season in Suriname, the authors of that study documented one way in which that year was atypical: leatherback turtles nested later in that season compared to 11 other years for which there are data (see figure 8, Mrosovsky et al., 1984a). Because the sand temperature becomes warmer towards the end of the season in Suriname, the atypical, late nesting by the leatherbacks in 1982 probably increased the percentage of leatherback hatchlings that were female. To be more

confident about estimates of natural sex ratios in Suriname, and other beaches in general, data from a number of years are needed. Also, since individual sea turtles do not usually nest in consecutive years, sex ratio estimates spanning more than a single year provide a more accurate sample of the entire nesting population.

A study lasting 5 years assessed the sex ratio and thermal environment of loggerhead turtles (*Caretta caretta*) nesting in the USA. It was estimated that approximately 90% of the hatchlings produced in southeast Florida were female (Mrosovsky and Provancha, 1992). Even in this case, one must consider the possibility that a string of 5 exceptionally warm years may have been responsible for this appreciable female bias. Several considerations argue against this. First, Wibbels et al., (1991) reported a female bias in juvenile loggerhead turtles inhabiting Florida coastal waters between 1984 and 1986. Presumably, these juveniles were born before 1986, the first year of Mrosovsky and Provancha's 5 year study; moreover, the juveniles probably included groups from a number of different years. Second, the sand temperatures at nest depth in Florida were generally well above the pivotal temperature (that temperature of constant incubation that results in an equal number of each sex; see Mrosovsky and Pieau, 1991, for definitions). Therefore, even somewhat cooler conditions would still have given female-skewed ratios. Third, during an additional 3 years of monitoring, sand temperatures have generally been above the pivotal level (J. Provancha, per. comm.).

Taken together, these points support the findings that the sex ratio of loggerhead hatchlings in Florida is strongly female biased. This is in contrast to sex ratios of green and leatherback turtles in Suriname, which were estimated to be closer to 1:1 in 1982 (Mrosovsky et al., 1984a). This makes it all the more interesting to discover whether or not 1982 was an anomalous year for sex ratio, or reflects a species difference on population structure, or represents a particular feature of sea turtles nesting in tropical regions. Therefore, further study of the sex ratios of turtles in Suriname is warranted.

There is also a practical reason for wanting more information about the sex ratios of turtle hatchlings in Suriname, and their seasonal sex ratio production profiles. A legal harvest of sea turtle eggs in Suriname is part of the national conservation programme. For two months of the season, the federal government grants permission to the Foundation for Nature Preservation in Suriname (STINASU) to collect eggs, which are sold at a set price in the local markets. The attractions of this system as a conservation measure are discussed elsewhere (Schulz, 1975; Reichart, 1982; Mrosovsky, 1983a). One potential problem, however, is that the timing of the harvest may alter the overall sex ratio, since the relative production of males and females differs over the season. The potential impacts of altered sex ratio in wildlife populations which are selectively harvested should be considered in management plans (Ginsberg and Milner-Gulland, 1994).

In the present work, we studied the natural sex ratio of green and leatherback hatchlings over the whole 1993 nesting season in Suriname, and part of the 1994 nesting season. The immediate aim was to arrive at more confident estimates of hatchling sex ratio than those based on previous data from a single year 1982. As a further step in that direction, we estimated sex ratios in an additional 12 previous years, by making use of correlations between sex ratio and rainfall. Our wider aim was to be able to compare sex ratios in different years, and to come closer to an understanding of overall average sex ratios of sea turtles, and their possible variation among different populations and species.

Materials and Methods

Study Site

The turtle populations in Suriname were the same as those studied in 1982, but the nesting beach had moved west some 20km. The North Equatorial Current constantly erodes the beach at its eastern end and adds sand to the western end, resulting in a net movement of about 2km/year (for more details, see Schulz, 1975). In 1993 and 1994, the beach began next to the Matapica Canal and ran some 8km west, ending in a sand spit separated from the mainland by a brackish lagoon. At the western tip of the spit, which is the most newly formed, there was very little vegetation cover; east of the spit, in older sections of the beach, low lying beach creeper (mostly *Ipomea-pes-caprae* and *Canavalia maritima*) ran parallel to the high tide line. The vegetation was separated from the high tide line by about 10m of open sand.

Nesting Frequency

The beach is used by nesting green and leatherback sea turtles. Although the nests of both species were found along the entire stretch of beach, in general the leatherbacks preferred the most western 3km of the spit, while the greens most often frequented the middle 3km of the beach. We surveyed two 1km subsections of the beach to obtain seasonal profiles of relative nesting frequencies over the different parts of the season. One subsection was located in the preferred green nesting area, the other in the preferred leatherback nesting area.

Beginning 3 March and ending 1 September 1993, we patrolled both subsections nearly every day. We recorded all nests laid the previous night. According to their position on the beach, the nests were categorized into one of 4 possible zones: a) vegetation, 45-100% vegetation cover; b) border, 5-45% vegetation cover; c) open, 0-5% vegetation; d) doomed, below the spring high tide line. Nests laid in the doomed zone were likely to be destroyed by high tides (hence the name). Nesting frequency was expressed as the number

of nests laid per half month period. There were a few gaps in the nesting survey because we were away from the beach on the following days: 27-30 March, 27-30 April, 18-21 May, 12 June, 30 June-4 July, 26-27 July, 17-20 August. In these cases, nesting frequency per half month interval was calculated by extrapolating from the number of nests laid on days that were patrolled, to the total number of days in the half month period.

Sand Temperature and Rainfall

Sand temperatures were monitored in two beach transects, one in each subsection of the beach used to survey nesting frequency. In the green turtle nesting subsection, the temperature transect consisted of sites in the vegetation, border, and open zones of the beach. At each of these sites, temperatures were measured at 30cm and 60cm depths. In the leatherback nesting subsection, there was very little vegetation, so the temperature transect was divided into the following 3 sites: high, middle, and low. In terms of distance from the spring high tide line, these sites corresponded roughly to those in the green transect. Temperatures were also measured at 30cm and 60cm depths at each site in the leatherback transect.

To assess which depth was more representative of mid-nest depth, we measured the distance from the surface of the sand to the top and to the bottom of several nests; the midpoint of these distances for each nest was the mid-nest depth.

We used two methods to obtain values for mean sand temperatures over 24 hr periods at each site. The first method depended on a digital thermistor and circuitry specially designed and adapted for recording sand temperatures on turtle beaches. These instruments memorized the maximum and minimum temperatures since last cleared. By calculating the mean of the maximum and minimum from a 24 hr period, we obtained a close approximation of the mean temperature over that period (see Chapter 2, for validation of this procedure and description of the apparatus).

The second method utilized type-T (copper/constantan) thermocouples (BAT-12, Sensortek, Inc.) to measure temperature at a single time of day. These readings were adjusted to give mean daily temperatures through a simple arithmetic transformation, based on a 24 hr thermal waveform. In total, 5 waveforms, profiling patterns of daily temperature variation, were generated by a series of round the clock readings, made at 3 hr intervals. The adjustments to the single readings were calculated by comparing that reading to the waveform of the nearest calendar date; this was at most 4 weeks later or earlier, and usually less.

Both thermistors and thermocouples were calibrated against good quality Hg thermometers, which themselves had been calibrated against Sybron/Taylor mercury Hg thermometers. The latter had certified calibration against platinum resistance thermometers that had been calibrated by the U.S. National Bureau of Standards. Calibration checks on all equipment were done before the start of the season, with two additional checks during the season, and one shortly afterwards.

Temperature transects were visited at least 4 times per half month, except in early March, when they were only visited twice. The thermistors and thermocouples were read at 07:00 ±30 minutes. Both temperature transects were monitored on the same days.

Rainfall, measured with a standard rain gauge, was recorded on those days that the nesting survey was conducted. Daily rainfall per half month was calculated by summing daily amounts and dividing by the number of days recorded. Daily rainfall per month was calculated in a similar manner.

Assignment of nests to sex ratio sample

For each half month period, the aim was to obtain a representative group of 10 clutches per species. At the beginning of each half month, we patrolled the beach every morning for nests laid the night before. Nests were located with a metal probe and marked with a stick and a numbered card. The first nests encountered were assigned to the sex ratio

study. This ensured that the nests were representative of the spatial distribution of nests on those days. It was necessary to patrol the beach on successive mornings in order to find enough nests to be marked. It was decided beforehand to mark more nests than needed, to allow for failure of some nests to hatch, predation, and the inevitable loss of some nest labels to heavy winds or exceptionally high tides. A total of 161 green turtle and 130 leatherback nests were marked, but only 79 and 27 respectively, were sampled.

The departures from these procedures were the following: a) two green turtle nests laid before we arrived at the beach were included in the sex ratio sample. They were located and marked by personnel of STINASU and the dates of laying recorded (23 February, 3 March); b) doomed clutches laid below the high tide line were not marked; c) sometimes, unexpectedly high tides would wash over marked nests. Daily observations were made of all nests susceptible to such wetting; d) 2 green turtle nests used in the sex ratio study had small (1x4x1cm) motion detectors placed at the top of the egg mass on the morning after laying.

Incubation duration and hatchling collection

After about 50 or 55 days of incubation, for green turtles and leatherbacks respectively, traps of wire netting were placed over the nests. Thereafter, these traps were checked daily around dawn. Any hatchlings encountered in the morning were considered to have emerged during the night; incubation duration was calculated as the number of days between the night the nest was laid and the night it emerged.

Upon discovery of an emergence, the mass of hatchlings was stirred and 10 turtles were selected at random. The remainder were either allowed to crawl to the water or taken for behavioural tests, and later released. In some cases, the random selection process was compromised (e.g., some hatchlings emerged outside the trap, or had been killed by a predator, etc.); these nests are flagged in Table 2. In addition, there were some occasions when we could not place a trap on a nest or the expected emergence date was on a day when we could not be on the beach. Some of these nests were dug up prior to emergence and 10 hatchlings below the surface were randomly selected for the sample. If the hatchlings were within 10cm of the surface, it was assumed they would have emerged the next night. Otherwise, no incubation duration was assigned to these nests.

Histology and Sexing

The gonads from sampled hatchlings were fixed in 8% buffered neutral formalin saline and transported to Toronto, with the required CITES documentation. The samples were prepared for histology as described by Mrosovsky et al. (1984a). Briefly, one gonad from each animal was cut in half transversely and one half was embedded in paraffin wax. 10 µm serial sections were made with a microtome from the cut end of the gonad, and the sections were mounted on slides for staining with PAS and Harris's heamatoxylin.

Sex was determined by examining the sections under a light microscope and classifying them by the criteria given by Yntema and Mrosovsky (1980) and Dutton et al. (1985). Briefly, female gonads are characterized by a thick, convoluted cortex with a PAS-

positive tunica albuginea between the medulla and the cortex. Male gonads are characterized by a very thin smooth cortex and the presence of seminiferous tubules in the medulla. A few green turtle hatchlings had gonads with both male and female traits, and were labeled intersexes. The gonads of leatherback turtles are less developed than other turtles at hatching (Rimblot-Baly et al., 1987); therefore, some were labelled as indeterminate. These indeterminate hatchlings may have been undifferentiated males or females, or they may have been intersexes.

1994 nesting season

From 7 May to 12 July 1994, we recorded sand temperatures in the same way, except that the green transect site was approximately 2 km west of the 1993 transect. This was because the preferred nesting area of green turtles had moved west between the 1993 and 1994 seasons. Daily rainfall was also recorded during this period. No hatchlings were taken for sexing in 1994.

Sources of data from previous years

Nesting distribution data for 12 years came from several sources. For 1969-75, the figures came from Schulz (1975). Data for 1979-1981 came from H. Reichart and J. Schulz (pers. comm.), for 1982 from Mrosovsky et al. (1984a), and for 1993 from the present study. All these figures are for green and leatherback turtles nesting on the same sand spit that has moved west since 1967 from Bigisanti to Krofajapasi, and then to Matapica.

Historical monthly rainfall data came from the Meteorologische Dienst Suriname, which monitored rainfall at the Matapica Station, near the head of the Matapica Canal. In some of the years, rainfall values for months in the nesting season were not recorded; if only one or two monthly values were missing, we substituted mean monthly totals from Schulz (1975), which were based on rainfall data collected from 1953-1971. Years in which more than two monthly totals were missing were excluded from the analysis. The final rainfall

data set included the following years: 1954 and 1957 (as reported by Schulz, 1975); 1970-75, 1980-81, 1985, 1991 (Meteorologische Dienst Suriname records); 1982 (Mrosovsky et al., 1984a); and 1993 (the present study).

Data analysis

Temperatures were grouped according to depth. For each depth we compared mean daily temperatures from the two different methods at different sites within and between transects, using repeated measures ANOVA, with Tukey-Kramer multiple comparisons test *post hoc*. On a few occasions (2.3 % of maximum number possible), some data from individual sites at individual depths were not recorded for various reasons (e.g., battery failure). If temperature data from one site at one depth were not recorded on one particular visit, all corresponding temperature data from the other sites for that day were excluded from the statistical comparisons.

Sex ratio is expressed as percent female, unless otherwise stated. For statistical analyses, sex ratios of individual clutches were subjected to an arcsine transformation (Zar, 1984).

Results

Sand Temperature

Sand temperatures varied over the season (Fig. 3.1). For both depths, at each site in each transect, the mean temperatures estimated by thermistors and thermocouples gave similar profiles, except in the 60cm border site in the green turtle transect ($p<0.05$). However, this single difference is probably not important, since the temperatures of the open and border sites at 60cm depth were not statistically different, for either method. Given that the two methods of temperature recording agreed well, for simplicity we report only data measured by the thermistors.

The mean mid-nest depths were 60.7cm ±2.1SEM for green turtles (n=8) and 63.7cm ±3.1SEM (n=11) for leatherbacks. Therefore, the temperatures measured at 60cm depth were more representative of the incubation temperatures experienced by the turtle eggs. At 60 cm depth, the average daily fluctuation of the sand temperature around the daily mean was ±0.22 ºC, based on daily maximum and minimum temperatures collected at the temperature transects in 1993 (n=307). Such a small variation around the mean probably did not have an effect on the sex ratio; for incubating loggerhead turtle eggs, a daily variation of ±1 ºC (which is 5 times greater than the 0.22 ºC variation we recorded) adds only ±0.1º C to the "constant temperature equivalent" (Georges et al., 1994).

There were no significant differences between temperatures recorded in the leatherback and green nesting transects, except that the vegetation site at 30cm and 60cm in the green transect was warmer than all other sites at corresponding depths ($p<0.001$). This vegetation site was higher above the water table than all the other sites, and this probably contributed to its thermal distinctiveness.

Figure 3.1. Mean sand temperatures of Matapica beach, Suriname in 1993. Only data recorded by the digital thermistors (see methods) are shown. Horizontal lines show pivotal temperatures for green and leatherback turtles in the Guianas (Mrosovsky et al., 1984b; Rimblot-Baly et al., 1987). See methods section for definition of zones.

FOR FIGURES IN CHAPTER 3, SEE Godfrey, M. H., Barreto, R. and Mrosovsky, N. (1996). Estimating past and present sex ratios of sea turtles in Suriname. Canadian Journal of Zoology 74: 267-277. Available here: http://members.seaturtle.org/godfreym/Godfrey_1996_CJZ.pdf

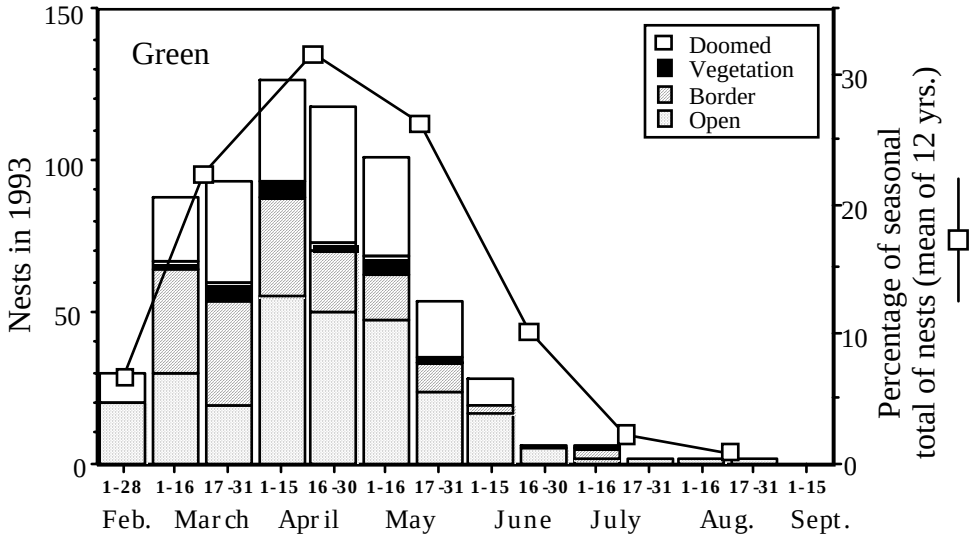
Nesting Frequency and Sex Ratio

For both species, the relative frequency of nests laid in the different months of the 1993 season follows the general pattern from 12 years (Fig. 3.2). The spatial distribution by different beach zones of nests that actually contributed to the sex ratio sample was similar to that for all nests laid in the areas surveyed (Table 3.1). The relatively high number of doomed nests is consistent with previous studies (Mrosovsky, 1983b; Mrosovsky et al., 1984a).

Sex ratios of green turtle nests produced in different zones were compared in each half month period, when there were enough nests in the sample to test for significance. There were no significant differences in the mean sex ratios in the open and border zones, except in the first half of March (61% female vs. 94% female, respectively; $p<0.05$, two-tailed t-test, with Bonferroni correction; Table 3.2). This slight difference may have been due to sampling bias, since there was no difference in mean sand temperatures between open and border sites at 30cm or 60cm depths in the second half of March, which corresponds to the thermosensitive period of nests laid in early March. In both the March 1-16 and April 1-15 periods, a single green turtle nest laid in the vegetation produced 100% females. Although this number is too small for statistical analysis, there does appear to be a trend to produce more females in the vegetation. This is consistent with the warmer sand temperatures in the vegetation zone in the green turtle preferred nesting area (Fig. 3.1). Nevertheless, since so few nests are laid in the vegetation zone and since there was no real difference in sex ratios of nests in the border and open zones (Table 3.1), the spatial distribution of nests on this beach does not strongly influence the overall seasonal sex ratio.

For both species, some of the nests sampled for sex ratio were washed over by high tides during incubation (Table 3.1). Of these, only 7 green turtle and 10 leatherback nests were washed over during the middle third of incubation, the

Figure 3.2. Mean nesting distribution of green and leatherback turtles at Matapica, Suriname. Vertical axis on the left is total number of nests laid in 1993; each half month period is divided into numbers of nests laid in each zone (histogram). Vertical axis on the right is the mean of 12 years of data (including the present study) on nest distribution (see methods for sources). Data for green turtle nests in February are based on mean nesting distribution data in Schulz (1975): on average, 6.5% of all nests in the season are laid in February. The percentage of doomed nests in February is based on the mean number of doomed nests for the other months in 1993.


Green
Doomed
Vegetation
Border
Open
Nests in 1993
Percentage of seasonal total of nests (mean of 12 yrs.)
150
100
50
0
30
20
10
0
1-28 1-16 17-31 1-15 16-30 1-16 17-31 1-15 16-30 1-16 17-31 1-16 17-31 1-15
Feb. March April May June July Aug. Sept.

