The ecology and sex determination of the pig-nosed turtle, *Carettochelys insculpta*, in the wet-dry tropics of Australia

By

J. Sean Doody
B.S. Zool., M.S. Biol. Sci.

A thesis submitted to the University of Canberra in fulfillment of the requirements of the degree of Doctor of Philosophy

Table of Contents

CHAPTER 1: GENERAL INTRODUCTION

CHAPTER 2: MOVEMENT PATTERNS AND ACTIVITY

Sex differences in activity and movements in the pig-nosed turtle, *Carettochelys insculpta*, in the wet-dry tropics of Australia (Copeia 2002:93-103)

CHAPTER 3: BEHAVIOR

Use of thermal springs for aquatic basking by the pig-nosed turtle, *Carettochelys insculpta* (ms *in press*, Chelonian Conservation and Biology)

CHAPTER 4: REPRODUCTION

Twice every two years: Reproduction in the pig-nosed turtle, *Carettochelys insculpta*, in the wet-dry tropics of Australia (ms to be submitted to Journal of Zoology)

CHAPTER 5: BEACH SELECTION

Beach selection in nesting pig-nosed turtles, *Carettochelys insculpta* (ms in review, Journal of Herpetology)

CHAPTER 6: EGGS AND HATCHLINGS

Embryonic aestivation and emergence behavior in the pig-nosed turtle, *Carettochelys insculpta* (Canadian Journal of Zoology 79:1062-1072).

CHAPTER 7: HATCHLING SEX RATIOS AND EMBRYONIC SURVIVAL

Early males and less late females: determinants of reproductive success and hatchling sex and embryonic survival in the pig-nosed turtle (*Carettochelys insculpta*) (ms prepared for submission to Oecologia)

CHAPTER 8: CAN TURTLES PREDICT OFFSPRING SEX?

Can turtles predict offspring sex? (ms prepared for Ecology)

CHAPTER 9: SYNOPSIS

A novel technique for gathering turtle nesting and emergence phenology data (Herpetological Review 31:220-222).

The Territory’s intriguing turtles (Australian Geographic 58:22-23).

LIST OF FIGURES

LIST OF TABLES

DECLARATION OF ORIGINALITY

This thesis is my original work and has not been submitted, in whole or part, for a degree at any university. Nor does it contain, to the best of my knowledge and belief, any material published or written by another person, except as acknowledged in the text. I attest that the content of this thesis, and the design and execution of the studies it describes, were my primary responsibility. Where papers derived from chapters are coauthored, it reflects a contribution from my supervisor, the technical officer employed on a broader project, or outstanding assistance from volunteers. They are included as authors on the ultimate publication under the University's Guidelines for Responsible Practice in Research. Other assistance I received in data collection and routine analysis is stated in the acknowledgments.

Signature…………………………….
Date………………………………….

COPYRIGHT

Signature…………………………….
Date………………………………….

ABSTRACT

Much of what we know about temperature-dependent sex determination (TSD) in reptiles stems from constant temperature incubation studies in the laboratory. In recent years, as TSD studies moved into the field it became evident that TSD was much more complex than previously thought. The present study attempted to reveal the complexity of TSD, as it relates to other features of the species' biology and physical characteristics tractable only in the field, such as fluctuations in incubation temperature and reproductive life history. To this end I studied the ecology of the turtle *Carettochelys insculpta*, a TSD species inhabiting the wet-dry tropics of northern Australia from 1996 to 1998. I tested hypotheses associated with movements, activity, behavior, reproduction, nest site choice, nest temperatures, embryonic survival, embryonic aestivation, hatchling sex ratios, and emergence in the species. Each of these was also considered in the context of the influence of the wet-dry tropics.

Compared to other turtles inhabiting lotic habitats, *C. insculpta* occupied considerably larger home ranges, covering up to 10 km of river. Of previously published factors influencing home range size, low productivity of the (micro) habitat may best explain the extensive home ranges in *C. insculpta*. Patchiness and low nutrient value of the chief food (aquatic vegetation) of *C. insculpta* may force turtles to cover large expanses of river to acquire sufficient energy for growth and reproduction. Females were more active, moved farther, and occupied larger home ranges than males. Home ranges of females comprised 1-4 activity centers, many of which were associated with thermal springs. I suggest that females may exhibit increased activity and movements relative to males because of sexual inequality in parental investment, where food is particularly limiting (e.g., in species with biennial

reproduction). Biennial reproduction in the population allowed the examination of the influence of reproductive condition on home range size, movements, and activity. Reproductive condition did not influence home range or activity, but gravid turtles moved father between successive sightings than non-gravid females. Individual data corroborate these findings, with females moving farther between successive sightings while gravid compared to while spent. Contrary to previous reports, turtles did not appear to move into estuarine areas or lowland floodplains during the wet season, but moved into the riparian forest and possibly into wetlands adjacent to the main channel in the vicinity of their dry season home ranges.

During the study I documented the turtles' use of small, localized thermal springs discharging from the river bottom. Dataloggers attached to the carapace to monitor ambient water temperatures recorded the frequency and duration of thermal spring use by individuals. Turtles used the thermal springs frequently during the winter (4-6 months) when river temperatures were lower than that of the thermal springs (8 = 29 ± 0.52° C). Turtles often utilized thermal springs for several consecutive hours, leaving the springs only to surface for air. Thermal springs may be derived from groundwater (which maintains a temperature equivalent to the annual mean air temperature), rather than from a specific geothermal heat source. Nine of 19 radio-telemetered adult females were seen to use thermal springs, of which seven were gravid and two non-gravid. Thus, gravid turtles may seek thermal springs more than non-gravid turtles. Frequency, duration, and timing of usage collectively suggest active thermoregulation as the primary function of thermal spring use. Utilization of thermal springs probably permits turtles to be more active in cooler months, which may enhance growth rates and accumulation of energy for reproduction. Onset of nesting along river stretches with thermal springs preceded nesting in a stretch not known to have thermal springs by 24 days. Thus, I speculate that by warming themselves on thermal springs in the months prior to nesting, turtles may have accelerated follicular development and nested earlier.

Female *C. insculpta* matured at ca. 6 kg body mass (38.0 cm carapace length, 30.5 cm plastron length). Turtles produced egg sizes and clutch sizes similar to that of other turtle species of similar size. Turtles reproduced every second year, but produced two clutches in each breeding year, ca. 40 days apart. Thus, it appeared that females were energy limited, possibly due to the low available energy content of the dry season diet (aquatic vegetation). Life history theory predicts that if some costly behavior is associated with reproduction, skipping years could reduce that cost and allow savings to be directed into future reproduction. The present study revealed no obvious accessory behavior in the population. Within years, clutch mass did not differ between early (first) and late (second) clutches. However, earlier clutches tended to have more and smaller eggs per clutch but than later clutches, a new finding for turtles that has been demonstrated in lizards and other animals. Because the study spanned both years with ‘big’ and ‘small’ wet seasons, I was able to examine how the magnitude of the wet season influenced reproductive characteristics. Following big wet seasons turtles produced larger, heavier, and more eggs per clutch than they did after small wet seasons. Relationships among body size, egg size, and clutch size were evident after two big wet seasons but not apparent after two small wet seasons. Collectively, annual variation in reproductive characteristics and current life history theory suggest that a big wet season is a plentiful time for the turtles.

I investigated beach selection of nesting pig-nosed turtles (*Carettochelys insculpta*) along a 63 km stretch of river in 1997 and 1998. I used three classes of beaches to examine beach choice: beaches with nests, beaches with only crawls, and beaches without nests or crawls. Across these beach types I compared aspect, solar exposure, temperature, substrate moisture, height, water depth at approach, and the height of cohesive sand. I located 82 nesting beaches with 221 nests, and identified 171 potential nesting beaches based on previously published criteria. Beaches with nests had a greater substrate moisture content and corresponding higher cohesive sand line (hereafter CSL) than beaches without nests. Beaches with nests also had a higher CSL than beaches with only crawls. Apparently, turtles could not excavate a nest chamber above the CSL due to loose substrate consistency causing sand to fall in on itself. Turtles could only nest at low elevations below the CSL on beaches with lower substrate moisture. Turtles apparently avoided nesting on these beaches due to the higher probability of nest flooding, as corroborated by a concurrent study. Beach temperatures increased with a seasonal increase in air temperatures, and were influenced by aspect and total angle of solar exposure. Temperatures did not differ among beaches with nests, beaches with only crawls, and beaches without crawls or nests. Therefore, there was no indication that turtles were manipulating offspring sex through choice of nesting beach. However, turtles may be manipulating sex by nesting in areas with particular thermal characteristics *within* beaches.

Two related aspects of hatchling emergence were studied. Using emergence phenology data, nest temperatures, historical weather data, and a developmental model, I tested the hypothesis that delayed hatching occurred in *C. insculpta*, and that such a delay would allow hatchlings to time their emergence to match the onset of the wet season. Hatchling *C. insculpta* emerged, on average, 17 days later than dates predicted from a developmental model. Combined with observations of hatchlings remaining in eggs until emergence, these results confirmed delayed hatching in nature. This delay was synchronized with initial river rises associated with the onset of wet season rains, and is consistent with published criteria for embryonic aestivation. On a diel scale, I generated predictions of two potentially competing models for nocturnal emergence in hatchling turtles, based on the knowledge that air temperatures decrease with season during the emergence period. A test of those predictions for *C. insculpta* produced ambiguous results. However, further analysis indicated that *C. insculpta*, and probably other nocturnally emerging turtle species, respond to a decline in diel temperature rather than an absolute temperature. The former would ensure nocturnal emergence, while the latter is experienced during the day as well as at night. Nocturnal emergence may be associated with nesting in open microhabitats.

The ‘decision’ of when and where to nest can influence both offspring survival and hatchling sex ratios in animals with temperature-dependent sex determination (TSD). Knowledge of how these maternal attributes influence the incubation environment is an important first step in hypothesizing why TSD evolved in a particular species. I studied the influence of nest site choice and timing of nesting on embryonic survival and hatchling sex ratios. Predation and flooding were the major sources of embryonic mortality. Embryonic survival was influenced by both lay date and nest site choice: In one year when nesting began later, nests laid later and at lower elevations were destroyed by early wet season river rises. In other years early nesting precluded flood mortality. However, turtles did not nest at the highest available elevations. I hypothesized that turtles were unable to nest at higher elevations because the sand was dry and not cohesive. A field experiment demonstrated that turtles were constrained to nest at lower elevations where they could construct a nest chamber. A mathematical model predicting hatchling sex from fluctuating temperatures was applied to temperature data from 102 natural nests. Results confirmed a type Ia pattern of TSD, whereby males are produced from cooler temperatures and females from warmer temperatures. The principal determinant of hatchling sex was lay date. Clutches laid earlier in the season produced mainly males, while later clutches yielded mostly females, due to seasonal ramping of air and sand temperatures. However, nest site choice also exerted an influence on hatchling sex. Female-producing clutches were deposited at higher elevations than male-producing clutches. The onset of nesting was not influenced by water temperatures, but may have been related to the magnitude of the previous wet season(s). Turtles nested earlier after two ‘big’ wet seasons and later following two ‘small’ wet seasons. This pattern indicates that the wet season is a plentiful time for the turtles. Adaptive ‘differential fitness’ models for the evolution of TSD have recently been reviewed and clarified. The differential fitness model that best fits *C. insculpta* is the ‘time-matching’ model, whereby one sex benefits more than the other from early hatching. Male *C. insculpta* hatched 2-3 weeks earlier then females, on average. Benefit to early hatching males and, therefore, the ultimate selective mechanism (e.g., growth, time to mature) is unknown. Obtaining such data will likely prove difficult in such a long-lived species.

A recent adaptive explanation for the evolution and maintenance of temperature-dependent sex determination (TSD) in reptiles rests upon the assumption that mothers can predict or manipulate offspring sex. I postulated that four physiological and behavioral criteria must be met in order for this assumption to be valid: (1) a strong correlation must exist between substrate temperatures during nest site choice and nest temperatures during the period of development when sex is determined in the egg (thermosensitive period = TSP). (2) Assuming that (1) is possible, mothers would need to be capable of correcting for temporal factors obscuring the predictable thermal characteristics of nest sites. This could be accomplished in two ways. By contracting nesting times mothers could assess the relative temperatures of alternate nest sites with some accuracy. A protracted distribution of nesting times could greatly reduce a mother's ability to distinguish between, for example, a cooler nest site at a warmer time and a warmer nest site at a cooler time. Alternatively, mothers would need to be able to incorporate temporal changes in nest site temperatures. (3) Sufficient variation in thermal profiles *among* nest sites, relative to the breadth of temperatures producing both sexes (pivotal temperatures), would be necessary. For example, if most nests produced both sexes, then depth of the eggs would be the deciding factor determining sex, leaving little opportunity for nest site choice to produce one sex or the other. (4) Mothers would need access to nest sites spanning a range of thermal profiles in order to produce either offspring sex. To this end, home range size relative to the number and location of nesting beaches should be important. I tested these four predictions in *Carettochelys insculpta*, a beach nesting turtle with TSD, using three years of field data on nest site choice, nesting times, thermal characteristics of nests, hatchling sex ratios, and movements of nesting turtles. A strong positive correlation existed between assessable substrate temperatures at nest site choice and mean daily TSP temperatures in all three years. However, the proportion of explained variation was highly variable among years, and low in 1998. Accordingly, the proportion of nests in which substrate temperatures at nest site choice predicted offspring sex correctly was low in 1998 (48-62 %, depending on treatment of the data). Nesting times were normally distributed, and combined with diel changes in nest site temperatures greatly reduce a turtle's ability to distinguish between sites that would produce different sexes. Considerable among-clutch variation in thermal profiles to produce variable sex ratios existed, agreeing with other studies on turtles. Radiotelemetry indicated that home ranges encompassed several nesting beaches with differing thermal profiles, indicating scope for producing the desired sex. However, the seasonal increase in air temperatures resulted in an overriding effect of mostly males being produced in early (first) clutches and mainly females being produced in late (second) clutches. Collectively, the results suggest that *C. insculpta* mothers would find it difficult to predict, and therefore, manipulate hatchling sex, supporting the conventional notion that TSD mothers have little or no control over offspring sex.

ACKNOWLEDGMENTS

I am thankful to the very many volunteers that contributed to the fieldwork during the three years in the NT, including A. Anselin, R. Alderman, K. Beggs S. Broomhall, A. Chariton, B. Christiansen, J. Davidson, C. Dean, M. Ewert, N. Freestone, S. Gentry, E. Guarino, L. Hateley, M. Heath, D. Hunter, J. Kirby, B. Kropp, I. Longo, J. Love, K. McCallie, N. McElhinney**,** M. McKenzie-Gay, K. Mikac, A. Miners, M. Pauza, A. Pepper, S. Thomson, R. Sims, S. Sims, J. Sites, M. Skelly, L. Snedden, A. Swindle, B. Taylor, R. Taylor, S. Thomson, D. Tolson, H. Webb, J. Webb, L. Webb, M. Welsh, G. West, and P. West. I am particularly grateful to J. Young's invaluable help in every phase of the fieldwork, and the study would not be half of what it is without her help. I thank R. Taylor for the raw data he collected for his honours, which he was unfortunately unable to complete. I am grateful to B. & E. Doyle, P. Hausler and the Douglas Daly Research Farm (DPI), P & A. Howie, L. & T. Dunn, J. & R. Lucas, J. O'Neal for logistical support. I also thank O. Price and NT Parks and Wildlife Commission for logistical support. In Canberra I thank A. Georges for advice and support in all phases of the study. Without his academic excellence none of this would have been possible, and he raised the level of my research dramatically. I thank my coauthors for their valuable contributions in the field and for their input to drafts of the manuscripts that comprise part of this thesis. I thank the various anonymous reviewers of manuscripts and K. Dodd, P. Doughty, R. Kennett, P. Pritchard, A. Rhodin, R. Sims, and A. Tucker for improving drafts of certain manuscripts or chapters. I thank W. Osborne and the other academics of the Applied Ecology Research Group for the many discussions that improved my way of thinking with regards to science and to my knowledge as a biologist and a

herpetologist. I thank my parents who helped from afar financially and with encouragement. Finally, I thank R. Sims for her unconditional support in every way possible.

CHAPTER 1: GENERAL INTRODUCTION

Background and Aims

Temperature-dependent sex determination (TSD), whereby offspring gender is determined by egg temperatures during incubation, is known among vertebrates in fishes, turtles, lizards, crocodilians, and the tuatara (see reviews in Ewert and Nelson, 1991, Ewert et al., 1994, Rhen and Lang, 1995). Over three decades have seen research into TSD since it was first discovered in 1966 (Charnier, 1966), and TSD continues to be a source of considerable research and controversy (Shine, 1999). During that time our knowledge of the mechanisms, causes, and consequences of TSD have progressed from the simple to the profoundly complex, as we attempt to uncover why TSD evolved.

Early on, it was established in the laboratory under constant temperature conditions that incubation temperature was the ultimate factor determining sex (Bull et al., 1982). Hydric conditions were implicated in a few studies (Gutske and Paukstis, 1983; Paukstis et al., 1984), but results have not proved reproducible (Packard et al., 1989; Packard et al., 1991; Hewavisenthi and Parmenter, 2000). As surveys for TSD in different taxa continued, several patterns of sex determination were uncovered (Ewert and Nelson, 1991). In some species, hotter temperatures produced males (e.g., crocodilians, Lang and Andrews, 1994) in others hotter temperatures produced females (e.g., turtles, Webb et al., 1986), and in others still males are produced from intermediate temperatures while females are produced from both hotter and cooler eggs (e.g., lizards, Harlow and Taylor, 2000). Thus, some species possessed one sex-determining threshold (called the pivotal temperature) and others had two. One branch of research has focused on identifying these pivotal temperatures using constant temperature incubation experiments (Mrosovsky, 1988;

Mrosovsky and Pieau, 1991). This lead to researchers to investigate geographic variation in pivotal temperatures within and among species (Mrosovsky et al., 1984; Vogt and Flores-Villela, 1992). Such information is important in determining whether or not geographic variation in pivotal temperatures would allow viable sex ratios over climatic clines in widespread species. A second branch of research has focused on determining at what point during development sex is determined. Studies on several species have arrived at a consensus that sex is determined (irreversibly) in the middle third of development (Bull, 1987; Mrosovsky and Pieau, 1991). Such information would be important in identifying ecological determinants of sex determination, and in answering the question of whether TSD mothers could predict or manipulate offspring sex.

Accordingly, studies of TSD began to move into the field. Clearly, eggs incubating in nature were not experiencing constant temperature conditions (Georges, 1992). How did the more realistic fluctuating temperatures influence sex determination? Could one simply take the mean of the fluctuations and successfully predict offspring sex? Although earlier studies did this, manipulation of temperature fluctuations in the laboratory revealed that the mean temperature was not a good predictor of offspring sex (Georges, 1989; Georges et al., 1994). Reasons for this inadequacy included the non-linear relationship between development and temperature, and presence of a developmental temperature minimum, below which no development occurs (Georges, 1989; Georges et al., 1994). To this end a statistic was developed called a constant temperature equivalent (CTE *sensu* Georges, 1989). The CTE could then be used to accurately predict offspring sex from a fluctuating temperature regime (Georges et al., 1994). However, field studies have yet to take advantage of this work.

In the meantime some progress was made on understanding how sex is determined in the field. For example, lay date can influence offspring sex (Harlow and Taylor, 2000), as can attributes of the nest site (Vogt and Bull, 1984; Janzen, 1994; Roosenberg, 1996). However, comprehensive studies of the determinants of sex determination in the field are lacking. A number of life history attributes would be expected to influence sex determination and its evolution. For example,

- What measurable attributes of a nest site influence offspring sex?
- How might clutch parameters such as reproductive frequency effect hatchling sex ratios?
- How are sex ratios influenced by the availability of nesting areas with particular thermal profiles?
- How might the movement of gravid females limit access to those sites? If lay date influences sex ratios, then what determines lay date?
- How might egg survival influence offspring sex – would sex ratios be optimal at the expense of embryonic survival?

Clearly, a complete understanding of TSD and its evolution require studying sex determination in an ecological context. For example, why does TSD evolve from GSD (genetic sex determination)? Although the prospects are encouraging for an adaptive significance for TSD in reptiles (Shine, 1999), a consensus adaptive explanation for the evolution of TSD in turtles is lacking (Burke, 1993, Ewert and Nelson, 1991, Ewert et al., 1994, Janzen, 1996, Janzen and Paukstis, 1991, Roosenberg, 1996). A main obstacle has been the difficulty in linking the incubation environment with fitness (Charnov and Bull, 1977), owed to delayed maturation and longevity (Shine, 1999). Another potential shortcoming is the paucity of field data on sex ratios and reproductive life histories.

Interpreting the ecology and evolution of T SD would likely be enhanced by an understanding of how TSD influences other life history aspects. For example, two adaptive hypotheses for the evolution or maintenance of TSD are based on knowledge of maturation time in a fish (*Menidia menidia*, Conover, 1984), and egg size in a turtle (*Malaclemys terrapin*, Roosenberg, 1996). Current theory suggests that knowledge of the survival consequences (i.e., differential fitness between sexes) is necessary to test evolutionary hypotheses of TSD (Janzen, 1996, Shine, 1999). However, quantifying variation in sex ratios and reproductive traits is a necessary first step in speculating on its origin.

The pig-nosed turtle (*Carettochelys insculpta*) is a monotypic species inhabiting freshwater systems in extreme northern Australia and southern New Guinea (Georges and Rose, 1993). The species is the sole surviving member of the Carettochelydidae, a widely distributed turtle family during the Tertiary (Georges and Wombey, 1993). Thus, its biology is of considerable interest worldwide due to its restricted geographic distribution and taxonomic position. Quantified data on the ecology of *C. insculpta* is limited to two studies on nesting and embryonic development (Georges, 1992, Webb et al., 1986). No study has investigated nest site choice or natural variation in sex ratios, and aside from anecdotal data (e.g., clutch size, egg size), the reproductive biology of this species is unknown.

The aim of the present study is to investigate the ecology of TSD in *C. insculpta*, with the broader goal of understanding what mechanisms drive natural variation in sex ratios in species with environmental sex determination, given the complex suite of factors that can influence offspring sex in the field.

Specifically, I asked the following questions:

- **What characterizes the reproductive biology of the species?**

  How do clutch characteristics vary among females, season, or year?

  At what size do females mature?

  How might the wet-dry tropics shape female reproductive biology?

- **How do mothers choose a nest site?**

  Are nesting beaches chosen randomly with respect to temperature?

  What physical attributes determine beach temperatures?

  What physical attributes determine nest site temperatures within beaches?

- **What are the determinants of reproductive success in the population?**

  Does nest site choice influence embryonic survival? If so, through what assessable attributes?

  Does timing of nesting (lay date) influence embryonic survival?

  Any seasonal or annual variation in reproductive success?

- **What are the determinants of hatchling sex?**

  Does nest site choice exert an influence on hatchling sex? If so, how so?

  Does timing of nesting or lay date affect hatchling sex?

  Any seasonal or annual variation in hatchling sex ratios?

- **Do movements and home range size influence sex ratios?**

  Is there scope for producing a particular offspring sex?

  How many beaches are within reach of a nesting turtle?

Are there sex differences in home range, movements, and activity?

Does reproductive condition influence home range, movements, and activity?

- **Can nesting turtles predict/manipulate offspring sex?**

  What assessable attributes of nest sites exist?

  Are mothers choosing those attributes non-randomly?

  Does variation in nesting times obscure the predictability of nest temperatures during the thermosensitive period?

- **What are the relationships between hatching, emergence, and embryonic aestivation?**

  Does embryonic aestivation occur in nature? If so, why?

  When and how do hatchlings emerge from the nest?

  What thermal cue(s) do emerging hatchlings use?

- **How do current hypotheses for the adaptive evolution of TSD fit the pig-nosed turtle system?**

  Which adaptive hypothesis for the evolution and maintenance of TSD best fits pig-nosed turtles?

  What can we learn about the evolution and ecology of TSD in reptiles from studying pig-nosed turtles?

This thesis is structured as a series of papers, each with its own introduction, materials and methods, and discussion. This will inevitably lead to some repetition, as several of the chapters have been published already. I have endeavored to keep the repetition to a minimum, by deletion of some passages in the materials and methods of the published papers and consolidation of abstracts and references. The thesis concludes with a synopsis drawing together, in integrated fashion, contributions of each chapter.

Pig-nosed Turtle Biology

Relatively little was known of the biology of *C. insculpta* prior to the present study. This is owed to its restricted distribution in Australia and New Guinea (Georges and Rose, 1993), and because it wasn't known to occur in Australia until 1970 (Georges and Wombey, 1993). However, three published studies have quantified aspects of ecology, distribution, reproduction and sex determination in the species. Webb et al. (1986), in a laboratory experiment demonstrated that *C. insculpta* exhibited TSD, whereby hotter eggs become females and cooler eggs become males. They also performed experiments to determine the influence of temperature on the metabolic rates of embryos, embryonic growth rates, and delayed hatching. Finally, they experimentally determined a trigger for hatching, leading them to hypothesize that delayed hatching was an adaptive response to early wet season flooding (Webb et al., 1986). The second study of *C. insculpta* investigated its dry season distribution and ecology in Kakadu National Park (KNP) (Georges and Kennett, 1989). In this study the investigators determined the known distribution of the species and estimated population densities in concentrated areas of billabongs. They also quantified the diet of *C. insculpta* in KNP, finding that the species was a dietary generalist, eating primarily aquatic vegetation, algae, and fruits, but also consuming macroinvertebrates and carrion (Georges and Kennett, 1989). They also concluded that *C. insculpta* nest during the dry season from July to October. Lastly, the authors documented clutch and egg sizes, nest site characteristics (height above and distance from water), and high predation of nests by goannas (*Varanus* spp.). A third study by Georges (1992) described the thermal characteristics of *C. insculpta* nests in the field. In this study nest sites were described, along with their daily temperature fluctuations. Georges (1992) also determined the basic determinants of nest temperature (solar radiation), and documented sex determination in the field.

A fourth unpublished study (Heaphy, 1990) of the ecology of *C. insculpta* in the Daly River quantified diet, growth rates, and dry season densities. Georges and Rose (1993) reviewed the known biology of *C. insculpta* and its implications for conservation and management. In this paper the authors identified potential threats to populations and to the species as a whole. The principal threats they identified to New Guinea *C. insculpta* were exploitation for meat and eggs, and mining and mineral exploration along rivers. In Australia major threats were pastoral and agricultural practices, erosion and siltation, and mining. With regard to conservation status, *C. insculpta* has been classified as "K" (= Insufficiently Known) in the Red Data Book (Groombridge, 1982). The species received an action plan rating of 1 (known threatened species in need of specific conservation measures) by IUCN/SSC Tortoise and Freshwater Turtle Specialist Group (IUCN, 1989).

The Study Site

I studied *C. insculpta* at the Daly River, in the Top End Region of the Northern Territory, Australia. The climate is typical of the wet-dry tropics of northern Australia (Taylor and Tulloch, 1985) with a mean monthly rainfall less than 7 mm from May to September, rising to a peak monthly average of 284 mm in February (Stn 014139/014941, Oolloo, 1962-1985). Mean monthly maximum air temperatures range from 30.9 C in June to 36.8° C in October. The Daly River winds through mainly savanna habitat in route to opening to the Timor Sea. The Daly River is a spring-fed system characterized by shallow depths and clear water during the dry season, and deep turbid water during the wet season. Water flows continuously through the study site throughout the year, with water levels rising to an average peak of 13.6 m (5.8 – 21.7 m) above dry season levels in March.

The study area was a 30 km stretch of the Daly centered around Oolloo Crossing (14° 04’ 40” S, 131° 15’ 00” E). Oolloo Crossing is a ford built from rocks on Oolloo Road, and is a popular primitive camping site. In the study stretch the river is roughly 50m wide, has a moderate flow, and the substrate is mainly bedrock interspersed with sand. Numerous thermal springs emanate from both the river bottom and its banks.

Numerous sand banks and beaches line the river in the study stretch, providing ample nesting habitat for *C. insculpta* (Georges, 1992). Substrate type in these nesting areas are primarily sand or sand with coarse gravel (Georges, 1992).

CHAPTER 2: MOVEMENT PATTERNS AND ACTIVITY

Sex differences in activity and movements in the pig-nosed turtle, *Carettochelys insculpta*, in the wet-dry tropics of Australia

(Copeia 2002:93-103)

J. S. Doody, J. E. Young, and A. Georges

INTRODUCTION

Knowledge of movement patterns of animals is fundamental to an understanding their life histories (Swingland and Greenwood, 1983; Gregory et al., 1987). Numerous studies have linked movements with functions such as food acquisition, aestivation, and reproduction, each of which influences lifetime reproductive success. In aquatic turtles, movements often differ between the sexes (e.g., Obbard and Brooks, 1981; MacCulloch and Secoy, 1983; Pluto and Bellis, 1988; but see Ernst, 1970; Jones, 1996; Carter et al., 2000). Possible reasons include sex related differences in habitat use (Plummer and Shirer, 1975; Plummer, 1977; Craig, 1992), or diet (reviewed in Lindeman, 2000), or differential reproductive strategies (e.g., nesting movements, Moll and Legler, 1971; Obbard and Brooks, 1980).

Morreale et al. (1984) generated a conceptual model termed the ‘reproductive strategies hypothesis’ to explain differential movement and activity between the sexes. The model, which derives support from studies of aquatic turtles (Brown and Brooks, 1993; Jones, 1996; Thomas et al., 1999), predicts that (1) during the mating season, activity and movement should be greater in males than females, and (2) during the nesting season, activity and movement of females should equal or exceed that of males. Assumptions underlying the predictions are: (i) males are more active to increase their chances of mating, (ii) males move farther to increase their opportunities for multiple matings, (iii) food resources used are similar between the sexes, and (iv) during nesting females make excursions associated with finding nest sites.

Although direct evidence is lacking for assumptions (i) and (ii), most studies have shown that males tend to move farther than females (reviewed in Gibbons, 1986, Gibbons et al., 1990; Tuberville et al., 1996). Also, these assumptions are consistent with current theory (Trivers, 1972; Maynard Smith, 1978; Andersson, 1994). Assumption (iii) is upheld in some species (Moll and Legler, 1971; Hart, 1983) but not others (Plummer and Farrar, 1981; Lindeman, 2000).

In most studies that have addressed assumption (iv) (reviewed in Gibbons, 1986; Congdon et al., 1987), reasons for the difference in movements between the sexes cannot be readily identified (e.g., Thomas et al., 1999). Most turtles mate in spring and autumn (Gregory, 1982; Ernst et al., 1994), yet many nest in summer (reviewed in Ernst et al., 1994). Differences in movement and activity between the sexes, accompanied by seasonal changes in female movements associated with nesting are indicative but potentially confounded. Females may be moving more in agreement with assumption (iv), or males may be moving less because females are unreceptive to mating at this time (Thomas et al., 1999). An unequivocal test of the idea that differential movements and/or activity between the sexes is due to females searching for a nesting area would require a comparative study of the movements among males, gravid females, and non-gravid adult females (e.g., a species exhibiting biennial reproduction). Comparison of the movements between gravid and non-gravid adult females is less likely to be confounded than comparing males to females (Shine, 1980; Schwartzkopf, 1993).

In this study, I examine dry season movement patterns, home ranges, and activity in a population of pig-nosed turtles (*Carettochelys insculpta*) in the Daly River of northern tropical Australia. I used radio-telemetry to test the hypotheses that sex and reproductive condition influenced home range size, movements, and activity of *C. insculpta* in ways predicted by the reproductive strategies hypothesis of Morreale et al. (1984). This species is ideal for such a study because it exhibits biennial reproduction, with approximately half of the adult females reproducing each year (unpubl. data), enabling a comparison between gravid and non-gravid adult mature females. I also consider influences on home range size, and compare our findings to those of other turtles, and in particular species inhabiting lotic habitats. Lastly, I examined a species-specific idea that Australian *C. insculpta* move into the lower estuarine floodplains during the wet season (Heaphy, 1990).

MATERIALS AND METHODS

I studied *C. insculpta* along an 11 km stretch of the Daly River near Oolloo Crossing (14° 04’ 40” S, 131° 15’ 00” E) in the Northern Territory, Australia, during the dry season (August–November) in 1996, and again during a single fly-over during the wet season of 1996/97. The climate is typical of the wet-dry tropics of northern Australia (Taylor and Tulloch, 1985) with a mean monthly rainfall less than 7 mm from May to September, rising to a peak monthly average of 284 mm in February (Stn 014139/014941, Oolloo, 1962-1985). The river averaged ca. 50 m across and ca. 1.5 m in depth (deepest holes are up to 4 m deep). Secchi disk clarity was 1-4 m during the dry season, but only a few centimeters during the wet season. Substrate was largely bedrock and sand, and flow was moderate during the dry season.

Turtles were captured during the day with dipnets from a boat, and their sex was determined by inspection of tail length. Each turtle was fitted with a numbered cattle ear tag on the rear edge of the carapace. Cattle ear tags allowed identification from the boat without recapture (numbers can be read without capture). Curved carapace length (CL) and plastron length (PL) was measured to the nearest 0.1 cm with calipers. Females were x-rayed for the presence of shelled eggs using a portable x-ray machine (ExcelRay®). Radiographs were developed in a makeshift darkroom in the field.

Twenty turtles were fitted with radio-transmitters (Sirtrack®). Of these, eight were females subsequently found to be gravid, seven were females that failed to reproduce in that season (I term them ‘non-gravid’ females), and five were males. Transmitters were mounted on aluminum plates (2.5 cm x 8 cm x 2 mm thick), and the unit was attached to the rear carapacial edge with surgical stainless-steel bolts, opposite the cattle ear tag. Bolts were fitted to two holes drilled through the edge of the marginal scutes. Wetsuit foam was used as a buffer between the transmitter mounting plates and the soft skin.