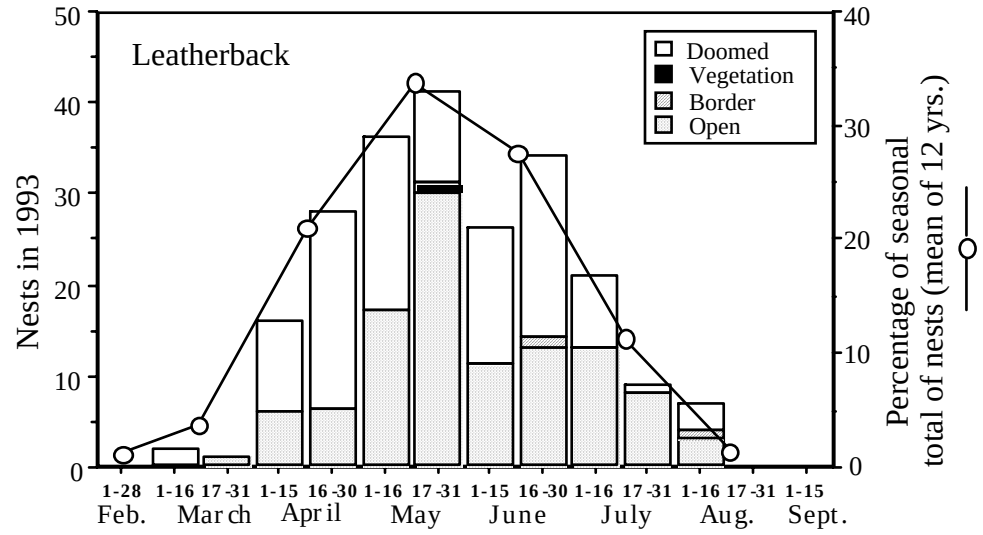

Leatherback
Doomed
Vegetation
Border
Open
Nests in 1993
Percentage of seasonal total of nests (mean of 12 yrs.)
50
40
30
20
10
0
40
30
20
10
0
1-28 1-16 17-31 1-15 16-30 1-16 17-31 1-15 16-30 1-16 17-31 1-16 17-31 1-15
Feb. March April May June July Aug. Sept.

Table 3.1. Distribution by zone of turtle nests at Matapica, 1993.

	Green turtle nests		Leatherback nests	
	Sampled for sex ratio	In nesting survey	Sampled for sex ratio	In nesting survey
Above high tide line	n=79	n=327	n=27	n=111
Vegetation	6.4%	5.6%	0%	0.9%
Border	31.6%	35.6%	0%	1.8%
Open	62%	58.8%	100%	97.3%
Washed over	12.7%		40.7%	
Below high tide line		n=194		n=110

Table 3.2. Percentage female hatchlings in samples of 10 per clutch from different zones.

	Feb.	March		April		May		June		July		Aug.
Zone	All	1-16	17-31	1-15	16-30	1-16	17-31	1-15	16-30	1-16	17-31	1-16
						Green turtle						
open		40[e]	60[e]	40	20	10	0	50	90(1)	100	100	100
		50[b]	80[f]	50	20	30	40	70	100			
		70	80[f]	70	30	30	50	90	100			
		80[c]	100	80	40	30	60(1)	90	100			
				90	50	60	60	90	100			
					80	70[f]	60	100	100			
						100	80		100			
bor-	70	70	60	70	30		14[g]	100		100		
der		89[de]	60	80[f]	40		80			100		
		90	70	90	70		100			100		
		100[c]	70	100	100							
		100[a]										
vege			100	100		0				100		
tation						60						
						Leatherback						
open				30	0[f](1)	0(1)	40	100[c]	100	100		
				90	40	33[h]	40	100	100[a]	100		
				100			50(3)	100	100	100		
							60(1)			100		
							70			100		
							86[g]			100		
										100		
										100		

Table 3.2 continued

NOTE: Values in parentheses give the number of intersexes, if any, in the sample from a green turtle clutch and the number of indeterminate gonads, if any, in the sample from a leatherback clutch. Because sex ratio for each nest was based on only 10 samples, the confidence limits for the sex ratio estimate of individual nests can be quite large. For example, for nests that were calculated to be 50% female, the confidence limits are ±32%, and for 80% female, ±28%. However, because there was a limit to the number of hatchlings we could sample, we decided to take fewer hatchlings from more nests, rather than take more hatchlings from fewer nests.

[a]Some hatchlings in clutch emerged outside trap

[b]Based on n=4

[c]Based on n=5

[d]Based on n=9

[e]A 1x4x1cm motion detector was just above clutch

[f]Some hatchlings in clutch killed by a predator

[g]Based on n=7

[h]Based on n=6

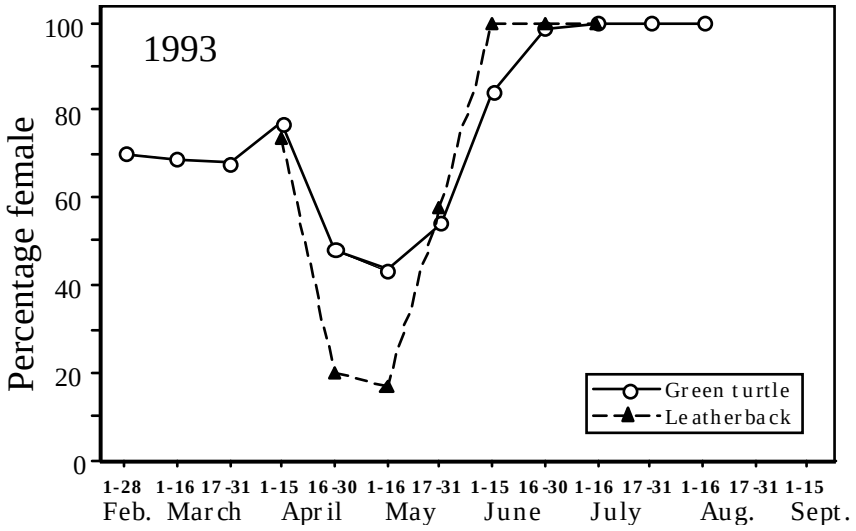
time which roughly corresponds to the thermosensitive period for sexual differentiation. The sex ratio of these 17 nests were compared to the sex ratio of dry nests, if any, laid within 3 days. The small sample sizes preclude statistical analysis, but trends toward male biases were evident only in washed-over nests with thermosensitive periods in May (n=1 for each species). Sand temperatures in May were close to pivotal (Fig. 3.1), which would permit cooling from washovers to have a masculinising effect. For nests laid in June and July, the sand temperature during the thermosensitive period was well above the pivotal temperature, so any wetting of nests by high tides would not be expected to reduce the numbers of females.

Although no leatherback hatchlings from nests laid in late July and early August were sexed, it was assumed that these nests produced 100% females, for two reasons: a) the sand temperature was very warm at this time (Fig. 3.1); b) all the nests that were sampled for sex ratio in early July had 100% female hatchlings.

Mean sex ratios of green turtle and leatherback nests laid within half month periods changed over the season. In April and May, the months with most rainfall, more males were produced, while in June, July, and August, almost all hatchlings produced were female (Table 3.2 and Fig. 3.3).

The sex ratio for the season as a whole was calculated by multiplying the mean sex ratio for each half month by the relative number of nests laid above the high tide line in that same time period. For green turtles, the overall sex ratio was 63.8% female. For leatherbacks, the overall seasonal sex ratio was 69.4% female.

Figure 3.3. Seasonal changes in sex ratios in 1993. Values are half month means for sex ratios of different clutches.


1993
Percentage female
100
80
60
40
20
0
Green turtle
Leatherback
1-28 1-16 17-31 1-15 16-30 1-16 17-31 1-15 16-30 1-16 17-31 1-16 17-31 1-15
Feb. March April May June July Aug. Sept.

Rainfall and its Relation to Sex Ratio

Rainfall was highest in May and June (Fig. 3.4). Temperatures were lower in these months (Fig. 3.1). Also, starting with nests laid in April (that is, those nests whose thermosensitive periods would have occurred a few weeks later), there were corresponding decreases in the percentage of female hatchlings produced (Fig. 3.3). In order to investigate this relationship further, correlations were calculated between monthly rainfall and monthly sex ratios for 1982 (data from Mrosovsky et al., 1984a) and 1993 (this study). For green turtles, there was a significant inverse correlation between monthly rainfall and the mean percentage of females produced by nests laid in the same month ($r=0.78$, $p=0.005$; Fig. 3.5). For leatherbacks, the strongest correlation was between mean sex ratio and rainfall in the month after those nests were laid ($r = 0.93$, $p=0.003$; Fig. 3.5). Using the regression lines in Fig. 3.5, and mean monthly rainfall data from 14 different years, it was possible to estimate the sex ratio in each month of these 14 years. Then, using the nesting distribution for each particular nesting season (or the mean distribution from 12 years, if the data for a particular season were not available, $n=4$), it was possible to calculate the sex ratio for each of the 14 years (Fig. 3.6). The means of these years are 68.3% female and 53.4% female, for green turtles and leatherbacks, respectively. By using Tukey's jackknife method (Sokal and Rohlf, 1981), with 2 subsets of data (months from 1982 and months from 1993), the calculated approximate standard error for the mean overall sex ratio of green turtles is ±1.72, and of leatherbacks is ±9.94. The 14 year mean for green turtles was significantly different than a 50% female sex ratio ($p=0.004$; one sample, two-tailed t-test).

In total, 13 monthly rainfall totals (11.6%) were missing from the 14 year data set, and thus mean values were substituted (see methods). The use of the mean values for these 13 months lacking rainfall data is unlikely to have much

Figure 3.4. Daily rainfall, expressed as an average per half-month, at Matapica, Suriname over the 1993 nesting season.

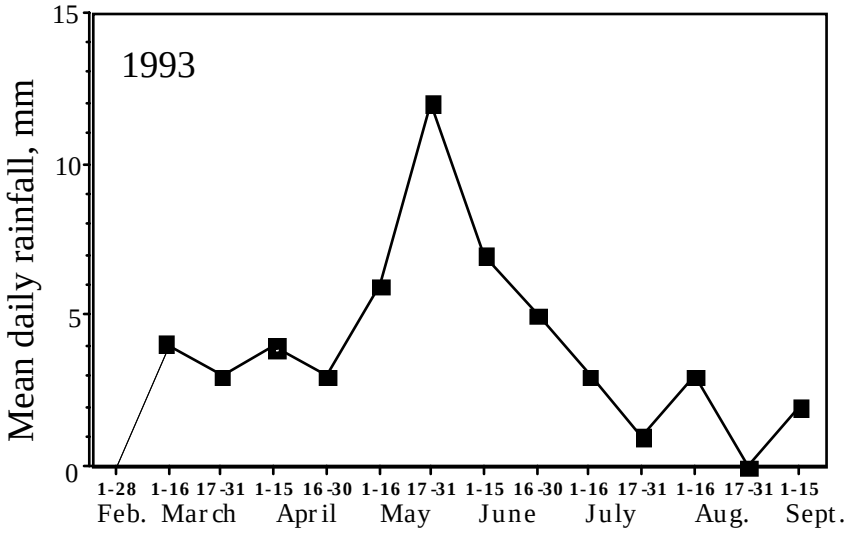
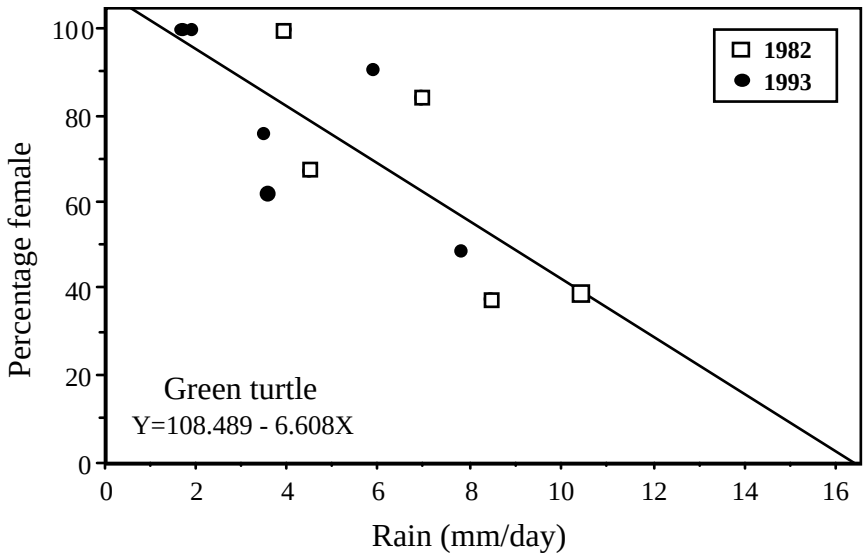

1993
Mean daily rainfall, mm
15
10
5
0
1-28 1-16 17-31 1-15 16-30 1-16 17-31 1-15 16-30 1-16 17-31 1-16 17-31 1-15
Feb. March April May June July Aug. Sept.

Figure 3.5. Top: Linear relationship between sex ratio of green turtle nests and monthly rainfall. Rainfall (expressed in mm/day) was calculated by summing the daily totals that were recorded in a given month and then dividing by the number of totals in that month. The equation for the regression line is y=108.489 - 6.608x ±1.78 SE. Bottom: Linear relationship between sex ratio of leatherback turtle nests and the amount of rainfall of the subsequent month. Rainfall was calculated as above. The equation for the regression line is y=112.493 - 10.861x ±1.99 SE.


Percentage female
Rain (mm/day)
1982
1993
Green turtle
Y=108.489 - 6.608X
0
20
40
60
80
100
0
2
4
6
8
10
12
14
16

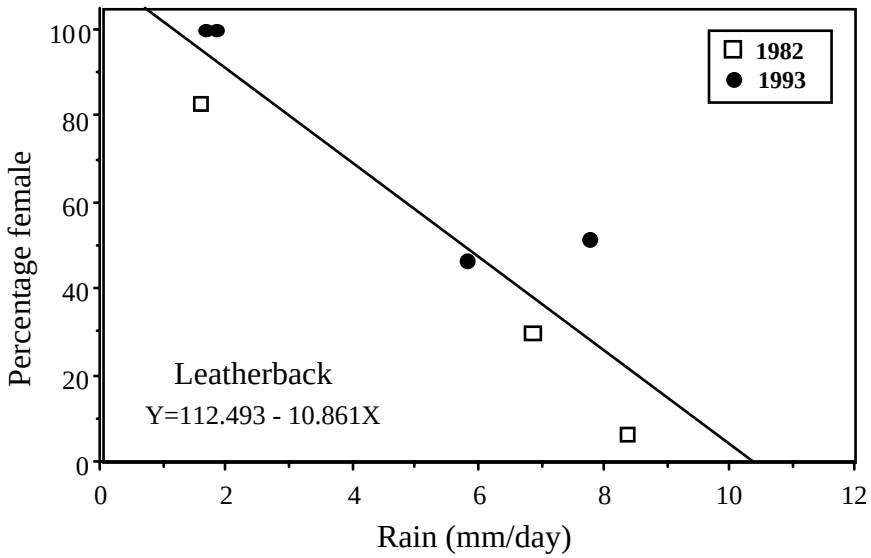
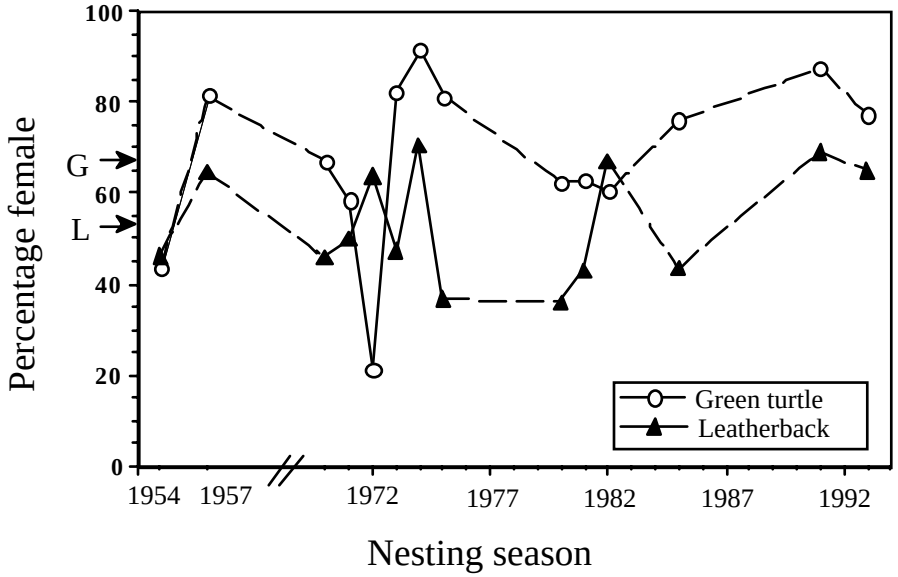

Percentage female
Rain (mm/day)
1982
1993
Leatherback
Y=112.493 - 10.861X
0
20
40
60
80
100
0
2
4
6
8
10
12

Figure 3.6. Overall sex ratio of leatherback and green turtle hatchlings produced at Matapica, Suriname in 14 different years. Monthly rainfall data was collected by Meteorologische Dienst Suriname at the Matapica Canal station, except for 1982 and 1993, in which case rainfall data is from Mrosovsky et al. (1984a) or the present study. Some monthly rainfall data were missing; in these cases, the particular monthly mean based on the other years was substituted. Sex ratio data are estimated from monthly rainfall data and equations from regression lines in Fig. 3.5. Monthly sex ratios for each species for a particular nesting season were multiplied by the corresponding seasonal nesting distribution of both species, unless this was not available, in which case the mean nesting distribution from 12 years was used, (see Fig. 3.2). Noncontiguous years are separated by dashed lines. Upper arrow on left vertical axis represents the mean sex ratio of green turtles, based on 14 years. Lower arrow represents mean sex ratio of leatherbacks. Data from 1994 are not included because we were not able to estimate the sex ratios for the whole nesting season.


100
80
G
60
L
40
20
0
Percentage female
1954
1957
1972
1977
1982
1987
1992
Nesting season
Green turtle
Leatherback

impact on the estimates of overall sex ratios for a number of reasons. First, 8 of the 13 missing values were for August or September, months at the tail of the nesting season. Because so few nests are laid in these months, any errors in estimates of the sex ratio at this time will have minimal impact on the overall sex ratio estimates. Second, using mean rainfall data for these months is not likely to have introduced errors because August and September are usually hot and dry, with little rainfall. Third, only in 4 years were mean rainfall values substituted for missing data from months other than August and September. If these 4 years are excluded from the analysis of overall sex ratio, the values for green and leatherback turtles respectively are 66.2% and 55.5% female. This is close to the 68.3% and 53.4% values estimated for the 14 years (considered as a whole).

Incubation Duration

Mean incubation duration varied over the season in a manner consistent with temperature and rainfall (Fig. 3.7). When incubation duration was plotted against the sex ratio of each clutch, it was possible to obtain a pivotal incubation duration (that duration at which a clutch produces 50% female sex ratio). In 1993, the pivotal incubation duration was 58.5 days for green turtles and 63.9 days for leatherbacks. These values are similar to those found by Mrosovsky et al. (1984a): 59.7 days and 66.0 days, for greens and leatherbacks, respectively.

1994 Nesting Season

For May, June, and July, mean sand temperatures at 30cm and 60cm depths were significantly cooler in 1994 in preferred nesting areas than in 1993 (Fig. 3.8, $p<0.05$). There was one exception to this finding: in the open zone in the preferred area of green turtles, the mean temperature at 30cm depth was not

Figure 3.7. Changes in mean incubation duration (±SEM) of turtle nests in 1993 season.

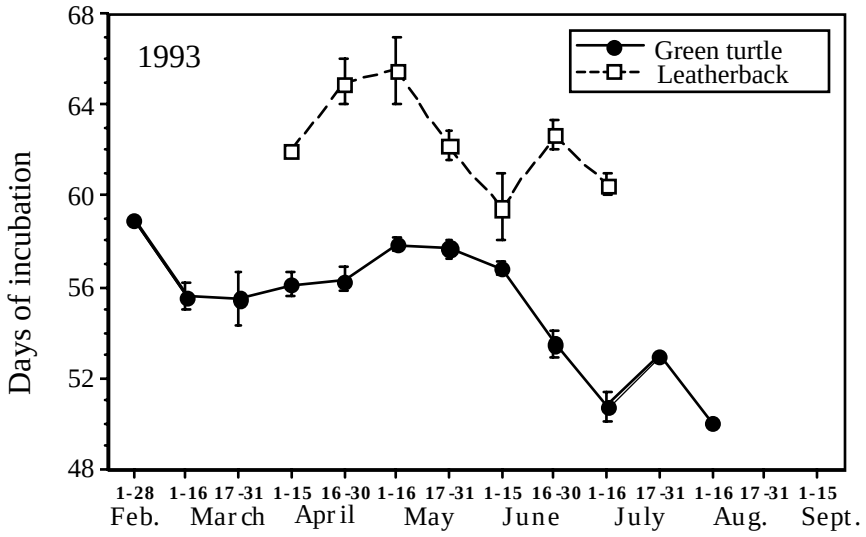
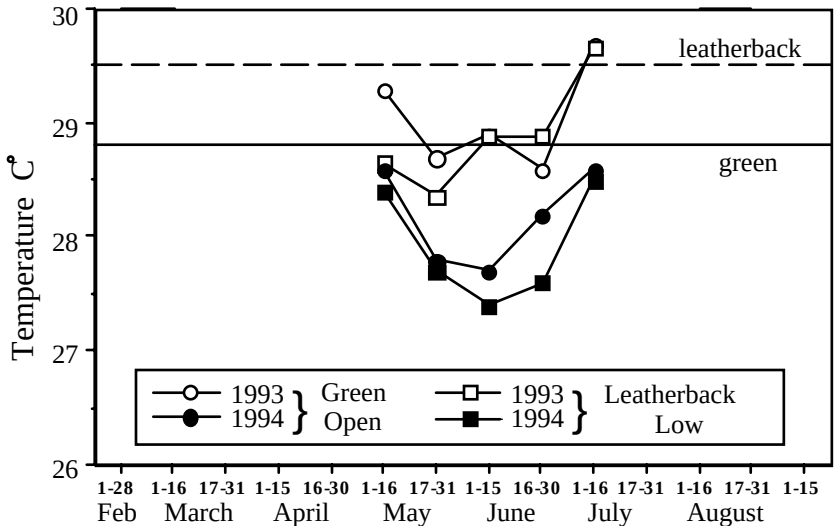

1993
Green turtle
Leatherback
Days of incubation
68
64
60
56
52
48
1-28 Feb.
1-16 17-31 March
1-15 16-30 April
1-16 17-31 May
1-15 16-30 June
1-16 17-31 July
1-16 17-31 Aug.
1-15 Sept.

Figure 3.8. Mean sand temperatures at the open and low sites of the green and leatherback temperature transects in 1994. Sand temperatures in 1993 from the corresponding months and sites are shown for comparison. Only data collected with digital thermistors at 60cm depths are plotted. Other temperature data were similar. Horizontal lines show pivotal temperatures, from Mrosovsky et al. (1984a) and Rimblot-Baly et al. (1987).


30
29
28
27
26
Temperature C°
leatherback
green
1993
1994
Green
Open
1993
1994
Leatherback
Low
1-28 1-16 17-31 1-15 16-30 1-16 17-31 1-15 16-30 1-16 17-31 1-16 17-31 1-15
Feb March April May June July August

significantly different between years. In 1994, for each depth, no significant difference was detected in the thermal profiles of zones either within or between transects. Although no hatchlings were sexed in 1994, it is likely that the mean sex ratio of nests laid in April, May, and June was less female-biased than in the same months of 1993, since sand temperatures were often below pivotal temperature, for both species. Mean daily rainfall values for these 3 months in 1994 also predicted male-biased sex ratios of turtle nests. When the rainfall values were combined with the regression lines in Fig. 3.5, green turtle nests in May, June, and July were estimated to produce about 20% female hatchlings, and leatherback nests laid in April, May, and June were estimated to produce 0% female hatchlings. However, we did not measure the monthly rainfall for the entire nesting season, and therefore we cannot estimate the overall sex ratios for the 1994 nesting season.

Discussion

Turtles nests in Suriname are subject to changes in incubation temperature at different times in the season, and this results in variation in sex ratio of the hatchlings. Rain in the middle of the nesting season results in more males: drier weather at the ends of the season results in more females. Rainfall also varies among seasons. Therefore, one would expect different sex ratios of turtle hatchlings in different years. Such a difference was found when data from two different years were compared: the relative number of female green and leatherback hatchlings in the present study (1993) was roughly 10% higher than in 1982. However, before considering the significance of these data, one should consider the precision of the sex ratio estimates of the present study (and perhaps all other studies).

In this study, it was assumed that all nests laid across the season were similar, with respect to size and hatching success. In Puerto Rico, the mean number of eggs of leatherbacks diminishes as the season progresses (Tucker and Frazer, 1994). So, in Suriname, it is possible that in the warmer last part of the nesting season, a clutch of 100% females produces fewer hatchlings in total than a clutch containing males that was laid in a

cooler earlier part of the season. Hewavisenthi (1994) reported that there was an inverse relationship between hatching success rate and clutch size for olive ridley (*Lepidochelys olivacea*) and green sea turtles. In addition, nest site may affect hatching success: nests laid in or near the vegetation may be more susceptible to invasion by roots (Whitmore and Dutton, 1985). Further caveats concerning the precision of estimating sex ratios of sea turtle populations have been made elsewhere (Mrosovsky and Provancha, 1992; Mrosovsky, 1994).

Nevertheless, despite the difficulties in arriving at precise estimates, it is very likely that sex ratios of hatchling green and leatherback turtles in Suriname vary from year to year. This is supported by the fact that the same methods were employed in 1982 and 1993, and yet the female bias in sex ratios of both species was 10% greater in 1993. In addition, sand temperatures in 1994 were cooler than in 1993, and this probably resulted in relatively more male hatchlings in 1994. Finally, hatchling green turtles from nests laid in April, May, and June in 1981 were more female biased than in 1982 (Mrosovsky et al., 1984a). In addition to our studies in Suriname, data from other countries suggest that yearly variations in sex production of hatchlings are common. In Tortuguero, Costa Rica, the sex ratio of green turtle hatchlings was estimated to be 67% female in 1980, 10% female in 1986, and 40% female in 1988 (Spotila et al., 1987; Horikoshi, 1992). In French Guiana, the sex ratio of leatherbacks in June varied from 0% to 87.5% female, in the years 1981 and 1983-85 (Rimblot-Baly et al., 1987). Juvenile green turtles caught off the Bahamas had different sex ratios when they were classified by size; those size classes probably corresponded to groups of hatchlings produced in different years (Bolten et al., 1992).

A bias in hatchling sex in one particular year may be countered by opposite bias in another year, giving a 1:1 sex ratio for the population as a whole. Indeed, Janzen (1994b) found in painted turtles (*Chrysemys picta*), a freshwater species with temperature-dependent sexual differentiation, that yearly variations in estimated hatchling sex ratios balanced out at 1:1 over 49 years. For sea turtles, sex ratios based on data from several years are probably the most accurate estimates of overall sex ratio. In the case of loggerheads nesting in

Florida, the overall 90% female sex ratio is based on data from 5 consecutive years (Mrosovsky and Provancha, 1992). The means of our present estimates for 14 different years are 68.4% female for green turtles and 53.6% female for leatherbacks in Suriname. It should be noted that these means are not from consecutive years, but from data that are scattered over a 40 year period. It is possible that the years for which we lack data were extreme. Had it been possible to include them, the estimate of overall sex ratio might have been different. Also, some of the above estimates for individual years are based on a mean nesting frequency. If more nests are laid on average, or if nesting frequency is different, in years with particular weather patterns and thermal profiles, that would possibly lead to errors in our overall estimates (cf. Limpus and Nicholls, 1988). However, it is more likely that variation in sex ratio across years was due to variation in rainfall patterns rather than variation in nesting frequency. Peak nesting intensity was relatively consistent across years the 12 years of available nesting data: 9 green turtle seasons were characterized by having a peak in nesting in April; 11 leatherback seasons had peak nesting in May (only 1982 was different, as was reported by Mrosovsky et al., 1984). Rainfall patterns during the nesting seasons were far more variable: of the 14 years with available rainfall data, the peak in rainfall was just as likely to occur in June, as it was in April or May. Two of the years had peak rainfall in either February or July. More data of course would increase confidence in the correlation between rainfall and sex ratio; one could also test the accuracy of the relationship by first predicting the sex ratio of some nests from the rainfall, and then checking those predictions by subsequent histological examination.

Nevertheless, it should be emphasized that the present overall sex ratio estimates are consistent with predictions based on pivotal temperatures. The pivotal temperatures for green and leatherback turtles from the Guianas are 28.75 °C and 29.5 °C, respectively (Mrosovsky et al., 1984a; Rimblot-Baly et al., 1987). Because of their lower pivotal temperature, green turtle eggs will differentiate into females at sand temperatures which, for leatherback eggs, are masculinising. A large proportion of the seasonal total of leatherback

and green turtle eggs normally incubate in the sand at the same time, despite the fact that the peaks of the nesting distribution of the two species are separated by one month (Fig. 3.2). However, green turtle nests laid in late April and early May produced more females than leatherback nests laid at the same time (Fig. 3.3). This tendency for green turtle nests to produce more females might not hold in every year. For example, in 1972, atypical wet weather at the beginning of the season resulted in relatively more male green hatchlings; atypical dry weather at the end of the season resulted in more relative female leatherback hatchlings. But for most years, the pattern is consistent: green turtle nests produce more females. The 14 year 68.4% female sex ratio estimate for green turtles nesting in Suriname was significantly different from a 1:1 sex ratio.

Assuming that estimates based on 14 years are reasonably accurate, one can begin to evaluate and apply this information. For example, there appears to

Figure 3.9. Seasonal changes in the relative number of hatchlings of each sex produced at Matapica, Suriname. Left two panels are based on the 1993 season. Right two panels are the mean sex production profiles of 14 years. Bars show standard deviations of the monthly mean sex production for 14 years.

FOR FIGURES IN CHAPTER 3, SEE Godfrey, M. H., Barreto, R. and Mrosovsky, N. (1996). Estimating past and present sex ratios of sea turtles in Suriname. Canadian Journal of Zoology 74: 267-277. Available here: http://members.seaturtle.org/godfreym/Godfrey_1996_CJZ.pdf

be a general overall pattern in sex production (Fig. 3.9). For both species, some female hatchlings are produced in every month of the nesting season, but male hatchlings are primarily produced only in nests laid in April and May. On average, 70.8% and 73.4% of all males produced in the season, for greens and leatherbacks respectively, are produced in those months. Because males come mostly from these months, conservation and management programmes that involve the manipulation of eggs in these months should consider the potential effects on the sex ratio of the population. In Suriname, some eggs are harvested during part of the season, while at other times doomed eggs are relocated to safer ground. If this harvest occurs only in April and May, for example, it is likely that hatchlings from male-producing nests of both species will be preferentially removed and a more female-biased sex ratio will result.

The value of knowing the overall sex ratios of sea turtles extends beyond applications in management policies. A growing database allows one to consider a basic question of sea turtle populations: what is the natural sex ratio in the offspring of species with temperature dependent sexual differentiation? In two cases, the present study and that of Mrosovsky and Provancha (1992), estimates based on a number of years have come up with female-biased sex ratios. It is unclear whether or not this is inconsistent with Fisherian theory, which predicts a 1:1 sex ratio in offspring at the end of parental investment, when offspring of the two sexes are equally costly (Fisher, 1930). It may be the case, with some sea turtle populations, that one or more of the assumptions of Fisherian sex ratio theory are not met (e.g. the populations are not at equilibrium; see Bull and Charnov, 1988; Mrosovsky, 1994, for discussion). The implications of the finding that there are differences in natural sex ratios among species must be examined. These differences range from about 9:1 female bias in loggerheads in Florida, to a roughly 2:1 female skew in greens in Suriname, to a close to 1:1 sex ratio in leatherbacks in Suriname. Such variation among turtle species, especially between two that nest on the same beach, suggests that there may be differences in

population structure and dynamics among these species. A question for the future is whether or not different populations of the same species have similar overall sex ratios.

Chapter 4:
Estimating the pivotal temperature of green sea turtles in Suriname

Introduction

Although TSD is thought to occur in all 7 species of sea turtle (Standora and Spotila, 1985), most of the research on TSD in sea turtles has been with one species, the loggerhead (*Caretta caretta*). For instance, there are many published studies devoted to the description of pivotal temperatures of different populations of loggerheads (Yntema and Mrosovsky, 1982; Limpus et al., 1985; Maxwell, 1987; Mrosovsky, 1988; Georges et al., 1994). On the other hand, green turtles (*Chelonia mydas*) have received relatively little attention in this area; only two studies have described pivotal temperatures of this species (Mrosovsky et al., 1984a; Standora and Spotila, 1985). According to these two studies, the pivotal temperature for Western Atlantic green turtles is somewhere between 28 and 30 °C. Each of these estimates has some limitations.

Standora and Spotila (1985) used nest temperatures from eggs incubating in the field to estimate the pivotal temperature. Although this arguably gives a more "natural" pivotal temperature, it is difficult to compare this to other data on pivotal temperatures derived from laboratory studies, in which the incubation temperatures remain constant. The pivotal temperatures reported by Mrosovsky et al. (1984a) was based on relatively small number of eggs, and the warmest of the 3 laboratory incubation temperatures used produced only 58% female hatchlings. It is desirable for pivotal temperature estimates to be based on more eggs and more incubation temperatures, especially those that produce 100% males and females, in order to pinpoint the full transitional range of temperature (that is, the span of constant incubation temperatures over which sex ratios change).

It is important to establish an accurate pivotal temperature for each species (and perhaps each population of species), because this knowledge can help in estimating sex ratios of hatchlings produced on a beach (see Chapter 1). Such information may aid in

understanding the importance of nest site selection and sex allocation of sea turtles. For instance, it appears that the pivotal temperature values from different populations and species of sea turtles are clustered around 29 °C (Mrosovsky, 1994). If it is the case that the pivotal temperatures of sea turtles are constrained around 29 °C , then in order to vary the sex ratios of their offspring, sea turtles must choose thermally specific sites on the beach or times of year to place their eggs. Indeed, it has been reported that the sex ratios of leatherback and green turtle hatchlings on a shared nesting beach are similar, despite the fact that the pivotal temperature for leatherbacks is almost 1 °C higher than that for green turtles (Mrosovsky et al., 1984a). The authors of that study argued that the majority of leatherbacks nested later in a nesting season than the majority of green turtles, causing the leatherback clutches to be exposed to higher sand temperatures during incubation. However, more data on pivotal temperatures and sex ratios are needed to properly evaluate this suggestion. Indeed, revised estimates of hatchling sex ratios of the two species nesting in Suriname suggest that there are interspecific differences in sex ratio, probably due to interspecific differences in pivotal temperature (Chapter 3), although the accurate pivotal temperature for this population of green turtles remains unclear.

Incubation of reptile eggs in the laboratory has been used not only to determine pivotal temperature, but also to try to understand the adaptive significance of TSD. One hypothesis suggests that, if there is random mating, a patchy environment, offspring fitness covarying with sex in a given patch, and neither mother nor offspring being able to choose which patch they enter, then environmental sex determination (of which TSD is a subset) will be selected for (Charnov and Bull, 1977). Apparent support for this hypothesis comes from laboratory incubation of eggs of the American alligator (*Alligator mississippiensis*), which exhibits TSD: female hatchlings produced at cool incubation temperatures have significantly more residual yolk in their abdomens than males produced at intermediate temperatures (Ferguson and Joanen, 1983). In addition, constant incubation temperatures that produce both sexes resulted in male offspring that were larger, but had less residual

abdominal yolk, than female offspring (Allsteadt and Lang, 1994). If greater residual abdominal yolk or greater mean size is a correlate of increased fitness, the findings would support the model of Charnov and Bull (1977).

The present study was undertaken for two reasons: 1) to provide more details about the pivotal temperature of green turtle eggs; 2) to determine whether at hatching there exist morphological differences between the sexes as a function of incubation temperature. Although the methods of establishing a pivotal temperature are relatively simple and straightforward, the logistics are rather daunting (most green turtles nest on remote tropical beaches) and this is probably the reason for the relative lack of studies performed to date.

Materials and Methods

Egg collection and transport

The green turtle eggs came from Matapica beach in Suriname. This is a dynamic beach that moves west with the predominant ocean current. The nesting season runs from February until early July, with a peak in April and May (Schulz, 1975).

On the night of 8/9 May, 1995, eggs were obtained from two separate clutches soon after being laid. At 10:30 PM, 90 eggs were taken from one clutch (designated Clutch N), and under supervision of the Suriname conservation officer, the remaining eggs were placed back in the original nest hole. At 00:30 AM, all 108 eggs were taken from another clutch (designated Clutch O). Eggs from each clutch were carefully placed in a Styrofoam box, with sand on the bottom, and a layer of cotton gauze followed by more sand at the top.

The egg boxes were carried by hand for approximately 6 km, to the head of the Matapica Canal. From there, they were transported by a small boat to Paramaribo, via the Matapica Canal and Tapoeripa Creek and the Commewijne and Suriname rivers. Upon arrival in Paramaribo at 7:00 AM, the eggs were transported to Zorg an Hoop airport, where they remained until 10:00 AM in an air conditioned room, to provide low temperature protection (Harry and Limpus, 1989). The eggs were then taken by a Cessna plane to

Georgetown, Guyana, arriving at approximately 11:30 AM. The eggs were kept in an air-conditioned waiting room in Georgetown until late afternoon, when they were transferred to a jet plane for a flight to Toronto. The eggs arrived in Toronto at 12:30 AM on the night of 9/10 May, and they were unpacked and placed in the incubators by 3:00 AM. At all times during the trip, care was taken to keep the eggs from being shaken or tipped, and the boxes were kept away from the X-rays machines at the airport security gates.

Laboratory incubation:

Each egg was randomly assigned a number and placed singly in a 500ml plastic container that contained 60ml of deionized water and an indented piece of sponge covered by moistened vermiculite. More moist vermiculite was carefully placed around the egg, such that only about 1/3 of the egg was exposed. An inverted plastic Petrie dish served as a cover for the plastic container; the latter had 8 small holes near its top, for air flow. Up to 16 eggs (in two layers) were placed on either 2 or 3 shelves of 5 incubators. About 65ml of deionized water were added to each egg container on days 17 and 41 of incubation.

A glass mercury thermometer (with 0.1 °C scale), encased in a tube of glycerol, was placed on each shelf such that the thermosensitive bulb end of each thermometer was roughly in the middle of the shelf. The thermometers were read once daily, and readings were done quickly so as to minimize temperature changes in the incubators. In addition, one digital thermistor probe, to record the daily maximum and minimum (see Chapter 2), was placed in each incubator, and checked each day for signs of large daily fluctuations. In order to assess for evaporative cooling, on day 30, a needle-thermistor probe was inserted into one egg, and its core temperature was compared to that of a vial of glycerol placed nearby. After allowing more than 24hrs for equilibration, 4 readings over 2 days were made. It was found that the egg core was cooler on average than the glycerol by 0.25 °C. Therefore, a correction factor of 0.25°C was subtracted from all temperature readings to allow for evaporative cooling.