Turtles were released at the point of capture within 24 hours. Markers were placed every 200 m for the 11 km stretch to facilitate the location of sightings. Locations of turtles were noted to the nearest 10 m by visual estimation of distance to markers.

Turtles were radio-tracked (using a Teleonics® TR4 receiver and Yagi antenna) by boat 6 days per week between 10 August and 1 December, 1996. This period included the nesting period (27 August – 30 September) and a post-nesting period (1 October – 29 November). Most (>95 %) observations were made during the day. In most cases I was able to see telemetered turtles. Date, time, location, microhabitat, activity, and depth were recorded with each fix. Turtles were scored as ‘active’ if first observed swimming or crawling along the river bottom, or ‘inactive’ if first seen sitting motionless on the river bottom (in association with logjams or other cover). Although this doubtless resulted in some error in assessing activity, the error would be expected to be similar between sexes. Microhabitat categories were: ribbonweed bed (*Vallisneria*), open sand flat, open rock flat, isolated log on sand/rock, and logjam. Ribbonweed is the primary dietary item of *C. insculpta* in the Daly River during the dry season (Heaphy, 1990; Welsh, 1999). Turtles were scored as using an isolated log when part of the shell was partially under the log. Logjams were two or more abutting logs. Depth was estimated to the nearest 0.3 m using a metered weighted line.

Linear home range was defined for each turtle as the range spanned between the farthest upstream and downstream locations (Plummer et al., 1997). The 95th percentile was then taken to decrease sensitivity to outliers. Home range area was calculated by multiplying linear home range by the average width of the river in the study area (50 m). Turtle observations were plotted against location to examine relative dispersion and to identify centers of activity. I hypothesized that three types of resources could explain clumped distributions (food = ribbonweed beds, nesting habitat = beaches, and thermoregulation sites = thermal springs). I therefore mapped activity centers against locations of these resources.

I also calculated mean distance moved as the linear distance between successive sightings for each fix. This served as an estimate of distance per move. I did not adjust for time between sightings because most turtles were sighted each day.

To examine where turtles spent the wet season when the Daly overflows its banks, I radio-tracked the 20 turtles from a low-flying airplane equipped with a Global Positioning System. The flyover was made on 5 February when the river was at ca. 8 m above typical dry season river levels. The river had been in continuous flood beginning in late December, and had reached a peak level of 18 m above normal dry season level on 5 January.

Among individuals, single-factor analysis of variance and analysis of covariance was used to determine the effects of body size, reproductive condition, and sex on home range size, movements, and number of beaches within a home range. Within individuals, I used paired t-tests to determine differences in home range, movements, and activity between the time females were gravid and the time females were spent (after eggs laid). All turtles were considered to have laid eggs by 15 October. This date is based on daily nest surveys conducted in a concurrent study on nest site choice (unpubl. data). I examined the independence of microhabitat use, activity, and sex by contingency table analysis. Assumptions of parametric tests were tested prior to analyses, and a 0.05 level of significance was used. Means are presented with their standard deviations, unless otherwise specified.

RESULTS

Home range size, movements, and activity. **–** Individual variation in number of fixes and home range size, movements, and activity are listed in Table 2.1. Asymptotes of change in linear home range size against number of fixes indicated that, on average, 24 fixes (observations) were needed for estimating linear home range size. After individuals with fewer than 24 observations had been discarded, the number of observations per individual did not influence linear home range size ($r^2 = 0.15$, $F_{1,17} = 2.90$, $p = 0.11$).

Linear home range size did not differ significantly between gravid and non-gravid adult females (Table 2.2), so the two classes were pooled for comparing the sexes. Females had significantly longer linear home ranges than males (Table 2.2). Consequently, home range area was also larger in females (8 = 43.7 ± 17.27 ha, N = 13) than in males (8 = 16.2 ± 6.58 ha, N = 5). Mean differences in linear home range size were influenced by sex, over and above the effects of carapace length (ANCOVA, $F_{1,15} = 6.20$, $p = 0.025$). Males, but not females, had significantly larger linear home ranges while females were gravid, compared to when reproductive females were spent (Table 2.3). Linear home range size of females remained larger than that of males during the two months after the nesting season, although the difference only approached significance ($F_{1,13} = 4.42$, $p = 0.057$). Home range overlap, defined as the proportion of all turtles sharing a particular stretch of river with a given turtle, was high in both females (96.8 %) and males (84.6 %).

Gravid females moved farther between sightings, on average, than non-gravid females, but the difference was not significant (Table 2.2). Although distance moved between sightings did not differ significantly between males and females, the difference approached significance, and on average females moved more than twice as far between sightings than males (Table 2.2). Both males and females covered longer distances between sightings while females were gravid, than when females were spent (Table 2.3). Females continued to cover greater mean distances than males during the two months following nesting (females, 8 = 334 ± 181.0 m; males,

Table 2.1. Descriptive data for individual *C. insculpta* obtained by radio-telemetry. Distance data are means ± 1 SD. Sample sizes are in parentheses when not equal to number of fixes. Data are not included for F01 and F54 due to low number of fixes. Activity (%) is defined the proportion of point locations in which turtles were 'active' (see Methods). CL = carapace length, n/a = not applicable.

turtle #	sex	CL (cm)	reproductive condition	# fixes	linear home range (m)	distance per move (m)	activity (%)
F01	f	44.6	non-gravid	11	-	-	-
F02	f	42.0	non-gravid	95	7950	329.5 ± 830.05	76 (62)
F03	f	41.4	non-gravid	51	6540	324.3 ± 759.10	75 (32)
F04	f	43.6	non-gravid	72	7810	391.0 ± 527.55	72 (53)
F05	f	42.4	gravid	99	9850	228.0 ± 279.68	64 (78)
F07	f	46.2	gravid	19	5870	489.0 ± 868.82	71 (7)
F08	f	43.0	gravid	91	7405	523.4 ± 1019.72	61 (76)
F12	f	44.6	gravid	61	13890	1031.5 ± 1707.11	26 (35)
F16	f	43.6	gravid	29	12630	1452.1 ± 1879.04	35 (20)
F40	f	44.4	gravid	52	2450	249.0 ± 460.73	62 (34)
F54	f	44.5	non-gravid	11	-	-	-
F64	f	40.9	gravid	31	9600	1297.3 ± 2370.78	53 (17)
F65	f	42.6	non-gravid	102	8460	672.7 ± 1147.30	70 (69)
F67	f	40.2	gravid	100	7280	775.3 ± 1324.20	74 (72)
F69	f	44.5	non-gravid	99	8250	463.0 ± 1052.73	74 (68)
M08	m	37.8	n/a	91	3955	259.2 ± 449.47	18 (79)
M52	m	37.5	n/a	89	3800	276.0 ± 434.16	61 (74)
M56	m	40.1	n/a	94	2185	212.4 ± 362.67	24 (80)
M62	m	40.1	n/a	96	4680	403.4 ± 658.59	10 (79)
M63	m	39.4	n/a	98	1535	210.0 ± 233.76	24 (84)

Table 2.2. Home range size, movements, and activity of male and female *C. insculpta*. Data are means ± 1 SD, or significance determined by ANOVA for home range and movements, or contingency analysis for activity. Numbers of animals are in parentheses.

group	linear home range length (km)	distance per move (m)	activity (%)
all females	8.3 ± 2.88 (13)	632.8 ± 399.16 (13)	67 ± 15.8 (13)
gravid females	8.6 ± 3.69 (8)	755.7 ± 465.23 (8)	56 ± 20.9 (4)
non-gravid females	7.8 ± 0.75 (5)	436.1 ± 143.70 (5)	73 ± 2.4 (5)
males	3.2 ± 1.32 (5)	272.2 ± 78.8 (5)	26 ± 8.9 (5)
females vs. males	$F_{1,18} = 4.49$, $p = 0.002$**	$F_{1,18} = 4.49$, $p = 0.066$	$X^2 = 33.79$, $p < 0.001$***
gravid vs. non-gravid females	$F_{1,13} = 4.84$, $p = 0.638$	$F_{1,13} = 4.84$, $p = 0.169$	$X^2 = 2.26$, $p = 0.133$

Table 2.3. Influence of female reproductive condition on home range size, movements, and activity of male and reproducing female *C. insculpta*. Data are means ± 1 SD.

variable	while females gravid	While females spent	N	significance
linear home range length (m)				
Females	5252 ± 2705.6	5561 ± 2965.4	4	t = -0.725, p = 0.260
Males	3052 ± 1239.3	1287 ± 824.8	5	t = 3.117, p = 0.018*
distance per move (m)				
females	715 ± 447.9	411 ± 200.2	5	t = 2.655, p = 0.028*
males	464 ± 188.1	166 ± 58.8	5	t = 3.494, p = 0.013*
activity (% active)				
females	64.8 ± 11.92	68.5 ± 4.06	3	t = -0.576, p = 0.312
males	32.2 ± 22.87	25.0 ± 18.68	5	t = 2.395, p = 0.037*

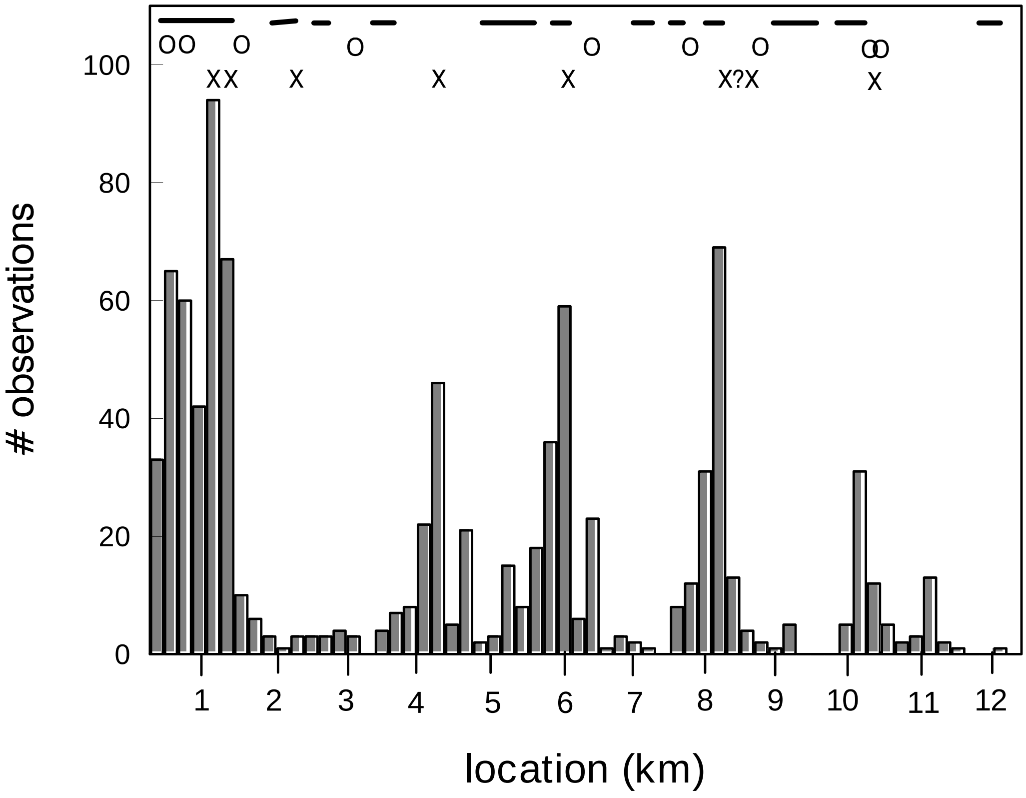


Figure 2.1. Combined observations or activity centers of female *C. insculpta*, showing locations of thermal springs (X), dense ribbonweed beds (-), and nesting beaches (O). Each column bar represents a 200 m stretch of river.

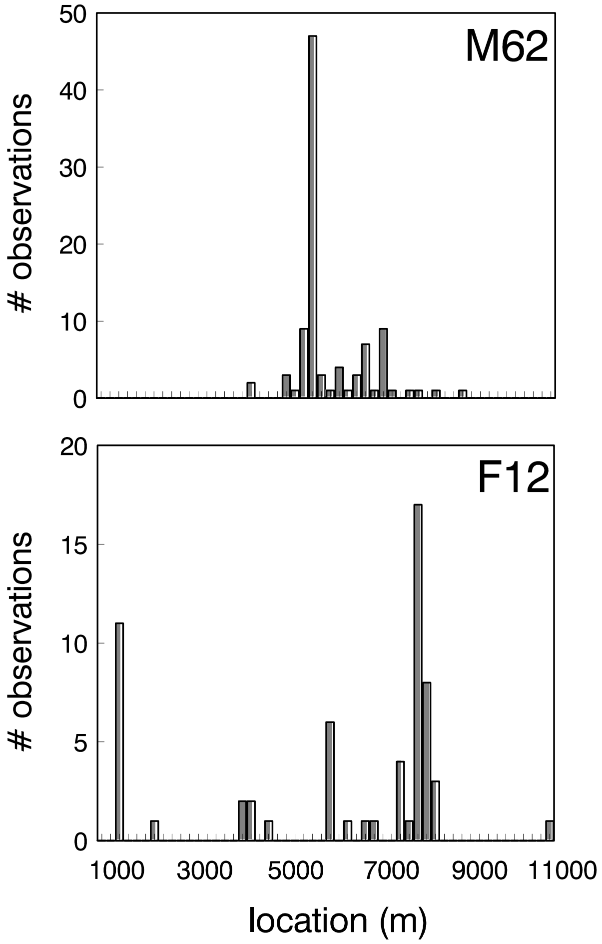


Figure 2.2. Typical examples of dry season activity centers for individual male (M62) and female (F12) *C. insculpta*, showing larger home range and greater number of activity centers in females.

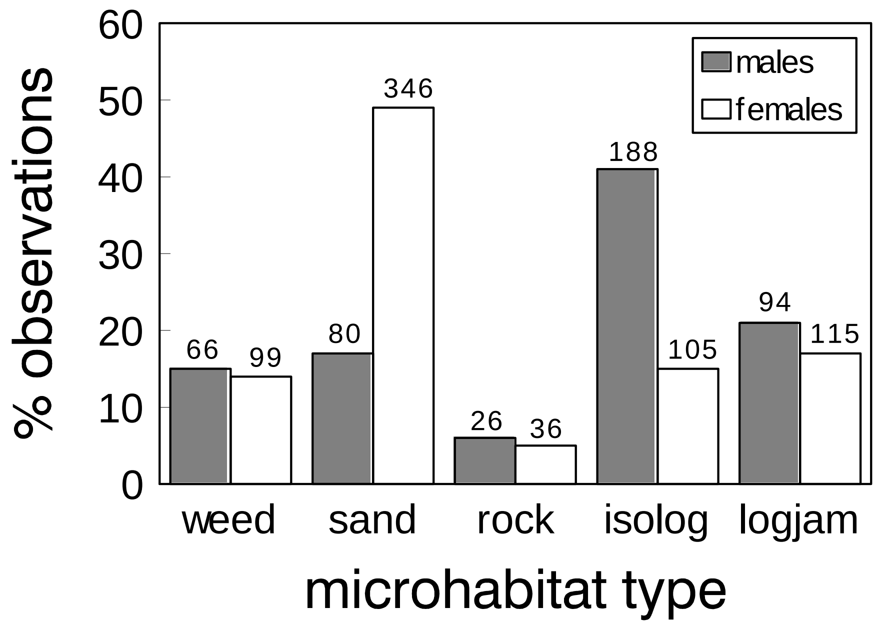


Figure 2.3. Influence of sex on microhabitat use by *C. insculpta*: weed = ribbonweed bed (*Vallisneria nana*), sand = sand flat, rock = rock flat, isolog = isolated log. Numbers of observations are shown above each column.

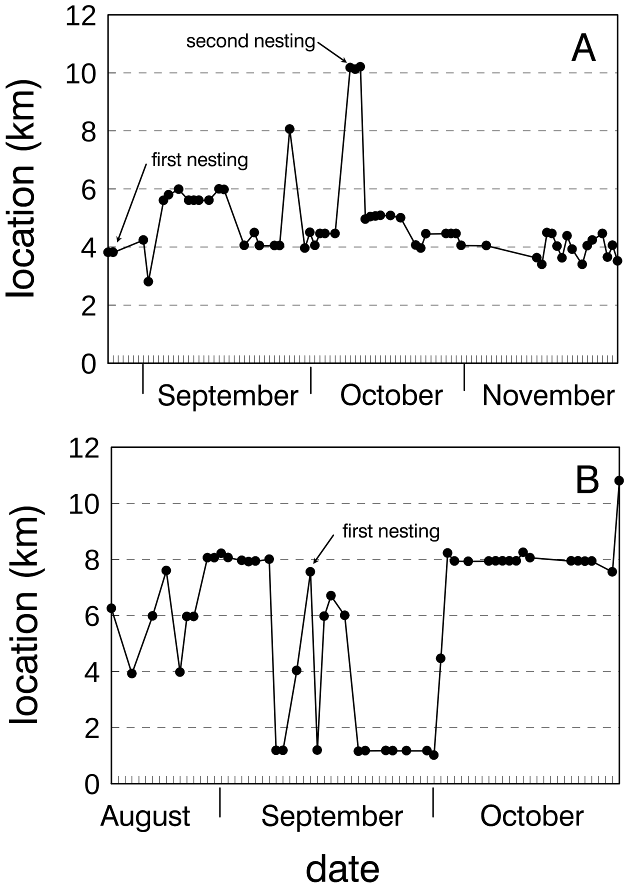


Figure 2.4. Examples of sequential movements of two individual *C. insculpta*, showing nesting events (A, B) and fidelity to thermal springs (B) at the one and eight km marks. Each dot represents a point location.

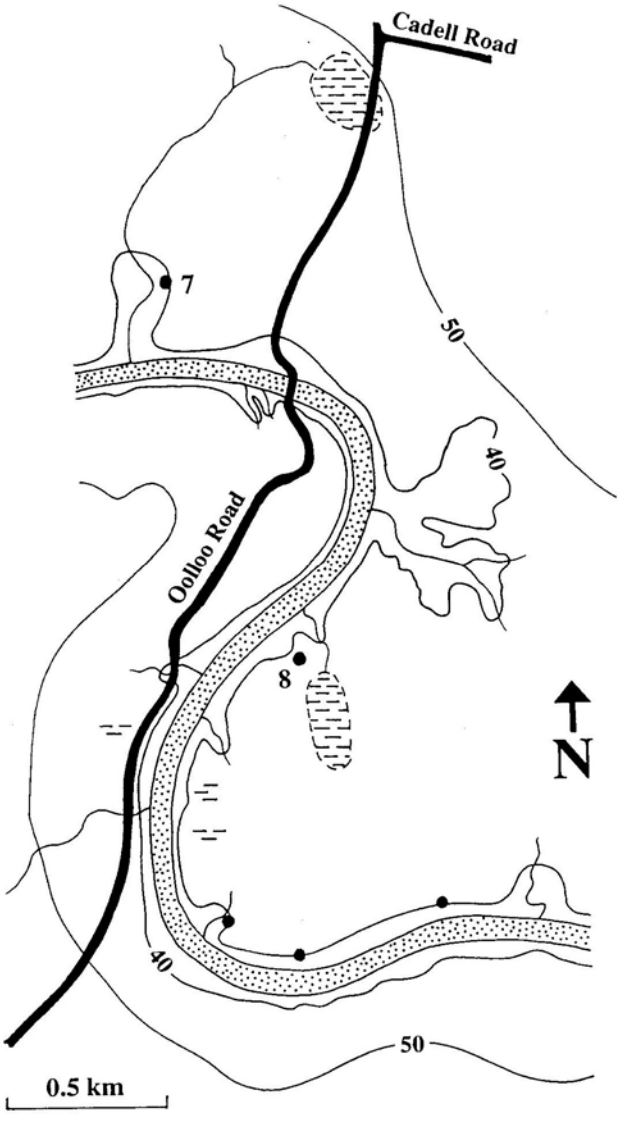


Figure 2.5. Point locations of 18 *C. insculpta* during the wet season when the Daly River (stippled area) was in flood. • indicates a turtle location or a group of 7 or 8 turtles. Note that two groups of turtles were near billabongs (surrounded by dashed lines), which become connected to the river during severe flooding. Numbered lines are contours.

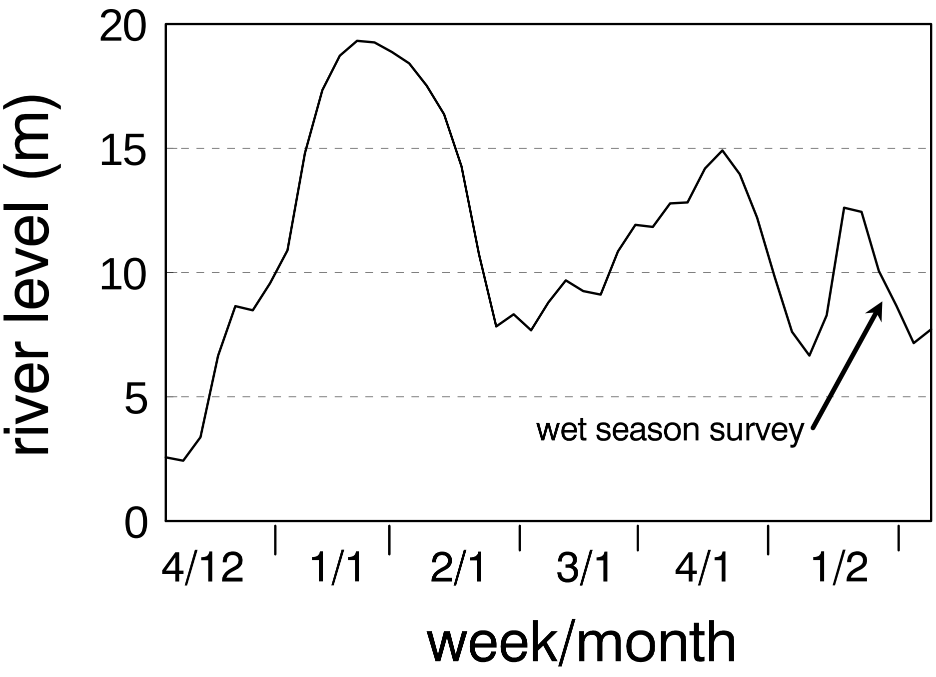


Figure 2.6. Flood levels during the study (1996) at Dorisvale Crossing, near the study area. The arrow represents the flood level when the aerial survey was conducted.

8 = 166 ± 58.8 m), although the difference only approached significance ($F_{1,14} = 4.67$, $p = 0.067$).

Females were more active than males during the day, when the majority of observations were made (Table 2.2). Activity was independent of reproductive condition among females (Table 2.2). In three cases with sufficient temporal data, there was no difference in activity of females while gravid, compared to when spent (Table 2.3). Males, but not females, were significantly more active when females were gravid, compared to when females were spent (Table 2.3). Greater activity in females persisted during the 2 months after the nesting season ($X^2 = 39.82$, df = 1, $p < 0.001$)

Activity centers and microhabitat use. – Pooled observations (point locations) for all radio-tracked turtles are compared to the locations of nesting beaches, ribbonweed beds, and thermal springs in Figure 2.1. Clustering was evident around thermal spring locations (Fig. 2.1; Fig. 2.4b), but may also be related to nesting beaches (Fig. 2.1). Most individual females had 1-4 discrete activity centers (areas with frequent usage), whereas males generally displayed one normally-distributed activity center (Fig. 2.2).

Microhabitats occupied by males and females are shown in Figure 2.3. The major difference between sexes was the greater tendency for females to use open sand flats and for males to use isolated logs on sand/rock ($X^2 = 27.36$, df = 1, $p < 0.001$). In all observations, isolated logs were found at shallower depths than sand flats ($F_{1,630} = 3.86$, $p < 0.001$). The type of microhabitat used by females was independent of reproductive condition ($X^2 = 1.05$, df = 1, $p = 0.90$). A three-way contingency analysis revealed that the difference in microhabitat use (open sand flat vs. isolated log) between the sexes was not independent of activity ($X^2 = 12.72$, Mantel-Haenszel = 3.76, df = 2, $p < 0.001$).

Mean depth at the time of observations did not differ significantly between gravid and non-gravid females ($F_{1,650} = 3.86$, $p = 0.08$), and so data were pooled for comparisons between sexes. Females were observed in deeper water than males (females = 1.47 ± 0.685 m, range = 0.2-4.0 m; males = 0.91 ± 0.470 m, range = 0.2 – 3 m; $F_{1, 1018} = 3.85$, $p < 0.001$).

Movements associated with nesting. - Seven gravid females were linked to their nesting locations. Most turtles (87.5 %) nested within the area they occupied 95 % of the time (i.e., their home range). The exception was the second nest of F08, who was linked to both of her nests for the year (Fig. 2.4). To lay her second nest, she apparently made a movement of six km, returning two days later to the area she had occupied prior to the foray (Fig. 2.4). Of 12 nesting events by 10 turtles with sufficient movement data (N > 24 fixes, Table 2.1), seven made upstream movements just before nesting, one moved downstream, two did not move (> 200 m), and two moved in both directions just before nesting.

The number of nesting beaches within a home range (defined as beaches utilized that year by nesting turtles) did not differ between gravid and non-gravid females ($F_{1,14} = 1.24$, $p = 0.28$). As expected due to range size, males had fewer beaches (1.4 ± 0.98 SD, range = 0-3, N = 5 turtles) within their home ranges than females (4.9 ± 1.57 SD, range = 2-7, N = 15).

Wet season locations. - Wet season locations, determined from the air in the fly-over on 5 February, 1997, were out of the main river channel (Fig. 2.5). The river was in flood on this day (fast flow and high) but was generally within its outer banks (< 12 m, Fig. 2.6). Of the 18 turtles for which a signal was received, most were in two groups consisting of seven and eight turtles (Fig. 2.5). Both of these two groups were near billabongs. Eight turtles appeared to be associated with small creeks within 300 m of the river (Fig. 2.5). These creeks, which are dry during the dry season were in flood on 5 February according to river stage data. Turtles not associated with creeks appeared to occupy the flooded riparian zone within 200 m of the dry season river boundary.

During the wet season fly-over, all turtles were found either within (N = 4) or downstream of (N = 14) their dry season home range. Turtles downstream averaged 1.8 ± 1.70 km from the closest point of their dry season home range (range = 1.0 – 6.4 km). The turtles that were found within their dry season home range comprised three (previously) gravid females and one male. All transmitters fell off the turtles (by necrosis of the marginal bones) by April 1997 and were retrieved. Of the 18 transmitters recovered, 82 % were found within the respective dry season home range of each turtle. Most transmitters (N = 12) were found in riparian forest out of the main channel, 15-39 m from the river's edge during the dry season. Five were found in the channel, 2 were found within a few meters of the river, and one was found in a creek 60 m from the river. One male died and was recovered with transmitter in riparian forest 45 m from the river. No other mortality was observed during the study.

Long-term Site Fidelity. – Of 150 *C. insculpta* marked in the study area in 1986-1988 by Heaphy (1990), 104 (69%) were recaptured during 1996-1998. The study area of the present study encompassed that of Heaphy (1990).

DISCUSSION

Sex differences and their significance. - In turtles, home range size, movements, and activity often differ between the sexes (Morreale et al., 1984). Most studies have found that movement and activity are greater in males than females (e.g., Pluto and Bellis, 1988; Rowe and Moll, 1991), whereas some studies have found the reverse (e.g., Gordon and MaCulloch, 1980; Ross and Anderson, 1990; Bodie and Semlitsch, 2000), and a few found no difference (e.g., Kramer, 1995; Jones, 1996; Carter et al., 2000). Current theory and available data on turtles suggests that differences in movement patterns and activity biased toward females can be explained by nesting excursions of those females (Morreale et al., 1984; but see Dodd, 1989). While this pattern is sometimes obvious, as when females make abrupt movements just before nesting and then return, an unequivocal test of this prediction requires simultaneous comparison of movements and activity between gravid and non-gravid females.

In the present study females were more active, moved farther, and occupied home ranges twice the size of that of males (Table 2). These differences are not likely to be attributable to food type, because dry season food types do not differ between the sexes (Heaphy, 1990; Welsh, 1999). This assumption (iii) of the reproductive strategies hypothesis (Morreale et al., 1984) is upheld, allowing us to address the model’s predictions.

The model predicts that during the nesting season (first half of the study period, i.e., late August through to mid-October) females should equal or exceed males in activity, movements, and home range size, based on the assumption that females make excursions associated with choosing a nest site (Morreale et al., 1984). Several studies convincingly support this prediction (Ernst, 1970; Moll and Legler, 1971; Pluto and Bellis, 1988). However, our study found that gravid females did not differ significantly from non-gravid females in home range size or activity (Table 2). Further, reproductive females did not possess larger home ranges while gravid compared to while spent, and the transition from gravid to spent was not associated difference in activity (Table 3). Consistent with this finding is the observation that gravid females generally nested within areas they already occupied; only one individual nested outside the area it otherwise occupied (Fig. 4). Finally, greater home range size, movements, and activity in females, relative to males, persisted after nesting was complete. Collectively, these results indicate that some factor other than nesting excursions must explain the differences between sexes in activity and movements in *C. insculpta*.

In theory, the difference between sexes could also be explained by males moving less during the nesting season, because females might not be receptive to mating (Morreale et al., 1984; Jones, 1996). Such data are difficult to obtain for turtles, but most mating activity reported occurs in spring and autumn (Gregory, 1982; Ernst et al., 1994). However, male *C. insculpta* actually had larger home ranges and moved farther (and thus, were probably more active) while females were gravid than they did while females were spent (Table 3). I observed male *C. insculpta* accompanying gravid females near beaches at night during the nesting season, and in some cases males emerged from the water and nuzzled the sand where females had emerged. This is in contrast with male *Graptemys flavimaculata*, which were more sedentary during nesting (summer) than in autumn (Jones, 1996). Such differences may reflect variation in the chronology of mating. Timing of mating is unknown in *C. insculpta*, though there have been unconfirmed observations of mating in June and July (Heaphy, 1990). Non-gravid females may be receptive during nesting in contrast to gravid females, and males may not be able to discriminate between the two female types. Or, females may become receptive just after laying.

An alternative hypothesis is that sexual size dimorphism accounts for the movement and activity differences (e.g., Schubauer et al., 1990). In the Daly River female *C. insculpta* are ca. 50 % larger than males (unpubl. data). However, ANCOVA indicated that home range size was influenced by sex, over and above any effect of body size. In general, vertebrates exhibit larger home ranges with larger body size, although this conclusion is largely based on across-species comparisons (Mace et al., 1983).

One possible explanation for the differences between males and females in activity and movements is related to energy acquisition. The study population exhibits biennial reproduction, with ca. half of females reproducing each year (unpubl. data). Assuming biennial reproduction in the population reflects a limiting food resource (Bull and Shine, 1979), females may need to maximize their time feeding relative to males, resulting in increased activity, movements, and larger home ranges. In this way differences in activity and movements between the sexes would reflect sexual inequality in parental investment involved with gamete formation (Trivers, 1972; Andersson, 1994). Among adult female vertebrates, home range area is related to access to food, with the quality and density of food, coupled with the animal’s energy requirements, being the major factors determining home range size (Mace et al., 1983).

If our hypothesis is validated by future work, an additional assumption should be included in the reproductive strategies model: that food (type, nutritional value, or abundance) is not particularly limiting to a measurable extent in reproductive output (e.g., biennial reproduction). This idea would be pertinent to turtles in general, because sex differences in movements and activity are not limited to aquatic species (e.g., Lue and Chen, 1999). A caveat, however, is that riverine turtles are habitat-constrained, having only two directions in which to forage. Confirmation of this phenomenon in *C. insculpta* would need to include (1) experimental evidence for phenotypic plasticity in clutch frequency (e.g., supplemental feeding), (2) a better understanding of the putative link between movement patterns and food availability, and (3) determination of activity patterns between sexes during the night. Our observations were biased towards daytime: males may have increased their activity during the night, relative to females. Turtles in the population are known to be active at night (Heaphy, 1990, pers. obs.).

Comparisons with other aquatic turtles. - *Carettochelys insculpta* occupied considerably larger home ranges than those reported for other lotic turtle species. Plummer et al. (1997) reviewed home range size for lotic turtles species, finding that most have home range areas of 0.5-4 ha, the maximum home range area being 11.6 ha (*Apalone mutica*). This figure is one-third of the mean home range calculated for *C. insculpta* (36 ha). The method could overestimate home range area in species that use one side of a large river (Plummer et al., 1997), because home range area was calculated by multiplying linear home range by width of stream. However, *linear home range* in *C. insculpta* (7.2 km) was also five times longer than the longest home range previously known (1.6 km, *Graptemys flavimaculata*, Jones, 1996). Further, stream width in the present study was ca. 50 m, and turtles were seen moving across the river in < 1 min. Thus, I am not likely to have overestimated home range area in the present study using this method.

Plummer et al. (1997) also reviewed factors influencing home range size in turtles, citing body size, sex, reproductive condition, season, habitat productivity, habitat type, stream size, and methods. Which of these factors might explain the unusually large home ranges of *C. insculpta*? Although interspecific comparisons are potentially confounded (e.g., by site, year, latitude), I can examine the apparent fit of these factors to home range size in *C. insculpta*.

At 5-11 kg and 37-45 cm carapace length (CL), Daly River *C. insculpta* are heavier and longer than most lotic species examined by Plummer et al. (1997). However, *C. insculpta* ranks near *Chelydra serpentina* in mass, and near *Apalone spinifera* in CL. Body size alone, therefore, cannot explain the extensive home ranges found in the present study. Although season may have influenced home range size in our study, I restrict my comparisons to dry season data, because I only tracked turtles once in the wet season. Stream size cannot explain the unusually large home range of *C. insculpta* in the present study. Using the regression equation of home range area against stream width (cf. Plummer et al., 1997), *C. insculpta* is predicted to have a home range size near 1.6 ha, compared to an actual home range area of 36 ha. Generally speaking, habitat type is not a factor, as our comparisons are restricted to lotic species. However, the distribution of microhabitats, particularly as related to productivity, could dictate home range size.