After day 45 of incubation, the incubators were checked twice daily for signs of hatching. An egg was considered hatched if the head and at least one flipper of the hatchling were outside of the shell. Signs of pipping (when the egg shell was first slit) were also noted. Incubation duration was calculated as the number of days between laying and hatchling. Once an egg was found hatched, the plastic container was removed from the incubator and hatchling was weighed to nearest 0.1g. The hatchling then was quickly killed, the residual abdominal yolk removed and weighed, and the gonads excised and placed in buffered 0.9 % formalin. The gonads were allowed to fix in the formalin for a number of days, after which they were prepared as outlined in Mrosovsky et al. (1984a). Briefly, one gonad from each hatchling was cut in half transversely, and embedded in paraffin wax. Serial sections (10 µm thick) were taken from the cut end of the gonad and mounted on slides.

Sexing:

The tissue sections were stained with Harris' haemotoxylin and periodic-acid-Schiff reagent (PAS), and examined under a light microscope. Male gonads were characterized by a thin smooth cortex and presence of immature semiferous tubules; female gonads were characterized by a PAS-positive tunica albuginea between the cortex, which was thickened and infolded, and the medulla, which was homogeneous with no sign of tubules (for more details, see Yntema and Mrosovsky, 1980; Miller and Limpus, 1981). Rarely, a gonad might exhibit characteristics of both testes and ovaries; such gonads were labelled intersexes, and treated as non-females in the calculations of sex ratio. All gonads of embryos that died at a late stage were examined for sex, although usually tissue from these embryos had degenerated beyond recognition.

Two experienced researchers independently evaluated sections from each gonad. In the few cases when identification of sex by the two disagreed, the two researchers re-examined the tissue sections together. If consensus could not be reached (this only occurred

in a few cases when the samples in question had deteriorated), no sex was assigned and those samples were excluded from further analysis.

Results

Only 7 of 90 eggs from Clutch N hatched (7.8% success rate), while Clutch O produced 67 hatchlings (62.0% success rate) (Table 4.1). The overall hatch rates were significantly different between clutches N and O (X^2=59.45, df=1, p<0.0001). Most of the unhatched eggs from Clutch N contained embryos that had died during development, suggesting the eggs in the clutch were fertilized. Of the unhatched eggs from clutch N, 2 were successfully sexed; from clutch O, 6

Table 4.1. Incubator temperatures and numbers of green turtle eggs on each shelf. Note that the actual temperatures for each shelf include the -0.25 °C correction factor for evaporative cooling.

Incubator no.	Shelf no.	Temperature ± range °C	Eggs set (N/O clutch)	Eggs hatched (N/O clutch)
2	2	30.6 ±0.4	4/10	0/5
	3	30.0 ±0.5	4/10	0/4
1	2	29.6 ±0.2	8/8	1/5
	3	29.2 ±0.2	8/8	1/6
3	1	28.8 ±0.2	8/8	1/7
	2	28.6 ±0.3	8/8	1/4
	3	28.4 ±0.4	6/8	0/4
4	1	28.4 ±0.1	6/8	1/6
	2	28.2 ±0.1	6/8	0/4
	3	28.2 ±0.3	8/8	1/5
5	1	27.8 ±0.2	8/8	0/4
	2	27.6 ±0.2	8/8	0/7
	3	27.6 ±0.2	8/8	1/6

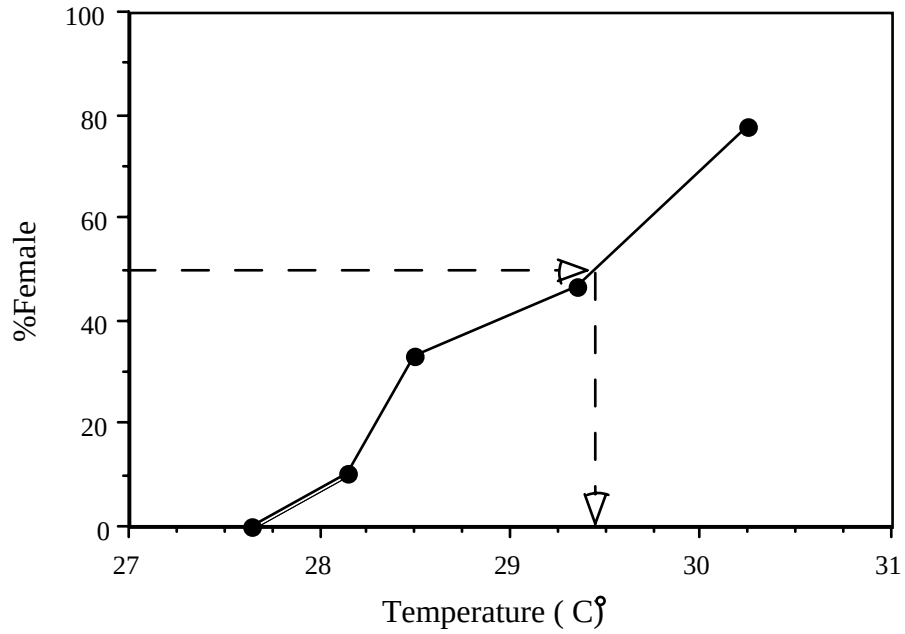
Table 4.2. Sex of green turtle hatchlings from different incubators.

Incubator no.	Temperature ± range °C	Number of males	Number of females	% female
2	30.2 ±0.8	2	7	77.8
1	29.4 ±0.4	8	7	46.7
3	28.5 ±0.4	12[a]	6	33.3
4	28.2 ±0.4	17	2	10.5
5	27.6 ±0.3	20	0	00.0

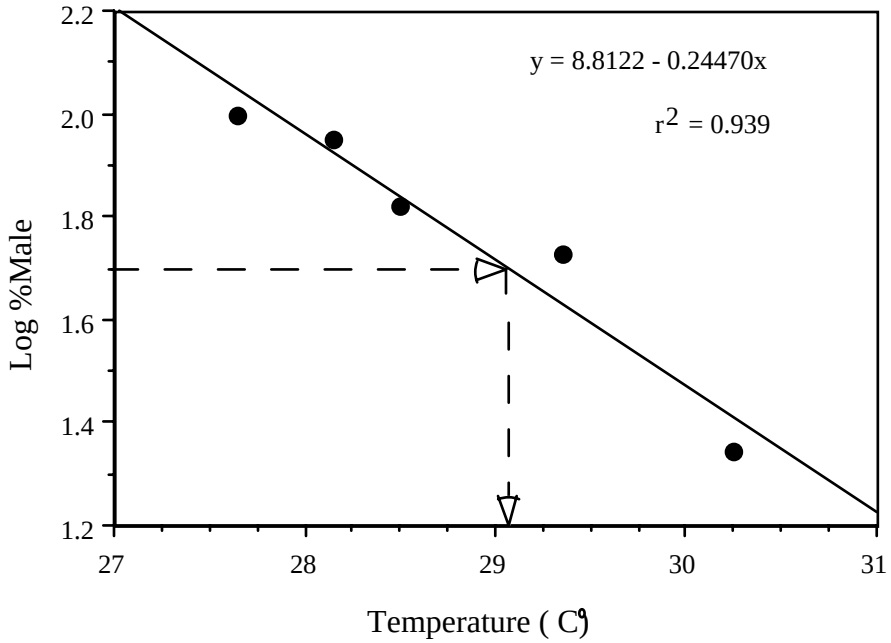
[a] Includes one intersex, which was treated as “not-female.”

Figure 4.1. Relationship between incubation temperature and sex ratio of green turtle eggs from Suriname. Arrows show the pivotal temperature (that constant incubation temperature which results in 50% female sex ratio). Upper graph (A) shows the pivotal temperature (29.4 °C) as determined the traditional method of interpolation between the 2 data points that bound 50% female. Lower graph (B) shows the pivotal temperature (29.1 °C) as determined by logistic regression. Mean sex ratios for each incubator were converted to % male before logarithmic transformation.

A


100
80
60
40
20
0
%Female
27
28
29
30
31
Temperature (C°)

B


y = 8.8122 - 0.24470x
r2 = 0.939
2.2
2.0
1.8
1.6
1.4
1.2
Log %Male
27
28
29
30
31
Temperature (C°)

unhatched eggs were accurately sexed. One hatchling from clutch O was rated as an intersex.

Hatchlings that were sexed were grouped by incubator, and mean sex ratio for each incubator was calculated (Table 4.2). The 5 temperature values for each incubator were plotted against mean sex ratio (Fig. 4.1A). From interpolating between the 2 points on either side of 50% female, the calculated pivotal temperature was 29.4 °C. If only those hatchlings from clutch O were included in the analysis, the pivotal temperature was estimated to be 29.2 °C. Those 9 hatchlings and embryos from clutch N that were sexed were all male, which makes it impossible to estimate the pivotal temperature for that clutch.

In addition to the traditional method of estimating the pivotal temperature, a logistic regression of the data was performed (sex ratio data were converted to % male for the logarithmic transformation). From this regression line, the estimate of pivotal temperature was 29.1 °C, as seen in Fig. 4.1B. The 95% confidence limits for this estimate is ±1.04 °C (based on an "inverse prediction," (Zar, 1984)).

The hatchling weights and residual abdominal yolk weights were grouped by incubator and sex (Table 4.3), and compared using nonparametric Friedman two-way ANOVA, with Kendall coefficient of concordance. No significant differences among the mean weights of hatchlings (coefficient of condordance=0.686, p=0.232), or their residual yolks (coefficient of condordance=0.302, p=0.753), from the different incubation conditions were detected.

Discussion

The poor survival rate of clutch N meant that the pivotal was based almost entirely on one clutch (Clutch O). This is far from ideal, since it is known that there are intraspecific differences in pivotal temperatures in turtles (e.g. Bull, et al, 1982a, b; Janzen, 1992; Bobyn and Brooks, 1994), even within the same

Table 4.3. Mean weights (grams) of hatchlings and their abdominal residual yolk, grouped by incubator and sex, and statistical significance as calculated from Friedman two-way ANOVA with Kendall coefficient of concordance.

Incubator	27.65 °C[a]		28.15 °C		28.5 °C		29.35 °C		30.25 °C		p
Sex	M	F	M	F	M	F	M	F	M	F	
Body (g)	30.3	--	30.1	31.9	31.5	30.2	29.5	29.4	27.1[b]	30.2[b]	0.23
n	(16)	(0)	(13)	(2)	(10)	(6)	(5)	(5)	(1)	(7)	
SD	1.3	--	1.6	0.8	1.7	2.5	1.2	1.7	--	1.4	
Yolk (g)	3.0	--	4.2	2.6	2.6	5.4	4.0	2.3	6.2	3.8	0.75
n	(16)		(12)	(2)	(10)	(6)	(5)	(6)	(2)	(7)	
SD	2.1	--	2.0	0.6	1.1	5.2	2.1	0.5	4.6	1.2	

NOTE: in a few cases, body weights and/or yolk weights were not recorded for hatchlings that were sexed.

[a]Data from this incubator were not included in statistical analysis because no females were produced at this temperature.

[b]Not included in statistical analysis because n<2 in one of the corresponding groups.

population (Mrosovsky, 1988). This also appears to be the case with the Suriname population of green turtles: eggs from 3 clutches incubated in 1983 were estimated to have a pivotal of 28.8 °C (Mrosovsky et al., 1984a), while eggs from the present experiment were estimated to have a pivotal of 29.4 °C, a difference of 0.6 °C. It should be noted that the incubation procedures in 1983 and 1995 were slightly different; for instance, the addition of water to the eggs during incubation was standardised in 1995, but not in 1983 (McLean et al., 1983). Other differences in incubation procedure (e.g. different amounts of sponge and vermiculite surrounding the eggs) may account for some of the difference between these two estimates. However, the difference of 0.6 °C between the studies may be an artifact of the traditional method of estimating the pivotal temperature, which is to interpolate between the values of sex ratio on either side of the 50% level (Mrosovsky and Pieau, 1991).

In addition to the traditional method of estimating pivotal, I also used logistic regression for the data from the incubators. The pivotal estimate from this analysis was 29.1 °C. When data from the previous study on pivotal temperature for this population (see Table 3 in Mrosovsky et al., 1984a) were subjected to a logistic regression, the pivotal estimate was 28.8 °C. No significant differences were detected between the regression lines of the two sets of data, when slopes (two tailed t=0.91, v=4, p>0.20) and elevations (two tailed t=1.75, v=5, p>0.10) were compared (Zar, 1984). Therefore, the two datasets were combined, and, based on a logarithmic regression analysis, the overall estimate of pivotal temperature for this population was 29.0 °C, with a confidence interval of ±0.95 (Zar, 1984). Use of this type of analysis in future pivotal studies will allow statistical comparisons to be made among datasets. Nevertheless, without standardization of incubation methods, it remains difficult to discern whether reported differences in pivotal temperatures are real or artificial (Mrosovsky, 1994).

The weights of the hatchlings and their residual abdominal yolks were not significantly different across the different incubators. This is in contrast to previous work in alligators and freshwater turtles (Ferguson and Joanen, 1983; Joanen et al., 1987; Allsteadt

and Lang, 1994; Brooks et al., 1991; McKnight and Gutzke, 1993), in which mass at hatching and residual abdominal yolk weights were significantly correlated to incubation temperatures. Such findings have been used to support the Charnov and Bull (1977) hypothesis that different incubation temperatures affect fitness differentially, although in the case of turtles, it remains to be seen whether variation in hatchling weight is truly an indicator of variation in fitness (cf. Bobyn and Brooks, 1994; Janzen, 1996). It could be the case that another character, not measured in this experiment, covaries with incubation temperatures and fitness. In any case, in order to substantiate changes in fitness, it is probably necessary to monitor the hatchlings for a long period of time, ideally at least until they begin to reproduce. This would be logistically challenging.

The estimate of the pivotal temperature based on 1995 data (29.4 or 29.1 °C, depending on the method of analysis used) for this population of green turtles is slightly (but apparently not significantly) higher than the 28.8 °C reported by Mrosovsky et al. (1984a), and within the range of 28-30 °C, as reported for Costa Rican green turtles by Standora and Spotila (1985). It falls close to 29 °C, as do the pivotals of sea turtles in all the published studies to date (Mrosovsky, 1994). Such conservatism in pivotal temperature has led to the suggestion that changes in sea turtle hatchling sex ratios must be achieved by changing behavioural patterns of nesting, such as laying eggs in temperature-specific areas of a beach or during temperature-specific periods of the nesting season (Mrosovsky, 1988; Bull et al., 1982b). However, it is also possible that variation in the transitional range of temperature (TRT) around the pivotal could result in differences in offspring sex ratios.

The TRT is the range of incubation temperatures that produce a mixed sex ratio. In the case of the present study, it appears that the TRT is about 3 °C (Fig 4.1), although the upper boundary of the TRT could not be precisely defined because the hottest incubator did not produce 100% female hatchlings (although shelf 2 in incubator 2, with a temperature of 30.6 ±0.4 °C, produced 5 females and no males). Nevertheless, this TRT is larger than that of leatherback turtles, which is only 1 °C, with a pivotal of 29.5 °C (Rimblot-Baly et al.,

1987). Thus, even though the pivotals of these two species are close, it is possible that their offspring sex ratios will differ greatly on a shared nesting beach. For example, sand temperatures at 30.0 °C will result in 100% female hatchlings for leatherbacks, but for green turtles, this will result in only 70% female (Fig. 4.1). Data from the field are consistent with leatherbacks having a narrower TRT. In Suriname nests laid at similar times, leatherback hatchlings on average were more male or female biased than green turtle hatchlings (see Fig. 3.3). When incubation temperatures are very cold or very hot, and outside of the TRT, there would be little difference between the two species. Nevertheless, variation in TRT is another possible way to achieve intra and interspecific differences in sex ratio.

Currently, the pivotal temperature is usually the focus of interest in studies of TSD in turtles. It has been suggested that, for some species, intraspecific pivotal temperature is inversely related to latitude of the nesting populations in North America (Ewert et al., 1994). Presumably, this would allow nesting populations at cooler latitudes to achieve similar sex ratios as those of a nesting population at warmer latitudes. However, there are a number of reports in which intraspecific changes in pivotal temperatures were not found to vary consistently with changes in latitude (Bull et al., 1982b; Schwarzkopf and Brooks, 1985; Mrosovsky, 1988). This has led to speculation that modification of behaviour, in terms of nest site selection and the timing of nesting, is the important factor that determines the sex ratio (Janzen, 1994a; Roosenburg, 1996). However, as shown in the present study, behavioural changes are not the only characteristic that affects sex ratio: the breadth of the TRT must also be taken into account.

Chapter 5:
Metabolically generated heat of developing eggs and its potential effect on sex ratio of sea turtle hatchlings

Introduction

Sexual differentiation of sea turtle hatchlings is influenced by the temperature at which the eggs are incubated (Raynaud and Pieau, 1985; Janzen and Paukstis, 1991b). For all species of sea turtles, the general pattern of this thermal effect is the same: at a certain constant incubation temperature, called the "pivotal temperature", an equal number of male and female hatchlings are produced; incubation below or above the pivotal temperature will result in more males or females, respectively (for definitions, see Mrosovsky and Pieau, 1991). The range of incubation temperatures in which both sexes are produced (called the "transitional range of temperature" or TRT) is usually not more than 2-3°C wide, centered around the pivotal temperature (Mrosovsky, 1994). At incubation temperatures outside the TRT, 100% male or female hatchlings result.

For sea turtles, the adaptive value of this system of environmentally influenced sexual differentiation is poorly understood (Mrosovsky, 1994; Bull and Charnov, 1988). More data on sex ratios of hatchlings in natural conditions are needed to provide a solid database upon which theories can grow. In addition, there are management and conservation implications of this system of temperature-dependent sexual differentiation: almost any management procedure involving sea turtle eggs has the potential to alter the sex ratio of the hatchlings produced. For instance, ex situ incubation in Stryofoam boxes, once widely used to protect the eggs and ensure high hatch rates, generally results in more male hatchlings because the temperatures in the boxes are usually cooler than those of the sand (Mrosovsky and Yntema, 1980; Mrosovsky, 1983c; Morreale et al., 1982). Of course, recognizing a change from the natural sex ratio implies that the natural sex ratio is already known. However, discovering natural sex ratios of sea turtles is an arduous task. This is because the nesting seasons on

individual beaches can stretch for several months, during which time weather patterns change, which influences sand temperatures and thus sexual differentiation of the hatchlings (see Appendix 1).

One method of monitoring the overall sex ratio of hatchlings produced at an individual beach is to record mean daily sand temperatures. If the pivotal temperature is known, sand temperatures can be used to generate estimates of sex ratio (see Fig. 1.2). By combining information on sand temperatures and nesting density, one can predict the sex of the hatchlings being produced at different times in the season. One difficulty with this procedure is that different zones of the beach may vary thermally. This problem can be overcome by monitoring sand temperatures in all the different zones.

Another potential problem is that data on pivotal temperatures are usually generated in laboratory conditions. In contrast to the constant temperature incubation common to laboratory incubation procedures, there can be daily variation in sand temperature on turtle nesting beaches, especially at shallow depths; this variation can complicate simple predictions of sex ratio from daily mean temperature values (Bull, 1985). However, compared to nests of freshwater turtles, those of sea turtles are quite deep and hence subject to less daily variation in sand temperature (see Chapter 3).

A third potential problem in using sand temperature as an index of sex ratio is that the eggs themselves produce metabolic heat during incubation (Carr and Hirth, 1961; Mrosovsky and Yntema, 1980; Standora et al., 1982; Spotila and Standora, 1985; Maxwell, 1988; Maloney et al., 1990; Binkley, 1996). It is unclear whether this self-induced warming overlaps with the thermosensitive period of sexual differentiation, which could potentially influence the sex ratio.

The latter problem could be avoided by discarding the method of using sand temperature to predict sex ratio and instead relying on direct assessment of sex of the hatchlings. However, this is not always desirable, since it requires killing hatchlings (as explained in Chapter 1). If it is the case that metabolic warming overlaps with the

thermosensitive period of sexual differentiation (roughly the middle third of incubation), then perhaps the amount of heat can be quantified. This amount could be used as a correction factor to be added to sand temperature data; this would make predictions of sex ratios from sand temperature more accurate.

However, the onset of metabolic heating may not necessarily overlap with the thermosensitive period of sexual differentiation. In order to quantify the amount of metabolic heat generated by incubating eggs and to relate this to the thermosensitve period of sexual differentiation, I monitored nest temperatures and nearby sand temperatures of leatherback sea turtles (*Dermochelys coriacea)* nesting at Matapica Beach, Suriname.

Materials and Methods

All work was done in the 1993 and 1994 nesting seasons, on Matapica Beach, Suriname. This dynamic beach is a major nesting area in Suriname for leatherback and green sea turtles (*Chelonia mydas*), and to a lesser extent, olive ridley (*Lepidochelys olivacea*) and hawksbill sea turtles (*Eretmochelys imbricata*) (for more details, see Schulz, 1975). Only natural nests of leatherback turtles were used in this study.

When freshly laid leatherback nests were encountered within 12 hours of deposition, about 30% - 50% of the eggs were gently removed, so that temperature sensors could be placed in the middle and side of the nest, at similar depths. All eggs (including small yolkless eggs, typical of leatherback nests) were returned to the nest, and the sand was replaced at the top and packed down. Approximately 1 meter away from each nest, parallel to the high tide line, a temperature sensor was buried in the sand at the same depth as the sensors in the nest. The sensors used were either Cu/Cn type K thermocouples, read with a Sensortek BAT 12 unit (Omega Inc., Stanford, Connecticut, USA), or digital thermistors (Chapter 2). The sensors were usually read every 2-4 days, between the hours of 10:00 and 14:00, local time. The daily instantaneous sand temperature values were recorded for the probes within the nest, as well as for the reference probe in the sand nearby. Metabolic

warming in each nest was calculated for each day by subtracting temperatures from the middle or side of each nest from the corresponding temperatures of the reference site. The aim of this was to generate a thermal profile of the nests throughout development.

During incubation, individual nests were excavated once and one or two eggs next to the temperature probes were removed. The eggs were opened and (if viable) staged according to Renous et al. (1989). The aim was to compare the amount of metabolic heat in a nest and the stage of embryonic development of the eggs. The thermosensitive period for sexual differentiation in leatherback embryos is between stages 23 and 27 (Desvages et al., 1993). Once a nest was opened and sample eggs removed, no more data were collected from that nest, lest the opening of the nest altered the thermal environment or affected development (cf. Hendrickson, 1958).

Results

Temperature probes were placed in a total of 7 leatherback nests in 1993; and 46 nests in 1994. Of these 53 nests, 22 were lost, because either they were washed away by excessive high tides, or they were dug up by other nesting turtles, or they were poached. In addition, upon excavation and inspection of the eggs around the probes, some further nests had no viable eggs surrounding the side and/or centre probes. Temperature data from probes that had no surrounding viable eggs were excluded from any analyses.

Metabolic warming was evident in the leatherback nests from very early stages and increased during development (Fig. 5.1). However, only after 25 days did the centre of the nests on average become more than 0.5 °C warmer than the sand. The eggs at the sides of the nests experienced less metabolic warming: only after day 45 did these eggs become more than 0.5 °C warmer than the sand (Fig. 5.1). Only 4 nests remained undisturbed until the very last stages of embryonic development, after which they were opened and the eggs surrounding the probes inspected. The temperature data from these nests (of which 1 had no viable eggs around its centre thermistor and another had no viable eggs around its side thermistor) are shown in Fig. 5.2.

From the eggs that were viable and staged, it is evident that the majority of eggs experienced <1 °C metabolic heat during the thermosensitive period for sexual differentiation (Fig. 5.3). During the thermosensitive period, there was no significant difference in mean amount of metabolic warming in those eggs in the centre of the clutches, +0.9 °C, and those on the side, +0.8 °C ($p=0.192$, two tailed t-test, unpaired). Three nests contributed data on embryonic stage of development during the thermosensitive period from both side and centre of the nest; a paired 2-tailed t-test did not reveal significant differences between means of the side and centre data from these 3 nests ($p=0.296$).

Figure 5.1. Mean temperature difference between developing leatherback sea turtle eggs and surrounding sand. Temperature data are from thermosensitive probes placed in the approximate centres and sides of clutches; each clutch had a reference temperature probe placed in the sand, at similar depths to the nest probes, and one metre away from the clutch. For each clutch, temperature data for side and for centre probes were collapsed into 5 day bins by averaging all temperatures recorded in those 5 days; shown is the mean temperature (±SEM) of all data from all clutches that contained viable eggs (see text). Values next to points refer to number of nests contributing to each point. Overall, temperatures were significantly warmer in the centre of the clutches than in the sides of the clutches (one-way ANOVA, unpaired, $p<0.001$). Student-Neuman-Keuls tests applied post-hoc revealed a significant difference only between means of the two groups on Day 51-55.

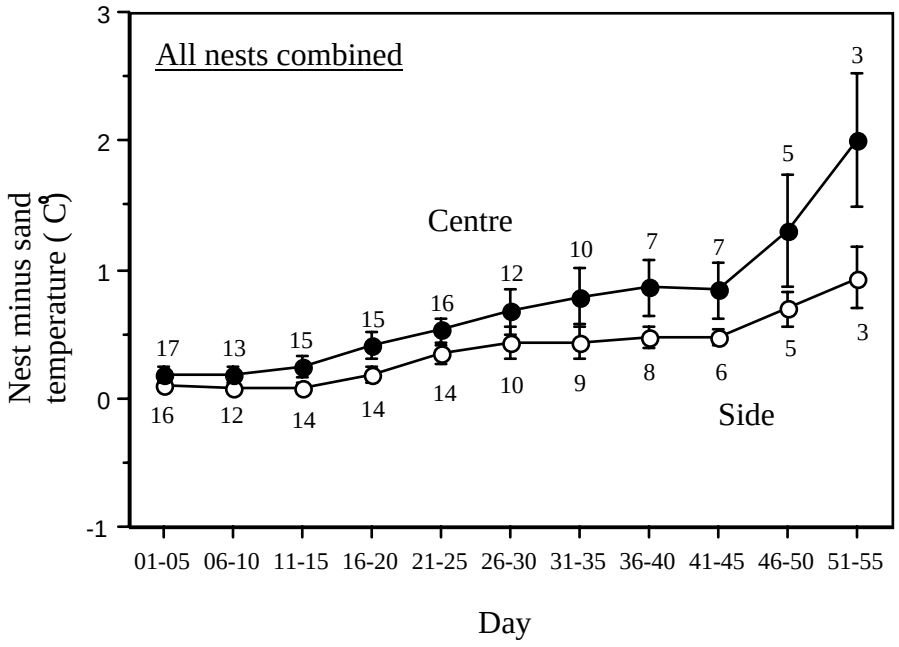

All nests combined
Centre
Side
Nest minus sand temperature (C)
Day
3
2
1
0
-1
01-05
06-10
11-15
16-20
21-25
26-30
31-35
36-40
41-45
46-50
51-55
17
13
15
15
16
12
10
7
7
5
3
16
12
14
14
14
10
9
8
6
5
3

Figure 5.2. Mean temperature difference (±SEM) between developing leatherback sea turtle eggs and surrounding sand, from nests that were not opened until just before hatching. Conventions are the same as in Fig 5.1. Centre temperatures were significantly warmer than side temperatures (one-way ANOVA, unpaired, $p<0.002$). Individual unpaired t-tests, 1-tailed, revealed significant differences between means of the centre and side data only in the last 2 time groups (Day 46-50 and Day 51-55).

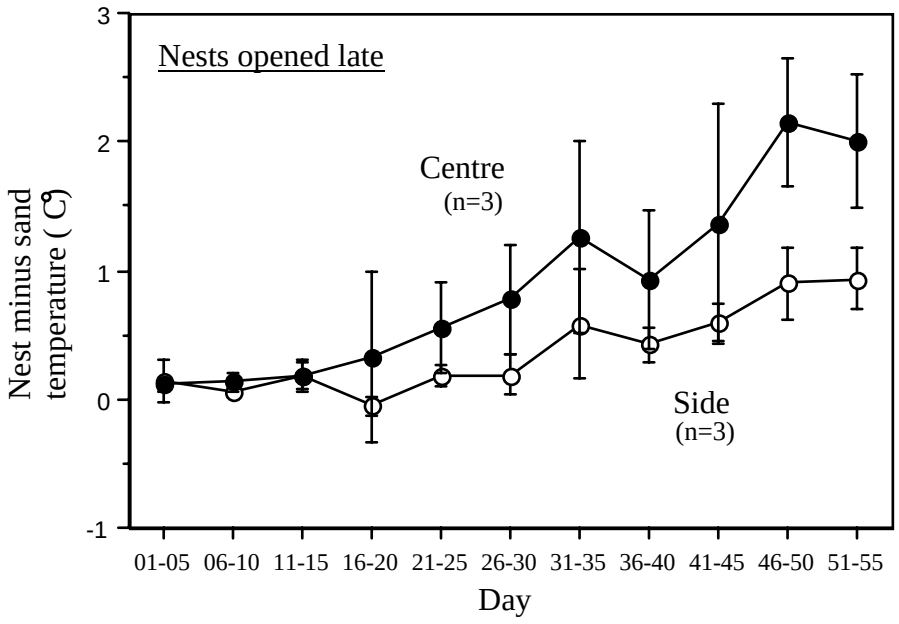
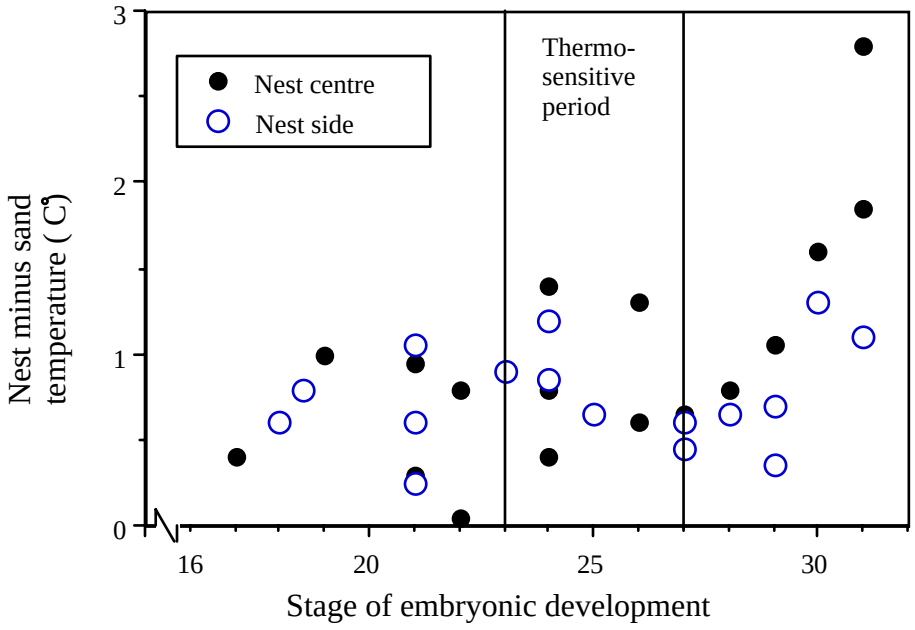

Nests opened late
Centre
(n=3)
Side
(n=3)
Nest minus sand temperature (C°)
3
2
1
0
-1
01-05
06-10
11-15
16-20
21-25
26-30
31-35
36-40
41-45
46-50
51-55
Day

Figure 5.3. The relationship between stage of development and amount of thermal difference between nest and surrounding sand. On a particular day, the temperatures in the nest (side and centre) and the nearby sand were recorded, and then the nest opened. Eggs located next to the temperature probes were opened and staged (if viable). These data are shown in the figure.


Nest centre
Nest side
Thermo-
sensitive
period
Nest minus sand
temperature (C°)
Stage of embryonic development
0
1
2
3
16
20
25
30

Discussion

As incubation of leatherback eggs progresses, mean rate of oxygen consumption increases (Thompson, 1993), indicating an increase in overall metabolism during development. This increased metabolism coincides with an increase of self-generated heat in sea turtle nests, as seen in Fig. 5.1. It is interesting to note that the profile of the increase in metabolic heat is similar to that of the increase in oxygen consumption (Thompson, 1993), although a strict comparison is probably not valid, given that the eggs monitored by Thompson were incubated at a constant temperature, while the eggs from this study came from nests laid at different times (and thus different thermal environments) during the season.

As expected, those eggs at the side of the nest experienced less metabolic heating on average than those at the centre (Figs. 5.1 and 5.2). However, in general, eggs on the sides of the nests were still warmer than the nearby sand. From Figs. 5.1 and 5.2, it appears that the eggs in the centre of the nests are >0.5 °C warmer than the surrounding sand by days 21-25 of incubation. If the total incubation period is roughly 60 days (see Chapter 3), and the thermosensitive period for sexual differentiation is roughly during the middle third of incubation (Renous et al., 1989), then one might conclude that metabolic warming can have an effect on the sex of the hatchlings. However, this analysis is simplistic, because groups of nests from different thermal zones and times of year were grouped. Some nests laid in cooler parts of the season would take longer to incubate, and so their thermosensitive period would occur later (in units of days) than that of nests laid in warmer parts of the season. Such grouping of nests is a common feature of previous studies of metabolic heat in sea turtle nests (e.g. Carr and Hirth, 1961; Maloney et al., 1990).

A more accurate estimate of the amount of metabolic warming that occurs during the thermosensitive period can be made from the data in Fig. 5.3. This is because eggs from different nests were opened and their embryonic stages were determined. This allowed direct comparison of nests that experienced different thermal environments. The mean amount of

metabolic warming experienced by incubating leatherback eggs during the thermosensitive period is 0.82 ±0.09SEM °C. Unfortunately, there are few data contributing to the thermosensitive period in Fig. 5.3 (more than 50% of all potential data were lost during the field work). From the data available, there was no difference in the metabolic heat of eggs from the centre and side of a nest, during stages 23 through 27, the thermosensitive period of sexual differentiation in leatherbacks (Desvages et al., 1993).

If there is differential heating of eggs in different parts of the nest (as suggested by Figs. 5.1 and 5.2), then one might predict that some eggs will be subjected to more feminising temperatures than others during incubation. However, it is unclear how often this may occur. For leatherbacks, the TRT is between 29.0 °C and 30.0 °C; above and below these temperatures, all female and male hatchlings are produced, respectively (Rimblot-Baly et al., 1987). Therefore, in order for metabolic warming to have an influence on the sex ratio of a nest, that nest must be incubating in sand that is at or just below the pivotal temperature: if the sand is warmer than 30.0 °C, then the hatchlings would differentiate into females regardless of metabolic warming. From Fig. 5.3, eggs were about 0.8 °C warmer than the sand during the thermosensitive period. This means that metabolic warming has the potential to alter the sex ratio when the sand temperature is between 28.2 °C and 30.0 °C. However, at Matapica, Suriname, sand temperatures during large parts of the leatherback nesting season are often well above 30 °C (Mrosovsky et al., 1984a; Chapter 3). Perhaps on other nesting beaches, the sand temperature at nest depth is closer to leatherback pivotal temperature for longer periods; in that case, metabolic warming might have larger effects on sex ratios.

Leatherback eggs have a small TRT relative to other sea turtles (Mrosovsky, 1994). This suggests that metabolic warming in developing eggs of other species, should it occur during the thermosensitive period, may have a greater impact. Studies on other turtle species should not ignore the potential impacts on sex ratios of metabolic warming.

Summary

1. Eggs are slightly warmer than the surrounding sand during incubation. Therefore, attempts to predict sex ratio from a combination of sand temperatures and pivotal temperatures may tend to underestimate the number of females produced, unless a correction factor is applied to account for this metabolic warming.

2. The effect of metabolic warming is not great, for the following reasons. One, sand temperatures are often well above or well below the TRT. This is especially true for leatherbacks, with their small TRT. Two, during the thermosensitive period, the amount of metabolic warming is not large (around 1 °C).

Chapter 6:
Rate of embryonic development as an index of sex ratio of sea turtle hatchlings.

Introduction

The presence of TSD in sea turtles (and other organisms as well) implies that the sex ratio of their offspring can be biased towards one sex. Uncovering offspring sex ratios has theoretical and practical applications (see Chapter 1), but to arrive at such data is not trivial. There are two general challenges in the task of estimating sex ratios in sea turtles: sampling and sexing.

Gross morphology cannot reliably indicate the sex of neonate turtles (Yntema and Mrosovsky, 1980; Whitmore et al., 1985; Rimblot-Baly et al., 1987). At present, there are only 2 methods of assigning sex to sea turtle hatchlings: histological analysis of the structure of the gonad (Yntema and Mrosovsky, 1980; Spotila et al., 1983; Miller and Limpus 1981; Mrosovksy et al., 1984a; Dutton et al., 1985), and analysis of relative hormone levels in the allantoic fluid remaining in the egg after hatching (Gross et al., 1995). The former is time consuming and requires sacrificing the hatchling (but see Appendix 2 for details on mitigation). The latter method requires sampling of the fluid before the eggs hatch, or removal of eggs from natural nests and final incubation of individual eggs in the lab, to guard against cross contamination of fluid from different eggs (which occurs during hatching in natural nests). Therefore, the allantoic fluid method is not practical for large scale projects, such as estimating sex ratios from an entire nesting season. At present, histology of the gonads is the only feasible method of sexing sea turtle neonates.

The other main challenge is sampling. For sea turtles, nesting seasons may span several months, during which fluctuations in weather can lead to changes in sand temperatures and thus sex ratios. In addition, the thermal environment of a nesting beach may vary from year to year, resulting in variations in sex ratio (Mrosovsky et al., 1984a;

Rimblot-Baly, 1987; Janzen, 1994b; also see Chapter 3). Finally, female sea turtles are not reproductively active in consecutive years; therefore, a representative sample of sex ratios of offspring from an entire nesting population should span several years. Therefore, direct observation of hatchling sex ratios requires sampling large numbers of nests over long periods of time, as well as histology, making long term studies difficult to complete. However, it is also possible to predict sex ratios from other variables measured on a nesting beach.

Sand and/or nest temperatures have been recorded at nesting beaches, and these may indicate the sex ratios of hatchlings produced there. In general, this technique is useful, especially in situations where the sand temperatures are outside the transitional range of temperature (TRT), which encompasses those middle trimester incubation temperatures that result in mixed sex ratios of turtle hatchlings (Mrosovsky and Provancha, 1992; Chan and Liew, 1995; Binkley, 1996). However, when the sand temperatures are close to pivotal (and thus in the TRT), it is more difficult to predict sex ratios. This is because the pivotal temperatures for many nesting populations remain undescribed, and thus sand temperatures cannot be converted to sex ratios. In addition, metabolic heat generated during development may increase nest temperatures, relative to sand temperatures, and this warming may affect the sex ratio of the nest. Monitoring of nest temperatures provide information on metabolic warming, but because temperature varies within a nest, measuring different locations within nests would be needed. Also, the equipment and methods of recording sand temperatures often vary from beach to beach, and study to study. Some methods and equipment are less reliable than others, and may give errors in the temperature readings. This is especially worrisome in the case of leatherback turtles; with this species, an error of 0.5 °C can change an estimate of sex ratio of a nest from male-biased to female-biased because the leatherback TRT spans less than 1 °C (Rimblot-Baly et al., 1987). For these reasons, it is desirable to use other measured variables to predict sex ratios.