In the Daly River, *C. insculpta* is primarily herbivorous during the dry season (Heaphy, 1990; Welsh, 1999). Welsh (1999) found that ribbonweed (*Vallisneria nana*) comprised 74-90 % of the total mass of dry season stomach contents of adult *C. insculpta* in the Daly. Ribbonweed is patchily distributed along the river (unpubl. data), so turtles may need to cover great lengths of river to forage and accumulate energy sufficient for reproduction. Data collected concurrent with the present study revealed that most *C. insculpta* in the Daly River exhibit biennial reproduction (unpubl. data). This fact, coupled with the relatively low available energy content of ribbonweed (Heaphy, 1990; Spencer et al., 1998), suggests that diet may limit reproduction in the population, as is apparently the case in the herbivorous sea turtle *Chelonia mydas* (Bjorndal, 1981). Large home ranges may, therefore, reflect movements between the scattered patches of the turtles' chief food during the dry season. This proposal is consistent with the finding that males had much smaller home ranges than females, given the greater relative energy demands of females. A study investigating the effect of supplemental feeding on the reproductive frequency of captive animals would provide a firmer basis for the above hypothesis.

Another possible reason for the extensive *C. insculpta* home ranges is related to method. Home range area can be underestimated in species inhabiting deeper rivers, relative to species occupying more shallow systems, because depth of water is not considered. Resources turtles use, are, in general, distributed in three-dimensional space. Food availability or abundance may covary with depth. In addition, depth may play a role in a turtle's perception of area, given that turtles swim through a range of depths. The Daly River averages ca. 1.5 m deep during the dry season, compared to much deeper systems in other studies of aquatic turtles (e.g., averaging several meters, Plummer and Shirer, 1975; Jones, 1996). This might also explain the long linear home range found in *A. spinifera* in a creek averaging 30 cm deep (Plummer et al., 1997). I recommend that future studies of home range in aquatic turtles should record and analyze depths as well as horizontal dimensions, as has been done in studies of marine mammals (e.g., Harcourt et al., 2000).

Activity centers and microhabitat use. - Visual inspection of combined point locations of females against locations of three potential resources reveals that turtles spent a considerable amount of time in areas near thermal springs (Fig. 1). During the dry season before the river warms to 30°C in September, turtles spend a substantial amount of time at thermal springs (Doody, 2000; Chapter 3). The two known thermal springs that were not associated with high turtle activity were small springs in shallow water (2 km, 8.5 km marks, Fig. 1). The activity peak near the 8000 m mark was associated with deep water – there may be a thermal spring at this location that I did not detect (Fig. 1, Fig. 2). Beach location may also have contributed to activity center location. Dense ribbonweed patches did not appear to be associated with centers of turtle activity, but may be important at a larger scale. Stretches upstream of the study area with little or no ribbonweed were associated with very few egg clutches in 75 km nest surveys (unpubl. data). The influence of sex and reproductive condition on activity centers could not be determined because the sample sizes were too small.

Males and females used microhabitats with similar frequencies, except for open sand flats and isolated logs (Fig. 3). In comparison with females, males used isolated logs more, sand flats less, were found at shallower depths, and were less active during the daytime when most observations were made. Observations and analyses indicate that these differences were interrelated because the males often sat motionless against submerged isolated logs in shallow (< 1 m) water. Thus, inactivity in males was probably responsible for sex differences in microhabitat, and thus depth of observations. Male *Graptemys flavimaculata* used shallower depths and more snags than did females, but this difference was not attributable to activity (Jones, 1996).

Wet season locations. - Turtles did not appear to leave their dry season home ranges and move into estuarine areas during the wet season (Fig. 5), despite the occurrence of *C. insculpta* in estuarine areas in Papua New Guinea (Georges and Rose, 1993). The wet season aerial survey indicated that turtles moved out of the river channel during flooding (Fig. 5, Fig. 6). Most turtles were clumped into two groups, each comprising males and gravid and non-gravid females. The reason for this clumping in not known, but each group was near (group 1 = within 200 m, group 2 = within 800 m) a billabong or river swamp (Fig. 5). Turtles may have used these billabongs when water levels were higher weeks earlier (Fig. 6), and then followed receding water toward the riverbanks. Alho and Padua (1982) found *Podocnemis expansa* residing in lakes when the Amazon and its tributaries were high, return to the river to nest when the water level dropped. Alternatively, clumping of *C. insculpta* could have occurred in response to some concentrated food source, such as flying fox colonies (Georges et al., 1989) or fig trees (Georges and Rose, 1993). The locations of the turtles at one point in the wet season relative to dry season home ranges indicated that turtles moved with the current downstream before leaving the river channel. Previous studies have reported downstream dispersal of freshwater turtles associated with periods of high water (Moll and Legler, 1971; Bury, 1972; Pluto and Bellis, 1988). However, few conclusions can be drawn from a single wet season survey. A radio-telemetry study during the wet season would be useful in completing our understanding of the movement patterns, diet, and other ecological attributes of *C. insculpta*.

CHAPTER 3: BEHAVIOR

Use of Thermal Springs for Aquatic Basking
by the Pig-nosed Turtle, *Carettochelys insculpta*

(in press, Chelonian Conservation and Biology)

J. Sean Doody, Rachel A. Sims, and Arthur Georges

INTRODUCTION

Thermoregulatory basking behavior is widespread among aquatic turtles, spanning marine, freshwater and semi-aquatic forms (e.g., Whittow and Balazs, 1982; Ernst, 1986; Krawchuck and Brooks, 1998). Moll and Legler (1971) recognized two forms. Aquatic basking occurs when turtles float close to the surface, taking advantage of the warmth of the surface stratum of water and perhaps benefiting directly from absorption of solar radiation. Atmospheric basking occurs when turtles climb onto emergent logs, rocks, or banks to bask directly in the sun’s rays. While less is known of the consequences of aquatic basking (Chessman, 1987), atmospheric basking has been shown to raise body temperatures of turtles (Moll and Legler, 1971; Standora, 1982; Crawford et al., 1983; King et al., 1998), though the elevated temperatures can be quickly lost when the turtles return to the water (Manning and Grigg, 1997). Thermoregulation is thought to be the primary function of basking behavior (Crawford et al., 1983), but there could be other attendant advantages such as removal of algae (Neill and Allen, 1954; Boyer, 1965) or ectoparasites (Cagle, 1950), or promotion of Vitamin D synthesis (Cagle, 1950). In turn, thermoregulation can influence ingestion (Kepenis and McManus, 1974; Parmenter, 1980), digestion

(Kepenis and McManus, 1974), retention rate (Spencer et al., 1999), intestinal motility (Studier et al., 1977), metabolism (Bennett, 1982; Jackson, 1971), activity (Gatten, 1974; Parmenter, 1981), and growth (Christy et al., 1973; King et al., 1998).

The pig-nosed turtle, *Carettochelys insculpta*, is a large freshwater cryptodire restricted to northern Australia and southern New Guinea (Georges and Rose, 1993). I initially observed individual *C. insculpta* spending much of their time resting on localized thermal springs discharging from the river bottom during the cooler months. As part of a larger study investigating the influence of reproductive condition on movements, I fitted temperature dataloggers and radio-transmitters to adult female *Carettochelys* (gravid and non-gravid). Our objectives were to determine how often and when turtles used thermal springs, and to determine the influence of reproductive condition on thermal spring basking in females. In some reptiles, gravid females bask more than non-gravid females (e.g. Charland and Gregory, 1990; Schwartzkopf and Shine, 1991). I also compared water temperatures of thermal springs to those in various microhabitats, and to the seasonal change in river temperature. Thus, I report a new behavior in turtles, and a rare and different form of aquatic basking. I also present evidence that access to thermal springs for thermoregulation may have influenced timing of nesting.

MATERIALS AND METHODS

Turtles were captured with dipnets from boats, by diving, and with hoopnets (after Legler, 1960) baited with wallaby (*Macropus agilis*) meat. Nineteen female turtles (9 gravid, 10 non-gravid) were fitted with radio-transmitters (Sirtrack® two-stage, waterproofed in epoxy). Radio-transmitters were mounted on aluminum plates bolted to the rear edge of the carapace, off-center. Neoprene was fitted between the plate and the turtle to reduce skin damage. Reproductive condition was determined in the field by radiography using a portable x-ray machine (EXCELRAY® 31-HR-100P, settings 60 KVP, 30 MA, 0.4 sec), and a makeshift darkroom. Each turtle was also equipped with a Stowaway® temperature datalogger, which was waterproofed with two balloons and attached to a cattle ear tag (Allflex®) with waterproof tape. The cattle ear tag was then fastened to the trailing edge of the carapace. Cattle ear tags were a proven marking technique in 1996-97. Datalogger packages were numbered for individual identification from a distance. Dataloggers recorded temperatures every 16 minutes for 20 days.

In most cases turtles were located during daylight hours, several times a week throughout the study, though a few were not located until after the study was completed. Two individuals were intensively tracked (up to 3 fixes per day) throughout the study. Turtles were recaptured when possible near the end of the 20-day period, and dataloggers were downloaded and re-launched for a second 20-day period. I also investigated the duration of thermal spring use by intensively monitoring a thermal spring for 2-4 continuous hours on four different days. In addition I made opportunistic observations of any turtles on thermal springs throughout the study.

Thermal springs were found opportunistically by noting cleared areas of sand on the river bottom or by discovering a group of turtles concentrated in a very restricted area. The cleared areas associated with thermal springs resulted from a combination of instability in the sand through which the water was passing, which prevented algal accumulation, and the activity of turtles. I confirmed the presence of a thermal spring by snorkeling, during which time a temperature was taken with a calibrated alcohol thermometer inserted 5 cm into the sand. The thermal springs were flagged to aid in future location. Turtles were determined to be using thermal springs when they could be seen on the cleared circle, or were within 10 m of a thermal spring as determined by radiotelemetry. To determine the overall thermal environment available to the turtles, Stowaway® dataloggers were placed in four microhabitats thought to bracket available water temperatures. These microhabitats were: (1) open, shallow pools (0.5 m deep) with low flow, (2) open bedrock flat (1 m deep) with moderate flow, (3) open sand flat (1 m deep) with fast flow, and (4) shaded deep pool (2.5 m deep) with low flow in a logjam. Dataloggers were attached to a star picket and sensors were fastened just above the river bottom, where *C. insculpta* spends the majority of its time (pers. obs.). Microhabitat dataloggers were calibrated and recorded temperatures once every hour for 75 days throughout the season in 1998. River temperatures were recorded every 15 minutes between May and November, 1996-98 using Datataker® Model DT500 dataloggers. River temperature data are presented as spline functions of mean daily temperatures.

Timing of nesting was determined in two ways, depending on the river stretch. In the 30 km stretch in which an intensive nesting study was being conducted, nesting surveys were conducted daily and actual nesting dates were obtained. In the other two stretches, which were adjacent and upriver, nests were located on surveys that were 8-10 days apart. Nesting beaches, as characterized by Georges (1992), were located with the aid of a motorboat. Nests were found by following tracks left by gravid turtles, by noting slight depressions in the sand, and by probing the sand.

RESULTS

Description of thermal springs. – I located 25 thermal springs in a stretch of river ca. 25 km in length. The substratum disturbance caused by emerging water in the thermal springs varied from a few cm in diameter to 70 cm in diameter for single springs, however aggregations of discharge points often resulted in a sandy area clear of algal growth up to 2 m in diameter (Fig. 3.1). Most thermal springs were in sandy substrates. Temperatures of thermal springs at 5 cm sand depth averaged 29.9 ± 0.52^{o}C (range = 29.5-31.0^{o}C; N = 10). The thermal springs may be derived from groundwater (which maintains a temperature equivalent to the mean annual temperature), rather than from some specific geothermal source of heating. Thermal springs ceased to influence water temperature 7 cm above the substrate. The temperature of many other springs in the river did not differ from that of the surrounding river water, and turtles were not seen to use these.

Frequency and duration of thermal spring use. – Turtles were seen resting on thermal springs (Fig. 3.1) a total of 157 times (females = 147, males = 4, unknown = 6). Of these, 136 sightings were of nine telemetered females. Turtles often remained on the thermal spring for several hours (Fig. 3.2). During 9.5 hours of intensive radio-tracking, I found two turtles to use thermal springs 79 % (Tag # F05) and 85 % (F49) of the time. Turtles used the thermal springs both by day and night (Fig. 3.2 & 3.3).

Gravid vs. non-gravid turtles. - Thirteen of 19 datalogger packages were attacked by freshwater crocodiles (*Crocodylus johnstoni*), as evidenced by teeth marks in the packages, resulting in datalogger failure, and loss of dataloggers. Nine of the 19 telemetered females were seen using thermal springs. Seven of these turtles were gravid. While this was suggestive of an association between reproductive condition

Figure 3.1. Thermal springs in the Daly River, which are difficult to detect, become conspicuous after being used by turtles. Turtle activity around the thermal spring harrows a circular area of sand, free of algae and debris (top). Bottom photo shows a male *C. insculpta* ‘basking’ on a thermal spring.

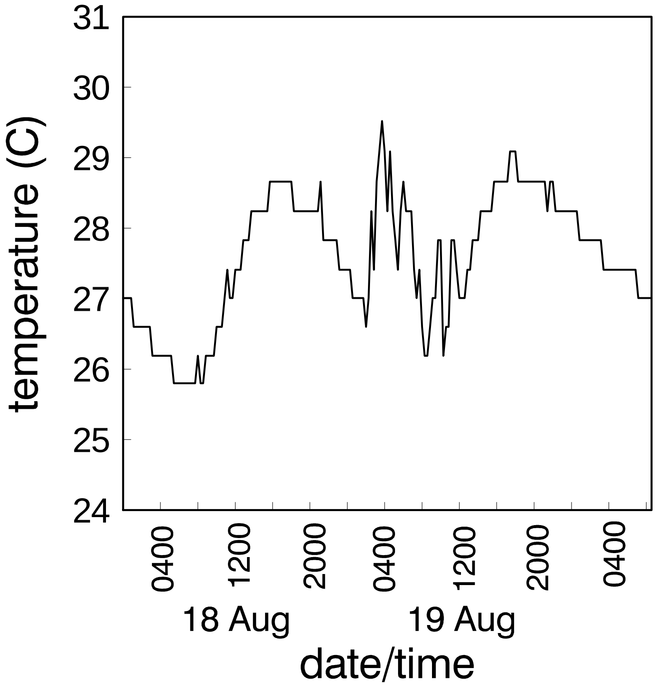


Figure 3.2. Example of a turtle using a thermal spring for an extended period of time (ca. 9 hours) during early morning. The figure shows a background sinusoidal variation in daily river temperatures for two days. This pattern is interrupted by thermal spring use between 0200 and 1100, 19 August. Data are ambient water temperatures recorded from a Stowaway® datalogger attached to the rear carapacial edge.

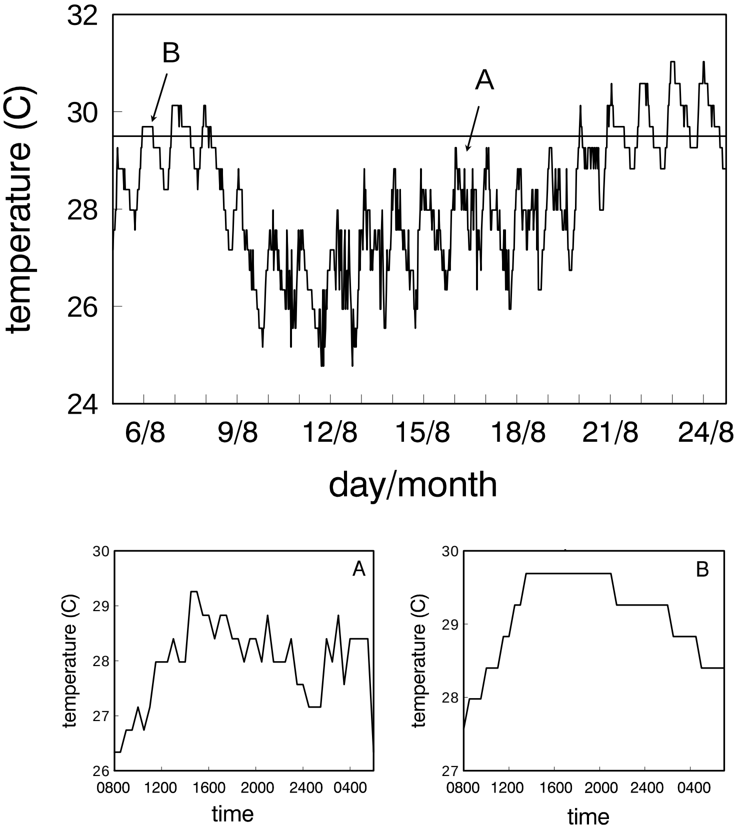


Figure 3.3. Top: A 20-day temperature trace from a female *C. insculpta*, showing thermal spring use. The straight line indicates the modal temperature of thermal springs in the river. Bottom: Note the spiked profile (A) indicative of thermal spring use when river/external body temperatures average below that of the thermal spring, compared to the step profile (B) exhibited when average river temperatures are similar to that of the thermal spring.

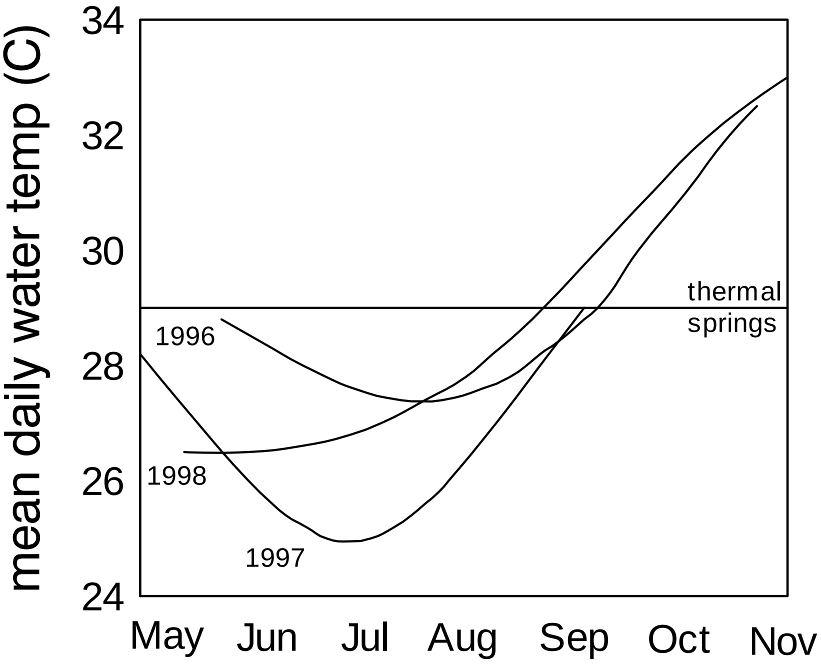


Figure 3.4. Mean daily water temperatures for the Daly River during 1996-98. The straight line represents the modal temperature of thermal springs in the river. Figure shows that *C. insculpta* could use thermal springs to thermoregulate for between 4 and 6 months a year.

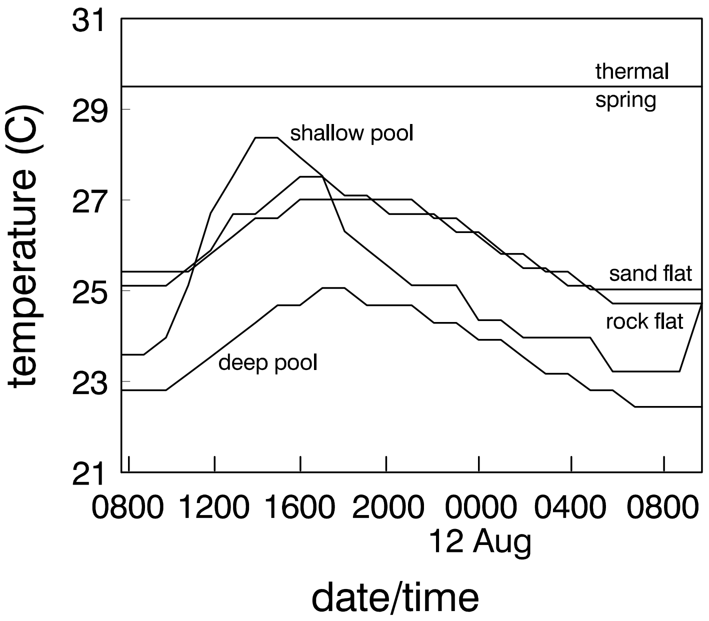


Figure 3.5. Example of environmental temperatures of different microhabitats available to *C. insculpta* in the Daly River on 11-12 August. Shallow pool was open, low flow, 0.5 m deep. Deep pool was shaded, low flow, 2.5 m deep. Sand flat was open, high flow, 1 m deep. Rock flat was open, moderate flow, 1 m deep. Straight line represents modal temperature of thermal springs.

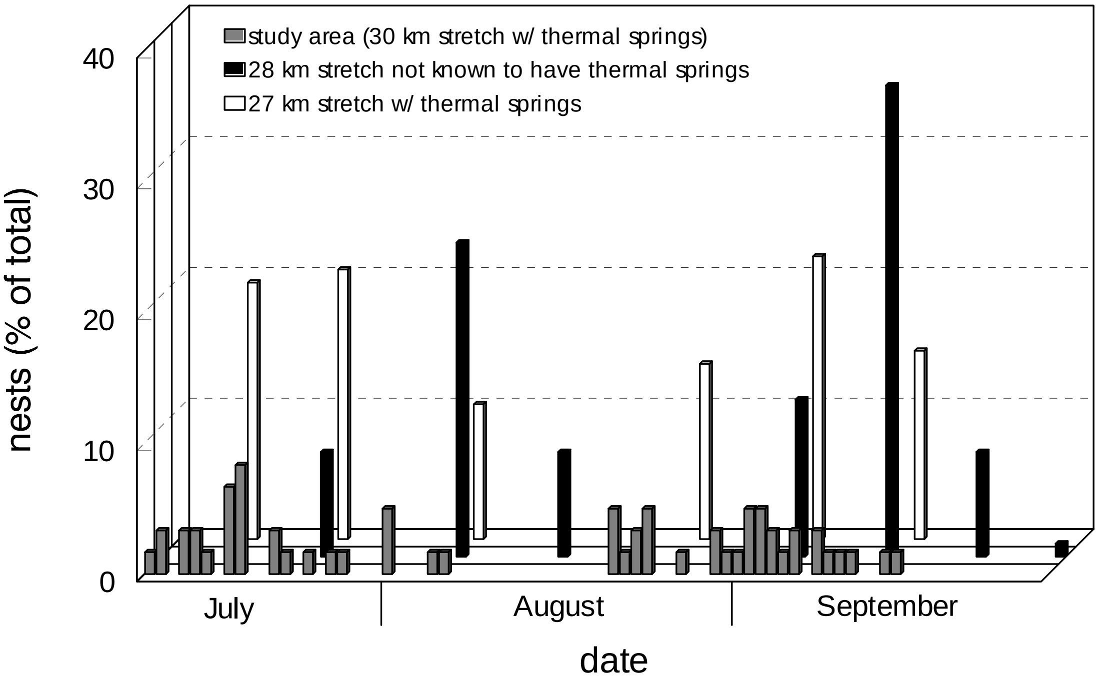


Figure 3.6. Differences in timing of nesting of *C. insculpta* in particular stretches of river. Gray bars represent lay dates in the study area. White bars represent dates found for nests in the stretch immediately adjacent and upstream of the study area. Black bars represent dates found for nests in the stretch adjacent to that represented by the white bars. Thermal springs are known from the stretches represented by the gray bars and white bars, but not from the stretch represented by the black bars.

Temporal patterns. –The behavior of turtle S51 (Fig. 3.3) in August provides an example of seasonal thermal spring use. Turtles used thermal springs when river temperatures were cooler than that of the thermal spring, but not when river temperatures were warmer than or averaged that of the thermal spring. Three other temperature traces (turtle F49, 10 days; F208, 9 days; S09, 9 days) showed a similar pattern of use. Seasonal change in water temperatures during 1996-98 (Fig. 3.4), compared to the modal temperature of thermal springs, indicates the seasonal periods when turtles are likely to be associated with the thermal springs. This pattern was supported by observations of turtles ceasing to use the thermal springs by 9 September in 1998.

Microhabitat temperatures - The shallow open pool exhibited the highest and most variable temperatures, while the deep shaded pool showed lower temperatures (Fig. 3.5). Between these two microhabitats, temperatures differed by 2-4° C (Fig. 3.5). The deep pool was generally 2° C cooler than the rock and sand flats, which were nearly identical (Fig. 3.5).

Timing of nesting. – In 1998, nesting began significantly earlier ($F_{1,121} = 47.62$, $p < 0.001$; Fig. 3.6) in a 27 km river stretch with numerous thermal springs (8 = 30 July) than in a 28 km stretch where thermal springs are not known to occur (8 = 23 August). A similar difference was noted in 1997 but data were confounded by sampling effort.

DISCUSSION

Thermal spring use. - The frequency (Fig. 3.3) and duration (Fig. 3.2) of thermal spring use, coupled with river temperatures during the cooler months (Fig. 3.4) suggest that thermal springs are important to *Carettochelys* for up to five months of the year. Turtles were regularly seen on thermal springs between July and September in 1996-98. Temperature profiles from four turtles provided evidence that turtles used thermal springs when river temperatures were cooler, but ceased using them when river temperatures rose (see Fig. 3.3). Indeed, once river temperatures exceeded those of thermal springs in September 1998, turtles were no longer seen on thermal springs, the cleared areas around them (Fig. 3.1) being no longer evident. A similar pattern was observed for four species of percid fish, which were attracted to a thermal effluent only during the cooler months of the year (Benda and Proffitt, 1973). I suggest that thermal spring basking is a complex behavior, as turtles would habitually seek out particular thermal springs. Some turtles were seen to use the same thermal spring for over a month, while others moved between different thermal springs. Five chelid turtle species common in the river (*Chelodina rugosa, Elseya dentata, Emydura victoriae, Emydura subglobosa, Emydura tanybaraga*) were not observed to use thermal springs.

Owing to datalogger failure I were unable to adequately test the hypothesis that gravid female *Carettochelys* used thermal springs more frequently than non-gravid females. However, of the 9 telemetered females recorded using thermal springs, seven were gravid. An additional three non-telemetered females (F60, S18, S53) seen using thermal springs were all gravid. Collectively, this suggests that gravid females may have used thermal springs more than non-gravid turtles, but this needs to be verified.

Observations of turtle behavior were difficult due to the turtles' wariness of approaching boats, and our diving was infrequent due to the threat of saltwater crocodiles (*Crocodylus porosus*). However, I made a few observations. Turtles were tolerant of one another when using thermal springs; as many as five were seen sharing a single spring. For nearly one month, two females simultaneously utilized the same thermal spring (F05, F49). In one case, however, a large female (F32) apparently displaced a smaller female (F49) from a thermal spring, resulting in F49 immediately moving 300 m downstream to another thermal spring. One telemetered female (S38) was seen to partially bury herself in the sand of a thermal spring, flip sand over her shell with her hindlegs, then bury down into the spring. However, it is difficult to rule out the possibility that the turtle was attempting to hide from us.

Microhabitat temperatures. - Environmental temperatures were similar among the sand and rock flat microhabitats, but warmer and more variable in the shallow pool, and cooler in the deep shaded pool (Fig. 3.5). Although turtles could potentially raise their body temperatures by using the shallow pools, in our three years of fieldwork *C. insculpta* was rarely seen in this microhabitat. This is not surprising, as this shallow sandy area provided no cover or food. *Carettochelys* is an extremely aquatic turtle, and is not known to exhibit atmospheric basking. Further, I have not seen this species engage in typical aquatic surface basking in three years of study. Turtles could, however, raise their body temperatures by sitting on thermal springs.

Potential consequences of thermal spring use. - Crawford et al. (1983) reviewed potential reasons for basking behavior in turtles, and concluded that thermoregulation was the primary factor influencing basking behavior in the turtle *Trachemys scripta*. I suggest the same for thermal spring basking in *Carettochelys*, although other reasons may exist. By warming themselves on thermal springs in winter when river temperatures are cooler, turtles could maintain higher metabolic, ingestion, and digestive rates (Jackson, 1971; Kepenis and McManus, 1974; Parmenter, 1980; Bennett, 1982), intestinal motility (Studier et al., 1977), retention rates (Spencer et al., 1998), activity levels (Gatten, 1974; Parmenter, 1981), and growth rates (Christy et al., 1973; King et al., 1998).

Studies manipulating food intake found that fed turtles basked more than unfed turtles (Gatten, 1974; Hammond et al., 1988). In some stretches of the Daly River, *C. insculpta* may facilitate digestion by basking on thermal springs after feeding, but this remains speculative. Alternatively, turtles may forage after achieving a preferred body temperature, taking advantage of the resistance to heat loss encumbered by their large body size (Bartholomew and Tucker, 1964). Regardless, thermal spring basking during the cooler months probably permits turtles to be more active a greater percentage of the year (Parmenter, 1980). Manning and Grigg (1997) questioned whether atmospheric basking in aquatic turtles was of thermoregulatory significance. Much of their argument rested on the fact that the turtles they studied (*Emydura signata*) spent only brief periods out of water raising their body temperature, only to return to the water and rapidly lose heat. They argued that the net result was that turtles were thermoconformers, their body temperatures effectively not uncoupled from water temperatures. I feel that the long periods *C. insculpta* spent basking on thermal springs during the cooler season, combined with their large body size (= slow cooling, see Bartholomew and Tucker, 1964), supports a thermoregulatory phenomenon.

Turtles inhabiting stretches of river with thermal springs nested, on average, 24 days earlier than turtles nesting in stretches not known to have thermal springs (Fig. 3.6). This may represent a consequence of frequent thermal spring basking. Increased basking could accelerate follicular development (Whittow and Balazs, 1982), and has been associated with earlier parturition in gravid viviparous reptiles (Shine, 1990; Schwartzkopf and Shine, 1991). Two studies found no difference in basking frequency and duration between male and female *Chrysemys picta* during extended periods prior to nesting (Lefevre and Brooks, 1995; Krawchuk and Brooks, 1998). However, *C. picta* females basked longer than males on days just prior to and during nesting (Krawchuk and Brooks, 1998). *Chelonia mydas* basked more during the breeding season, and less as the nesting season progressed (Balazs, 1980). Gravid *Podocnemis expansa* are known to bask only 2-3 weeks prior to, and during nesting (Mosquiera Manso, 1960), a behavior presumed to play a role in egg maturation (Pritchard and Trebbau, 1984). Finally, in a laboratory experiment *Trachemys* (= *Pseudemys*) *scripta* females basked more than males in spring/summer, but not in autumn/winter (Hammond et al., 1988).

This putative connection between thermal spring basking and timing of nesting in *C. insculpta* may be spurious, as I cannot be certain that thermal springs did not exist in the stretch of river where I found no thermal springs. However, once used by turtles thermal springs are conspicuous (Fig. 3.1), and I did not detect thermal springs in the stretch in over 20 nest surveys in 1996-98. Although some other factor may have caused the differential timing, I can find no other differences between the stretches, except the availability of ribbonweed (*Vallisneria nana*), the preferred food of *C. insculpta* in the Daly (Heaphy, 1990; M. Welsh, unpubl. data). The paucity of ribbonweed in the upper stretch may have caused a delay in nesting by limiting energy uptake, thereby slowing follicular development. Christens and Bider (1987) found that the onset of nesting in the turtle *Chrysemys picta* was affected by mean air temperatures the previous year rather than temperatures in the current year, suggesting that productivity and food availability were implicated. Lastly, just prior to nesting, at least some gravid *C. insculpta* aggregate, moving from beach to beach in groups of up to 12 animals (pers. obs.). It is possible that the behavior of this group influences the exact timing of nesting, thus affecting data independence.

In summary, the apparent importance of thermal springs to *C. insculpta* warrants further research. In particular, internal body temperatures, monitored by temperature-sensitive radio-transmitters, are needed to accurately characterize the thermal biology of the turtles. The present study recorded ambient water temperatures around the turtles, which are only indicative of body temperatures. Determining the ultimate importance of the thermal springs to *Carettochelys* would be challenging but worthwhile. For example, Christy et al. (1973) found that, compared to those in nearby natural sites, *Trachemys scripta* inhabiting thermal effluent areas near a fossil-fuel generating plant grew faster and attained a larger body size. However, because the thermal effluent area was extensive, observed growth differences could be due to either direct effects of temperature (i.e., increased metabolic and digestive rates) or indirect effects (increased productivity of food items). Due to their point-source nature, thermal springs would be expected to elicit only direct effects on turtles. Laboratory studies manipulating body temperature and examining its influence on digestive efficiency and growth would be revealing, while investigating effects on timing of reproduction would be logistically difficult.

CHAPTER 4: REPRODUCTION

Twice Every Two Years: Reproduction in the Pig-nosed Turtle, *Carettochelys insculpta*, in the Wet-dry Tropics of Australia

(ms prepared for submission to Journal of Zoology)

J. Sean Doody, Arthur Georges, and Jeanne E. Young

INTRODUCTION

Reptiles are ideal for the study of reproductive output and trade-offs between offspring size and number (Elgar and Heaphy, 1989; Gregory and Skebo, 1998). This is partly owed to their general lack of parental care after hatching or birth. Among reptiles the lack of parental care is most pronounced in turtles, which offer no maternal contribution after provisioning yolk to the eggs.

Patterns of reproduction in turtles have emerged, such as a negative correlation between body-size adjusted clutch size and egg mass, and the negative correlation between clutch frequency and latitude (Elgar and Heaphy, 1989; Iverson et al., 1993). However, interpreting these patterns in a natural context can be complicated by environmental factors and by physical constraints (Olsson and Shine, 1997a). Reproductive output can be driven by food availability and rainfall (James and Whitford, 1994), or by morphological constraints such as size and shape of the abdominal cavity (Vitt and Congdon, 1978; Shine, 1988, 1992), or size of the pelvic girdle through which eggs pass (reviewed in Clark et al., 2001). In particular, local environmental conditions can often dictate the specifics of reproductive output through their effects on rates of resource acquisition (Kuchling, 1999), and investigating the interactions between those conditions and reproduction is necessary to develop an understanding of how reproductive output contributes to life-history evolution (van Noordwijk and de Jong, 1986; Bernardo, 1996; Roosenberg and Dunham, 1997).

In theory, turtles facing annual variation in environmental conditions resulting in low energy acquisition can: (1) adjust reproductive output by reducing clutch frequency (Turner et al., 1984; Bjorndal, 1985; Iverson, 1991a), clutch size and/or egg size (Swingland and Coe, 1978; Roosenberg and Dunham, 1997), or (2) defer laying eggs completely (Nieuwolt-Dacanay, 1997; Kuchling, 1999). However, few studies on turtles have linked environmental variation to reproductive characteristics (reviewed in Kuchling, 1999).

Phenotypic plasticity in reproductive traits is likely to be more pronounced in species exposed to high or extreme annual or seasonal variation in environmental factors dictating food acquisition. One such system is the wet-dry tropics of northern Australia, which is characterized by a mean monthly rainfall of < 7 mm from May to September, rising to a peak monthly average of 284 mm in February.

I studied the ecology and sex determination in the pig-nosed turtle (*Carettochelys insculpta*) for four years in the wet-dry tropics of northern Australia. In particular, I was interested in the previously unknown female reproductive biology. Because the study spanned both years with ‘big’ and ‘small’ wet seasons, I was able to ask: How might the wet-dry climate shape the reproductive biology of this unique species? Specifically, how did reproductive characteristics relate to the magnitude of the wet season prior to reproduction? The study was also designed to answer fundamental questions on reproduction in *C. insculpta*, such as: What variation exists in reproductive frequency, clutch size, clutch mass, and egg size? What relationships exist among clutch characteristics? At what size do females mature?

Two findings on the reproductive biology of female *C. insculpta* were of particular interest: biennial reproduction with double clutching, and a seasonal change in egg and clutch size. I discuss these findings within a broader context of current life history theory.

MATERIALS AND METHODS

I captured turtles for reproductive examination nearly every day from August to October 1996 and from July to October in 1997-98. Turtles were captured with dipnets from a motorboat, by snorkeling, and with baited hoopnets. Captured turtles were measured, weighed, and females were x-rayed for the presence of shelled eggs using a portable x-ray machine (EXCELRAY®). Radiographs were developed in a makeshift darkroom in the field. Turtles were marked with passive transponder (PIT) tags and with cattle ear tags, the latter of which were attached by drilling a hole in the rear carapacial edge. Cattle ear tags allowed identification from the boat. Turtles were held in a large tub (2000 l) and released within 24 h of capture.

Clutch frequency (within and among years) was determined by (1) compiling x-ray records of individuals throughout the 3-year study, and (2) by determining the proportion of gravid turtles during the period in which turtles were found to be gravid. Radiograph histories were considered to be sufficient for determining within-year clutch frequency when the interval between successive radiographs for an individual was < 12 days. This number was based on data from two turtles in which both the egg-shelling date and the subsequent nesting date were known: In five turtles, x-rays revealed that the eggs were in the process of being calcified, as evidenced by faint images of the eggs (see also Turner et al., 1986). Fresh nests with known laying dates were subsequently found for two of these turtles, 17 and 18 days after the eggs were being calcified. Thus, I estimated conservatively that x-rays every 12 days would ensure that a complete reproductive history was known. Repeated x-rays for the same individuals allowed us to estimate minimum egg retention times for some individuals. Size at maturity for female *C. insculpta* is defined herein as the size of the smallest gravid female.

In Australia *C. insculpta* nests on sandy banks and beaches adjacent to the river (Georges, 1992; Georges and Rose, 1993). I surveyed for nests daily by boat, and nests were found by noting tracks in the sand and probing for the eggs with a spring steel rod. Eggs were carefully removed from each nest and counted, weighed, and measured. *Carettochelys* lays eggs that appear spherical, but are actually slightly oblong or elongate. I measured egg length and width with calipers to the nearest 0.05 mm. Eggs were then returned to their original depths and positions in the nest. Data logger probes were placed in most nests to measure continuous temperatures for a concurrent study.

Mean inter-nesting intervals were determined by subtracting the mean/median nesting date of first clutches from that of second clutches. This was appropriate because a distinct bimodal temporal distribution of nests was found (unpubl. data).

I used mean monthly river levels as an index of the magnitude of each wet season during the study years. River stage data were from Dorisvale Crossing (60 km upstream of the study area), and are routinely collected by Northern Territory Water Resources. To assess how typical the size of the wet season was, these data were compared to 38-year averages of mean monthly river levels calculated for the years 1960–1998.

I also analyzed data on the onset of nesting, egg size, and clutch size collected by AG during 1986. These data were not included in all analyses due to missing information (e.g., first clutch vs. second clutch). Methods for finding nests and handling eggs in 1986 were identical to those described above.

RESULTS

Number of nests and radiographed turtles. - A total of 210 adult females were x-rayed 491 times during the study. One hundred and ninety-one nests were found between 1996 and 1998 (see Table 4.1 for breakdown by year).

Size at maturity. - Size distribution of reproductive females is given in Figure 4.1. The smallest mature female *C. insculpta*, based on radiography, measured 38.0 cm (CL), 30.5 cm (PL), and 5.9 kg (mass).

Clutch frequency. - Proportions of gravid turtles each year, determined by radiography between the dates of the first and last gravid turtles, are given in Table 4.1. Twenty-eight of 34 (82 %) turtles with adequate x-ray profiles followed a pattern consistent with biennial reproduction, while five turtles (15 %) skipped more than one year, and one turtle (3 %) nested in consecutive years. Of 20 turtles with sufficient x-ray records to determine clutch frequency *within* a year, 16 (80 %) nested twice in a year while 4 (20 %) nested once. See methods for criteria used to determine which radiograph records were sufficient.

Clutch size, egg size, clutch mass, and clutch number. - Data on egg size, clutch size, and clutch mass in *C. insculpta* are presented in Table 1. Clutch size distribution for

Table 4.1. Annual variation in reproductive characteristics of *C. insculpta*. Data are means ± 1 SD, except egg size data, which are grand means ± 1 SE. Sample sizes (number of clutches) are in parentheses. Inter-nesting intervals are presented as means/medians.

Attribute	1996	1997	1998	all years
# nests	65	51	75	191
% gravid	48 (87)	34 (61)	37 (62)	41.3 (210)
inter-nesting interval (days)	36/36 (57)	46/48 (38)	42/46 (56)	40/42 (145)
egg length (mm)	39.2 ± 0.15 (47)	39.1 ± 0.74 (47)	40.2 ± 0.18 (69)	39.6 ± 0.21 (156)
egg width (mm)	38.3 ± 0.15 (47)	38.8 ± 0.14 (47)	38.9 ± 0.10 (69)	38.7 ± 0.07 (156)
egg mass (g)	34.0 ± 0.42 (44)	35.5 ± 0.34 (44)	35.9 ± 0.25 (69)	35.2 ± 0.20 (153)
clutch size	9.8 ± 2.46 (50)	10.7 ± 2.62 (45)	10.6 ± 2.34 (69)	10.40 ± 2.47 (164)
clutch mass (g)	327.0 ± 98.41 (44)	376.1 ± 94.30 (40)	369.1 ± 99.20 (69)	360.9 ± 7.79

Table 4.2. Influence of clutch (first vs. second) on reproductive attributes in *C. insculpta* in Data are means ± SD for clutch size and clutch mass, and grand means ± SE for measures of egg size. Numbers of clutches are in parentheses. Significance is from single-factor ANOVA. * denotes $p < 0.05$; **, $p < 0.01$.

Attribute	year	first clutch	second clutch	significance
clutch size	1996	9.4 ± 2.54 (20)	10.2 ± 2.39 (30)	$F_{1,49} = 4.04$, p = 0.250
	1997	11.5 ± 2.34 (24)	9.8 ± 2.66 (21)	$F_{1,44} = 4.07$, p = 0.025*
	1998	11.1 ± 2.31 (35)	10.1 ± 2.29 (34)	$F_{1,68} = 3.98$, p = 0.068
egg mass (g)	1996	34.4 ± 0.58 (14)	34.0 ± 0.53 (29)	$F_{1,42} = 0.21$, p = 0.652
	1997	34.5 ± 0.44 (19)	36.4 ± 0.45 (21)	$F_{1,39} = 8.84$, p = 0.005**
	1998	35.4 ± 0.39 (31)	36.3 ± 0.32 (38)	$F_{1,68} = 2.98$, p = 0.091
egg length (mm)	1996	39.3 ± 0.26 (14)	39.2 ± 0.18 (33)	$F_{1,46} = 0.13$, p = 0.716
	1997	39.5 ± 0.16 (19)	38.7 ± 1.41 (21)	$F_{1,39} = 0.31$, p = 0.583
	1998	40.0 ± 0.25 (31)	40.4 ± 0.25 (38)	$F_{1,68} = 1.02$, p = 0.316
egg width (mm)	1996	38.4 ± 0.24 (14)	38.3 ± 0.20 (33)	$F_{1,46} = 0.13$, p = 0.716
	1997	38.4 ± 0.18 (19)	39.2 ± 0.17 (21)	$F_{1,39} = 10.33$, p = 0.003**
	1998	38.5 ± 0.14 (31)	39.1 ± 0.12 (38)	$F_{1,68} = 10.34$, p = 0.002**
clutch mass (g)	1996	295.9 ± 103.21 (14)	341.5 ± 94.34 (30)	$F_{1,43} = 2.10$, p = 0.155
	1997	393.2 ± 78.28 (19)	360.5 ± 106.26 (21)	$F_{1,39} = 1.21$, p = 0.279
	1998	393.7 ± 87.82 (31)	349.1 ± 104.46 (38)	$F_{1,67} = 2.73$, p = 0.104

Table 4.3. Summary of annual variation in reproductive patterns and flood mortality of *C. insculpta*, and magnitude of the wet season during 1996-98. Timing of nesting, flood mortality, and hatchling sex ratio data are unpublished.

attribute	1986	1996	1997	1998
‘big’ wet season	no	no	yes	yes
early clutch size larger than late clutch size	no	no	yes	yes
early clutch eggs smaller than late clutch eggs	n/a	no	yes	yes
tradeoff between clutch size and egg size	no	no	yes	yes
clutch size influenced by female body size	n/a	no	yes	yes
early nesting	no	no	yes	yes
flood mortality	yes	yes	no	no
female-biased hatchling sex ratios	yes	yes	no	no

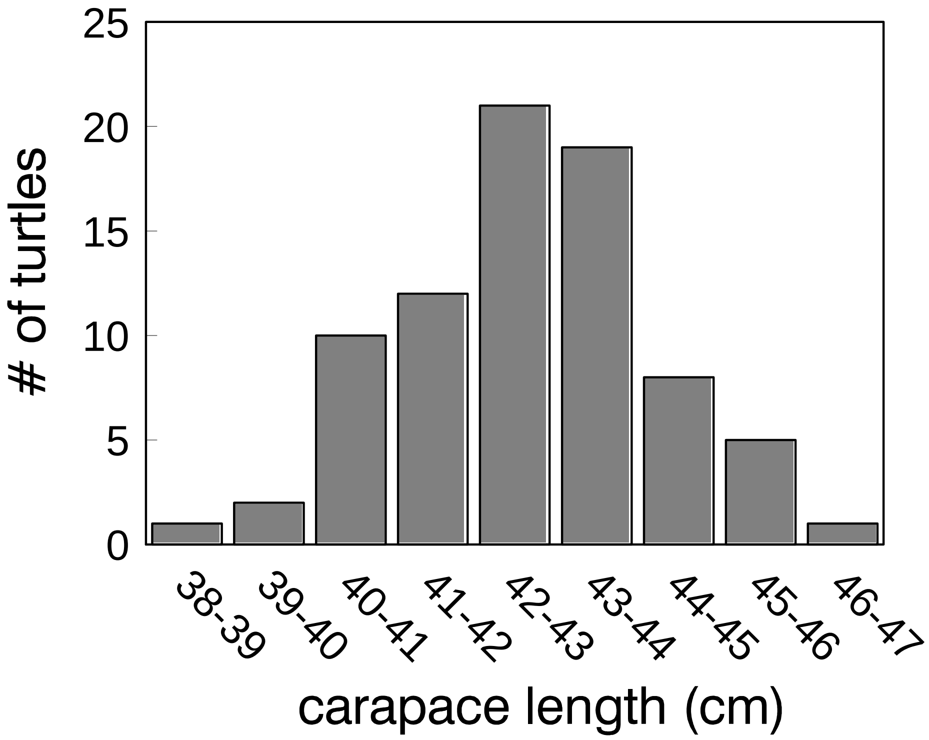


Fig. 4.1. Frequency distribution of mature female *C. insculpta*, based on radiograph data.

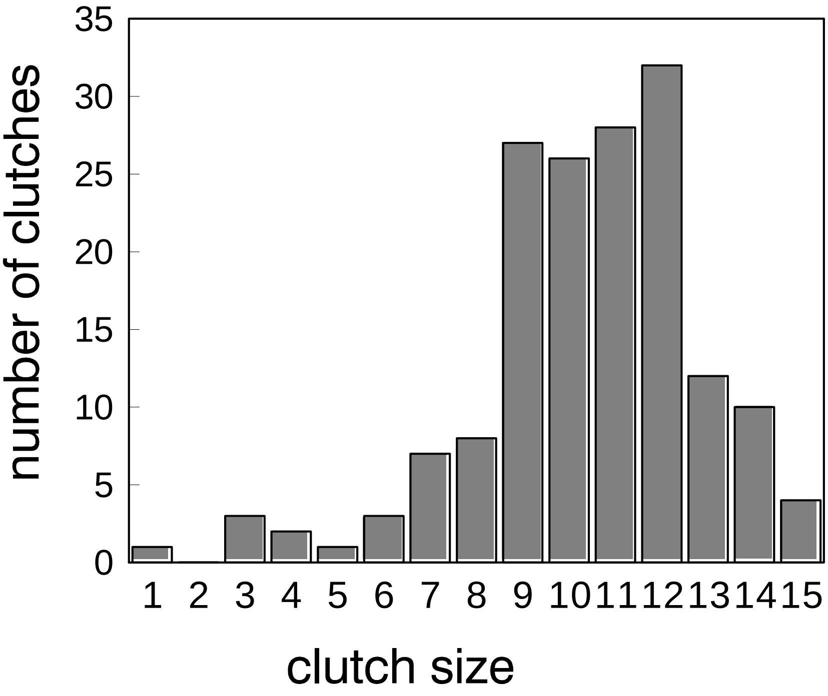


Fig. 4.2. Clutch size distribution of *C. insculpta* for 1996-1998 combined.

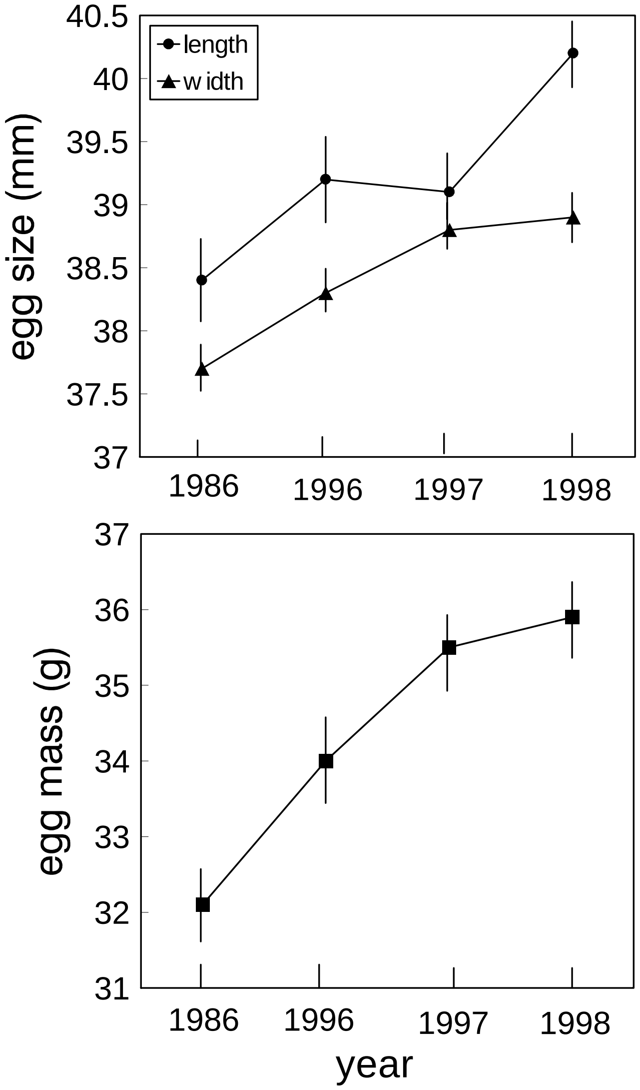


Fig. 4.3. Annual variation in egg dimensions of *C. insculpta* for the years 1986 and 1996-1998. Bars represent ± 1 SE.

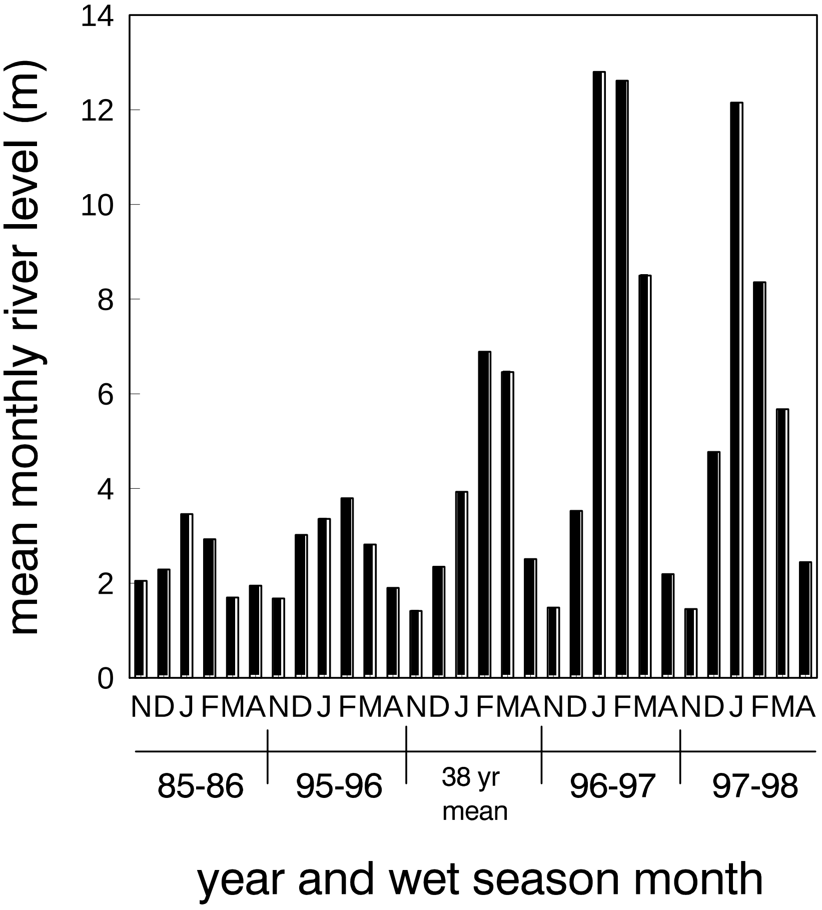


Fig. 4.4. Annual variation in the magnitude of the wet season, as indexed by mean monthly river levels prior to each year of the study (1995-96, 1996-97, 1997-98), in the year preceding the first year of the study (1994-95), in the year prior to data collection in 1986, and a 38-year average (1960-1998). Note that 1985-86 and 1995-96 were ‘small’ wet seasons and that 1996-97 and 1997-98 were ‘big’ wet seasons.

1996-1998 combined is shown in Figure 2. Taken from radiographs, clutch size generally increased with carapace length (1996, $F_{1,49} = 2.97$, $p = 0.091$; 1997, $F_{1,21} = 4.50$, $p = 0.047$; 1998, $F_{1,21} = 7.97$, $p = 0.011$). Clutch size did not differ between radiographs and nests ($F_{1,265} = 1.36$, $p = 0.25$). Clutch size did not differ significantly among years ($F_{3,190} = 1.27$, $p = 0.286$).

A two-factor ANOVA revealed no significant effect of year ($F_{2,158} = 2.14$, 0.12) or early vs. late season clutch ($F_{1,158} = 2.85$, $p = 0.09$) on clutch size taken from nests. However, a significant interaction was found ($F_{2,158} = 3.60$, $p = 0.03$). In 1997 and 1998 clutch size was significantly higher in the first clutch, while in 1996 clutch size did not differ between first and second clutches (Table 4.2).

Mean egg size differed significantly among years (Table 4.1; Fig. 4.3). However, egg dimensions differed in different ways. Egg length was significantly different among years ($F_{3,178} = 4.22$, $p = 0.007$), and a Tukey's HSD showed a significant difference between 1986 and 1998 ($p = 0.007$; Fig. 4.3). Egg width varied significantly among years ($F_{3,176} = 10.16$, $p < 0.001$). Generally, eggs were wider in years following 'big' wet seasons (1997, 1998) than in years following 'small' wet seasons (1986, 1996) (Fig. 4.3). A Tukey's HSD revealed wider eggs in 1986 than in 1996 ($p = 0.034$), 1997 ($p < 0.001$), and 1998 ($p < 0.001$), and wider eggs in 1998 than in 1996 ($p = 0.026$). Egg mass also differed significantly among years ($F_{3,175} = 17.34$, $p < 0.001$; Table 4.1; Fig. 4.3). Eggs were lighter in 1986 than in all other years (1996, $p = 0.009$; 1997, $p < 0.001$; 1998, $p < 0.001$), and eggs were also lighter in 1996 than in 1997 ($p = 0.033$) and 1998 ($p < 0.001$) (Fig. 4.3). No measure of egg size differed significantly between 1997 and 1998 (Table 4.1; Fig. 4.3).

I was not able to examine the influence of female body size on egg size because I could only link a few nests to their respective females, and because egg size in radiographs is confounded by error associated with eggs positioned at different (unknown) depths in the turtle. However, egg mass was significantly negatively correlated with clutch size in years after big wet seasons (1997, $r = -0.320$, $p = 0.044$; 1998, $r = -0.254$, $p = 0.035$), but not in years after small wet seasons (1986, $r = 0.165$, $p = 0.411$; 1996, $r = 0.098$, $p = 0.514$).

Mean egg mass was higher in later (second) clutches than in earlier (first) clutches in 1997 and 1998, although the difference only approached significance in 1998 (Table 4.2). No significant difference in mean egg size between early and late clutches existed in 1996 (Table 4.2). Egg width was significantly larger in later clutches than in earlier clutches in 1997 and 1998, but not in 1996 (Table 4.2). Egg length did not differ between early and late clutches in any year (Table 4.2).

Clutch mass did not differ significantly among years ($F_{3,176} = 2.55$, $p = 0.057$; Table 4.1). However, the difference approached significance, and clutch mass was larger in years after big wet seasons (1997, 1998) than in years following small wet seasons (1986, 1996). Clutch mass did not differ between early and late clutches in any year (Table 4.2).

Magnitude of the wet season. - The magnitude of the wet season during the study, as indexed by mean monthly river levels, is shown in Figure 4.4. A ‘small’ wet season characterized 1986 and 1996, while ‘big’ wet seasons occurred in 1997 and 1998 (record rainfall totals for the catchment in both years). Also shown is the 38-year average level for each month during the years 1960-1998, which is intermediate in magnitude.

Inter-nesting intervals and egg retention. - The estimated inter-nesting interval, based on the time elapsed between mean nesting dates of the first and second clutch, was 41.3 days (see Table 1 for breakdown by years). Two turtles retained their first clutch for a minimum of 52 days each. One of these turtles laid her first clutch when most turtles were laying their second clutch. The other turtle and her nest were not recovered.

DISCUSSION

Certain reproductive attributes of female *C. insculpta* were not unusual among turtles. Female *C. insculpta* matured at around 82 % of their maximum size (CL). This is high but just within the range found for other turtles species (reviewed in Shine and Iverson, 1995). The eggs and clutches produced by *C. insculpta* in the present study were similar in size to those produced by other turtle species of similar size and at similar latitudes (see Iverson et al., 1993). Comparing clutch frequency (CF) of *C. insculpta* to other turtles is more difficult, because data are not available for many species, and because of difficulty in interpretation. For example, CF = 1 for both *C. insculpta* and four species of *Clemmys*, despite the fact that *Clemmys* species generally lay once clutch per year (reviewed in Forsman and Shine, 1995) and *C. insculpta* lays two clutches every other year. Nevertheless, CF in *C. insculpta* is consistent with the prediction of multiple clutching (within years) in tropical species (Fitch, 1981).

Biennial reproduction and multiple clutches within years. – In contrast to the above features, other *C. insculpta* female reproductive traits were unusual among turtles. Both individual and population data strongly suggest that female C. insculpta in the

Daly River follow a biennial breeding cycle. Indeed, only one individual produced eggs in consecutive years. Gregory (1982) concluded that biennial (or less frequent) reproductive cycles were not known in oviparous reptiles. However, sea turtles are known to possess a multi-year vitellogenic cycle and skip years (Moll, 1979; Limpus and Reed, 1985; Johnson and Ehrhart, 1994; Miller, 1997), and a few studies have since recorded biennial cycles in oviparous reptiles (e.g., Cree et al., 1992; Kuchling, 1993). Among freshwater turtles, only *Erymnochelys madagascariensis* is known to have a biennial cycle (Kuchling, 1993). Although other studies on freshwater turtles have reported some individuals to skip years, in those studies most individuals in each population reproduced annually (reviewed in Kuchling, 1999).

According to Kuchling (1999) female animals exhibiting multi-year cycles either (1) fail to initiate or sustain a vitellogenic cycle (Kuchling and Bradshaw, 1993), or (2) fail to ovulate despite a normal annual vitellogenic cycle (Moll, 1979). I have no data to indicate which might be the case for *C. insculpta*. In either case, multi-year reproductive cycles presumably reflect an energy accumulation problem at some stage of the cycle (Bull and Shine, 1979). I suggest that the energy accumulation problem lies in the dry season diet of the population. During the dry season Daly River *C. insculpta* consume mainly aquatic vegetation (*Vallisneria nana*, Heaphy, 1990; Welsh, 1999), which is low in available energy content (Spencer et al., 1999; Tucker, 2000).

Bull and Shine (1979) reviewed animals exhibiting biennial reproduction, producing an adaptive hypothesis for why animals would skip opportunities to reproduce. They hypothesized that in these animals an energy-expensive behavior associated with reproduction exists, such that animals could increase lifetime reproductive success (LRS) by skipping years and putting the savings into future reproduction. Examples of these accessory behaviors were brooding, live bearing, and migration. Animals exhibiting this pattern were generally long-lived, and were food- or season-limited (Bull and Shine, 1979). While brooding and live-bearing are not features of turtles, *C. insculpta* could theoretically expend energy in migrations associated with nesting. However, the results of a concurrent movements study were equivocal. Most gravid *C. insculpta* nested within their home ranges, and did not occupy significantly larger home ranges than non-gravid females (Chapter 2). However, females moved farther between fixes while gravid, than while spent (Chapter 2). An ultimate test of this idea would require knowledge of energy expenditure associated with searching for a nesting beach and nest site (e.g., Congdon and Gatten, 1989).

Alternatively, biennial reproduction in the population may simply reflect phenotypic plasticity in clutch frequency. A negative energy balance caused by poor habitat quality or low available energy may have forced biennial reproduction (Congdon et al., 1987). Well-fed, captive green sea turtles produce eggs annually (Wood and Wood, 1980), compared to wild individuals that usually skip years (Mortimer and Carr, 1987; Limpus and Nicholls, 1988). However, such plasticity is not inconsistent with the savings hypothesis of Bull and Shine (1979).

<u>The wet season and reproduction: boom or bust</u>? - Numerous studies on lizards, mostly desert-adapted species, have shown that annual variation in reproductive output is associated with rainfall amounts which dictate food availability (reviewed in Ballinger, 1977; Judd and Ross, 1978; Vitt et al., 1982; Dunham, 1981; Trauth, 1983; Ferguson et al., 1990; James and Whitford, 1994; Smith et al., 1995; Abell, 1999). Less evidence exists for environmental control of reproductive output in turtles. In

the herbivorous turtles *Geochelone gigantea* and *Gopherus agassizzii*, reproductive output (clutch size) is strongly influenced by primary production, which is in turn influenced by rainfall (Swingland and Coe, 1978; Swingland and Lessells, 1979; Henen, 1997). Two other species have been shown to abort reproduction in dry years (Nieuwolt-Dacanay, 1997; Kuchling, 1999). Other studies suggest links between rainfall and reproductive output (Tucker, 2000) and still others report no annual variation in reproductive characteristics (Rowe, 1994). Nearly all of these studies were in temperate climates.

Is the wet season a favorable time for *C. insculpta* females? The wet season in the Top End is generally thought to be a plentiful time for many reptile species, based on seasonal studies of diet, activity, growth, and energetics of lizards and snakes (Madsen and Shine, 1996; Shine and Madsen, 1997; Christian and Green, 1994; Christian et al., 1995; Griffiths and Christian, 1996; Christian et al., 1999a; Christian et al., 1999b; but see Vitt, 1982; James and Shine, 1985). However, the opposite argument is reasonable for *C. insculpta*. In the dry season *C. insculpta* prefer to eat aquatic vegetation (*Vallisneria nana*), which although patchy and relatively nutrient-poor (Heaphy, 1990; Tucker, 2000), is in abundance in the Daly. During the wet season the river is often in continuous flood, precluding the turtles from eating that vegetation. Radiotelemetry during a fly-over in the wet season indicated that turtles were not in the river channel where the *Vallisneria* beds are located, but were in flooded riparian forest (Chapter 2). Further, river levels fluctuate, and the strictly aquatic nature of *C. insculpta* means that turtles would be constrained in many cases to follow those fluctuations.

Alternatively, turtles may experience increased food uptake during the wet season, taking advantage of the availability of food such as fruits. Populations of *C.*

insculpta in the Alligator River system have been shown to have catholic diets (reviewed in Georges and Rose, 1993). Six juvenile *C. insculpta* in the Daly River grew significantly faster during the wet season than did seven juveniles during the dry season (Heaphy, 1990).

Are bigger floods better than smaller floods? Annual variation in the magnitude of the wet season can have considerable impacts on reproduction. For example, the strength of the Southern Oscillation, a correlate of wet season magnitude in the Australasian region where sea turtles feed, predicted the number of nesting sea turtles (*Chelonia mydas*) in the Great Barrier Reef, Australia (Limpus and Nicholls, 1988). The present study indicates that the magnitude of the wet season influences the female reproductive biology of *C. insculpta* in the Daly River in several ways. The study spanned one ‘small’ wet season and two very ‘big’ wet seasons, and I analyzed data from 1986, which followed a small wet season (Fig. 4.4). Several reproductive patterns aligned themselves according to this difference in wet season magnitude, including the onset of nesting (unpubl. data), egg size, clutch mass, a seasonal decrease in clutch size/increase in egg size, the tradeoff between clutch and egg size (not standardized for body size), and the influence of body size on clutch size (Table 4.2; Table 4.3; Fig. 4.4). Clutch size and egg size were smaller after small wet seasons, and many studies have shown that these attributes are influenced by food uptake in reptiles and other animals (e.g., Ford and Seigel, 1989; Drent and Daan, 1980; Rohr, 1997). In addition, nesting began 4-5 weeks earlier after big wet seasons compared to small wet seasons (unpubl. data). Direct evidence of food uptake influencing the laying date is lacking for reptiles, but body condition influenced lay date in the sand lizard (Olsson and Shine, 1997b). In birds, lay date advances in years of high food availability, findings confirmed by food supplementation experiments (see reviews in Drent and Daan, 1980; Rowe et al., 1994). A concurrent study found that late nesting in *C. insculpta* can be disadvantageous in two ways. First, late nesting in 1996 resulted in 20 % nest mortality due to early wet season flooding (unpubl. data). Second, late nesting led to a female-biased hatchling sex ratio, compared to sex ratios near unity following big wet seasons in 1997 and 1998 (unpubl. data). Based on these findings, and current life history theory, I hypothesize that a big wet season has a positive impact on *C. insculpta* reproduction, relative to a small wet season.

Because *C. insculpta* appear to need two years to accumulate enough energy to produce eggs, the magnitude of the *two* wet seasons prior to nesting may be important, rather than just the preceding wet season. Examining the two previous wet seasons gives the same result – after big wet seasons turtles produce larger eggs earlier in the year, exhibit tradeoffs between clutch and egg size, show seasonal changes in clutch and egg size, and generate female-biased hatchling sex ratios.

Although collectively our data indicate that a big wet season is advantageous for turtle reproduction, direct evidence is lacking, and a study of the wet season diet and/or field energetics of *C. insculpta* would be required to test our supposition. Not all of our findings support the idea of ‘bigger is better’ in terms of the influence of wet season magnitude on turtle reproduction. In the lizard *Sceloporus woodi* seasonal shifts in, and tradeoffs between, egg and clutch size were only evident in a drought year (DeMarco, 1989), a finding opposite to ours. Variation in energy accumulation among females can obscure clutch size-egg size tradeoffs (van Noordwijk and de Jong, 1986; Bernardo, 1996). I suggest that annual variation in energy uptake can have a similar obscuring effect. However, the specific impact of this effect based on previous studies is equivocal. This may be owed to the complexity of ‘capital’ vs.