Other environmental variables that are known to be correlated with sand temperatures and/or sex ratios of turtles include mean air temperatures, density of vegetational cover, beach albedo, and rainfall (Janzen, 1994a, b; Hays et al., 1995; Chapter 3; see Fig. 1.2). However, some of these variables are not easily measured, or their relationships to sex ratio have not been extensively studied. One of the more promising of the measured variables that indicates sex ratio is incubation duration.

Incubation duration, which is defined as the time between laying and hatching of the nest, indicates developmental rate. It is known that developmental rate of turtle eggs is affected by temperature in a consistent manner (Ackerman and Prange, 1972; Bustard and Greenham, 1968; Mrosovsky, 1988; Mrosovsky et al., 1992; Lewis-Winokur and Winokur, 1995): the warmer the incubation temperatures, the faster the development. Because sex ratio and developmental rate are affected by temperature, it should be possible to describe the direct relationship between these two variables, and from this, predict one from the other.

Incubation duration is an easily measured variable on nesting beaches, and such data are often already collected for beaches that have been monitored as part of conservation programmes. The purpose of this chapter is to summarize the relationship between sex ratio and incubation duration in a number of species, and describe the usefulness of this relationship in estimating sex ratios of sea turtle hatchlings.

Materials and Methods

Data on sex ratio and incubation duration of individual nests are available for 3 species of sea turtles. For green turtles, data on 169 nests come from Mrosovksy (1982), Mrosovsky et al. (1984a), and Chapter 3. For loggerhead turtles, data on 76 natural nests come from Mrosovsky et al. (1984b) and Mrosovsky and Provancha (1989, 1992). For leatherback turtles, data on 48 natural nests come from Mrosovsky et al. (1984a) and Chapter 3. In all cases, only information from natural nests are included. In almost all cases, the sex

ratio of each nest was determined by sampling 10 hatchlings (for details, see studies cited above).

For each species, nests were grouped by length of incubation, and mean sex ratios were tabulated. Each incubation duration and corresponding mean sex ratio were plotted against each other, and a best fit sigmoidal curve was fitted through all the points using Inplot 2.2 software (GraphPad, Inc., San Diego, California). Briefly, the fit is nonlinear regression based on the Marquardt method, and the equation of the curve is a four parameter logistic equation. The slopes at the 50% female level of all 3 species were compared using analysis of covariance, followed by individual t-tests, 2 tailed (Zar, 1984).

Results

For loggerhead and green turtles, the pivotal incubation duration (that incubation period at which 50% female hatchlings are produced) is close to 59.5 days (Table 6.1 and Figs. 6.1 and 6.2). For leatherbacks, the pivotal duration is 65.7 days (Fig 6.3). The slope of the curve for leatherbacks is roughly twice as steep (-0.26) than the slopes for green turtles (-0.15) and loggerheads (-0.10). Analysis of covariance revealed a significant difference among the slopes of the

Table 6.1. Estimated pivotal durations and slopes of fitted nonlinear regression lines for the 3 populations of sea turtles studied. Coefficients of determination (r^2) for each curve are also reported.

Population	n	Pivotal duration	±SEM	Slope	±SEM	r^2
Loggerheads, S.E. USA	76	60.16	1.08	-0.10	0.02	0.77
Green turtles, Suriname	169	59.37	0.38	-0.15	0.02	0.92
Leatherback, Suriname	48	65.66	0.32	-0.26	0.05	0.94

NOTE: n refers to the number of nests contributing to the curves. In the loggerhead dataset, 1 nest with 67 days incubation duration was 100% female. There is no justification for excluding this nest from the analysis; however, if it were excluded, the already high r^2 is improved to 0.87, and the pivotal duration changes to 59.67 days ± 0.74 SEM.

Figure 6.1. Relationship between sex ratio and incubation duration for loggerhead turtle nests from North Carolina, Georgia, and Florida, U.S.A. Incubation duration for nests in the field is defined as the time between when the eggs are laid and when the hatchlings emerge from the nest. Points are means of sex ratio of nests with a particular duration. Numbers next to points represent the numbers of nests contributing to each point. The sigmoidal curve was fitted with InPlot 2.2 software (GraphPad, Inc. San Diego, Ca.), with upper and lower asymptotes specified as 100% and 0% female, respectively. The pivotal duration (that incubation duration which results in 50% female sex ratio) is 60.2 days. The slope of the curve is -0.10.

FOR SOME FIGURES IN CHAPTER 6, SEE Godfrey, M. H. and Mrosovsky, N. (1997). Estimating the time between hatching of sea turtles and their emergence from the nest. Chelonian Conservation and Biology 2: 581-585. Available here: http://members.seaturtle.org/godfreym/Godfrey_1997_CCB.pdf

Figure 6.2. Relationship between sex ratio and incubation duration for green turtle nests laid in Suriname. Specifications for curve fitting are the same as described in the legend of Fig. 6.1. The pivotal duration is 59.5 days, and the slope of the curve is -0.15.

FOR SOME FIGURES IN CHAPTER 6, SEE Godfrey, M. H. and Mrosovsky, N. (1997). Estimating the time between hatching of sea turtles and their emergence from the nest. Chelonian Conservation and Biology 2: 581-585. Available here: http://members.seaturtle.org/godfreym/Godfrey_1997_CCB.pdf

Figure 6.3. Relationship between sex ratio and incubation duration for leatherback turtle nests from Suriname. Details of curve fitting are the same as in previous figures. The pivotal duration is 65.7 days. The slope of the curve is -0.26.

FOR SOME FIGURES IN CHAPTER 6, SEE Godfrey, M. H. and Mrosovsky, N. (1997). Estimating the time between hatching of sea turtles and their emergence from the nest. Chelonian Conservation and Biology 2: 581-585. Available here: http://members.seaturtle.org/godfreym/Godfrey_1997_CCB.pdf

curves ($F_{2,42}$=62.45, p<0.001). The slope for the leatherback curve was significantly different from that of the green turtle curve (t=4.90, v=24, p<0.001) and from that of the loggerhead curve (t=3.05, v=29, p<0.005).

Discussion

The steeper slope of the curve defining the relationship between sex ratio and incubation duration in leatherbacks, relative to the slopes of the curves of loggerhead and green turtles, is consistent with the narrower TRT of leatherbacks. The broader the TRT, the greater the range of temperatures that will produce mixed sex ratios, and hence the greater the range of incubation durations that produce mixed sex ratios. In addition, the r^2 values of the curves are all high (>0.9; except for loggerheads, for which r^2= 0.77). High r^2 suggest that incubation duration could be a good predictor of sex ratio.

That the pivotal durations of loggerheads and green turtles are similar (within 0.8 days), while the leatherback pivotal duration is roughly 6 days longer, may be explained in the differences in mean egg and hatchling sizes. Leatherback eggs are on average larger than green or loggerhead eggs which tend to be very similar (Hirth, 1980). Leatherback hatchlings weigh more than greens and loggerhead turtle hatchlings (Hirth, 1980). Presumably, development takes longer for leatherback embryos because they are larger at hatching. Because the thermosensitive period of sexual differentiation in sea turtles is thought to be the middle third of development (Yntema and Mrosovsky, 1982; Desvages et al., 1993), and because leatherbacks take longer to develop during incubation, it is reasonable to assume that their pivotal incubation duration is longer than those of turtles with smaller hatchlings.

Previous studies have made inferences about sex ratios of turtle hatchlings from incubation durations either indirectly, by converting durations to temperature and then relating temperature to sex ratio (Mrosovsky and Yntema, 1980; Standora and Spotila, 1985), or directly (Mrosovsky et al., 1984a; Eendebak, 1995). In the case of the latter studies, the

numbers of sexed turtles contributing to the curves relating sex ratio and incubation duration were smaller than those presented here, and no coefficients of determination were given, making it difficult to evaluate the strength of the relationships.

In the present study, it would appear that the curves from Figs 6.1, 6.2, and 6.3 can be used to predict sex ratios. It should be noted that each point on each graph is the mean of several nests; hence, predictions made by reading off the curves should give mean sex ratios for a number of nests. In the case of individual nests, one might expect some variation in sex ratio despite similar incubation durations. This is because incubation duration reflects the thermal environment during the whole of development, whereas sex ratio only represents the thermal environment during the middle third of incubation. Some nests that were warm during the thermosensitive period of sexual differentiation (and thus produced mainly females) might be exposed to heavy rain or high tidal washes during the latter stages of development, which would lengthen the incubation duration without affecting the sex ratio. However, turtle nests are laid deep in the sand, and are usually not exposed to thermal variation greater than ±0.5 °C (Morreale et al., 1982; see also Chapter 3). According to Georges et al. (1994), a ±1 °C daily fluctuation around the mean temperature will result in an incubation duration that is equivalent to that of eggs incubated at a constant temperature that is 0.1 °C warmer. Therefore, on average, incubation durations of sea turtle nests should reflect sex ratio.

This method of estimating sex ratio from incubation duration requires previous knowledge of sex ratio from a number of nests from a particular population. For many populations, no such data exist. However, it may still be possible to predict sex ratios from incubation durations, without having to sample a large number of nests. All that is needed are data on laboratory incubation of some turtle eggs (often obtained when investigating the pivotal temperature of a population) and data on the average time it takes for turtles to emerge from the nest after hatching from the eggs (the HE interval). Then, the following equation could be employed:

Incubation duration in the field	=	Incubation duration in the laboratory	+	Time from hatching to emergence from nest (HE interval).

From a study of pivotal temperature in the laboratory, one can also calculate the pivotal duration in the laboratory . To the curve relating sex ratio and laboratory incubation duration, the HE interval can be added to derive a field pivotal incubation duration and a corresponding curve relating sex ratio and field incubation duration (Fig. 6.4). This derived curve can then be used to predict sex ratios of nests whose incubation durations are known.

Unfortunately, the HE interval in sea turtles has not been well described. In general, it is difficult to assess when the hatchlings have pipped from their eggs without invading the nest. Opening the nest to see if the eggs have pipped, and replacing the sand, could well alter the HE interval. For instance, the compaction of the replaced sand might be different, or allowing light into the nest may affect the behaviour of the hatchlings. What is required is a different, non-invasive approach to estimating the mean HE interval for sea turtle hatchlings. Such an approach is outlined in the following chapter.

Figure 6.4. Hypothetical derived curve relating sex ratio to incubation duration in the field, obtained by adding the HE interval to the laboratory curve of sex ratio vs. incubation duration. See text for more details.

FOR SOME FIGURES IN CHAPTER 6, SEE Godfrey, M. H. and Mrosovsky, N. (1997). Estimating the time between hatching of sea turtles and their emergence from the nest. Chelonian Conservation and Biology 2: 581-585. Available here: http://members.seaturtle.org/godfreym/Godfrey_1997_CCB.pdf

Chapter 7:
Estimating the time between hatching of sea turtles and their emergence from the nest

Introduction

Sea turtles lay their eggs deep in the sand of tropical or temperate beaches. The eggs are left behind to incubate in the sand, eventually producing hatchlings which emerge from nests and scramble to the ocean. Several important physiological and behavioural events occur during this pre-emergence period. These include temperature-dependent sexual differentiation during the middle third of incubation (Yntema and Mrosovsky, 1982; Raynaud and Pieau, 1985), and the nocturnal emergence of hatchlings from the nest, which appears to be gated by changes in sand temperature (Gyuris, 1993; Mrosovsky, 1968).

After hatching from the egg but before emerging from the nest onto the beach, the newborn turtles remain in the sand for a few days. This is an important stage for the hatchlings, allowing time for closing and straightening the plastron and for reabsorbing of the remnants of the yolk sac, as well as for achieving behavioural synchrony in their efforts to emerge from the nest and scramble towards the sea together, apparently as an anti-predator behaviour (Carr, 1973). Indeed, the greatest metabolism of residual yolk occurs while the hatchlings are still in the nest (Kraemer and Bennett, 1981). The interval between pipping from the egg and eventually emerging from the nest has not been extensively studied. Even the duration of the interval between hatching and emerging is not firmly established because all previously published studies that estimated this interval relied on some sort of manipulation of the nest, for example by digging into the nests prior to emergence to see if the eggs had hatched, or by placing a glass pane on one side of the nests (Table 7.1).

Table 7.1. Estimates of time between hatching and nest emergence (HE interval) of sea turtles.

Species	HE interval (days)	Sample size (nests)	Method	Reference
Caretta caretta	6	1	excavation	Caldwell, 1959
Caretta caretta	4-6	5[a]	plastic pole in nest, excavation	Kraemer and Richardson, 1979
Caretta caretta	5	23	temperature probe in nest	Webster and Gouveia, 1988
Caretta caretta	5.7-5.9	18	temperature probe in nest, excavation	Neville et al., 1989
Caretta caretta	4-7	7[a]	glass-sided nests	Christens, 1990
Chelonia mydas	>4	??[a,b]	excavation	Hendrickson, 1958
Chelonia mydas	7	1[a]	glass-sided nest	Carr and Ogren, 1960
Eretmochelys	4-6	2[a]	glass-sided nests	Diamond, 1976
imbricata	6	1[a]	microphone, artificial nest	Raj, 1976

[a]Relocated or artificial nests

[b]Not stated

Rather than rely on such interventions for estimating the time it takes for the turtles to reach the surface of the sand after hatching, we have developed a technique that dispenses with any manipulations of the nest at all, or the introduction of any recording devices. This method depends on the use of data from naturally incubated nests, as well as data from laboratory incubated eggs. Specifically, we derived the interval between hatching and emergence by formulating an equation and solving for the variable that represents the interval, as follows. It was assumed that in natural nests

$$TOT = LAB + HE\ interval,$$

where TOT is the total incubation period in days from laying until emergence at the surface of the sand, as seen in natural nests; LAB is the number of days from laying to hatching as seen in laboratory conditions; and HE interval is the time between hatching and emergence from the sand. Values for TOT and LAB were derived, respectively, from studies conducted in the field and in the laboratory. However, because developmental rate varies with ambient temperature, it is necessary to compare values for TOT and LAB from eggs that developed at similar temperatures. An index of nest temperature during development is the sex ratio of hatchlings from the nest, since the direction of sexual differentiation of sea turtle hatchlings is determined by prevailing incubation temperature (Raynaud and Pieau, 1985). To standardize the rate of development, duration values were chosen for TOT and LAB from eggs that produce 50% of each sex. These values came from published studies on the sex ratios of loggerhead turtles.

Materials and Methods

Data on sex ratios and incubation durations for natural nests come from a number of studies on sea turtles (Mrosovsky et al., 1984b; Mrosovsky and Provancha, 1989, 1992). We are most grateful to Jane Provancha, Sally Hopkins, and Jim Richardson for making available more detailed data than were reported in the above publications. Data are from natural loggerhead nests laid in the North Carolina, South Carolina, Georgia, and Florida, U.S.A.

For our purposes, we define incubation duration in natural nests as the number of days in between the date of laying (day 0) and the date of the emergence of hatchlings from the nest. In all cases, freshly laid nests encountered in the morning were scored as being laid on the previous night. The location of each nest was marked, and several days before expected emergence, wire traps were placed over the nest at the surface of the sand, and checked daily in the early morning for hatchlings. If hatchlings in a trap were encountered, the nest was scored as having emerged the night before (the end of the incubation period).

The data on sea turtle eggs incubated in the laboratory also come from clutches laid in North Carolina, Georgia, and Florida, U.S.A. (Mrosovsky, 1988). We defined incubation period for the eggs incubated in the laboratory as the number of days between the date of laying (day 0) and date of hatching. A turtle was considered hatched if its head and at least one flipper were outside of the egg shell. In a few cases, for example if a turtle appeared wedged against the side of the container in which the egg was placed during incubation, turtles with their heads and most of their flipper exposed were scored as hatched, even though the tip of their flipper was still inside the egg.

For the incubation durations in the field, we obtained information from 76 loggerhead clutches. For the incubation durations in the laboratory, we obtained information from 389 eggs from 6 different loggerhead nests. Data were grouped into 1 day bins that spanned the range of incubation durations. For each analysis, we fitted a sigmoidal curve to the plots of incubation duration vs. sex ratio (% female), using Inplot 2.2 software (GraphPad, Inc.). From each of these best fit sigmoidal curves, we calculated the average incubation duration

that would result in 50% females. The duration giving 50% of each sex has been designated the pivotal incubation duration (Mrosovsky et al., 1984a).

To estimate the number of days that sea turtle hatchlings spend digging to the surface of the sand following hatching from the egg (HE interval), we calculated the difference between the pivotal incubation durations estimated from natural nests (TOT) and laboratory conditions (LAB), using the equation:

TOT - LAB = HE interval.

Results

Incubation durations in natural nests in the field (TOTs) were longer than those of eggs incubated in the laboratory (LABs) (Figs. 7.1 and 6.1). These differences reflect the time in which hatchlings dig up to the surface of the sand. At pivotal incubation duration, that is the number of incubation days that results in a 1:1 sex ratio, for loggerheads the difference between the TOT and LAB was 4.1 days (Fig. 7.2).

Discussion

For loggerhead turtle hatchlings, we estimate about 4 days on average are spent in the nest after hatching and before emerging on the beach surface. Our definition of hatching obviously affects our estimates of the HE interval. Gutzke et al., (1984) have suggested that in reptiles, pipping (when the eggshell is first slit) is better than hatching as an index of the end of the incubation period, because it shows less variability. Pipping was not systematically monitored for all the laboratory incubated eggs; however, we have noted that time to pipping can be shorter than time to hatching: in two clutches of loggerhead eggs incubating at onstant temperatures in a different study, hatching on average occurred 0.8 days

Figure 7.1. Sigmoidal curve fitted to data from loggerhead eggs incubated in the laboratory. Individual eggs (from 6 separate clutches) were incubated at different constant temperatures. The sex ratio of groups of eggs were sorted in one day intervals, according to incubation duration, which for laboratory incubation was defined as the number of days between laying and when the head and at least one flipper of the hatchling was outside of the egg shell. Values next to each point refer to numbers of eggs that contributed to each point. The curve was fitted by nonlinear regression, with Inplot 2.2 software (GraphPad, Inc., San Diego, California, U.S.A), with 0% and 100% female specified as lower and upper asymptotes. The laboratory pivotal incubation duration (i.e. that duration which results in 50% females) is 56.1 days.

FOR FIGURES IN CHAPTER 7, SEE Godfrey, M. H. and Mrosovsky, N. (1997). Estimating the time between hatching of sea turtles and their emergence from the nest. Chelonian Conservation and Biology 2: 581-585. Available here: http://members.seaturtle.org/godfreym/Godfrey_1997_CCB.pdf

Figure 7.2. The sigmoidal curves from time between laying and hatching in the laboratory vs. sex ratio (Fig. 7.1) and time between laying and emerging from the nest vs. sex ratio (Fig. 6.1) are replotted together. At 50% female sex ratio, the curves differ by 4.1 days. This is the HE interval for loggerhead nests.

FOR SOME FIGURES IN CHAPTER 6, SEE Godfrey, M. H. and Mrosovsky, N. (1997). Estimating the time between hatching of sea turtles and their emergence from the nest. Chelonian Conservation and Biology 2: 581-585. Available here: http://members.seaturtle.org/godfreym/Godfrey_1997_CCB.pdf

after pipping (n=185, range 0-2.5 days, data to be published elsewhere). This may be an overestimate, because eggs were inspected only twice a day. Pending further studies in sea turtles, if the interval between pipping and emergence is required, then we suggest a value of 5 days rather than 4 days as a mean.