‘income’ breeding (Bonnet et al., 2001), and/or to a diversity of functional responses (i.e., energy allocation) in reproductive characteristics to environmental variation among different taxa. Finally, some of the trends I have outlined may be spurious due to sample size; our data spanned four years only.

Seasonal changes in clutch size and egg size. - An unusual finding of the present study was that later (second) clutches contained fewer but larger eggs than earlier (first) clutches of each female (Table 4.2), despite no difference in clutch mass between the two clutches. This pattern is not previously known in turtles (but see seasonal decrease in clutch size, reviewed in Tucker and Frazer, 1994). Seasonal changes in clutch and egg size occurs in some lizards (reviewed in Nussbaum, 1981) and have been reported for other animals (e.g., Wolda and Kreulen, 1973). Nussbaum (1981) discussed theoretical underpinnings of this pattern, in which he critiqued an existing hypothesis (parental investment model) and proposed another (bet-hedging model).

The parental investment model predicts that females are selected to increase egg size, at the cost of clutch size, late in the season in order to produce larger and competitively superior hatchlings at a time when food for hatchlings is low and juvenile density is high (Ferguson et al., 1982; Ferguson et al., 1990; see also review of similar models in Nussbaum, 1981). The key assumption of this optimal offspring model is a late season reduction in offspring fitness (Landa, 1992), related to food availability. No data exist on diet or food availability in hatchling *C. insculpta*. However, juveniles are reported to have a catholic diet (Welsh, 1999), including ribbonweed (*Vallisneria nana*), which is abundant relative to body size of juveniles (pers. obs.). There is little change in late season environment compared to early season at the Daly River (mean inter-nesting interval = 6 weeks); dry season conditions persist throughout this period, and river levels are stable (Chapter 6). Lastly, the difference in timing between early and late clutches is reduced by both a seasonal increase in air temperatures (increasing developmental rate and reducing incubation period), and by embryonic aestivation in the egg (Chapter 6). Thus, there is no indication that the observed decline in clutch size in *C. insculpta* supports the parental investment hypothesis.

The bet-hedging model claims that the amount of food available to females for the production of late season clutches is unpredictable, and that selection has favored conservatively small clutches in the late season to insure that each egg is minimally provisioned (Nussbaum, 1981). Nussbaum argued that when faced with an optimal clutch size that is fractional, lizards are likely to opt for the smaller integral clutch size and adjust their investment by increasing egg size. Nussbaum outlined five predictions of this model: (1) both small and large clutches will have variable egg sizes among years, depending on annual variation in resource levels, (2) within years, small clutches will have larger eggs than large clutches because unanticipated resources are divided among fewer eggs (Smith and Fretwell, 1974), (3) the largest mean difference among egg sizes should occur between eggs of very small and very large clutches sampled at the same time and place, (4) large-egged clutches will seldom consist of sufficient surplus yolk, compared to clutches of equivalent size with average-sized eggs, to provision an additional egg of minimal size, and (5) this model would most likely apply to species that cannot afford to miss an opportunity for to reproduce in the late season, i.e., those with very high adult mortality, even in the absence of reproduction.

Prediction (1) is upheld in *C. insculpta*, with egg sizes of small and large clutches varying among and within years (Fig. 3). Presumably, these differences are at least partially due to resource uptake. Predictions (2) and (3), involving a tradeoff between clutch and egg size, are evident in two of three years (Table 4.2). Prediction (4) could not be examined in the present study, as yolk content was not determined. Prediction (5), that the model would most likely apply to species with high adult mortality that cannot afford to skip opportunities for reproduction, is not consistent with *C. insculpta*. Although adult mortality data are unavailable for *C. insculpta*, turtles are generally characterized by high adult survival (Gibbons, 1987; Iverson, 1991b; Shine and Iverson, 1995). The present study has demonstrated that *C. insculpta* in the Daly River do skip opportunities for reproduction.

Nussbaum (1981) noted that the bet-hedging model would be falsified if a late-season increase in egg size was, on average, large enough to account for one or more additional eggs of the smaller size, thereby providing evidence that clutch size was sacrificed for egg size. For an average late season clutch of *C. insculpta*, the percent increase in egg size would have to exceed the critical value of 10 % (clutch size of 10). In 1997 and 1998 the mean percentage late-season increase in egg size was 9.5 % and 9.7 %, respectively. Thus, according to Nussbaum (1981), there is evidence that clutch size is sacrificed for egg size in *C. insculpta*. Therefore, I cannot reject the bet-hedging model. However, some of the model’s predictions are not consistent with our data for *C. insculpta*.

In summary, the present study suggests that (1) considerable phenotypic plasticity exists in reproductive traits of *C. insculpta*, manifested in clutch characteristics influenced by annual variation in the magnitude of the wet season, and that (2) the species exhibits biennial reproduction, which may be a result of the low available energy content in the dry season diet. A study of the field energetics in both wet and dry seasons, coupled with information on the wet season diet, would provide confirmation of the latter of these hypotheses. Finally, investigating why the turtles nest twice every second year rather than once each year needs attention.

CHAPTER 5: BEACH SELECTION

Beach Selection in Nesting Pig-nosed Turtles, *Carettochelys insculpta*

(submitted to Journal of Herpetology)

J. Sean Doody, Peter West, and Arthur Georges

INTRODUCTION

In oviparous animals, the choice of nest site can have a profound influence on embryonic development and survival by moderating the incubation environment (reviewed in Packard and Packard, 1988; Janzen, 1994; Wilson, 1998). Surviving offspring are also affected via the influence of incubation environment on phenotypes (Allsteadt and Lang, 1994; Shine and Harlow, 1996). In reptiles with temperature-dependent sex determination (TSD), one such phenotype is sex (Bull, 1980; Ewert and Nelson, 1991). The scope exists for TSD mothers to influence hatchling sex through the maternal trait of nest site choice (Janzen, 1994; Roosenberg, 1996; but see Schwartzkopf and Brooks, 1987).

The pig-nosed turtle, *Carettochelys insculpta*, is a beach-nesting turtle that inhabits rivers and billabongs in northern Australia and New Guinea (Georges and Rose, 1992). The species has TSD (Webb et al., 1986). In theory, the manipulation of hatchling sex through nest site choice could be accomplished on two different spatial scales: by choosing a beach with a particular thermal profile (Vogt and Bull, 1984; Roosenberg, 1996), or by choosing among sites within a beach, each differing in thermal characteristics (Janzen, 1994).

In this paper, I report on the broad scale option of choosing among beaches I address the following questions: (1) What variation in thermal environment exists among beaches? (2) Do mothers select beaches with a particular thermal profile? (3) What physical factors (e.g., aspect, solar exposure) determine beach temperatures? (4) Do mothers select beaches randomly with respect to those factors? (5) Does a mother select a beach with a particular moisture content? I also examined beach attributes such as height above water and the presence of vegetation to better understand nest site choice in the species.

MATERIALS AND METHODS

I characterized potential nesting beaches and surveyed for nests during 2-day boat trips during the dry season (July to October). There were eleven trips in 1997 and nine trips in 1998. My criteria for potential nesting beaches were based on prior knowledge of the species (Georges, 1992; Georges and Kennett, 1993; Doody and Georges, unpubl. data). These criteria were: sandy banks and beaches adjacent to the water, little or no vegetative cover, and a minimum height of 0.25 m above water. Each beach was mapped, and I measured aspect, slope, and solar exposure for each. I also recorded the presence of any vegetation both on the beach and in the water at the beach edge. To investigate whether turtles might prefer nesting near deeper water, I measured the water depth 2 m from the beach. Finally, for each beach I estimated the maximum height in which a nest chamber could be constructed, based on the friability of the sand. This was accomplished by attempting to construct a nest chamber by hand at 15 cm depth at the highest point on the beach. If I could not make a chamber (the sand fell in on itself due to low moisture content) I moved progressively lower and repeated the procedure until I was able to construct a chamber. I then measured the height above water of this ‘cohesive sand line’(hereafter CSL).

I used a compass to measure aspect, and a clinometer to measure slope and solar exposure. I define solar exposure as the total angle of exposure received by each beach, as dictated by treelines in the directions of sunrise and sunset. Aspect was coded symmetrically about due S to facilitate statistical analyses.

To estimate the relative thermal environment of each beach I took spot sand temperatures one meter above water at 50 cm depth. Although *C. insculpta* nests at depths of 10-25 cm, at 50 cm there is little diel variation in sand temperature (unpubl. data), allowing rapid assessment of thermal profiles of beaches without time of day confounding the data. This allowed me to gather large amounts of data over tens of kilometres in a day. I measured beach temperatures twice in 1997 and 5 times in 1998. To determine the relative range of sand temperatures on beaches I also placed minimum-maximum thermometers one meter above water at 16 cm depth on 33 beaches. These thermometers measured temperatures from 5-14 September 1998.

To estimate sand moisture for each beach I collected ca. 500-700 g of substrate in plastic containers with lids. Substrate samples were weighed, oven-dried at 105 °C for 48 hours, and re-weighed as dry samples.

I located nests by noting tracks in the sand, and by searching each beach using a probe made of spring steel (Blake, 1974). I counted crawls and attempted nest excavations (conical pits) on each beach. Upon discovery each suspected nest was excavated for confirmation. At the end of the nesting season, beaches were classified as: those with nests, those with crawls but no nests, and those without crawls or nests. Although I undoubtedly missed a few nests, I was confident in placing beaches into these categories, because crawls remain visible for longer than the survey interval (rainfall is rare during the nesting season). I avoided double-counting crawls by raking beaches each after each visit. I avoided double-counting nests by marking each nest site with a wooden stake, or by removing eggs (for concurrent experiments).

All analyses were single factor ANOVA or linear regression with a significance level of 0.05. Where necessary data were transformed prior to analyses.

RESULTS

Number of beaches and nests. - A total of 117 beaches in 1997 and 54 beaches in 1998 were designated as potential nesting beaches along the same 63 km stretch. I found 90 nests on 40 beaches (34 %) in 1997 and 131 nests on 42 beaches (56 %) in 1998. Temporal variation in timing of nesting is indicated in Figure 5.1. Turtles began nesting mid-July in 1998 and late July in 1997 (Fig. 5.1). In both years nesting ended in late September (Fig. 5.1).

Beach selection. - Beaches with nests were similar to beaches without nests with respect to temperature, height, aspect, and water depth at approach (Table 1). However, beaches with nests had higher moisture content and a higher CSL than beaches without nests (Table 1). Percent substrate moisture (arcsine transformed) was significantly positively related to minimum beach temperature (F = 1.33, df = 1,23, p = 0.261, r^2 = 0.057) but was not related to maximum beach temperature (F = 1.04, df = 1, 23, p = 0.319, r^2 = 0.045).

Although not quantified, beaches with submergent vegetation dominating the edge were not used by nesting turtles. This was corroborated by observations of the

Table 5.1. Comparison of physical attributes between beaches with *C. insculpta* nests and beaches without nests. FSL = friable sand line. Beach temperatures were spot temperatures taken at 50 cm depth. Data are means ± 1 SD. Sample sizes are in parentheses. Significance is based on single-factor ANOVA. * denotes significant at 0.05, ** = 0.01.

attribute	beaches with nests	beaches without nests	significance
temperature (°C)			
1997	29.4 ± 2.09 (35)	28.9 ± 2.50 (72)	$F_{1,106} = 1.35$, p = 0.248
1998	28.1 ± 1.77 (26)	28.6 ± 2.18 (8)	$F_{1,33} = 0.41$, p = 0.525
substrate moisture (%)			
1998	6.1 ± 4.51 (21)	3.0 ± 3.39 (13)	$F_{1,33} = 4.51$, p = 0.042*
height (m)			
1997	2.37 ± 1.466 (40)	2.02 ± 1.214 (73)	$F_{1,112} = 1.88$, p = 0.173
aspect (coded)			
1997	20.0 ± 10.85 40)	19.0 ± 10.91 (40)	$F_{1,79} = 0.20$, p = 0.660
CSL height (cm)			
1997	89.2 ± 63.44 (38)	60.6 ± 36.75 (75)	$F_{1,112} = 9.19$, p = 0.003**
water depth at approach (m)			
1997	0.98 ± 0.424 (36)	0.98 ± 0.488 (67)	$F_{1,102} = 0.00$, p = 0.988

Table 5.2. Comparison of physical attributes between beaches containing *C. insculpta* nests and beaches containing only crawls. FSL = friable sand line. Data are means ± 1 SD. Sample sizes are in parentheses. Significance is based on single-factor ANOVA. ** denotes significance at 0.001.

attribute	beaches w/nests	beaches w/crawls only	significance
temperature (°C)			
1997	29.4 ± 1.86 (37)	29.6 ± 2.36 (32)	$F_{1,68} = 0.21$, p = 0.650
height (m)			
1997	2.39 ± 1.480 (38)	2.08 ± 1.328 (37)	$F_{1,74} = 0.88$, p = 0.350
aspect (coded)			
1997	20.9 ± 11.28 (34)	20.0 ± 10.90 (40)	$F_{1,73} = 0.11$, p = 0.741
total angle of solar exposure (°)			
1997	131 ± 20.9 (38)	129 ± 23.3 (33)	$F_{1,70} = 0.30$, p = 0.587
CSL height (cm)			
1997	132.2 ± 114.47 (40)	66.6 ± 40.76 (35)	$F_{1,74} = 10.34$, p = 0.002**

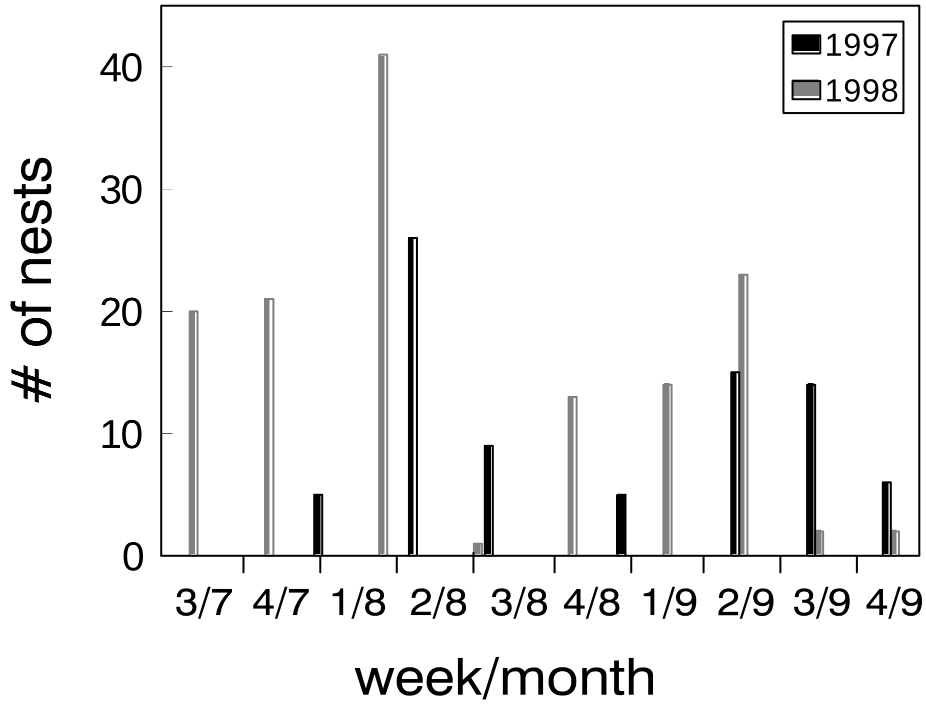


Fig. 5.1. Temporal variation in number of *C. insculpta* nests found during 63 km trips along the Daly River in 1997 and 1998. Note the bimodal distributions.

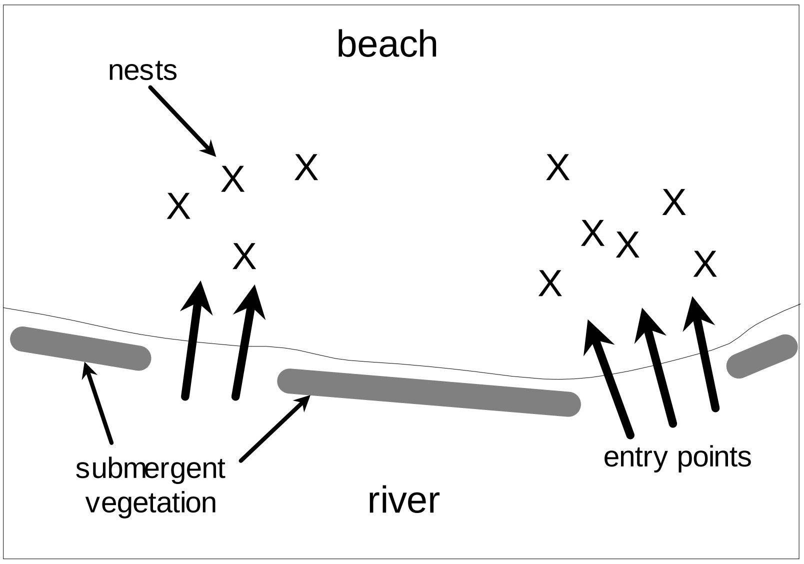


Fig. 5.2. Diagram of how nesting turtles avoided exiting the water in places with submergent aquatic vegetation. These observations corroborate those of nesting turtles avoiding beaches where the entire edge is dominated by submergent vegetation.

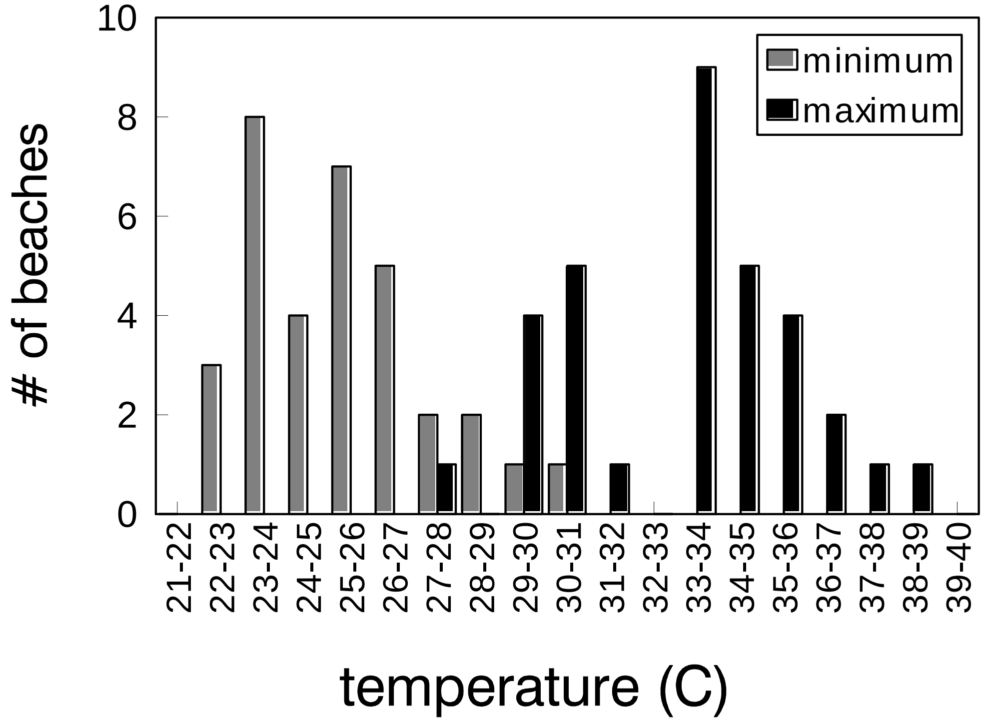


Fig. 5.3. Maximum and minimum substrate temperatures from 33 *C. insculpta* nesting beaches in 1998. Data are from Minimum-maximum thermometers buried at nest depth (16 cm) one meter above water.

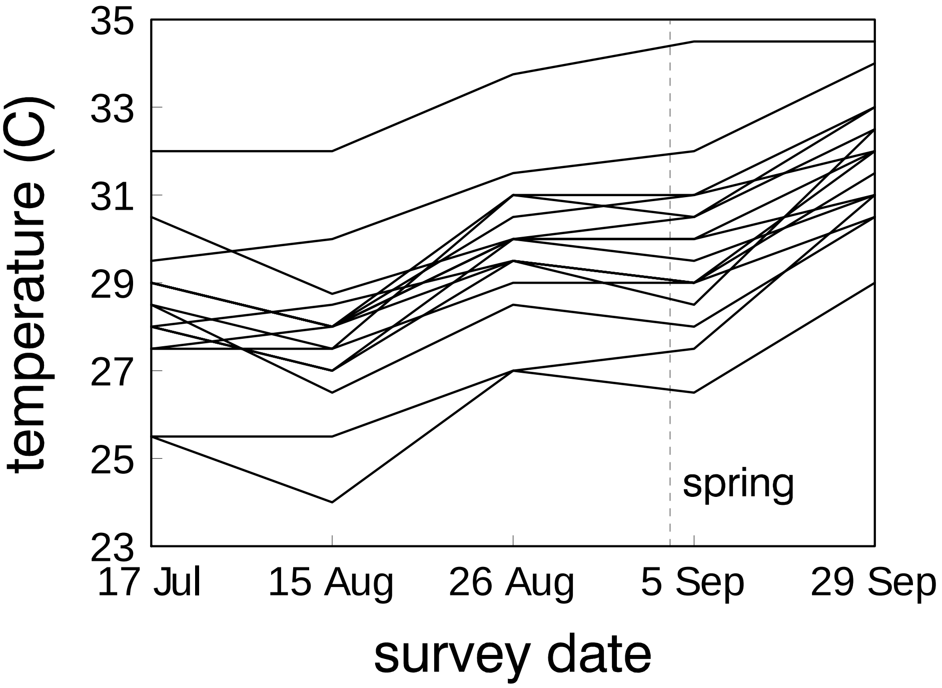


Fig. 5.4. Seasonal increase in *C. insculpta* nesting beach temperatures with the onset of spring. Samples were spot temperatures taken 1 m above water, at 50 cm depth in the substrate.

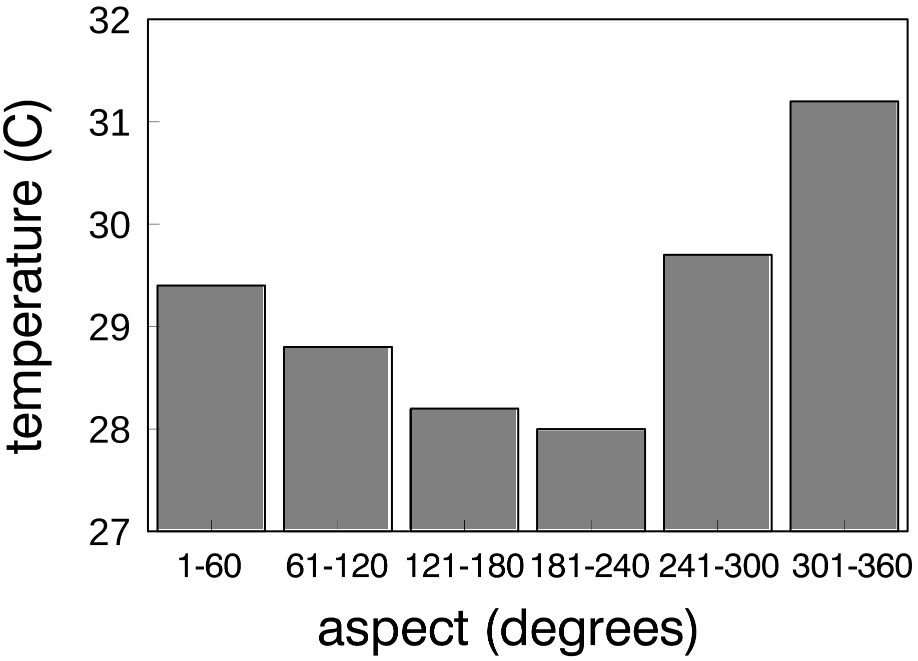


Fig. 5.5. Influence of aspect, or direction of the slope of the beach, on beach temperature. Temperatures were taken 1 m above water at 50 cm depth.

lack of turtle crawls in areas with such vegetation within a nesting beach (Fig. 5.2). It was also evident that beaches < 0.25 cm above water were not used by nesting turtles.

I found 35 (30 %) beaches with only crawls in 1997, and 8 (14 %) with only crawls in 1998. Beaches with nests had a higher CSL than beaches with only crawls (Table 5.2). Other comparisons between beaches with nests and beaches with only crawls revealed no significant differences in: beach temperature at 50 cm, maximum height, aspect, or solar exposure (Table 5.2).

Beaches selected by nesting turtles comprised four basic types: trapped sand around logs (22 = 47 %), sandy banks (13 = 28 %), large sandbars along river bends (8 = 17 %), and rocky areas with trapped sand (4 = 8 %). These are formed during wet season flooding, and most are ephemeral among years (unpubl. data).

<u>Beach temperatures and their determinants</u>. - Maximum (8 = 33.4 ± 2.63 SD °C) and minimum (8 = 25.6 ± 2.04 SD °C) beach temperatures were obtained for 33 beaches in 1998 (Fig. 5.3). Maximum and minimum temperatures were not related to solar exposure (Max. F = 0.71, df = 1,29, p = 0.407, r^2 = 0.025; Min. F = 0.01, df = 1,29, p = 0.937, r^2 = 0.000). Both maximum and minimum temperatures were generally positively related to temperatures taken at 50 cm depth (Max. F = 4.13, df = 1,32, p = 0.051, r^2 = 0.118; Min. F = 6.77, df = 1,32, p = 0.014, r^2 = 0.179).

Beach temperatures taken at 50 cm depth showed a marked seasonal increase with the onset of spring (Fig. 5.4; F = 32.21, df = 4, 144, p < 0.001). Aspect, arbitrarily divided into 60° intervals, significantly influenced 50 cm beach temperature (F = 3.66, df = 5, 108, p = 0.004), with north-facing beaches exhibiting the warmest temperatures (Fig. 5.5). Solar exposure significantly positively influenced 50 cm beach temperatures in 1997 (F = 8.35, df = 1, 108, p = 0.005, r^2 =

0.072) but not in 1998, though the result approached significance (F = 3.58, df = 1,28, p = 0.069, $r^2 = 0.117$). Aspect did not influence solar exposure (F = 1.60, df = 1,112, p = 0.209, $r^2 = 0.014$).

DISCUSSION

Beach selection. - Generally, beaches utilized by nesting *C. insculpta* in the present study agreed closely with previous observations (Georges, 1992; Georges and Rose, 1993). Turtles nested on beaches and banks largely free of vegetation. However, nesting was not restricted to clean fine sand, as previously reported. Although most nest sites were predominately sandy, turtles nested in a variety of substrate types ranging from gravel to loamy sand. Turtles avoided nesting on the lowest elevation beaches - the lowest maximum height of a nesting beach in the study was 0.47 m. However, turtles often crawled on these beaches. This reluctance to nest is consistent with flood mortality quantified for lower nests for the species (Chapter 7; Doody and Georges, unpubl. data) and for other riverine turtle species (Doody, 1995; Plummer, 1976; Roze, 1964). Turtles also avoided nesting on beaches that were dominated by submergent vegetation (e.g., *Vallisneria*) along the beach edge (Fig. 5.2). In general, turtles did not crawl onto the beach when the submerged edge was not sandy (Fig. 5.2). This is supported by observations of turtles ‘sniffing’ the sand prior to crawling on the beach to nest at night (JSD, pers. obs.). It would appear that *C. insculpta* are not very visually-oriented, and so use underwater cues to choose a potential nesting beach. This is in contrast to nest site choice in the more visually-oriented freshwater crocodile (*Crocodylus johnstoni*) at the site, which locates sandy areas some distance from the water’s edge and disconnected from it (pers. obs.).

How did turtles choose a nesting beach? Our results indicated that turtles chose beaches randomly with respect to aspect, height (but see above), temperature, and water depth at approach (Table 5.1). However, two related attributes I quantified differed between beaches with nests and other beach types. Beaches with nests had a greater substrate moisture content and corresponding higher CSL than the other beach types (Fig. 5.1; Fig. 5.2). Apparently, turtles could not excavate a nest chamber above the CSL due to loose substrate consistency causing sand to fall in on itself. For example, in 1998 I found 20 beaches without nests that had numerous crawls and attempted nest constructions (conical pits in sand with looses consistency). Similarly, in 1997 I found 59 crawls and 30 such pits on one beach late in the nesting season. Turtles could only nest at low elevations below the CSL on beaches with low substrate moisture. Turtles apparently avoided nesting on these beaches due to the higher probability of nest flooding (Chapter 7; Doody and Georges, unpubl. data). In riverine turtles like *C. insculpta* height of the nest site may be the primary determinant of reproductive success due to flooding (Roze, 1964; Plummer, 1976; Doody, 1995). Countering this in *C. insculpta* is the constraint of cohesive sand, given that the species does not exhibit body-pitting like other beach-nesting reptile species (e.g., the crocodile *C. johnstoni*, sea turtles, the freshwater turtle *Podocnemis expansa*).

Although substrate moisture was higher on beaches with nests than on beaches without nests, further data are needed to determine whether substrate moisture was inherently important to nesting turtles, over and above the constraint imposed by cohesive sand. Comparisons of other attributes between beaches with nests and beaches with only crawls revealed that turtles were not selecting beaches according to those attributes once they exited the water (Table 5.2).

Determinants of beach temperatures. - Relative beach temperatures, as estimated with spot samples at 50 cm depth, increased with season (Fig. 5.4). This increase was associated with an increase in air temperatures with the onset of spring (Chapters 6 & 7). This temporal effect influences timing of nesting, embryonic survival, and hatchling sex, because *C. insculpta* have an extended nesting period (Fig. 5.1; Chapters 4 & 7). The primary spatial determinant of beach temperature measured in the present study was aspect, or direction of the slope of the beach. North-facing beaches exhibited the hottest temperatures (Fig. 5.5). In general, the total angle of solar exposure, measured between shading treelines at sunrise and sunset directions, positively influenced beach temperatures. These findings are similar to those of Janzen (1994), who found that vegetational cover, as influenced by aspect and solar exposure, predicted hatchling sex ratio in painted turtles (*Chrysemys picta*). Similarly, aspect of nest sites influenced incubation period in the turtle *Malaclemys terrapin* (Burger, 1976b).

Implications for manipulating offspring sex. - In the present study beach temperatures varied in a predictable manner, driven by aspect and solar exposure. A concurrent movements study revealed that gravid *C. insculpta* occupied linear home ranges averaging 8.6 km in length (Chapter 2). Given the above, the meandering path of the river, and the density of potential nesting beaches per river km (0.86-1.86), turtles would generally have the opportunity to select beaches that were hotter or cooler. However, turtles did not take advantage of this opportunity. In particular, turtles nested on beaches with temperatures covering the full range of what was available, provided that the beach was relatively free of shading vegetation. Thus, although I did not determine offspring sex, if *C. insculpta* mothers were manipulating offspring sex through nest site choice they were not doing so on an among-beach scale, as suggested for the turtle *Malaclemys terrapin* (Roosenberg, 1996). It is possible that *C. insculpta* mothers are manipulating or predicting sex on a finer scale, by nesting in spots with a particular thermal profile, *within* beaches (Janzen, 1994). Field studies linking physical attributes, temperatures (both at nesting and those during the sex-determining period), and offspring sex are needed to ultimately determine whether or not turtles (or indeed, other reptiles) can manipulate offspring sex (Janzen, 1994; Weisrock and Janzen, 1999).

CHAPTER 6: EGGS AND HATCHLINGS

Embryonic Aestivation and Emergence Behavior in the Pig-nosed Turtle, *Carettochelys insculpta*

(Canadian Journal of Zoology 79:1062-1072)

J. Sean Doody, Arthur Georges, Jeanne E. Young, Matthew D. Pauza,
Ashe L. Pepper, Rachael L. Alderman, and Michael A. Welsh

INTRODUCTION

Emergence from the nest can be a critical life history stage for hatchling turtles (Kuchling, 1999). For example, during emergence and in their brief crawl to the water, sea turtle hatchlings can incur high mortality (e.g., Hendrickson, 1958; Diamond, 1976; Pritchard and Trebbau, 1984). Thus, studies on the behavior of emergence are needed because of the potential for emergence success to shape both individual emergence behavior and population age structure.

On a diel scale, hatchlings of several species of turtle emerge primarily at night (e.g., Anderson, 1958; Witherington et al., 1990; Gyuris, 1993). Nocturnal emergence in turtles is said to be adaptive, reducing the probabilities of heat stress, desiccation, and predation by visually-oriented predators (Hendrickson, 1958; Bustard, 1967; Stancyk, 1982). Support for the heat stress mechanism comes from observations of scorched hatchlings that emerged during the day (Carr and Ogren, 1959; Hughes and Richard, 1974; Diamond, 1976), while the predation mechanism has received little support (Witherington and Salmon, 1992; Gyuris, 1994). At a minimum, emergence would be detrimental during much of the day for species that nest in areas free of vegetation cover, because substrate temperatures can exceed 60°C in some areas (e.g., Georges, 1992).

Thermal cues have been proposed as determinants of nocturnal emergence in sea turtles. Earlier work suggested that a threshold in absolute temperature triggered nocturnal emergence (Hendrickson, 1958; Bustard, 1967; Mrosovsky, 1968), while more recent studies have implicated a change in temperature (Hays et al., 1992; Gyuris, 1993).

The pig-nosed turtle (*Carettochelys insculpta*) is a monotypic species found in New Guinea and in the wet–dry tropics of northern Australia (Georges and Rose, 1993). In Australia, *C. insculpta* nests in open sandy riparian areas from mid-July to late October (dry season), and hatches from early October to early December (late dry to early wet season) (Georges and Rose, 1993; Georges et al., in press). Although hatching has been studied in the laboratory (Webb et al., 1986), nothing is known about emergence behavior in this species, and thus in the family Carettochelydidae.

On a seasonal scale, Webb et al. (1986) hypothesized that *C. insculpta* exhibits delayed hatching in the field after finding delayed hatching and hatching in response to anoxia in the laboratory. They suggested that such delays would allow hatchlings to synchronize timing of emergence with the more favorable conditions of the wet season.

I investigated the emergence behavior of *C. insculpta* during the years 1996–1998. I used emergence phenology data, nest temperatures, historical weather data, and a developmental model to test or examine the following three hypotheses associated with emergence:

(1) embryonic aestivation (delayed hatching) occurs in *C. insculpta* in nature;

(2) embryonic aestivation in *C. insculpta* results in synchronization between hatching/emergence and the onset of the wet season;

(3) the cue *C. insculpta* hatchlings use to emerge nocturnally is an absolute nest temperature threshold, or alternatively is a change in nest temperature. I generated predictions for the two models and tested those predictions, with the ultimate goal of identifying a general thermal cue for nocturnally-emerging turtles.

I also examined other behavioral aspects of emergence in *C. insculpta*, asking:

(1) Do hatchlings emerge in response to rainfall? (2) Do sibling hatchlings emerge simultaneously, in small groups, or singly? (3) Is emergence synchronized among nests within a nesting area? These questions have been difficult to answer for turtles because of logistical problems in observing emergence (Ehrenfeld, 1979; Christens, 1990). However, a novel remote data-collection technique allowed us to gather large amounts of emergence data with relative ease. I also review emergence data for other turtle species to elucidate for comparison with our results, and to elucidate any existing patterns among species.

MATERIALS AND METHODS

I studied *Carettochelys insculpta* along a 30 km stretch of the Daly River in the Northern Territory, Australia. The study stretch centered on Oolloo Crossing (14°04'40"S, 131°15'00"E). The climate is typical of the wet–dry tropics of northern Australia (Taylor and Tulloch, 1985) with a mean monthly rainfall of less than 7 mm from May to September, rising to a peak monthly average of 284 mm in February (Stn 014139/014941, Oolloo 1962–1985). Mean monthly maximum air temperatures range from 30.9°C in June to 36.8°C in October. Most data were collected in 1998, but some data (e.g., timing of nesting, observations on flooding) were collected in 1996 and 1997.

A standard station for monitoring sand, water, and air temperatures was set up on a small nesting bank used by *C. insculpta* in May of each of three years (1996–1998). Temperatures were monitored at 15 min intervals at the sand surface and at 10 cm depth intervals to a depth of 50 cm. Water and air temperatures were taken in the shade. Temperatures were recorded with four-wire RTD probes fitted to a datalogger (Datataker Model DT500) calibrated against a thermometer certified as accurate by the National Authority of Testing Agencies. Rainfall gauges were placed at each nesting beach and checked daily. River rises were recorded from mid-October to mid-December of 1996–1998.

I inspected nesting areas daily for turtle tracks throughout the nesting season. Nests were located by probing the sand with a 2 mm diameter spring steel rod (after Blake, 1974). Temperatures in 44 nests were monitored with either Datataker® DT500 multi-channel dataloggers (N=37) or Stowaway® single-channel dataloggers (N=7). Temperatures were recorded at 15 min intervals by the Datataker dataloggers and at 1 h intervals using the Stowaway dataloggers. Typically, three temperature probes were fitted to each nest: one immediately below the deepest egg, one in the core of the nest, and one immediately above the shallowest egg. When Stowaway dataloggers were used, often only core temperatures were recorded. The probes were fitted as soon as possible after discovery of the nest, usually within 1–3 days. The depth of each egg was measured before its removal and eggs were returned to their original positions and orientations after deployment of datalogger probes.

Nests were subsequently inspected each day throughout the period when hatching and emergence were considered likely (October–December). Emerging hatchlings leave a distinctive hole and tracks in the sand. After checking each nest, I cleared the sand surface and sprayed it with non-toxic paint to avoid double counting. For 17 nests, emergence dates and times were recorded by Trailmaster® infra-red camera systems (Doody and Georges, 2000). Each system consisted of a transmitter box, a programmable receiver box with LED readout, and an automatic camera (Olympus®). Boxes were placed on either side (and just to the river side) of each nest, and the camera was attached to a metal stake (1.7 m long), which was driven into the sand. Emerging hatchlings were photographed as they crossed the beam, and both the receiver box and the photographs displayed the date and time of each emergence event. I also determined emergence dates for 46 nests without camera systems by monitoring nests daily throughout emergence. The sand was smoothed out just over the nest after each emergence to discriminate between emergence events. Incubation period is defined here as the number of days elapsed between nesting date and emergence date. For 10 nests the actual emergence date was not known and the date was estimated as the median within a known range of possible dates (Table 1). Emergence temperatures were determined by inspecting data logger traces for temperatures corresponding to dates and times recorded by the camera systems.

Because *C. insculpta* is known to exhibit delayed hatching after completion of embryogenesis (Webb et al., 1986), it is difficult to determine the endpoint of embryonic development in natural nests without being invasive. I calculated this parameter from temperature traces using a method of summation (deCandolle, 1855; Reibish, 1902; Georges, unpubl. data). Gaps in the temperature traces, typically only the first few days between finding the nest and fitting the probes to it, were filled by cross regression with traces from other nests on the same beach or with traces from the standard monitoring station. The relationship between incubation temperature and developmental rate (Beggs et al., 2000; Georges et al., unpubl. data) was integrated along each temperature trace to estimate when embryo head width attained its maximum. A period of some days, obtained by correcting for average terminal incubation period, was added to account for maturation period (at 30°C it is 10 days from attainment of maximum size to yolk internalization) (Georges et al., unpubl. data). Thus for each nest, I obtained a date at which emergence could occur, and a date at which emergence *did* occur. To confirm that *C. insculpta* was exhibiting delayed hatching rather than hatching and delayed emergence, I carefully excavated to the top eggs of each nest, up to three times during the period between predicted earliest hatching and observed hatching.

RESULTS

Embryonic aestivation, emergence, and the onset of the wet season

Embryonic aestivation. - Table 6.1 lists the predicted date of earliest emergence and shortest incubation period, and the observed emergence date and incubation period. Data were available for 37 nests. Observed incubation period ($\bar{X}$ = 86 days) was significantly greater ($F_{1,70} = 48.74$, $p < 0.001$) than shortest possible incubation period ($\bar{X}$ = 69 days). Observed incubation period ($r^2 = 0.71$, $F_{1,34} = 84.52$, $p < 0.001$) and shortest possible incubation period ($r^2 = 0.48$, $F_{1,34} = 31.65$, $p < 0.001$) decreased with emergence date (Fig. 6.1). Inspection of the top eggs of each nest at various times after the predicted hatching date confirmed that turtles were exhibiting delayed hatching rather than hatching and delayed emergence.

Timing of emergence and rainfall. - Hatchlings emerged from 16 October to 26 November (N = 63 nests). Hatchlings emerged at a greater frequency on nights after rainfall in the previous 24 h (0.92) than expected (0.60) on nights when no rainfall occurred ($X^2 = 9.14$, df = 1, $p = 0.003$, $N = 63$). In three of 17 nests, it appears that emergence occurred *during* rainfall because the sand was visibly wet in emergence event photographs. In one nest, hatchlings emerged as the river rose and flooded the nest chamber (Fig. 6.2). Rainfall in 1998 appeared to be typical in frequency and magnitude (NT Water Resources 1999).

Timing of emergence and the onset of the wet season. - The mean onset of the wet season, as indexed by date of first river rise (> 0.3 m) each year during 1960–1996, was 17 November (range = 25 October – 30 November; Fig. 6.3). These rises were associated with a decrease in water clarity that persisted throughout the wet season. Using 1998 emergence data, and extrapolating timing of emergence data from timing of nesting data for 1996–1997, the mean first and last emergence dates were 30 October and 10 December, respectively (Fig. 6.3).

Emergence behavior and the cue for nocturnal emergence

Emergence times. - Sixty-seven *C. insculpta* hatchlings from 17 nests on seven beaches were photographed as they emerged from the nest (Fig. 6.2). On average, 4.1 ± 1.91 SD (N = 17; range 2–8) hatchlings emerged from each nest. Emergence occurred at night with the exception of two hatchlings that emerged from one nest at approx. 1800 hours. Actual times of emergence ($\bar{X}$ = 2348 hours ± 147.4 min SD; N = 67; range 1756–0456 hours) were normally distributed (Fig. 6.4). Hatchlings that

Table 6.1. Predictions of *C. insculpta* incubation (inc.) period by developmental model, compared to observed incubation periods.

nest #	beach	date laid	earliest poss. emerg. date	observed emerg. date	shortest poss. inc. period (days)	observed inc. period (days)
1	pandanus	11 Jul	24 Sep	16 Oct	75	97
2	oolloo	12 Jul	22 Sep	16 Oct	72	96
3	bonfire	12 Jul	6 Oct	16 Oct	87	96
4	triangle	14 Jul	3 Oct	21–27 Oct	82	99
5	moyes	14 Jul	12 Oct	8 Nov	91	107
9	snag	18 Jul	10 Oct	26 Oct	85	100
10	experimental	17 Jul	20 Sep	16–25 Oct	66	91
11	experimental	17 Jul	21 Sep	16–25 Oct	67	91
12	experimental	17 Jul	24 Sep	16–18 Oct	70	91
13	experimental	19 Jul	1 Oct	16 Oct	75	89
14	experimental	19 Jul	24 Sep	18 Oct	68	91
15	experimental	19 Jul	24 Sep	16 Oct	68	89
16	experimental	19 Jul	23 Sep	16–20 Oct	67	89
17	experimental	19 Jul	20 Sep	16–25 Oct	64	89
18	triple A	22 Jul	3 Oct	27 Oct	75	97
21	pandanus	22 Jul	8 Oct	30 Oct	80	100
24	oppsalt	2 Aug	5 Oct	27 Oct	66	86
26	rapids	5 Aug	30 Sep	29 Oct	58	85
27	rapids	5 Aug	30 Sep	29 Oct	58	85
29	big bend	1 Aug	15 Oct	29 Oct	77	89
30	big bend	1 Aug	14 Oct	29 Oct	76	89

Table 6.1. Continued. Predictions of incubation (inc.) period by developmental model, compared to observed incubation periods.

31	big bend	1 Aug	11 Oct	20–25 Oct	73	93
32	big bend	1 Aug	9 Oct	5 Nov	71	109
33	moyes	21 Aug	28 Oct	17 Nov	70	88
34	moyes	21 Aug	26 Oct	16 Nov	68	87
35	moyes	22 Aug	19 Oct	8 Nov	60	78
37	oppsalt	24 Aug	20 Oct	30 Oct	59	66
41	salty extens.	23 Aug	29 Oct	8 Nov	69	77
43	big bend	21 Aug	21 Oct	5 Nov	63	76
47	triple A	31 Aug	23 Oct	5–7 Nov	55	66
48	triangle	1 Sep	29 Oct	5 Nov	60	65
50	pyramid	3 Sep	27 Oct	14 Nov	56	72
52	pyramid	2 Sep	31 Oct	24–26 Nov	61	83
53	pyramid	3 Sep	1 Nov	10–17 Nov	64	68
58	moyes	6 Sep	2 Nov	16 Nov	62	71
60	salty extens.	6 Sep	2 Nov	13 Nov	62	68
68	pyramid	5 Sep	1 Nov	17 Nov	62	73

Table 6.2. Primary emergence times and nesting habitats of various turtle species gleaned from the literature.

species	primary emergence	nesting habitat	investigator(s)
Caretta caretta	night	open	Witherington et al. (1990); Hays et al. (1992)
Chelonia mydas	night	open	Hendrickson (1958); Gyuris (1993)
Lepidochelys olivacea	night/early morning	open	Hughes and Richard (1974)
Eretmochelys imbricata	night	open	Diamond (1976); Limpus (1980)
Dermochelys coriacea	night	open	Carr and Ogren (1959)
Apalone mutica	night/early morning	open	Muller (1921); Anderson (1958)
Podocmenis expansa	night/early morning	open	Alho and Padua (1982); Rose (1964)
Malaclemys terrapin	day	vegetated	Burger (1976a)
Trachemys scripta	day	vegetated	Tucker (1997)
Emydoidea blandingii	day	vegetated	Congdon et al. (1983); Butler and Graham (1995)
Chelydra serpentina	day	open/ vegetated	Congdon et al. (1999)
Graptemys pulchra	nocturnal	open/ vegetated	Anderson (1958)
Graptemys oculifera	nocturnal	open/ vegetated	Anderson (1958)
Carettochelys insculpta	nocturnal	open	this study

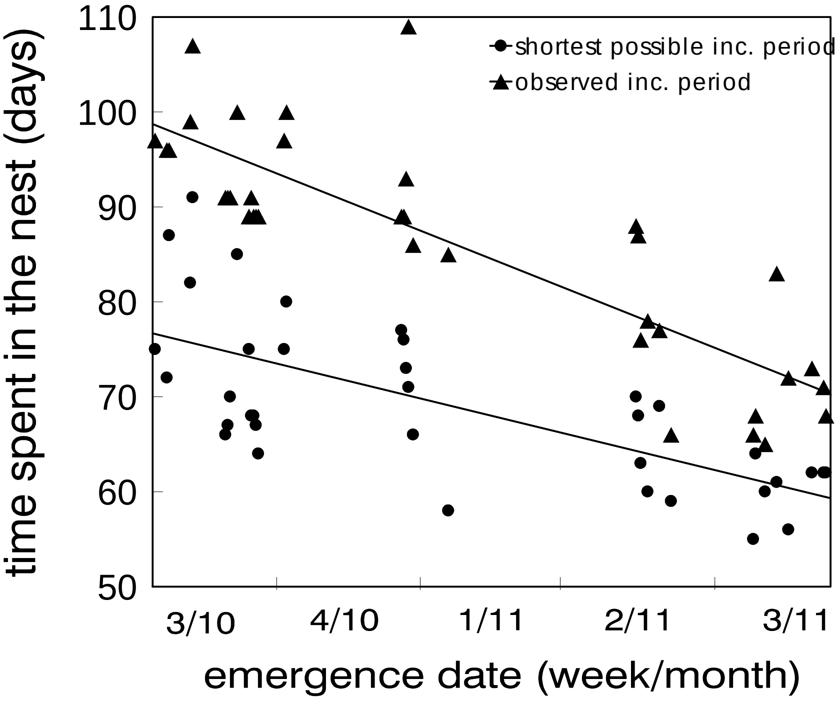


Fig. 6.1. Evidence for embryonic aestivation in *C. insculpta*. Observed incubation (inc.) period and shortest possible incubation (inc.) period regressed against emergence date. Shortest possible incubation period was calculated using the developmental model.

Fig. 6.2. Photographs of hatchling *C. insculpta* emerging from the nest, as taken by remote cameras mounted above. In each photograph, a single hatchling (positioned between infra-red transmitter and receiver boxes in each photo) has broken the infra-red beam, triggering the camera. Photograph on left shows data logger probes emanating from the nest. Photograph on right shows emergence associated with a river rise and subsequent flooding of the nest chamber. Note clear exit hole in this photo.

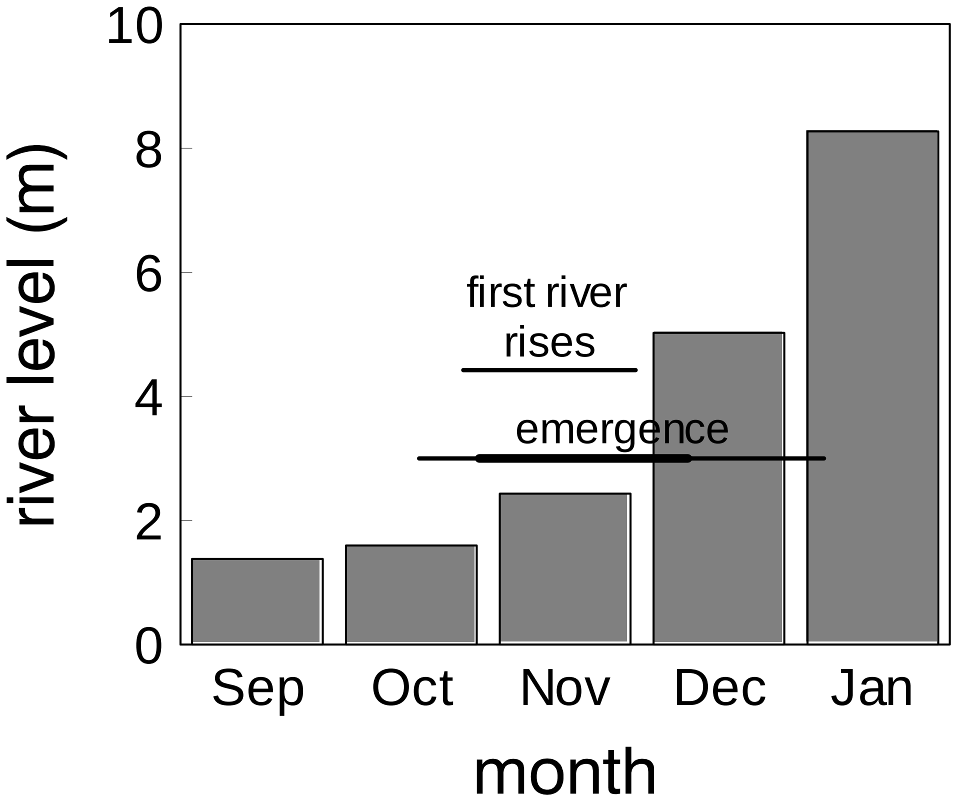


Fig. 6.3. Timing of emergence in *C. insculpta* is consistent with the first river rises of the wet season. Mean monthly river-stage data (histograms) and period during which the first river rises occur (extent of the upper horizontal bar) are from the years 1960–1996. Emergence data are from 1996–1998; in 1996–1997 these data were extrapolated from nesting dates. For emergence, the thickened line spans the range of mean first and last emergence dates for the three years, while the thinner line indicates the total range.

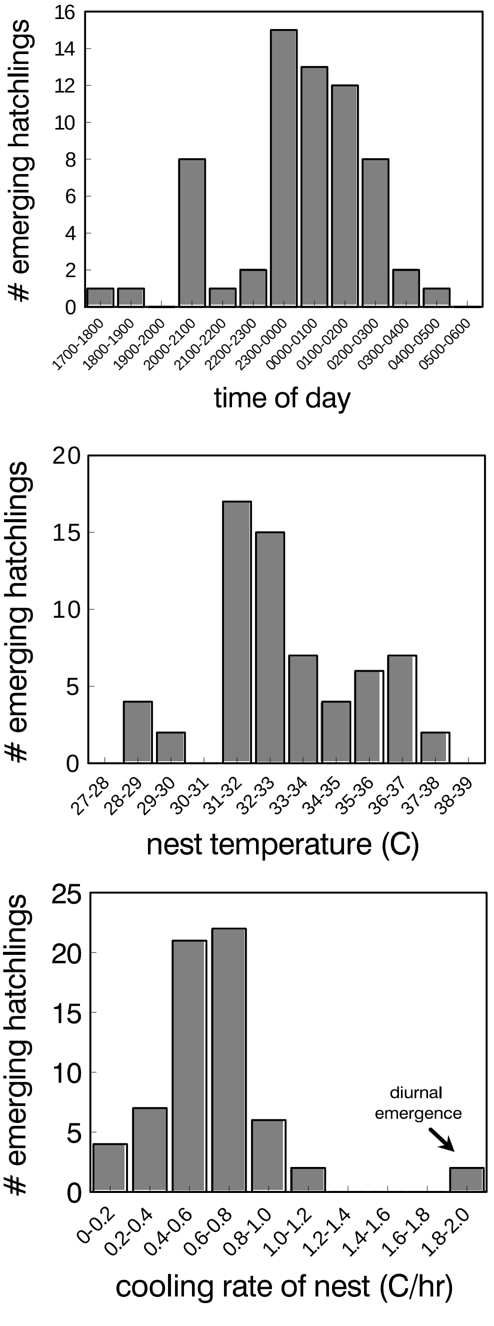


Fig. 6.4. Emergence time, temperature, and cooling rate of nests in relation to emergence of *C. insculpta* hatchlings. Cooling rate applies to the 3 h period prior to emergence.

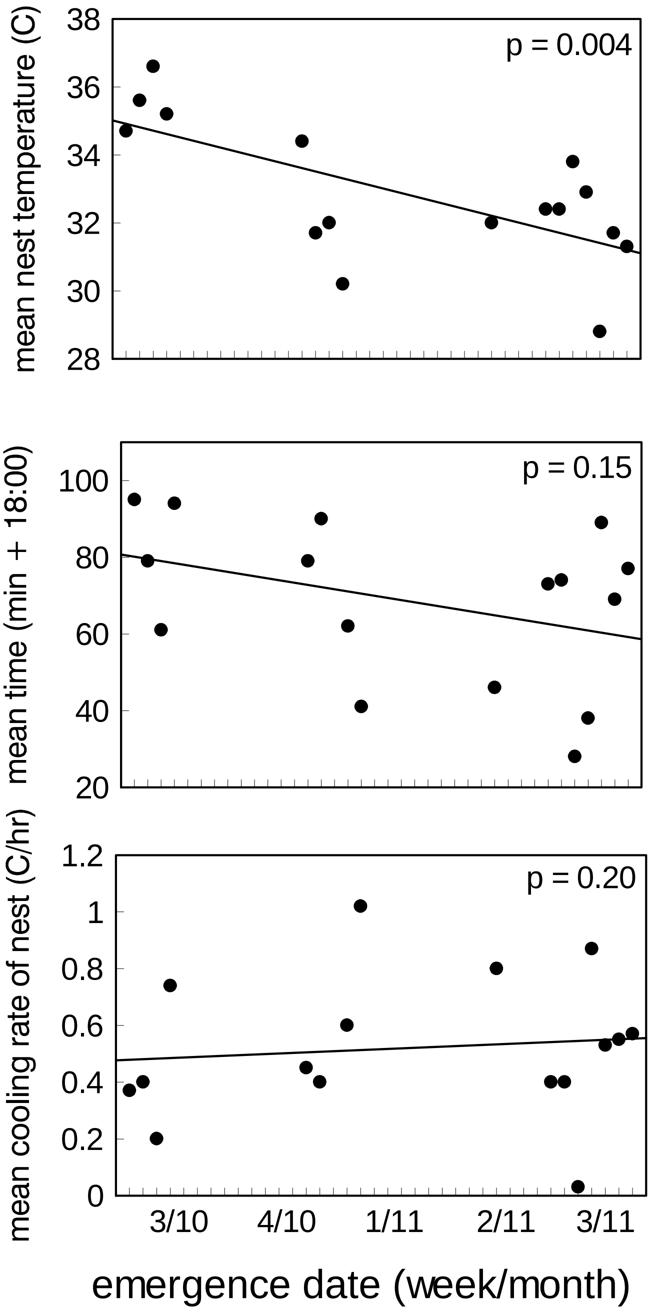


Fig. 6.5. Tests of the three predictions generated from hypotheses for nocturnal emergence. Mean emergence time (A), mean nest temperature at emergence (B), and mean cooling rate of nests preceding emergence (C), plotted against emergence date. Cooling rates were measured across the 3 h period prior to emergence.

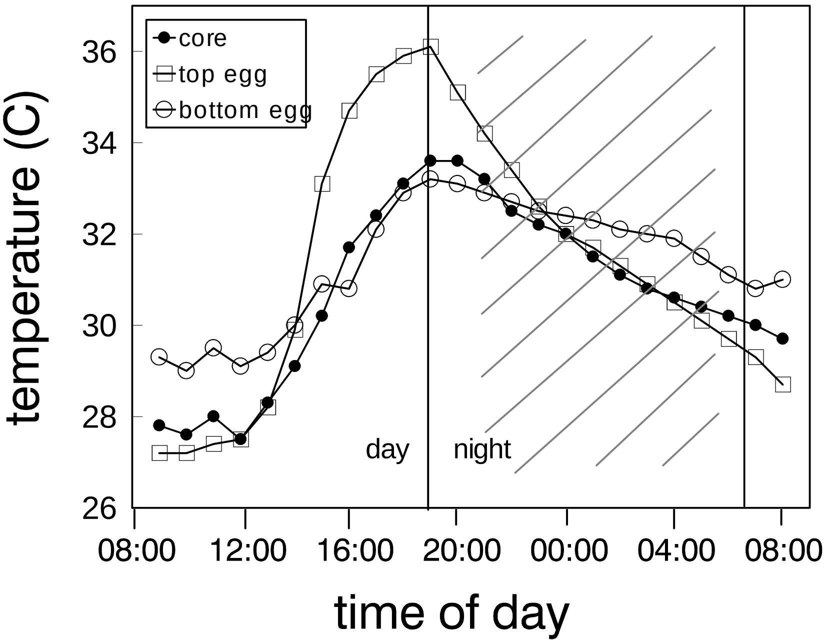


Fig. 6.6. Typical temperature trace of a *C. insculpta* nest relative to day/night. Data taken from Georges (1992).

emerged later in the season did so earlier in the night when considering either the first emergence for each nest ($r^2 = 0.796$, $F_{1,19} = 6.92$, $p = 0.007$) or all emergences ($r^2 = 0.277$, $F_{1,56} = 22.96$, $p < 0.001$).

Emergence temperatures. - Nest temperatures at emergence were normally distributed (Fig. 6.4). The mean nest temperature at emergence was 33.0 ± 2.28°C ($N = 64$, range 28.8–37.0). Hatchlings that emerged later in the season emerged at cooler nest temperatures (Fig. 6.5), for both the first emergence from each nest ($r^2 = 0.621$, $F_{1,19} = 31.15$, $p < 0.001$) and for all emergences ($r^2 = 0.586$, $F_{1,56} = 79.21$, $p < 0.001$). Emergence temperature was not related to emergence time, when considering either the first emergences for each nest ($r^2 = 0.058$, $F_{1,19} = 1.17$, $p = 0.292$) or all emergences ($r^2 = 0.05$, $F_{1,56} = 2.72$, $p = 0.105$). Temperatures began to decrease earlier in the day as the season progressed ($r^2 = 0.796$, $F_{1,15} = 58.61$, $p < 0.001$). All hatchlings emerged when nest temperatures were decreasing. Figure 6.4 shows the number of hatchlings emerging against the cooling rate of the nest during the 3 h preceding emergence. The two outliers in this figure emerged during the day after an afternoon rain-shower that resulted in a rapid decrease in nest temperature. Rate of cooling during the 3 h before emergence was not related to emergence time (first emergence, $r^2 = 0.008$, $F_{1,19} = 0.15$, $p = 0.701$; all emergences, $r^2 = 0.019$, $F_{1,56} = 1.08$, $p = 0.303$) or emergence temperature (first emergence, $r^2 = 0.012$, $F_{1,19} = 0.24$, $p = 0.631$; all emergences, $r^2 = 0.02$, $F_{1,56} = 0.94$, $p = 0.336$). Cooling rate did not change with season (Fig. 6.5) for either the first emergence for each nest ($r^2 = 0.00$, $F_{1,19} = 0.02$, $p = 0.998$) or all emergences ($r^2 = 0.01$, $F_{1, 56} = 0.30$, $p = 0.590$).

Other behavior. - In 49 of 62 nests (79%) all siblings within a nest emerged on the same night. Siblings that emerged on different nights generally did so on two nights, usually separated by one or two nights. Outliers included one nest in which siblings emerged on two nights 20 days apart, and another in which siblings emerged on four different nights. Siblings generally emerged through the hole that the first emerging hatchling created, but in six nests multiple holes were made.

Siblings emerged singly, not in groups. Only nine of 67 photographs showed more than one hatchling on the surface at one time. Considering only nests in which all hatchlings emerged on the same night, and removing three outliers, the (grand) mean emergence time between siblings was 12.0 ± 3.57 min SE ($N = 14$ nests; range 0.7–46.3 min). A single-factor ANOVA revealed that rainfall in the previous 24 h did not significantly influence the mean emergence time between siblings ($F_{1,16} = 4.54$, $p = 0.613$).

Emergence from nests on the same beach the same night was observed on six occasions (in groups of 7,7,4,2,2,2 nests). Most synchronous emergence among nests within a beach was explained by nesting date (i.e., in 15 of 19 nests, nesting dates were within 2 days of the other nest(s) emerging that night).

DISCUSSION

Timing of emergence, embryonic aestivation, and onset of the wet season. - Hatchling *C. insculpta* were more likely to emerge after rainfall. Emergence associated with rainfall has been documented for sea turtles (Carr, 1984), and a few freshwater species (Hammer, 1969; Alho and Padua, 1982; DePari, 1996; Kuchling, 1999). Hatchling turtles of some species may depend on rainfall to soften or degrade the nest chamber so they can emerge (DePari, 1996). However, *C. insculpta* clutches are deposited in sand, and hatchlings are likely to be able to emerge without such softening. This is supported by our observations of hatchlings from eight nests that emerged following rainless periods of 2–4 days. Butler and Graham (1995) found that rainfall during the previous 24 h was not necessary for inducing emergence in *Emydoidea blandingii*. Similarly, DePari (1996) found an imperfect association between rainfall and emergence in *Chrysemys picta*, and Tucker (1997) found no association between the presence or magnitude of rainfall and the emergence of *Trachemys scripta* hatchlings.

For Australian *C. insculpta*, however, rainfall also signals the onset of the wet season, which follows a long period of extremely dry conditions (e.g., mean monthly rainfall for May–Sept. = 7 mm). After finding delayed hatching and hatching in response to anoxia in *C. insculpta* eggs in the laboratory, Webb et al. (1986) hypothesized that similar delays in nature would allow hatchlings to emerge and disperse under the more favorable conditions of the wet season (flood waters). The present study supports both of these hypotheses.

First, delayed hatching was observed in nearly all nests (Fig. 6.1). On average, hatchlings spent 17 days in the ground at a hatchable stage, according to the developmental model that predicted the earliest date of completed development (Table 6.1; Fig. 6.1). The laboratory findings of Webb et al. (1986) indicated that turtles spent this time in the egg, rather than as hatchlings. This was confirmed in our study by (1) inspection of eggs after the predicted hatching date and (2) spontaneous hatching of eggs that were removed for sex determination for a concurrent study. Webb et al. (1986) also found that after yolk internalization *C. insculpta* embryos cease developmental growth and metabolic rate decreases precipitously. Thus, using the terminology of Ewert (1985), I conclude that *C. insculpta* possesses embryonic aestivation.

Second, historical river-stage data for 37 years and emergence data for three years indicate that most hatchlings emerged as river levels were rising (Fig. 6.3). Thus, embryonic aestivation may have evolved as a mechanism for optimizing timing of emergence and hence fitness, provided that hatchling survival or growth is favored under wet season conditions. From the present study I cannot distinguish between the two proposed survival mechanisms (namely, reduction in predator detection by reduced water clarity, and lower hatchling densities due to higher water volume) proposed by Webb et al. (1986). I found that river rises of > 0.3 m were invariably associated with a reduction in water clarity. Water clarity of 1–4 m during the dry season was reduced to a few centimeters by December.

The primary benefit of delayed emergence in turtles is said to be the ‘sanctuary offered during a period when growth benefits are likely to be outweighed by predation or mortality resulting from harsh environmental conditions’ (Gibbons and Nelson, 1978). In a review of turtles known to possess different types of developmental arrest, Ewert (1985) concluded that, in species with embryonic aestivation, late incubation is often associated with hot and dry conditions. Although these conditions persisted throughout incubation and aestivation in *C. insculpta*, it is unclear how these conditions might affect hatchlings in the river. It seems more likely that embryonic aestivation in *C. insculpta* has evolved to allow hatchlings to exploit early wet season survival or growth benefits rather than to avoid any particular stress of late dry season conditions.

Emergence behavior and the cue for nocturnal emergence. - Hatchling *C. insculpta* emerged primarily at night. Nocturnal emergence in turtles is said to be adaptive, reducing the probability of heat stress, desiccation and predation (Hendrickson, 1958;

Bustard, 1967; Stancyk, 1982). Support for heat stress mechanism comes from observations of hatchlings that emerged during the day and were scorched (Hughes and Richard, 1974; Diamond, 1976), and possibly in the behavior of *Malaclemys terrapin* hatchlings that head for cover immediately after diurnal emergence (Burger, 1976a). But how do hatchlings in the nest know when it is night, assuming that they do not penetrate the surface? What signal could they use to emerge nocturnally?

Thermal cues were first implicated as the trigger for nocturnal emergence in sea turtle hatchlings, based on indirect evidence of inhibition of activity at some temperature threshold (Hendrickson, 1958; Bustard, 1967, 1972; Mrosovsky, 1968, 1980; Heath and McGinnis, 1980; O'Hara, 1980). Earlier models suggested that hatchlings could avoid diurnal emergence by emerging below some absolute temperature (Hendrickson, 1958; Bustard, 1967; Mrosovsky, 1968). More recently, Witherington et al. (1990) suggested that a rapid decrease in temperature may be an important thermal cue for *Caretta caretta*. In support of this, Hays et al. (1992) found that cooling rates of sand at 15 cm were linked to emergence times in that species. They added that diel and seasonal variations in sand temperatures made it doubtful that a single absolute temperature cue could reliably ensure nocturnal emergence. Gyuris (1993) also challenged the absolute temperature threshold hypothesis, producing a 'thermal gradient model' to explain nocturnal emergence in *Chelonia mydas*. That work showed that the difference between sand temperatures at the surface and 10 cm depth was a more reliable predictor of darkness than an absolute temperature threshold. For the purpose of this discussion, cooling rates and thermal gradients are lumped into a 'decreasing temperatures' model. Both are a way of describing a pulse of cooler temperatures moving down through the sand, measured as a decrease in temperature at any given depth.

A goal of the present study was to determine which of these two models (absolute temperature threshold, and decreasing temperatures) could best explain nocturnal emergence in *C. insculpta*. I generated the following predictions for each model based on the knowledge that air temperatures decline as the season progresses throughout emergence in our study population (because of an increase in cloud cover and rainfall). If turtles were responding to an absolute temperature, a seasonal decrease in air temperatures would be likely to result in (1) a shift of emergence to times earlier in the night so that hatchlings could emerge at the same temperature(s). On the other hand, if turtles were responding to a *change* in temperature, then the seasonal decline in air temperatures might result in (2) a concordant decline in emergence temperatures but no change in emergence times, because (3) the cooling rate of sand does not change with season.