These estimates depend on various assumptions about the comparability of field and laboratory incubation conditions. One concerns temperature fluctuations occurring in natural nests. Georges et al. (1994) found that an increase in the amplitude of the diel temperature cycle during laboratory incubation caused loggerhead eggs to become more female biased without a concordant reduction in incubation duration. However, it is unlikely that this would have affected our estimates, for the following reasons. First, natural sea turtle nests are generally placed deep beneath the surface of the sand, and thus are subject to little daily variation in temperature, often less than 1 °C overall (Morreale et al., 1982; see also Chapter 3). In addition, we maintained relatively constant temperatures in all incubators during artificial incubation, usually with less than 0.5 °C variation. Even if the temperature of the incubating eggs cycled around the mean as much as ±1.0 °C per day, according to model of Georges et al., (1994), such variation during incubation corresponds to only a 0.1 °C increase in the constant temperature equivalent. This small increase in temperature would have only a slight effect on sex ratio.

Another assumption of the present method of estimating the HE interval is that the eggs incubating in the laboratory were healthy and developing at similar rates, for a given temperature, as eggs in the sand on natural beaches. Etchberger et al. (1991) were able to lengthen by about 4 days the incubation period of freshwater turtle (*Trachemys scripta*) eggs independent of changes to the sex ratio by chronically decreasing the amount of oxygen that was circulated through the incubators. These results were dependent upon extremely low levels of O_2 in the incubators (8%), which resulted in extremely low hatch rates. In contrast, our method of incubating eggs in the laboratory was designed to allow circulation of air throughout all egg containers, with the tops of the eggs exposed above the substrate (see

Mrosovsky, 1988, for diagram). Our method is capable of giving high hatch rates (e.g. Mrosovsky, 1988), which would not have been expected if there had been oxygen deprivation (Ackerman, 1980; Etchberger et al., 1991). In addition, in a different study using the same incubation methods, oxygen levels in the incubators remained between 20% and 20.9% (measured with a Servomex Oxygen Analyser #572), which suggest that eggs previously held in these incubators were not oxygen deprived.

In terms of eggs incubating in the field, it is unlikely that the oxygen levels are so different from the laboratory incubation conditions as to contribute to a lengthening of the field incubation period. There are 2 reasons for this view. First, from the study by Etchberger et al. (1991), the hypoxic conditions needed to lengthen the incubation period produce a low survival rate (11%). Ackerman (1980) noted also a positive relationship between relative oxygen levels and hatching success for sea turtle eggs incubated in artificial nests. In contrast, for our study, the nests from which samples were collected in the field generally had high hatching success rate, which suggests that conditions were not hypoxic in the nests. Second, measured levels of O_2 in natural sea turtle nests remain between 14% and 19% for most of the incubation period, and drop to roughly 5% in the last few days before hatching (Ackerman, 1977). Presumably, this acute condition (only a few days) of hypoxia would not result in a delay in incubation of days, since chronic hypoxia (over the 60 day incubation period) resulted in a lengthening of incubation by only 4 days for *Trachemys scripta* eggs (Etchberger et al., 1991). Of course, caution should used when comparing results from different species, but it does suggest that the conditions between laboratory and field incubation are not greatly dissimilar.

A further assumption is that sex ratio is correlated with incubation duration. This assumption is supported by the high coefficients of determination of the fitted curves. Regression lines fitted to the data also had similar coefficients of determination, and gave an overall value of 3.9 days for the HE interval at 50% female. If the correlation between sex ratio and incubation duration is not high, and greatly influenced by a few points in a small

data set, then pivotal duration will become less reliable. Perhaps such factors account for the value of about 55 days pivotal incubation duration for naturally incubated green turtle clutches from Tortuguero, Costa Rica (Standora and Spotila, 1985, n=9 nests, r^2=0.48). In the absence of information on laboratory incubation of Costa Rican green turtle eggs, it is not possible to estimate the HE interval for this population of turtles. Webster and Gouveia (1988) used the relationship between nest temperature and sex ratio of naturally incubated and laboratory reared turtle eggs to predict a 5-6 day HE interval for loggerheads. Overall, the data from that study are difficult to evaluate, as they are available in abstract form only. One possible source of error in their estimates is their use of laboratory incubation data from Mrosovsky and Yntema (1980), which was based not only on small numbers of eggs, but also had variable corrections added to them to compensate for different transit times.

Returning to our own data, it is clear that not all the points fall perfectly on the fitted lines (see figures 7.1 and 6.1; see also figures 12 and 13 in Mrosovsky et al., 1984a), but some scatter is to be expected, because the sex ratios from natural nests were estimated not from full clutches but from samples of about 10 eggs per clutch. Sampling error in the sex ratio would contribute to some of the variation in the data. In addition, we would expect there to be some differences in the pivotal temperatures in individual clutches, and such variations should translate into differences in pivotal durations (Mrosovsky, 1988). This is due to the strong relationship between sex ratio and incubation duration, which is an indicator of developmental rate. Consider two clutches that have different pivotal temperatures. If turtle embryos from the first clutch differentiate into females at lower temperatures than those from the second clutch, then the pivotal duration from the first clutch (with a lower pivotal temperature) should be longer than that of the second (with a higher pivotal temperature).

All this is another way of saying that in contrast to previous work, the present method is suitable for estimating the mean HE interval of large groups of nests, rather than of individual clutches. It is interesting to note that there is a general correspondence between

our HE interval estimate and those obtained by methods involving intervention and/or translocation of individual clutches (Table 7.1). This suggests that such interventions do not have major effects on the HE interval.

Mean values can be useful in comparing the HE interval in distinct situations, such as in different seasons. For example, it is possible that the HE interval is different at different temperatures. From Fig. 7.2, there is a suggestion that at lower temperatures (longer incubation durations) the HE interval is longer than at higher temperatures. More data are needed to examine this potential difference. For the present time, it should be stressed that the present estimate of mean HE interval comes from eggs incubated near pivotal. The mean HE interval may not be the same under different incubation conditions.

In some types of sand, sea turtles may have more difficulty digging to the surface after pipping. In the case of beach nourishment, a common technique used to stem beach erosion, sometimes the introduced sand is different from the naturally occurring sand and may initially be more compacted (Crain et al., 1995). This could lead to an increase in the digging up time, not only because the hatchlings must work harder to reach the surface, but also because greater exertion produces greater amounts of lactate in the body. These high levels of lactate would likely require a longer period of repose for the hatchlings just beneath the surface, to allow for degradation of the lactate (Dial, 1987). The longer the HE interval, the more residual yolk is likely to be utilized during this pre-emergence period, and the less yolk is left for the post emergence period. This in turn might curtail the 24 hr. post hatching frenzy, which is thought to be important in assisting the newly hatched turtles to move away from a predator-filled shoreline towards a safer pelagic environment (Wyneken and Salmon, 1992).

In conclusion, our estimates of the hatching to emergence interval are based on large sample sizes as well as on certain assumptions. However, until a method is developed of directly measuring the behaviour of the hatchlings in individual nests without disturbance,

the present procedure may provide the most extensively based and most accurate estimate of the time it takes a sea turtle to reach the surface after hatching.

Chapter 8:
General Discussion and Future Directions

The general aim of this thesis was to investigate the impact of TSD on the overall sex ratios of sea turtle hatchlings. To do this, new methods had to be developed to assist in estimating offspring sex. These include a simplified technique of monitoring daily mean sand temperatures and the use of indirect indices of sex ratio, such as rainfall and developmental rate of eggs. The additional investigations into the role of metabolic warmth generated during incubation of the eggs, the pivotal temperature, and transitional range of temperature, contribute to more accurate estimates of sex ratios of sea turtle hatchlings. In addition to focussing on these technical matters, I estimated the seasonal sex ratios of leatherback and green sea turtles in Suriname from 14 different years.

One of the salient findings about these sex ratio estimates of sea turtle hatchlings in Suriname is the extent of variation from year to year. This is also true of at least one population of freshwater turtles (Janzen, 1994b), and is consistent with some reports of sex ratios of sea turtle hatchlings from limited parts of different seasons (Mrosovsky et al., 1984a; Rimblot-Baly et al., 1987; Spotila et al., 1987; Horikoshi, 1992). This variation contrasts with that of loggerhead sea turtle populations in Southeast USA and Brazil (Mrosovsky and Provancha, 1992; Marcovaldi et al., in review). The original question of whether or not the sex ratios of sea turtle hatchlings are different from 1:1 has not been fully resolved. More data need to be generated on offspring sex ratios of different species and different populations of sea turtles. This also would make it possible to compare various traits among populations that have similar or different sex ratios, which in turn may help in understanding the adaptive value of TSD.

With regard to adaptive value, it may be the case that TSD evolved under conditions that no longer exist at present, but TSD has been maintained in some species because it works as adequately as GSD (Mrosovsky, 1980). However, if this is the case, one would

expect the sex ratios of species with TSD to be similar to sex ratios of species with GSD (except in situations where the assumptions of the Charnov-Bull model (1977) are met, and biases towards one sex are selected for). GSD tends to result in 1:1 offspring sex ratios (Maynard Smith, 1980). Can the same be said of TSD? As yet, there still are not enough data to fully answer this. From studies that are based on data from 5 or more years, there are suggestions that 3 populations of turtles have biased sex ratios of hatchlings (Chapter 3; Mrosovsky and Provancha, 1992; Marcovaldi et al., in review); however, there are 2 other studied populations that have offspring sex ratios that are close to 1:1 (Janzen, 1994b; Chapter 3). Clearly, more data on offspring sex ratios are needed to more fully resolve this question.

In the future, more emphasis should be placed on long-term studies of offspring sex ratios. One reason is because sea turtles generally do not nest in consecutive seasons. Thus, the mean sex ratio from a single season may not represent the entire nesting population, and may provide only a "snapshot" of the overall sex production of the whole population. This is particularly critical because of environmental variation across different years. Even data from 5 consecutive years may not be enough to obviate this problem. In the case of a nesting population of painted turtles, offspring sex ratio estimates from 49 years were balanced out at 52.2% female overall. There were yearly fluctuations above and below a balanced sex ratio in this population; however, there was at least one 5 year period in which sex ratios were strongly female biased in consecutive years, and another 4 year period when they were heavily male biased. Had sampling been confined to these sets of years, or even 10 years including one of these sets, the overall estimate would have been different from 1:1. This example illustrates the importance of long term studies comprising many years.

In addition to information on sex ratios, more data are required on population dynamics of these species. Most studies assume that the offspring sex ratio of a single nesting population represents the sex ratio of the whole breeding population. Population genetic studies based on the mitochondrial DNA (mtDNA) of sea turtles suggest that there is

little gene flow among turtles nesting on different beaches, and thus nesting populations are independent units (Bowen et al., 1992, 1993; Allard et al., 1994; Bass et al., 1996; Dutton, 1996). However, because mtDNA is usually inherited maternally, data from mtDNA do not uncover male-mediated gene flow across populations.

In the case of green sea turtles, studies of nuclear DNA (nDNA) from different nesting populations suggest there is very little segregation among nesting populations, except for a split between Atlantic and Pacific ocean basins (Karl et al., 1992). These data support the idea that males produced on different nesting beaches are moving among groups of nesting females. This in turn suggests that the functional breeding populations are not restricted to a single nesting beach. In the case of leatherbacks, it has been found that there is very little difference in nDNA haplotypes among nesting populations in the Caribbean (Dutton, 1996). For instance, estimates of genetic distance (based on 3 different methods) between leatherbacks that nest in Suriname and Trinidad range from 0.006 and 0.119, and the *Nm* (the number of migrants per generation) is greater than 4 for all 8 populations assayed (Dutton, 1996). Strictly speaking, a proper estimate of the sex ratio for a breeding population of leatherbacks would include data from Suriname and Trinidad, as well as other nesting groups linked by significant male-mediated gene flow. This means that a sex ratio bias on one nesting beach may be balanced by an opposite bias on another nesting beach within the larger breeding population. This is further argument for pursuing studies of offspring sex ratios in different nesting populations and over large areas.

Nevertheless, data on sex ratios of individual nesting areas are important for management and conservation. As sea turtles rookeries are often subject to active management, there is the possibility that the thermal environment of the developing eggs will be altered by these programmes. For instance, relocating eggs to a central hatchery may increase hatching success, but it can change the sex ratio of the eggs if the temperature of the hatchery is different from natural nesting areas. Knowledge of the thermal environment of the natural nesting sites, and of the natural sex ratio, is useful as a yardstick against which

alterations can be assessed. Thus, data collected on sex ratios serve at least two purposes: management and advancement of our understanding of the significance of TSD.

This thesis has emphasized the need to discover overall sex ratios in different populations. And yet, there is also the potential gain of information from studying TSD in individuals. The spectre of rapid global warming from increased levels of "greenhouse" gases in the atmosphere has led some to hypothesize that species with TSD (such as sea turtles) may be threatened with extinction because increased global temperatures can result in all-female offspring (e.g. Mrosovsky et al., 1984a; Janzen, 1994b). However, others have asserted that such pessimism is unwarranted (Deeming and Ferguson, 1989; Davenport, 1989); they submit that, in response to increased global temperatures, animals with TSD would either alter their pivotal temperatures and/or alter their nest site selection, such that extreme biases in sex ratio are avoided. Unfortunately, there are few data available with which to evaluate these claims.

In the case of oviposition, sea turtles nest sites are usually defined in terms of distance from the high tide line. Sea turtles generally appear to place their nests randomly on a nesting beach, whether one is looking at groups or individuals (Eckert, 1987; Bjorndal and Bolten, 1992; Hays and Speakman, 1993; Hays et al., 1995). There have been some indications that sea turtles select nest locations by using certain cues, such as sand temperature gradients (Stoneburner and Richardson, 1981), an uneven beach surface (Hays et al., 1995), and dark silhouettes (Salmon et al., 1995). However, in these cases, the studies have been based on small sample sizes and/or limited time scales. As yet, there has been no detailed study of the long-term nesting patterns of individual sea turtles. Perhaps individuals alter their long-term patterns of nest site selection in response to environmental changes (such as a general warming of sand temperature). One way of addressing this question is to look at beaches that have been subject to hurricanes, since beach vegetation is usually knocked down and destroyed from the heavy winds and salt spray associated with hurricanes. Presumably, loss of vegetation will contribute to higher sand temperatures and thus more

female hatchlings. Perhaps in these circumstances, sea turtles would alter their nesting patterns in response to this environmental change.

Recently, it has been suggested that diamondback terrapin (*Malaclemys terrapin*) females will choose thermally specific nest sites on the basis of the size of their eggs (Roosenburg, 1996). This species exhibits TSD with MF pattern (low temperature males, high temperature females, see Fig 1.1). Also, diamondback egg size varies greatly among clutches (but not within clutches), and egg size determines hatchling size and eventually size at age of first reproduction. In addition, there is sexual dimorphism at age of reproduction, with females being more than twice the weight of males. Roosenburg (1996) has suggested that female diamondbacks will lay smaller eggs in cooler nest sites (producing small males) and larger eggs in warmer nest sites (producing large females). Although the differences in mean egg weight across different nest sites was small (ca. 10%), this study suggests that maternal effects are an important component of TSD. However, even in reptile species that do not display TSD, nest site selection also can affect the shape, activity levels, and other phenotypes of the offspring (e.g. Shine and Harlow, 1996). This makes it difficult to understand the evolutionary significance of TSD, in terms of nest site selection. Nevertheless, it would be interesting to look at individual sea turtles and their processes of nest site selection. It may be the case that there is some interaction between changes in egg size and sand temperature across a nesting season, and that females will adjust their placement of eggs accordingly.

In the case of alteration of pivotal temperatures in response to climate change, one can look for evidence among populations of a single species that are located in different latitudes. In the northern hemisphere, one might expect more northerly populations of turtles to have lower pivotal temperatures, relative to more southerly populations, because mean temperatures are inversely related to latitude. However, studies have found no such change in pivotal temperatures across latitudes (Bull et al., 1982b; Mrosovsky, 1988). In fact, in the case of snapping turtles (*Chelydra serpentina*), there is a positive relationship between

latitude of the population and pivotal temperature (Ewert et al., 1994), which appears to be counter intuitive. However, these studies relied on relatively small numbers of clutches, and were not carried out over a number of years. There has yet to be a study in which possible variation in pivotal temperature of one population (or even one individual) is monitored over a long period of time.

Clearly, there is still a long way to go before reaching a satisfactory understanding of TSD and its adaptive value in sea turtles. Perhaps a new theoretical model is needed, given the difficulty in applying the Charnov-Bull (1977) model to sea turtles. It is unclear what the benefits are of this system of sexual differentiation, given that slight changes in incubation temperatures during embryonic development have such a profound effect on adult sea turtles that inhabit a variety of habitats vastly different from beaches where they hatched. In order to develop new models, a variety of different types of studies are needed. These range from estimating overall sex ratio in populations to uncovering specific details in the processes of nest site selection and its effects on offspring sex ratios. Although the challenges in doing these studies are great, the ultimate reward in gaining an understanding of TSD in sea turtles is even greater.

Appendix 1:
Estimating hatchling sex ratios

When confronted with the information that hatchling sex is affected by incubation temperature, one of the first questions that springs to mind is "What is the natural sex ratio of sea turtles?" A second question that soon follows is "What is the optimal sex ratio, for conservation?" Both questions are closely related, and at the present time neither can be easily answered. No one has designed and completed a long-term study that shows whether such manipulation is beneficial or detrimental. Until more information is made available, and in the light of some possible negative consequences of interference (Lovich, 1996), for the time being (though not necessarily in the future), it is assumed that the safest course of action is to maintain natural hatchling sex ratios.

Knowledge of the natural sex ratio of nesting sea turtle populations is an important component of any management plan. Such information provides a baseline against which the impacts of certain conservation techniques could be evaluated. These include: a) nest relocation, to higher up on the beach or to a hatchery, either of which may be thermally different from the original site; b) limited egg harvest, which may result in the disproportionate removal of one sex from the beach; c) beach renourishment, which may alter the thermal characteristics of a beach by introducing a different type of sand.

Estimating sex ratios requires the synthesis of two types of information. First, the sex of the hatchlings must be determined. The chapter by Merchant covered different methods that can be used, such as direct histological examination, comparing nest temperatures to pivotal temperatures, etc. Second, data on sex must be combined with information on the nesting patterns of a population. It is necessary to know where the turtles are nesting on the beach, and when they nest, as there is spatial and temporal variation in sand temperature.

Spatial variation

On a turtle beach, there may be distinct zones that are thermally different. Some zones have vegetation, others do not. Nests that are laid under dense vegetation are likely to be cooler, and thus produce more males than those laid in the open zone, which are likely to be warmer (cf. Spotila et al., 1987). The distance from the high tide line may affect the depth of the water table, and thus influence temperatures at nest depth. In addition, if a nesting beach is long, then ideally all the subsections should be sampled to account for any thermal variation along the beach. Finally, if the aim is to estimate the hatchling sex ratio of the entire breeding population, then information from all the nesting beaches in the range of the meta-population should be included. Genetic information is likely to be required to ascertain the extent of the breeding population (note that despite the apparent segregation of females by distinct nesting beaches, these groups of females may still be part of a larger interbreeding population if the males move and mate freely among the different groups).