Our data fully support only one of these predictions: cooling rate did not change with season (Fig. 6.4). A seasonal decrease in air temperatures was associated with both lower emergence temperatures and with earlier emergence (Fig. 6.4). Our data, therefore, were not sufficient to reject either model. However, subsequent analyses revealed that as the season progressed temperatures began to drop earlier in the evening, probably because of increased cloud cover or rainfall. This would explain the apparent failure of the prediction of ‘no change’ in emergence times with season generated for the decreasing temperatures model.

I also found evidence against the fit of the absolute temperature model to *C. insculpta,* and, indeed, to other shallow-nesting turtles. In *C. insculpta,* higher nest temperatures are roughly symmetrical about dusk (Fig. 6.6), though rates of heating are faster than cooling rates because there is a time lag in the pulse of heat moving down through the substrate. If an absolute temperature threshold existed, it would be reached twice in a 24 h period, once during the day and once at night (Fig. 6.6). In other words, nest temperatures are no higher during the day than at night. Thus, no absolute temperature threshold can serve as a nocturnal cue for hatchling *C. insculpta*. Other nest temperature data available in the literature indicate that the eggs of other shallow-nesting turtle species similarly do not experience an appreciable decline in temperatures until near dusk (e.g., *Chelydra serpentina* in north-eastern and central USA, Wilhoft et al., 1983; Packard et al., 1985; *Emydura macquarii* and *Chelodina longicollis* in south-eastern Australia, Thompson, 1988; Palmer-Allen et al., 1991; *Podocnemis unifilis* in western Brazil, de Souza and Vogt, 1994; *Kinosternon subrubrum* and *Pseudemys floridana* in south-eastern USA, Bodie et al., 1996). Thus, it appears that in most turtle nests a decline in temperature can serve as a reliable cue for nocturnal emergence, rainfall events notwithstanding. In agreement with this hypothesis, emergence in *C. insculpta* was restricted to times when nest temperatures were decreasing (Fig. 6.4).

Our results suggest that emerging *C. insculpta* and other nocturnally-emerging species are likely to be responding to either a particular rate of nest-cooling, or simply a decrease in nest temperature. The latter alone could prevent diurnal emergence on hot, rainless days. As noted by Hays et al. (1992), nest-cooling as an emergence cue would explain the occasional diurnal emergence reported in sea turtles after rainfall (Carr, 1984; Witherington et al., 1990).

The models are not necessarily mutually exclusive. There may be a temperature threshold above which activity is inhibited (e.g., in the present study no hatchlings emerged at nest temperatures >37°C), setting an upper limit on emergence temperatures, in addition to a nocturnal cue of decreasing temperatures. Manipulation of the thermal environment of eggs, particularly using constant temperatures, would be useful for revealing the importance of a decrease in temperature to nocturnal emergence.

A few species of turtles, particularly sea turtles, deposit eggs in deeper nests by ‘body pitting’, or making a form into the substrate prior to constructing a nest cavity. In these nests only the topmost eggs may experience appreciable declines in diel temperatures (e.g., see Fig. 6.5 in Maloney et al., 1990). However, because social facilitation is known in these species (Carr and Ogren, 1960; Carr and Hirth, 1961), it seems intuitive that the uppermost hatchlings could make the ‘decision’ of when to emerge, with hatchlings from deeper in the nest following their lead. This idea is supported by experiments with *Chelonia mydas* by Bustard (1967), who found that by removing the topmost hatchlings from the nest he could induce the others to emerge in daylight. Carr and Hirth (1961) reported that the entire group of hatchlings moves upwards as they dig away at the roof of the nest chamber. In this scenario hatchlings from the bottom would move up into a zone experiencing temperature declines associated with nighttime. Mrosovsky (1968) documented that the uppermost hatchlings emerge from a depth of about 10 cm. This is very similar to the depth to the top egg in *C. insculpta* nests. I suggest, therefore, that hatchlings of turtles that emerge primarily at night do so from a depth that allows them to perceive a decline in temperatures associated with night-time.

Other behavior. - Timing of emergence (diel) data for turtles are sparse (Table 6.2), and are biased in favor of sea turtles and turtles nesting in open habitats (free of vegetation) where nests are easier to find. Despite few data, a pattern may exist. In Table 6.2, the eight species emerging in open habitats do so at night (and early morning), while the three species emerging in (at least partially) vegetated habitats emerge during the day. Data regarding the two *Graptemys* species are difficult to interpret because hatchlings emerged near the vegetated edge of large open sandbars. Although these two species would eventually have to traverse open sand, they appeared to have the option of moving into vegetation. It is worth noting that two of the three day-emerging species, *Malaclemys terrapin* and *E. blandingii*, headed for vegetation immediately after emergence or release during midday (Burger, 1976a; Butler and Graham, 1995). Species or populations that nest in vegetated areas may be freed of the ‘constraint’ of nocturnal emergence by being able to remain in vegetation until conditions are suitable for moving to the water. While these findings are consistent with an adaptive explanation for nocturnal emergence, emergence data are needed for more species to facilitate a comparative study of any potential adaptive advantage.

Sibling *C. insculpta* usually emerged on the same night, but in several nests emergence spanned two or more nights, roughly agreeing with studies on sea turtles (e.g., Peters et al., 1994; but see Hays et al., 1992), *Chelydra serpentina* (Congdon et al., 1987) and *M. terrapin* (Burger, 1976a). Congdon et al. (1983) found that roughly half of *E. blandingii* hatchlings emerged the same night, while Butler and Graham (1995) found that sibling *E. blandingii* emerged over a period of several days.

Carr and Hirth (1961) suggested that mass emergence, often observed in sea turtles, would be advantageous, because emerging hatchlings stimulate one another to crawl more quickly to the ocean. Sibling *C. insculpta* that emerged the same night did not emerge simultaneously in one group or a few groups, but generally trickled forth from the nest one at a time, usually separated by at least one minute. These data, combined with the short distance (<4 m) hatchlings traverse to the water, do not support adaptive mass emergence within or among clutches in *C. insculpta*. However, data presented in our study are from one year only. In years when rainfall events are more intense and coincident with mature hatchlings in the nest, hatching synchrony within and among nests may be more evident. For example, in 1986 seven mature clutches of *C. insculpta* eggs were placed in artificial nests. None hatched following a rainshower of 29.2 mm on 10 November, but four of the seven nests hatched after a rainshower of 52.2 mm on 19 November (Georges, unpubl. data). No rainfall events of this intensity were experienced during the majority of emergence events in the present study.

Emergence in small groups has also been documented in sea turtles (e.g., Christens, 1990; Witherington et al., 1990), and *E. blandingii* is known to emerge singly (Butler and Graham, 1995). The logistical difficulty in monitoring emergence has resulted in a paucity of such data, especially for freshwater turtles (Ehrenfeld, 1979; Christens, 1990; Kuchling, 1999). Future studies may find single emergence in other freshwater species.

In summary, C. insculpta hatchlings exhibit embryonic aestivation in nature, a characteristic that has probably evolved to synchronize emergence with the onset of wet season conditions. On a diel scale, I erroneously predicted that season would not influence emergence times under the decreasing temperatures model, because I was unaware that temperatures begin falling earlier in the day later in the season. Absolute nest temperatures were no cooler at night than during the day. Based on published nest temperature data, temperatures in turtle nests worldwide begin to decrease late in the evening. I suggest that in nocturnally-emerging species this decrease triggers emergence. As emergence data for turtles are scarce, few comparisons can be made at this time. However, it is hoped that the remote photographic technique I used will be adopted for investigations into the emergence behavior of other turtle species.

CHAPTER 7: HATCHLING SEX RATIOS AND EMBRYONIC SURVIVAL

Early males and less late females: Determinants of reproductive success and hatchling sex in pig-nosed turtles, *Carettochelys insculpta*

(prepared for submission to Oecologia)

J. Sean Doody, J. E. Young, and A. Georges

INTRODUCTION

Oviparous animals lacking parental care can provision their offspring in two ways: by providing material to the egg to meet the needs of the developing embryo, and by influencing the incubation environment through nest site choice (Roosenberg, 1996; Bernardo, 1996). In choosing a nest site there are two primary considerations. First, natural selection should favor mothers that choose nests sites that maximize offspring survival. Second, in species with environmental sex determination (ESD), selection should also favor mothers whose nest site choice tends to produce a balance in offspring sex ratio not markedly different from unity over their reproductive lives (Fisher, 1930).

Several factors potentially influence hatchling sex ratios in animals with ESD, including attributes of the mother, embryo, and environment. The mother can influence where she lays, when she lays, and the depth of the nest site. Influential attributes of the embryo include the relationship between temperature and developmental rate (Georges, 1989), the value of the pivotal temperature that separates male-producing temperatures from female-producing temperatures (e.g., Mrosovsky, 1988; Mrosovsky and Pieau, 1991), and the period during incubation when sex is influenced by temperature (the thermosensitive period, Bull, 1987; Mrosovsky and Pieau, 1991). Environmental factors include the magnitude of fluctuations in temperature (Georges, 1989; Georges et al., 1994), seasonal trends in temperature (Vogt and Bull, 1984), and stochastic events such as rainfall which temporarily depress nest temperatures. On a broader temporal scale, overall climate will be influential (Vogt and Bull, 1984). We would expect natural selection to shape distributions of the above attributes of the mother and embryo to produce balanced sex ratios, provided (i) that the attributes have a genetic underpinning, and (ii) no conflict exists between optimizing offspring sex ratios and optimizing offspring survival (Schwartzkopf and Brooks, 1987).

Understanding the influence of the incubation environment on offspring sex ratios is a prerequisite to answering why temperature-dependent sex determination (TSD) has evolved in reptiles. The most popular explanations for the evolution of TSD involve models derived from notions of differential fitness of male and female offspring incubated under particular thermal regimes (reviewed in Shine, 1999). These models link incubation temperature, phenotype, and fitness, and posit that TSD can enhance maternal fitness by enabling the embryo to develop as the sex best-suited to the particular environmental conditions it experiences during incubation (Shine, 1999). According to these models, incubation temperatures influence differential fitness between the sexes, primarily as a result of nest site choice or timing of nesting (reviewed in Shine, 1999; Harlow and Taylor, 2000). However, evidence for these models is meager (Harlow and Taylor, 2000).

In reptiles with TSD such as turtles, nest site choice can influence hatchling sex ratios (Vogt and Bull, 1984; Janzen, 1994; Roosenberg, 1996) and embryonic mortality (Wilson, 1998; Weisrock and Janzen, 1999). Timing of reproduction can also have strong fitness consequences, probably because this trait is related to maternal quality (Olsson and Shine, 1997b). In birds, for example, lay date advances in years of high food availability, findings confirmed by food supplementation experiments (reviewed in Drent and Daan, 1980; Rowe et al., 1994). Seasonal trends in hatchling sex ratios are known in a few reptile species (Vogt and Bull, 1984; Webb and Smith, 1984; Mrosovsky et al., 1984, 1985; Mrosovsky, 1994; Harlow and Taylor, 2000).

How do nest site choice and timing of nesting influence offspring sex and embryonic survival? For example, are there trade-offs, such that turtles choosing a particular nest site are maximizing one of these attributes at the expense of the other? No study on reptiles with TSD has examined the combined effects of timing of nesting and nest site choice on both sex ratios and embryonic survival, despite the potential importance of interpreting these in a combined context (Schwartzkopf and Brooks, 1987; Weisrock and Janzen, 1999). Functional links between nest site attributes, nest temperatures, and fitness-related traits of neonates in natural nests have rarely been studied (Weisrock and Janzen, 1999), and even fewer studies have established these causal links for a robust sample of natural nests (but see Shine and Harlow, 1996; Harlow, 2001). Such a sample could be especially useful to an interpretation of the possibilities for sex ratio evolution (Bulmer and Bull, 1982).

The objective of this study was to quantify the relationships between three maternal traits (nest site choice, timing of nesting, nest depth) and two fitness-related traits of offspring (hatchling sex and embryonic survival). I used *C. insculpta* because this species exhibits TSD, and because a prior study of the population documented variation in the thermal properties of nests, laying a foundation for studying determinants and consequences of that variation (Georges, 1992). I used four years of data from natural nests, environmental data, and a field experiment to elucidate factors determining nest site choice and timing of nesting, and to test the hypotheses that nest site choice and timing of nesting influence hatchling sex ratios and nest survival. I determined patterns of field sex-determination in our study system and briefly discuss their implications for differential fitness models for the evolution or maintenance of TSD.

MATERIALS AND METHODS

In northern Australia, *C. insculpta* nests during the dry season, from July to October (Georges and Rose, 1993). On the Daly River *C. insculpta* nests on isolated sandy beaches and banks varying in size from a few square meters to several hundred hectares (Georges, 1992). The turtles lay two clutches each year but alternate years (Chapter 4). Eggs hatch in September-November, and beginning in mid-November beaches become inundated by river rises associated with early wet season rains (Chapter 6).

<u>Timing of nesting and nest site choice</u>. - I searched for nests daily during the nesting seasons of 1996-1998. I accessed nesting areas by boat, and located nests by noting tracks and probing for the eggs with a spring steel rod. Because I swept the beaches clean after each survey, I was confident that I missed very few nests. For each nest I recorded laying date and measured the following attributes of the nest site: height above water, distance to water, aspect, and slope. I also recorded depth to the top egg and depth of the nest chamber. To assess whether turtles were choosing these variables, I divided 15 beaches into grids with square meter cells by demarcating lines

in the sand. I measured the same attributes for the center of each cell that were measured for each nest (height and distance from water, slope, aspect). Attributes of nest sites and available sites were then quantitatively compared for each beach. Aspect was coded symmetrically about due north to facilitate statistical analyses. Finally, for each beach I estimated the maximum height in which a nest chamber could be constructed, based on the cohesiveness of the sand. I did this by attempting to construct a nest chamber by hand to 15 cm depth at the highest point on the beach. If I could not make a chamber (the sand fell in on itself due to low moisture content) I moved progressively lower and repeated the procedure until was able to construct a chamber. I then measured the height above water of this ‘cohesive sand line’.

<u>Nest height experiment</u>. - After finding that turtles did not nest at the highest elevations on most beaches (see Chapter 6), I hypothesized that this was because the sand was not cohesive (turtles could not make a chamber due to dry sand falling in on itself). To test this hypothesis I conducted an experiment on a nesting beach in 1998. This beach was chosen because turtles had begun attempting to nest there on the highest areas, but appeared unable to make a chamber, as evidenced by > 20 cone-shaped pits.

I divided the beach into bands, each 1 m wide and perpendicular to the river. Every other band was then wetted with river water every 10 days, and the alternate bands served as controls. By wetting the bands I created cohesive sand, despite the first few cm on the surface drying out in a few days. Specifically, I hypothesized that nests deposited in the wetted bands would be higher above water than those in control bands, because (1) the constraint of loose sand was removed, and (2) because turtles benefit through nesting at the highest sites by reducing the probability of nest flooding. The beach was checked for nests every day throughout the nesting season, and the height above water was measured for each subsequent nest. As with other nesting beaches, I estimated the maximum height of cohesive sand by attempting to construct a ‘nest chamber’ by hand at a depth of 15 cm.

<u>Nest temperatures</u>. - Continuous temperatures were monitored in 102 nests with either Datataker® DT500 multi-channel dataloggers or Stowaway® single-channel dataloggers. Temperatures were recorded at 15 min intervals by the Datataker dataloggers and at 1 h intervals using the Stowaway dataloggers. Typically, three temperature probes were fitted to each nest: one immediately below the deepest egg, one in the core of the nest, and one immediately above the shallowest egg. Three probes were used to facilitate a study modeling the relationship between fluctuating temperatures and hatchling sex (Georges et al., unpubl. data). When Stowaway dataloggers were used, often only core temperatures were recorded. The probes were fitted as soon as possible after discovery of the nest, usually within 1–2 days. The depth of each egg was measured before its removal and eggs were returned to their original positions and orientations after deployment of datalogger probes.

To quantify what nest temperatures produce what offspring sex, I used a mathematical model that predicts hatchling sex from fluctuating nest temperatures (Georges et al., unpubl. data). The model produces constant temperature equivalents (CTE’s – see Georges, 1989; Georges et al., 1994) for each day during embryonic development. The model also determines the cumulative contribution to development for each day throughout the developmental period. From this I demarcated the thermosensitive period (TSP), or the period of development during which sex is determined for each temperature trace. I estimated the TSP to be the middle third of development, based on experimental data for other turtle species (Bull, 1987; Mrosovsky and Pieau, 1991). Once the TSP was determined, I took the average daily CTE during the TSP and compared it to hatchling sex (male, mixed, female) determined for that nest. I used the temperature trace recorded at the core of each nest, and I used only data from nests from which the lay date was known. Because dataloggers were typically employed 1-2 days after the lay date, I used a model to backfill the temperature trace to the lay date.

Embryonic mortality. - Flood mortality was estimated through (1) actual observations of nest flooding, and, when a clutch had already been removed for determining hatchling sex (2) by comparing observations of nest site flooding to hatching dates of the respective nest in the laboratory. I used an observation of flood mortality in one nest to estimate flood mortality of nests that were removed prior to flooding. For example, if a nest with a height of 1 m above water was flooded, then all nests with heights < 1 m that would have been in the ground (had I not removed them) were considered to have been flooded. To determine which nests would still have been in the ground, I used a typical late season incubation time of 61 days (Doody and Georges, unpubl. data).

To ensure that I would obtain hatchling sex from nests, I installed flat wire covers (20 cm X 20 cm hardware wire) at the surface of each nest site to protect it from predatory monitor lizards (*Varanus* spp.). I monitored nests for predation at least every other day throughout incubation. Although I protected nests, if a predator attempted to excavate a nest, as evidenced by diggings around the cage, I scored the nest as destroyed by a predator. In this way I could estimate mortality data without sacrificing sex ratio data. I also noted clutches of eggs that failed to hatch due to intrinsic reasons (e.g., infertility, developmental problems). Because I removed many clutches prior to their natural hatching date, my predation estimates are conservative. However, because most predated nests are taken soon after laying (unpubl. data), my predation estimates should be realistic.

Hatching and hatchling sex. - When a nest contained eggs that were near-term, as estimated by knowledge of lay date and previous incubation data (Georges, unpubl. data), I removed the clutch and housed it in a makeshift field laboratory until hatching. In this way I could isolate eggs to facilitate determination of sex in relation to depth in the nest. In 1996 and 1997 I obtained hatching dates in the field laboratory. In 1998 I obtained actual emergence dates in the field using remote camera systems set up on the nests (Doody and Georges, 2000; Chapter 6).

Hatchlings were measured, weighed, and killed with intercranial injection of pentobarbital or ethanol and stored in 10 % formalin. In 1996 all hatchlings were sacrificed, thereby obtaining the sex of every hatchling, while in 1997-1998 I only killed a few hatchlings from each nest to determine whether a nest contained all males, all females, or mixed sexes. For example, if the hatchling from a top egg in the nest was found to be a male, then all eggs in the nest were deemed to be males, because deeper eggs are cooler (Burger, 1976b; Wilhoft et al., 1983), and cooler *C. insculpta* eggs become males (Georges, 1992).

The right gonad, kidney, and associated ducts were removed, embedded in wax, sectioned, and dyed with haemotoxylin and eosin. The sex of each gonad was assessed by examination under a light microscope according to criteria established by Miller and Limpus (1981). Where necessary, the second gonad was examined.

Environmental data collection. - A standard station for monitoring water temperatures was set up on a small beach used by nesting *C. insculpta* in each of the study years (1996–1998). The station monitored water temperatures from May–November each year. Temperatures were recorded in the shade at approx. 0.5 m depth with four-wire RTD probes fitted to a datalogger (Datataker Model DT500) calibrated against a thermometer certified as accurate by the National Authority of Testing Agencies. To document seasonal changes in air temperatures I used data from a nearby weather station (Douglas Daly Research Farm, Dept. of Primary Industries and Fisheries). Associated changes in sand temperatures were recorded by taking monthly temperature samples 50 cm below the surface on each beach. I chose 50 cm because temperature at this depth is not confounded by time of day.

To examine any association between timing of nesting and the preceding wet season(s), I used mean monthly river stages as an index of the magnitude of the wet season. River stage data were obtained for Dorisvale Crossing (60 km upstream of the study area) for the years 1995–1998. I used only wet season river stages, because dry season river stages were nearly identical among years during the study. I used timing of nesting data from the three study years, and from 1986 from the same population (Georges, unpubl. data).

RESULTS

Number of nests. - One hundred ninety-one nests were found during the three years (1996, N = 65; 1997, N = 51; 1998, N = 75).

Timing of nesting. - Biennial reproduction is evident in the bimodal nesting distribution exhibited each year (Fig. 7.1). Daily nest surveys revealed that nesting ceased for 1-2 weeks between clutches.

Timing of reproduction, based on nesting dates, differed significantly among years (ANOVA; $F_{2,150} = 37.19$, $p < 0.001$). In 1996, nesting began 4 and 5 weeks later than in 1997 and 1998, respectively (Fig. 7.1). The onset of nesting was not associated with mean daily water temperature in the weeks or months just prior to nesting (Fig. 7.2). In the warmest year (1996) nesting began late, in the coolest year (1997) nesting began earlier, and in the year with intermediate temperatures nesting began earliest (Fig. 7.2).

The onset of nesting was negatively correlated with the magnitude of the wet season ($r = - 0.947$, df = 1, $p = 0.053$), as indexed by mean monthly river levels (Nov-April) during the wet season preceding nesting. Turtles nested earlier following 'big' wet seasons (1997, 1998) than they did following 'small' wet seasons (1986,1996) (Fig. 7.3). I also examined the onset of nesting against the magnitude of the two wet seasons preceding nesting, because *C. insculpta* in the Daly nest every second year (Chapter 4), indicating that turtles need two years to complete vitellogenesis. For this I found a similar result but the association was not significant ($r = - 0.819$, df = 1, $p = 0.181$).

Within years, lay date was generally correlated with clutch size (-), egg mass (+), egg width (+), but not with egg width, and not usually with clutch mass (Table 7.1).

Table 7.1. Clutch correlates of lay date in *C. insculpta* during 1996-98. * = p < 0.05, ** = p < 0.01, *** = p < 0.001.

clutch attribute	1996	1997	1998
clutch mass	r = 0.281 p = 0.079	r = - 0.030 p = 0.068	r = - 0.128 p = 0.426
clutch size	r = 0.296 p = 0.060	r = - 0.450 p = 0.005**	r = - 0.214 p = 0.180
egg mass	r = - 0.005 p = 0.977	r = 0.658 p < 0.001***	r = 0.310 p = 0.048*
egg length	r = 0.007 p = 0.965	r = - 0.180 p = 0.286	r = 0.203 p = 0.204
egg width	r = 0.050 p = 0.758	r = 0.607 p < 0.001***	r = 0.431 p = 0.005**

Table 7.2. Nest site attributes for *C. insculpta* nests in 1996-1998.

nest site attribute	mean ± 1 SD (range)	N
height above water (m)	0.97 ± 0.437 (0.35-2.88)	178
distance from water (m)	2.45 ± 1.259 (0.59-9.10)	180
slope (°)	17.18 ± 9.889 (0-50)	177
aspect (coded about due N)	13.92 ± 10.025 (0-36)	178
nest chamber depth (cm)	21.47 ± 1.773 (14.6-26.2)	166

Table 7.3. Comparisons of *C. insculpta* nest site attributes with availability of those attributes within a nesting beach. See text for methods. Data are means ± 1 SD, and statistics are from single factor ANOVA. Sample sizes are in parentheses. Height above water and distance to water are in cm, slope is in degrees, and aspect is coded about due north.

beach/attribute	nest sites	available sites	significance
Lower Beach			
height above water	73.4 ± 20.66 (14)	85.5 ± 31.08 (122)	F = 0.99, p = 0.160
distance to water	232.0 ± 96.41 (14)	416.5 ± 247.13 (123)	F = 7.63, p = 0.007**
slope	11.1 ± 4.87 (14)	12.9 ± 9.48 (120)	F = 0.48, p = 0.490
aspect	9.5 ± 2.90 (14)	10.0 ± 3.35 (120)	F = 0.33, p = 0.569
Moyes Beach			
height above water	92.7 ± 23.06 (13)	180.2 ± 102.06 (107)	F = 9.44, p = 0.003**
distance to water	264.5 ± 145.23 (13)	745.0 ± 532.28 (107)	F = 10.42, p = 0.002**
slope	9.8 ± 6.16 (13)	11.1 ± 8.57 (103)	F = 0.28, p = 0.596
aspect	26.85 ± 1.72 (13)	23.44 ± 8.87 (103)	F = 1.90, p = 0.171
Dover Beach			
height above water	101.3 ± 23.11 (11)	93.9 ± 43.45 (28)	F = 0.29, p = 0.594
distance to water	236.1 ± 67.80 (11)	289.3 ± 177.09 (28)	F = 0.92, p = 0.342
slope	13.4 ± 6.15 (11)	20.9 ± 7.40 (28)	F = 8.82, p = 0.005**
aspect	9.6 ± 3.32 (11)	8.6 ± 3.49 (28)	F = 0.74, p = 0.395
Neils Beach			
height above water	98.00 ± 32.46 (10)	92.3 ± 48.09 (53)	F = 0.13, p = 0.720
distance to water	259.1 ± 48.88 (10)	256.7 ± 139.86 (53)	F = 0.00, p = 0.958
slope	19.4 ± 2.59 (10)	18.8 ± 8.91 (53)	F = 0.05, p = 0.822
aspect	4.5 ± 0.97 (10)	3.8 ± 1.10 (49)	F = 3.53, p = 0.065

Table 7.4. Influence of timing of nesting, nest site attributes, and nest depth on nest temperatures in *C. insculpta*. Results are from multiple regression analysis. *** denotes > 0.001.

attribute	df	X^2	p
lay date	1	132.305	0.0001***
height	1	0.485	0.4860
lay date X height	1	0.0796	0.7779
distance	1	0.000	0.9922
slope	1	0.2953	0.5869
aspect	1	0.5792	0.4466
chamber depth	1	0.0506	0.8220

Table 7.5. Statistical results from stepwise discriminant function analysis of embryonic survival and hatchling sex, as explained by lay date, nest site attributes, and nest depth in *C. insculpta*. Hatchling sex outcomes were male, female, and mixed sexes. * = significance at $p < 0.05$, *** = $p < 0.001$.

attribute	total embryonic survival			hatchling sex		
	partial r^2	$F_{1,113}$	p	partial r^2	$F_{2,95}$	p
lay date	0.148	20.636	0.000***	0.510	52.125	0.000***
height	0.000	0.006	0.940	0.079	4.262	0.017*
distance	0.000	0.000	0.988	0.013	0.653	0.523
slope	0.009	1.071	0.303	0.024	1.201	0.305
aspect	0.000	0.028	0.866	0.014	0.664	0.517
depth	0.000	0.018	0.893	0.008	0.397	0.674

Table 7.6. Statistical results from stepwise discriminant function analysis of embryonic (nest) survival as explained by lay date, nest site attributes, and nest depth in C. insculpta. Flood survival data are from 1996 only. * = significance at $p < 0.05$, *** = $p < 0.001$. Depths were not available for nests taken by predators, as most nests were taken the morning after laying.

attribute	flood survival (1996)			predation survival		
	partial r^2	$F_{1,33}$	p	partial r^2	$F_{1,145}$	p
lay date	0.384	22.449	0.000***	0.038	5.770	0.018*
height	0.358	20.056	0.000***	0.030	4.643	0.033*
distance	0.004	0.119	0.732	0.001	0.168	0.683
slope	0.003	0.097	0.757	0.010	1.439	0.232
aspect	0.021	0.759	0.390	0.001	0.088	0.768
Depth	0.001	0.030	0.864	n/a	n/a	n/a

Table 7.7. A spatial factor, height of the nest site above water, influences hatchling sex when lay date is held constant, or nearly so. Higher nest sites are warmer, producing more females.

beach	nest #	lay date	height (cm)	hatchling sex
experimental	11	17 July	116	male
experimental	12	17 July	116	male
experimental	13	19 July	114	male
experimental	10	17 July	174	mixed
experimental	14	19 July	174	mixed
big bend	29	1 August	87	male
big bend	32	1 August	86	male
big bend	30	1 August	93	mixed
big bend	31	1 August	94	mixed
spring	46	20 September	61	mixed
spring	42	18 September	97	female

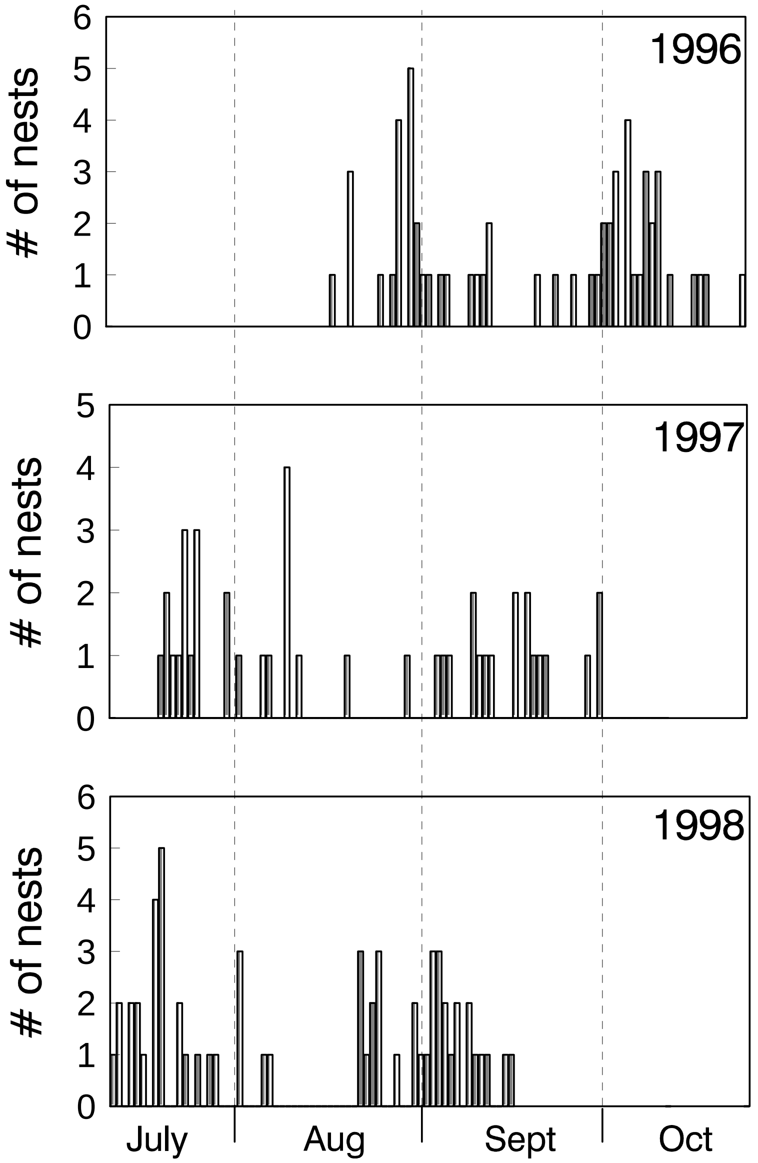


Figure 7.1. Annual variation in timing of nesting of *C. insculpta* during 1996-1998, showing a five-week maximum difference in the onset of nesting between years. Data are from daily surveys during the dry season.

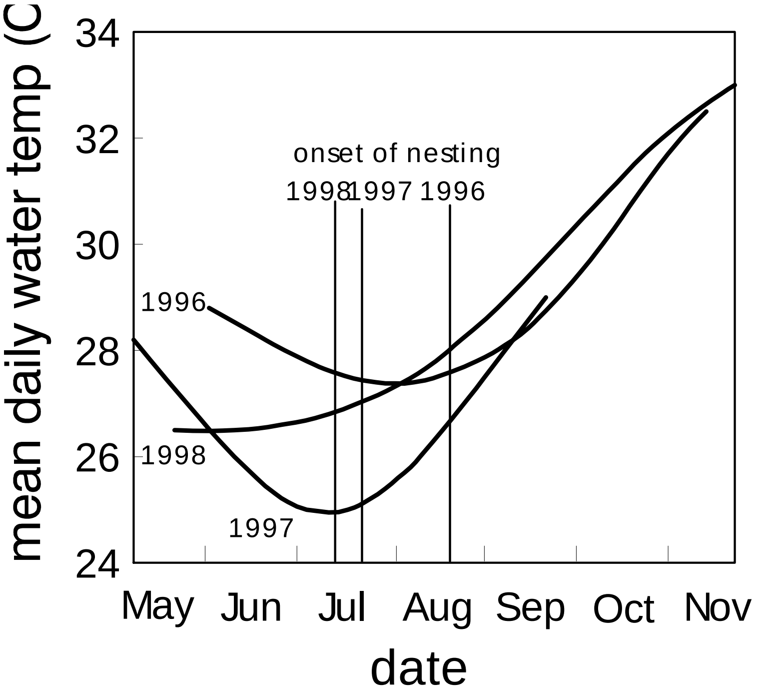


Figure 7.2. Lack of association between the onset of nesting and water temperatures for *C. insculpta* during 1996-1998. Water temperature curves are spline functions. The onset of nesting in each year is indicated by the dashed lines.