Temporal variation

Over a nesting season, which may last several months, there are likely to be changes in weather. For instance, rainy seasons can affect sand temperatures at nest depth, which in turn can affect sex ratio. Therefore, a proper estimate of the sex ratio should be based on samples taken throughout the nesting season. In practice, it is often easier to divide up the season into discrete units of time, such as month or half-month periods, and estimate the mean sex ratio for each of those periods (e.g. Chapter 3). Also, it is important to remember that sea turtles tend to nest every second or third year (or more). Therefore, an estimate of hatchling sex ratios from a single season may represent nest site selection by only a third or so of the total adult population. Ideally, estimates of hatchling sex ratio should be based on data from several consecutive years. Of course, some variation in sex ratios from year to year is to be expected, since weather and nesting frequency can (and do) change. By collecting data on sex ratios from a number of years, it is possible to determine an average of hatchling sex ratio. If it is possible to determine sex ratios in only a single year, then it is

desirable to consider whether or not that year was thermally typical. Meteorological records can be used for this purpose.

Nesting frequency and constructing an estimate of sex ratio

In general, more turtles nest in the middle of the season than at its beginning or end. This change in nesting frequency must be integrated with the information on how sex ratio varies over the season. The aim is to combine sex ratio information for specific periods of the nesting season with data on the relative numbers of nests laid during that same time. For instance, a sex ratio profile of a hypothetical nesting beach is shown in Fig. A1.1. The nesting season spans 3 months, and marks the transition from the dry to the rainy season. The relative nesting frequency in each month is shown on the right, with the majority of the nests being laid in June. The mean sex ratio from several sampled nests laid in each month is also shown on the right, presented in % female. Combining the two sets of data from all three months produces an overall seasonal sex ratio of 57% female. However, if sampling is restricted to one month (e.g. June), then the estimate of sex ratio would be inaccurate (e.g. 40% female). Also, data from one beach or one year may well not be representative of the average long term population sex ratio (see Fig A1.2). Finally, it may be the case that clutch size or hatching success varies from beach to beach (or over time). If the variation is large, it would important to take these factors into account when calculating the sex ratio.

In summary, understanding relationships between temperature and sex ratio on a beach enables protection to be organized in such a way that conserves both sexes. For instance, when relocation of eggs is necessary, it helps managers avoid impacts on sex ratios. However, measures taken in one place or time should be assessed in the context of the spatio-temporal variation of sex ratio (and nesting patterns) in the population as a whole.

Figure A1.1. Hypothetical example of turtle nesting frequency and sex ratio in a single season at a single nesting beach.

FOR FIGURES IN APPENDIX 1, SEE Godfrey, M. H. and Mrosovsky, N. (1999). Estimating hatchling sex ratios. In Research and Management Techniques for the Conservation of Sea Turtles, eds. Eckert, K. L., Bjorndal, K. A., Abreu-Grobois, F. A. and Donnelly, M.), pp. 136-138: IUCN/SSC Marine Turtle Specialist Group. Available here: http://members.seaturtle.org/godfreym/Godfrey_1999_Manual.pdf

Figure A1.2. Requirements to constitute an adequate sample. If sampling is confined to one area only (dashed circle at top left), this does not represent the full spatio-temporal variation, or changes in nest density.

FOR FIGURES IN APPENDIX 1, SEE Godfrey, M. H. and Mrosovsky, N. (1999). Estimating hatchling sex ratios. In Research and Management Techniques for the Conservation of Sea Turtles, eds. Eckert, K. L., Bjorndal, K. A., Abreu-Grobois, F. A. and Donnelly, M.), pp. 136-138: IUCN/SSC Marine Turtle Specialist Group. Available here: http://members.seaturtle.org/godfreym/Godfrey_1999_Manual.pdf

Appendix 2:

Saving Sea Turtle Eggs in Suriname

It is conservatively estimated that in Suriname approximately 40% of leatherback nests and about 20% of green turtle nests are laid below high tide line (Mrosovsky, 1983c; Mrosovsky et al, 1984a). If left alone, the eggs from these nests are subsequently washed away. These "doomed" eggs, as they have been called, provide considerable opportunity for augmenting output of turtle rookeries. Transplanting them to areas of the beach not threatened by high tides, although hard work, is a conservation technique that has minimal technical requirements and can be performed in isolated areas. During the 1993 nesting season of green and leatherback turtles at Matapica, we participated in the translocation of doomed eggs, alongside our scientific studies.

Our efforts can be divided into two categories: an "assist" category and a "save" category. The former includes those nests we moved or helped to move, but would probably have been translocated by the STINASU workers without our help. The latter includes those relocated nests that would have not been moved had we not been patrolling sections of the beach every morning and some evenings. There are various reasons why STINASU personnel could not save all doomed eggs: the field workers were not on the beach that day, we encountered doomed nests which would have been washed away by the tides before the STINASU patrol, etc. The numbers of nests we moved are as follows:

	Eggs		Clutches	
	Assist	Save	Assist	Save
Leatherback	1608	2487	19	32
Green turtle	8425	5674	65	44
Olive ridley	234	93	2	1

According to the literature, the hatch rates of translocated nests for leatherback, olive ridley and green turtles are 68.7%, 50% and 58%, respectively (Whitmore and Dutton, 1985;

Schulz, 1975). Thus, the total number of hatchlings produced as a result of our work approximates the following:

	Assist	Save
Leatherback hatchlings	1105	1701
Green hatchlings	4886	3291
Olive ridley hatchlings	117	46
total	6108	5046

In the course of our studies on sex ratios in sea turtles we collected 770 green turtle hatchlings from 79 nests and 260 leatherback hatchlings from 27 nests (see Chapter 3). If the number of hatchlings taken for our sex ratio study are subtracted from "save" category, the totals for each species are as follows:

	Save
Leatherback hatchlings	1449
Green hatchlings	2521
Olive ridley hatchlings	46
total	4016

Thus, we saved 3.9 as many hatchlings as we collected for scientific work. In addition to the 6108 hatchlings we helped the STINASU staff to save, our independent efforts produced a net gain of 4016 hatchlings from this beach. Also, although not quantifiable, it is possible that our presence on the beach acted as an additional deterrent to poachers.

Conclusions

1) STINASU's egg relocation program can be supplemented by visiting researchers.

2) It is possible to combine collecting of hatchlings for scientific study with direct conservation benefits (see Appendix 3 for work completed in Suriname during the sex ratio study in 1993).

Appendix 3:
Beach vegetation and sea finding orientation of turtle hatchlings.

Introduction

When sea turtle hatchlings emerge from a nest at night, they immediately move toward the ocean. This seaward orientation is guided by visual cues: sea turtles tend to move away from dark silhouettes and towards the brightest area along a horizon (Salmon et al., 1992; Mrosovsky and Shettleworth, 1968, 1974). On the beach, this corresponds to moving away from the treeline and towards the open horizon over water.

Green (*Chelonia mydas*) and leatherback (*Dermochelys coriacea*) sea turtles often share the same nesting beaches, as is the case at Matapica, Suriname. These animals lay a substantial percentage of their nests below the spring high tide line where they are likely to be washed away: about 32% for leatherbacks (Mrosovsky, 1989) and about 25% for greens (Mrosovsky et al., 1984a). As part of Suriname's conservation programme, these doomed nests are relocated to beach sites above the spring high tide line, even though relocation often reduces hatching success (Schulz, 1975; Limpus et al., 1979; Eckert and Eckert, 1990; but see Whitmore and Dutton, 1985; Wyneken et al., 1988). Moving doomed eggs is a simple way to increase overall hatchling production on this beach.

In 1993, conservation workers succeeded in transplanting most doomed nests higher up the beach into areas of dense low-lying vegetation. This boosted the proportion of neonates that emerged in vegetation, as typically only a small percentage of natural green and leatherback nests are laid in this area: 13% and 0% respectively (Whitmore and Dutton, 1985).

To assess what effects this nest relocation procedure may have on their seafinding behaviour, we studied the ability of hatchlings to orient towards the ocean when surrounded by vegetation,

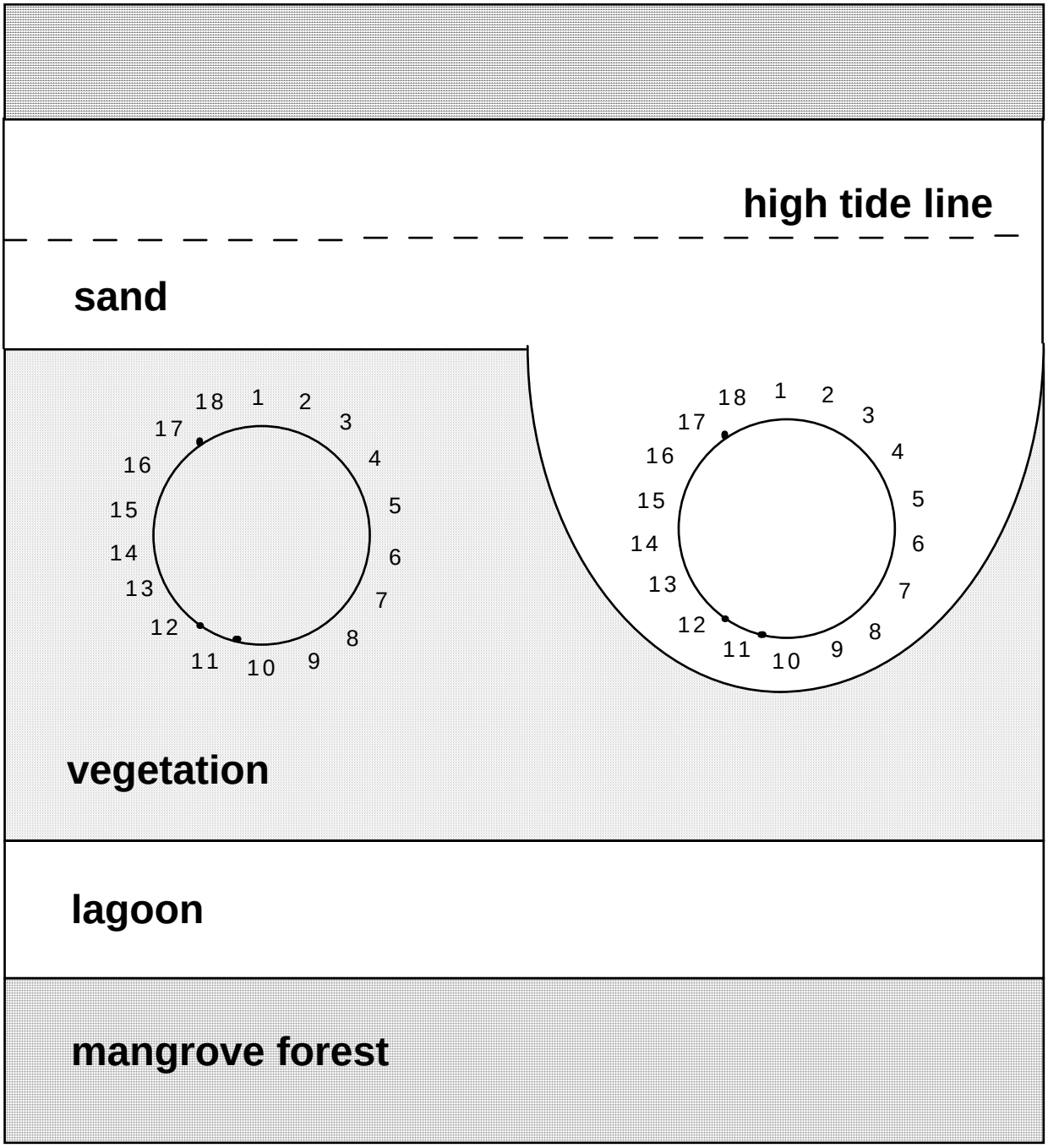
Materials and Methods

Experiments were carried out from 21 July to 10 September 1993 on a beach in Suriname running east-west between 1-8 km west of the Matapica Canal. The beach is located on a sand spit which drifts west at the rate of about 2 km per year (Schulz, 1975), and is separated from the coastal mangrove forest by a brackish lagoon. The width of the spit is approximately 75 m. Above the high water line there is a strip of open sand about 8 m wide. Beyond this is an area of dense vegetation consisting mainly of *Ipomea-pes-caprae* and *Canavalia maritima* (see Fig. A3.1).

Two orientation arenas were used, each consisting of a circular trench 6m in radius, dug 40 cm deep and 20 cm wide and divided into 18 equal segments by small sheet metal barriers. Segment #1 was the most seaward, and other segments followed in numerical order clockwise. Both arenas were built in the vegetation and were equidistant from the ocean, but one had all vegetation cleared from its center and from the sand north of it (Fig. A3.1). The centers of the arenas were approximately 16m from the spring high tide line, and separated from each other by about 40m. Within the arenas the ocean was not visible at hatchling eye level.

We gathered naive hatchlings from natural nests that had been marked after laying and had wire traps placed over them 4-5 days before expected emergence. The traps were checked each morning and turtles were collected and

Figure A3.1. Diagram of the orientation arenas used in this study. The radii of the arenas are 6m. The centres of the arenas are separated by approximately 40m. Arena segment numbers are indicated. The arenas are located in a typical section of beach at Matapica, Suriname.


high tide line
sand
18 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
18 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
vegetation
lagoon
mangrove forest

kept indoors in covered plastic buckets until that night, when the experiments were performed.

Experiments took place 2-3 hours after sundown. The hatchlings were kept outdoors uncovered for at least an hour before any tests were run, to ensure that they would be adapted to ambient light. Each experiment consisted of quickly placing equal numbers of hatchlings in the center of both arenas, and then allowing them to move in any direction until they fell into the trench. During tests we remained seated at a distance (at least 20m) from the two arenas to avoid interfering with hatchling orientation. At the end of each test the trenches were checked, and the number of hatchlings in each segment and those remaining in the arena (if any) were recorded. The turtles were then released on the berm from where they proceeded toward the ocean. The position of the moon, if visible, relative to the arenas was recorded for each run.

Only one species was tested at any one time. Preliminary tests, in which hatchlings were placed in the center of the vegetation arena with a bright light shining from segment #1 (most seaward), showed that green turtle hatchlings needed between 15-25 minutes to reach the trench, whereas leatherbacks needed around 45 minutes. Because leatherback turtles moved more slowly, the duration of each run was different depending on the species tested. Green turtle hatchlings were deposited in the middle of the two arenas simultaneously, and were allowed 30 minutes to move about. Leatherback turtles in the vegetation arena were given 60 minutes, while those tested in the open arena were tested for only 30 minutes, timed to coincide with the second half of the vegetation arena run. The test time of the leatherbacks in the open arena was cut to reduce exposure to ghost crabs (*Ocypode* spp.).

Rayleigh's test (Zar, 1984) was used to check for significant orientation within each group of animals. When both groups in a pair showed significant

Figure A3.2. The results of the orientation experiments on green hatchlings. Each row represents two groups tested at the same time in the open and vegetation arenas. If the moon was visible at the time of testing, a moon symbol indicates its direction. Each turtle icon represents an individual tested and final location at the end of the trial. The number in the centre of the arena represents the turtles remaining there at the end of the test. Mean angle of dispersion of each group is indicated by the line originating at the middle of the circle, where 0^{o} is defined as the middle of segment #1 (most seaward). Line length is inversely proportional to dispersion; lines reaching the perimeter of the circle correspond to r=1. The r-vector (r), group mean angle (a), and significance of orientation (p value) were calculated by the Rayleigh test.

FOR FIGURES IN APPENDIX 3, SEE Godfrey, M. H. and Barreto, R. (1995). Beach vegetation and seafinding orientation of turtle hatchlings. Biological Conservation 74: 29-32. Available here:

http://members.seaturtle.org/godfreym/Godfrey_1995_BiolConserv.pdf

Figure A3.3. The results of the orientation experiments on leatherback hatchlings. Conventions are the same as in figure 2.

FOR FIGURES IN APPENDIX 3, SEE Godfrey, M. H. and Barreto, R. (1995). Beach vegetation and seafinding orientation of turtle hatchlings. Biological Conservation 74: 29-32. Available here:

http://members.seaturtle.org/godfreym/Godfrey_1995_BiolConserv.pdf

orientation, Watson's U^2 test (Zar, 1984) was used to look for differences in direction.

Results

We tested four paired groups of leatherback and green turtle hatchlings (summarized in Figs. A3.2 and A3.3). We observed a clear difference in orientation between all animals tested in the open sand arena and in the vegetation arena. Animals in the open arena showed a strong seaward orientation. Animals in the vegetation arena were more evenly distributed around the circumference and their orientation was rarely significant. When the Rayleigh test did show significant orientation by the hatchlings tested in the vegetation, it tended to be landward. In these cases the Watson U^2 test revealed that the direction was different from that of the hatchlings in the open arena ($p<0.001$ in all instances).

Open sand and vegetation sites also differed in the time needed by the hatchlings to reach the arena trenches, evidenced by the numbers of hatchlings that remained in the centre. In the vegetation arena, 18% of the greens and 69% of the leatherbacks failed to reach the perimeter, whereas only 6% and 3% remained in the center of the open arena (Figs. A3.2 and A3.3).

Discussion

There was a difference in the seafinding of the hatchlings tested in the open sand and vegetation. In the open arena, both green and leatherback turtles oriented towards the sea, although there appears to be a slight difference in direction between the species (also observed by Mrosovsky and Shettleworth, 1975). When placed in dense vegetation, hatchlings tended to scatter rather than orient towards the ocean. Dense vegetation presumably obscured the visual cues normally used for sea-finding, specifically silhouettes along the horizon and photic gradients (Salmon et al, 1992; Mrosovsky and Shettleworth, 1968, 1974). The former corresponds to the mangrove forest treeline, and the latter to the

brighter sky above the ocean (Fig. A3.1). At turtle eye level the beach vegetation created a visually homogeneous environment where the usual orientation cues were absent.

In some cases, hatchlings confronted with uniform vegetation oriented away from the ocean (Figs. A3.2 and A3.3). This landward orientation might be explained by the use of slope cues in the absence of adequate photic information. Salmon et al (1992) found that loggerhead hatchlings oriented down inclines, though this response was weakened in the presence of even very dim light. The beach at Matapica slopes down on either side of the high water line, thus in both arenas, segment #1 was at a higher elevation than segment #10. It is interesting to note that this landward orientation was strongest on moonless nights, when ambient light levels were lowest.

Animals in the vegetation arena took longer to reach the trench, particularly leatherbacks, the majority of which remained in the arena after each test was finished. This may be because the hatchlings became entangled in the vegetation, but this is not likely to be the only cause. Disorientation played the major role: preliminary tests using an artificial light source demonstrated that even in dense vegetation leatherback hatchlings were able to reach the arena trench in about 45 minutes. Had the animals been able to properly orient, the testing interval would have allowed ample time for turtles to reach the trench. The greater mobility of green hatchlings in vegetation may be attributable to a species difference in sampling for orientation cues (Mrosovsky and Shettleworth, 1975). This could be tested further in a similar study of hawksbill (*Eretmochelys imbricata*) hatchlings, as they frequently nest in vegetation (Witzell, 1983). This may reveal the principal orientation cues hawksbills make use of in finding the sea.

Regardless of the causes and mechanisms responsible, these results show that the seaward movements of green and especially leatherback turtles are hampered when nests are in dense vegetation. Because these animals spend more time on the beach, their exposure to predators increases, and there is the added threat of desiccation for a hatchling remaining on the beach after sunrise. On several different occasions we noticed stray hatchlings in the

middle of the day wandering through the vegetation near our field camp some 70 meters from the spring high tide line. It is probable that some hatchlings that emerge in the vegetation never reach the sea. Thus, published hatch rates of relocated nests may not reflect the proportion of hatchlings that are actually added to the population, if a significant number of nests are reburied in vegetation.

Nevertheless, these results should not be taken as an argument against nest relocation as an effective conservation tool. Rather, this study serves as a means to refine this technique. Caution is needed in selecting relocation sites. Where the doomed eggs are placed is important, not just their removal from the tidal zone.

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