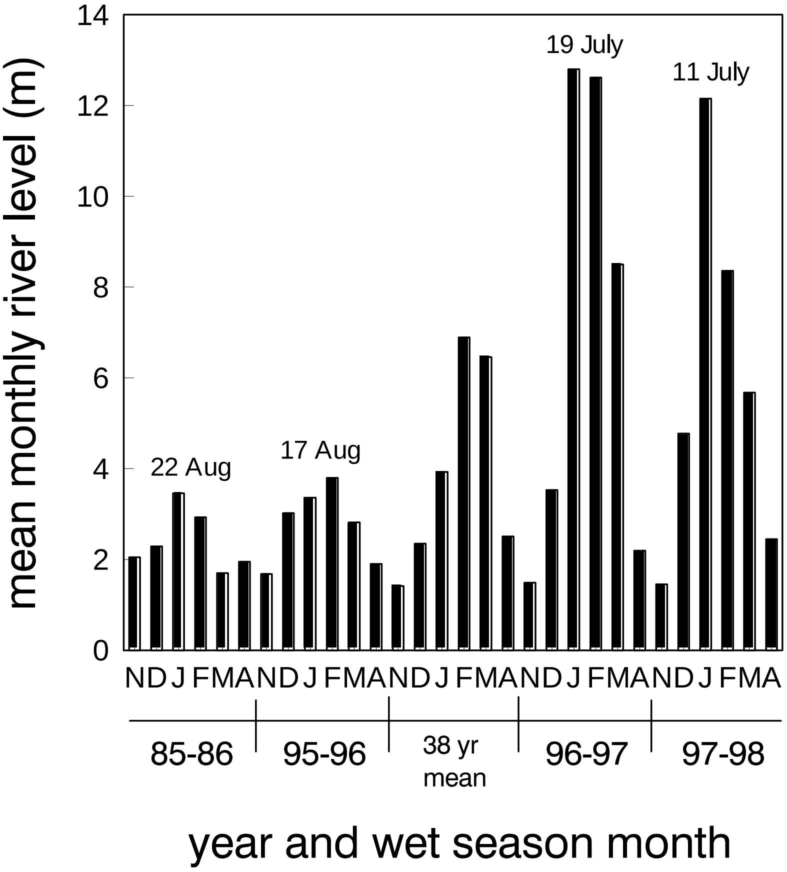


Figure 7.3. The onset of nesting may covary with annual variation in the magnitude of the previous wet season in *C. insculpta*. Here the magnitude of the wet season is indexed by mean monthly wet season river levels prior to each year of the study (1996-97, 1997-98), in a previous unpublished study (1986), and a 38-year average (1960-1998). Nesting season initiation dates are given above the respective year. Note that 1985-86 and 1995-96 were ‘small’ wet seasons and that 1996-97 and 1997-98 were ‘big’ wet seasons. Data are from Dorisvale Crossing and are routinely collected by NT Water Resources.

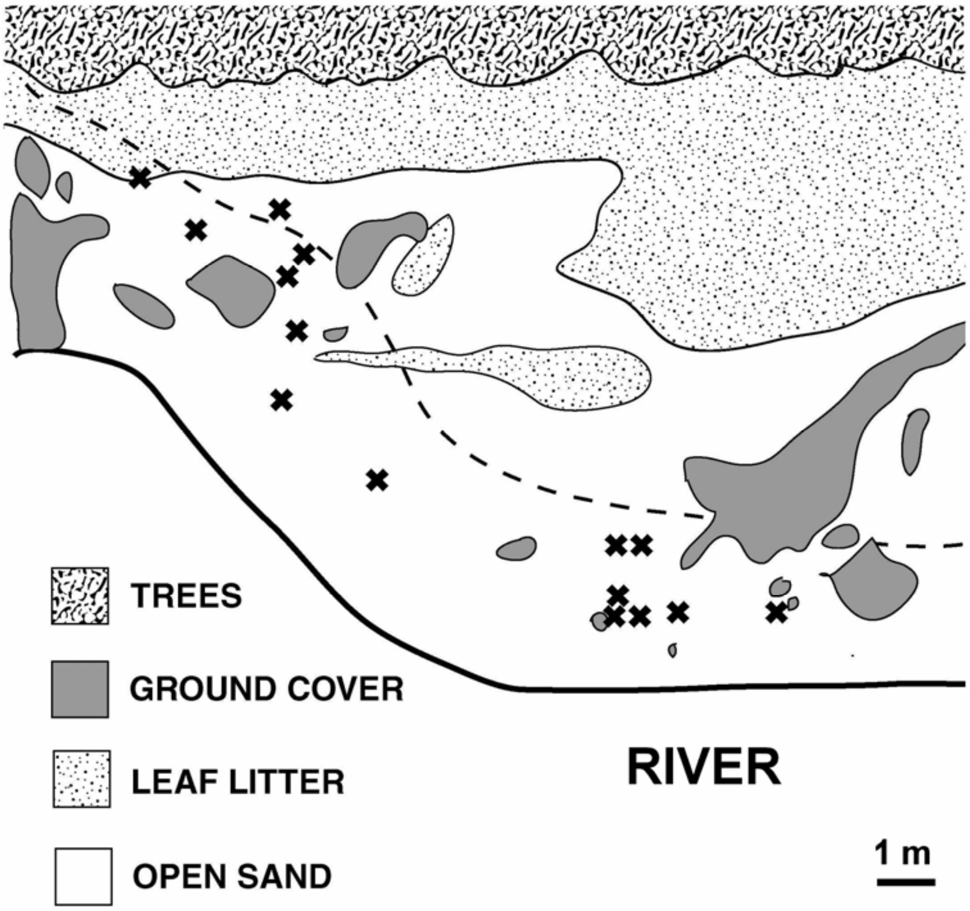


Figure 7.4. Top view of a nesting beach used by *C. insculpta*, showing location of nests. Note that estimated extent of cohesive sand (dashed line) agrees with maximum height of nest sites. Shaded areas = low vegetation. Stippled areas = sparse leaf litter.

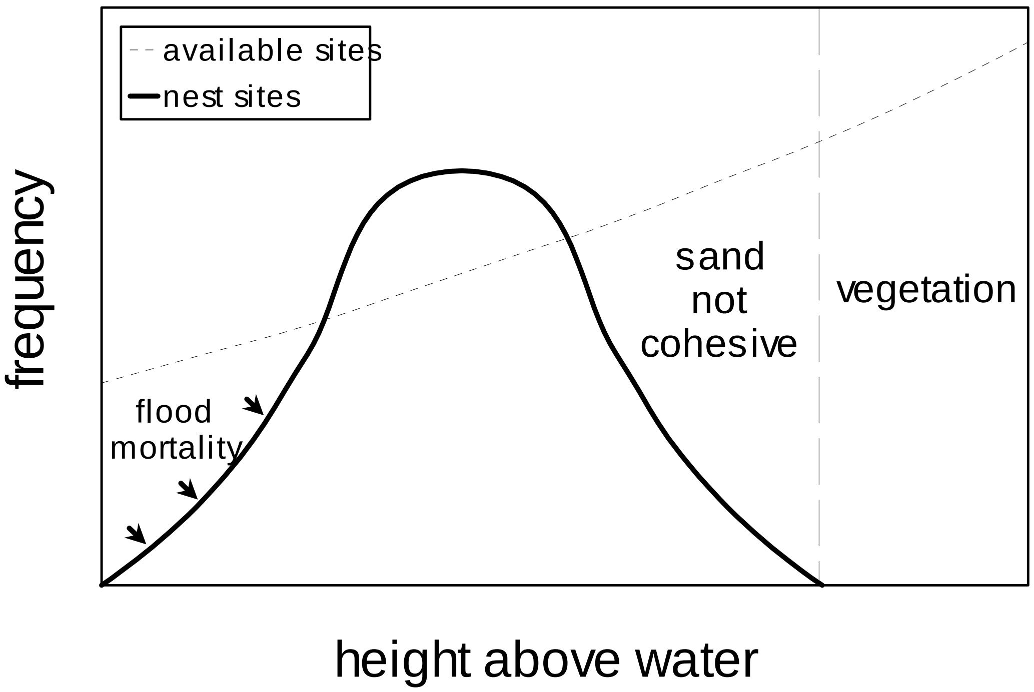


Figure 7.5. Graphical model for heights of nest sites chosen by *C. insculpta*. A typical distribution of nest heights on a beach is compared to heights of available sites. Eggs deposited at the lowest elevations are prone to flood mortality. The majority of the highest available sites, although associated with the lowest probability of nest flooding, are effectively unavailable because the sand is not cohesive. Turtles did not nest in vegetated areas.

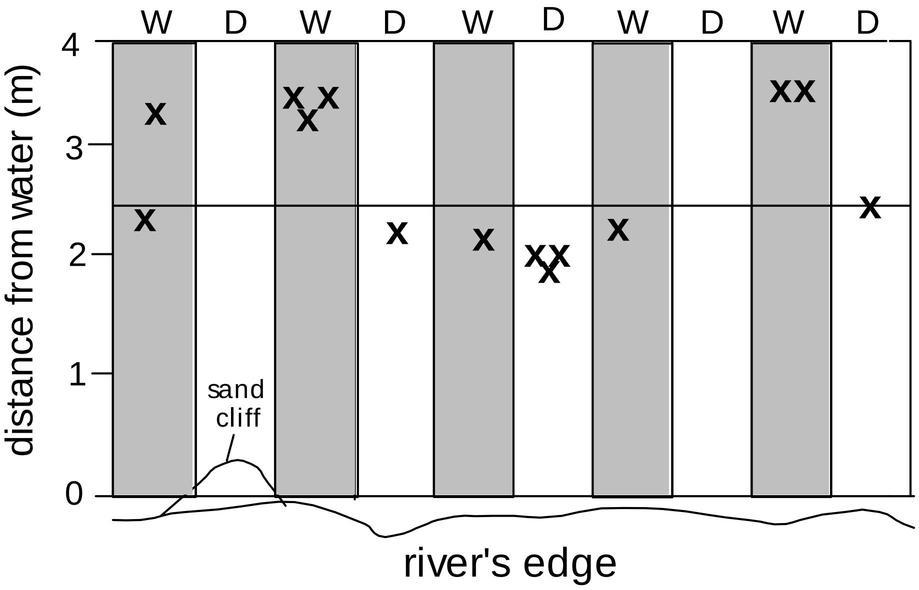


Figure 7.6. Experimental layout (top view) of the ‘nest height’ experiment, showing alternating experimental and control ‘bands’. W = wetted, experimental bands (shaded), D = dry, control bands. Experimental bands were manipulated by adding river water every 10 days throughout the nesting season. X’s denote *C. insculpta* nests deposited during the experiment. The horizontal line demarcates the highest elevation with cohesive sand, as estimated by attempting to construct a ‘nest chamber’ by hand at 15 cm depth. A cliff of sand at the water’s edge in the second band from the left may have influenced the probability of turtles nesting in that band.

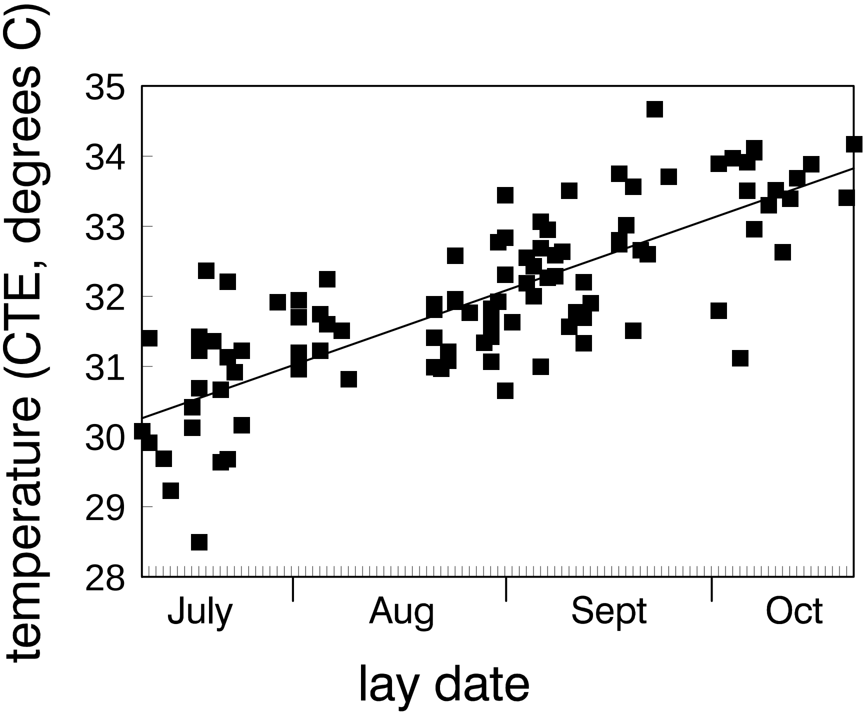


Figure 7.7. Lay date influences nest temperatures, reflecting a seasonal increase in air and sand temperatures during the nesting season of *C. insculpta*. Temperatures are CTE's from the TSP (middle third of development). See text for explanation of CTE's.

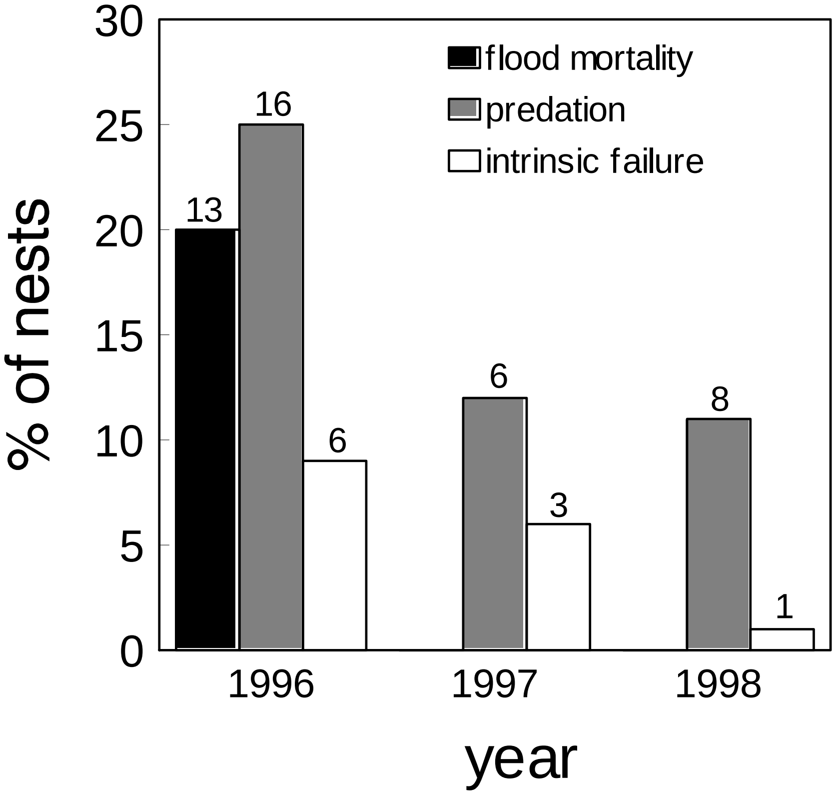


Figure 7.8. Annual variation in mortality of *C. insculpta* nests during 1996-1998. Intrinsic failure refers to clutches that failed to hatch due to inherent causes (e.g., infertility, developmental problems).

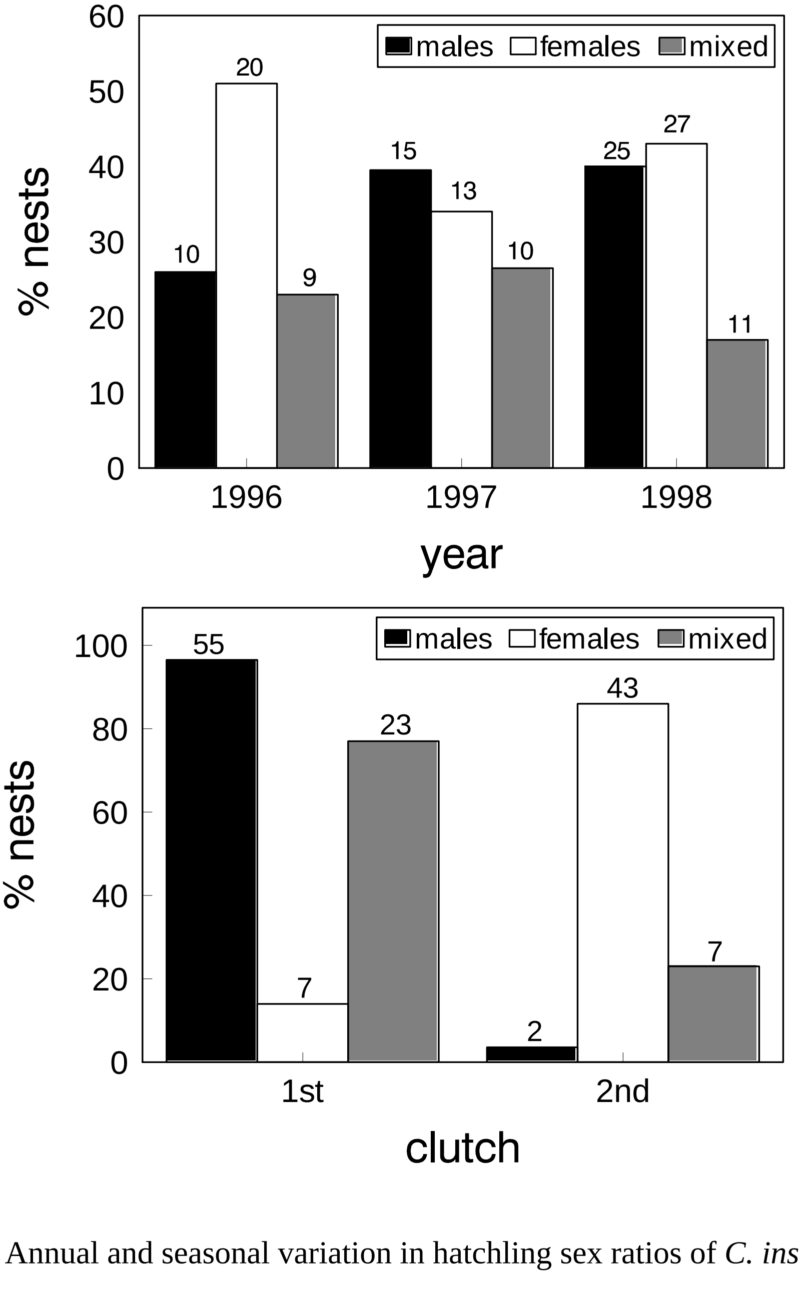


Figure 7.9. Annual and seasonal variation in hatchling sex ratios of *C. insculpta* nests during 1996-1998 (top graph). Mainly males are produced in the first clutch and mostly females produced in the second clutch (bottom graph). Samples sizes (number of nests) are presented above bars.

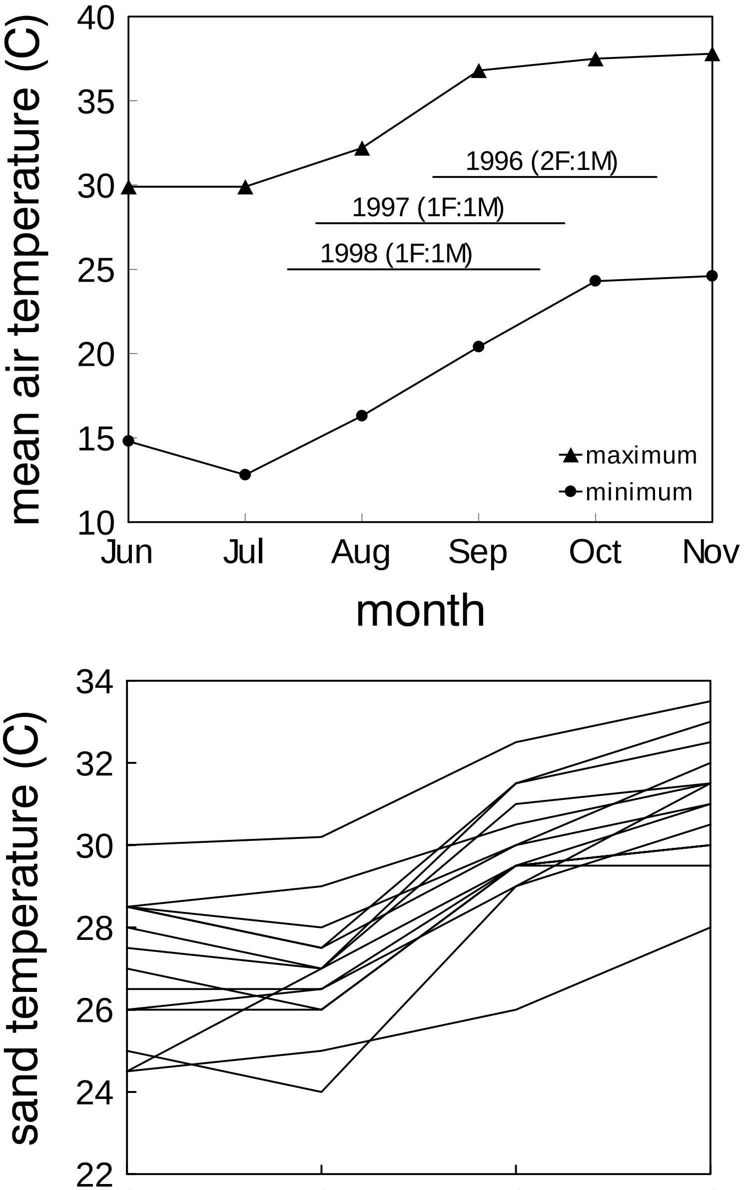


Figure 7.10. Timing of nesting and season determines sex ratios of hatchling *C. insculpta*. Top graph shows timing of nesting (horizontal lines) and corresponding sex ratios for each year during 1996-1998. Mean maximum and minimum air temperatures for each dry season are indicated. Bottom graph shows associated increase in sand temperatures, taken monthly from 50 cm depth on 13 different beaches.

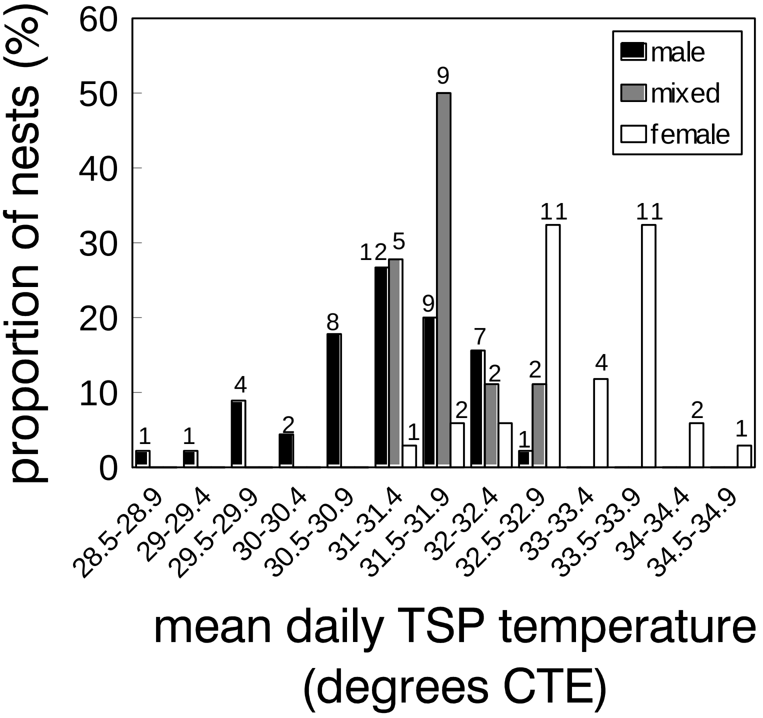


Figure 7.11. Hatchling sex production as a function of the mean daily temperature during the thermosensitive period (TSP) in *C. insculpta*. Temperature data are constant temperature equivalents (CTE's). The TSP was found to occur during the middle third of development in a concurrent laboratory experiment (unpubl. data).

Nest site choice. - Means for nest site attributes and nest depth for the three years combined are listed in Table 7.2.

Of the 15 beaches on which availability of nest site attributes was quantified, four beaches were chosen for statistical examination of nest site choice, due to the number of nests deposited on each (N = 14,13,11,10 nests; Table 7.3). Within beaches, turtles nested closer to water and at lower elevations than mean available sites on some beaches, but not others (Table 7.3). Turtles nested randomly with respect to slope and aspect on all beaches, except in one case where steeper slopes were chosen (Table 7.3).

Turtles did not nest at the highest elevations available (Fig. 7.4), apparently because the sand was not cohesive at these sites on most beaches. A typical, hypothetical distribution of nest heights is shown in Figure 7.5. The figure illustrates how the distribution of nest heights is apparently shaped by (1) natural selection through flood mortality on eggs laid at lower sites, and (2) the physical constraint of loose, dry sand precluding the construction of a nest chamber at higher sites. This sand was drier, due to a vertical moisture gradient through the sand. Turtles avoided nesting in vegetated sites (Fig. 7.5).

Height of nest sites was influenced by year ($F_{2,168} = 16.23$, $p < 0.001$). In 1998 nests were deposited at higher sites than in 1996 or 1997 (Tukey's HSD, $p < 0.001$ in both cases). Distance of nest sites from water mirrored height relationships among years ($F_{2,179} = 8.69$, $p < 0.001$), with nest sites farther from water in 1998 than in 1996 or 1997 (Tukey's HSD, $p < 0.001$ in both cases). Turtles chose steeper slopes in 1998 than in 1996 ($F_{2,176} = 11.77$, $p < 0.001$). Slopes were not significantly different between 1997 and the other two years. Aspect of nest sites differed significantly among years ($F_{2,177} = 5.26$, $p = 0.006$), with more east-facing nest sites in 1998 than in 1997 ($p = 0.008$).

Nest height experiment. - Fourteen nests were deposited during the nest height experiment, nine in the experimental (wetted) bands and 5 in the control (dry) bands (Fig. 7.6). Nests in the experimental bands were deposited significantly higher above water than those deposited in the control bands (ANOVA; $F_{1,13} = 4.75$, $p = 0.001$). Our estimated upper limit of cohesive sand agreed closely with the highest nests deposited in the control bands (Fig. 7.6).

Nest temperatures. - Daily nest temperatures during the TSP, expressed as CTE's, are given in Chapter 8 (Table 8.2), along with sexes produced in each nest. Continuous temperatures were successfully monitored in 102 nests for which sex was determined (1996, N = 30; 1997, N = 29; 1998, N = 43).

Multiple regression revealed that nest temperatures (CTE's) during the TSP were significantly related to lay date but not to nest site attributes or nest depth (Table 7.4). Nests laid later in the season were hotter (Fig. 7.7).

Hatching and emergence dates. - Analysis of variance indicated that eggs in nests producing all males hatched, on average, 12 days before eggs in all-female nests (1996, $F_{1,28} = 3.49$, $p = 0.07$; 1997, $F_{1,23} = 9.23$, $p = 0.006$). In 1998, mean emergence date was earlier ($\xi = 19$ days) in all-male nests than in all-female nests (ANOVA, $F_{1,29} = 57.50$, $p < 0.001$).

Embryonic survival. - Sources of mortality in order of importance were predation, flooding, and intrinsic causes (Fig. 7.8). Step-wise Discriminant Function Analysis indicated that embryonic survival was influenced by lay date, but not by nest site choice or nest depth (Table 7.5).

Observed nest predators were the monitor lizards *Varanus panoptes* and *V. mertensi*. Most predation occurred on the morning after eggs were laid. Step-wise Discriminant Function Analysis indicated that the probability of nest predation was influenced by nest site choice and by lay date (Table 7.6). Destroyed nests were at higher elevations that surviving nests ($F_{1,177} = 5.55$. $p = 0.02$). A weaker effect of lay date was found ($F_{1,167} = 3.14$, $p = 0.078$), with destroyed nests being laid 13 days later than surviving nests, on average. Nest depth and other nest site attributes did not influence predation survival.

In 1996, 13 (20 %) nests were destroyed by a single flood on 22 November. In three of these nests mortality was observed, and mortality was estimated to have occurred in the other 10 nests that were removed for sexing prior to the flooding date. This flood was associated with heavy rainfall during the early wet season. Step-wise discriminant function analysis indicated that flood survival was explained by lay date and height of the nest site, which together explained 74 % of the variation (Table 7.6). Nests deposited at low elevations later in the season experienced flood mortality. Nest depth and other nest site attributes did not influence nest survival (Table 7.6). No flood mortality was incurred in 1997-1998; in these years hatching preceded early wet season river rises, due to earlier nesting.

A proportion of nests each year experienced complete embryonic failure for presumably intrinsic reasons (e.g., infertility, developmental problems) (Fig. 7.8).

Hatchling sex ratios. - Hatchling sex was determined in 140 nests (1996, N = 39; 1997, N = 38; 1998, N = 63). Hatchling sex ratios differed annually, with a preponderance of females in 1996, compared to sex ratios near unity in 1997 and 1998 (Fig. 7.9). In a given year 17-26 % of nests contained both males and females (mixed nests, Fig. 7.9).

Hatchling sex was not independent of clutch number (Contingency analysis; $X^2 = 83.73$, df = 2, $p < 0.001$). Most early (first) clutches produced males, while most later (second) clutches produced females (Fig. 7.9). This seasonal difference in sex production was due to the seasonal increase in both air and sand temperatures throughout the dry season when eggs were incubating (Fig. 7.10).

The distribution of sexes produced in field nests as a function of mean daily temperature during the thermosensitive period, expressed as constant temperature equivalents, is given in Figure 7.11. Males were produced at cooler temperatures (ξ = 31.2 ± 0.92 °C SD; range = 28.5-32.8 °C, N = 45), mixed sexes resulted from intermediate temperatures (ξ = 31.8 ± 0.46 °C SD; range = 31.1-32.7 °C, N = 19), and females were produced at hotter temperatures (ξ = 33.1 ± 0.75 °C SD; range = 31.4-34.7, N = 35) (Fig. 7.10). Two far outliers were removed prior to analyses.

Step-wise discriminant function analysis revealed that hatchling sex was determined primarily by lay date, which explained 51 % of the variance in sex production (Table 7.5). However, the analysis also found a significant influence of nest site choice on hatchling sex. Nest sites were significantly higher above water in clutches producing females than in those producing mixed nests or males (Table 7.5). This is corroborated by nests at higher elevations producing more females than nests deposited at lower sites when lay date was the same (Table 7.7). Nest depth and other nest site attributes did not influence hatchling sex (Table 7.5).

DISCUSSION

Timing of nesting. - Considerable annual variation in the onset of nesting was found in the present study. More than five weeks (39 days) separated the onset of nesting in 1996 and 1998 (Fig. 7.1). This annual variation is high for turtles (e.g., in *Chelydra serpentina*, 22 days, Congdon et al., 1987; 14 days, Obbard and Brooks, 1987; 10 days, Iverson et al., 1997; in *Emydoidea blandingi*, 6 days, Standing et al., 1999; 23 days, Congdon et al., 1983; in *Chrysemys picta*, < 15 days, Congdon and Gatten, 1989; in *Terrapene ornata*, 12 days, Doroff and Kieth, 1990), and possibly for reptiles in general (but see Olsson and Shine, 1997b). I might expect that temperature would best explain the annual variation in the onset of nesting. In particular, I hypothesized that turtles would nest earlier in years with warmer air or water temperatures (Congdon et al., 1983; Congdon et al., 1983; Congdon et al., 1987; Obbard and Brooks, 1987; Joanen and McNease, 1989; Iverson and Smith, 1993; but see Christens and Bider, 1987), due to enhanced egg maturation (Wittow and Balazs, 1982; Congdon et al., 1987; Adolph and Porter, 1993). The presence of thermal springs appeared to be associated with *C. insculpta* nesting earlier in some stretches river (Chapter 3). However, in the three years of our study warmer water temperatures in the months prior to nesting did not lead to earlier nesting (Fig. 7.2).

In a review of timing of reproduction in Australian lizards, James and Shine (1985) concluded that there was no clear association between food habits and timing of reproduction. However, they were interested in interspecific patterns at a large temporal scale: breeding during the wet season vs. dry season. At a smaller scale and within a species, the magnitude of the wet season may dictate the initiation of reproduction. If the wet season represents a high resource accumulation period for the turtles, as in many other reptiles in the wet-dry tropics (Madsen and Shine, 1996; Shine and Madsen, 1997; Christian and Green, 1994; Christian et al., 1995; Griffiths and Christian, 1996; Christian et al., 1999a, 1999b), then timing of nesting in *C. insculpta* may be related to energy acquisition during the previous wet season. Although based on only four data years, I found early nesting after two ‘big’ wet seasons (1997, 1998 = record wet seasons for the catchment), contrasting with late nesting following two ‘small’ wet seasons (1986, 1996). Direct evidence of food uptake influencing the laying date is lacking for reptiles. However, indirect evidence exists: sand lizards that oviposited first were those that grew rapidly and were in the best physical condition prior to the breeding season (Olsson and Shine, 1997b), and water snakes fed ad libitum during follicular development gave birth earlier than free-living snakes (Weatherhead et al., 1999). In birds, in years of high food availability lay date advances, findings confirmed by food supplementation experiments (see reviews in Drent and Daan, 1980; Rowe, Ludwig, and Schluter, 1994).

Late nesting for *C. insculpta* can be disadvantageous in two ways. First, later nesting in 1996 resulted in 20 % nest mortality due to early wet season flooding (Fig. 7.7). Second, late nesting led to a female-biased hatchling sex ratio, compared to sex ratios near unity following two big wet seasons in 1997 and 1998 (Fig. 7.9). A concurrent study of reproductive output also found larger clutches of larger eggs following the same two big wet seasons, and smaller clutches of smaller eggs after the same two small wet seasons (Chapter 4).

Thus, the magnitude of the wet season may dictate energy accumulation in *C. insculpta*. The wet season diet of the population is unknown, but continuous flooding of the river channel would generally prevent turtles from feeding on their principal dry season food (aquatic vegetation, *Vallisneria nana*, Heaphy, 1990; Welsh, 1999). A telemetry fix from a low-flying aircraft during wet season flooding in February 97 revealed that turtles were in flooded riparian forest out of the river channel (Chapter 2). Confirmation of the link between wet season magnitude and timing of nesting in *C. insculpta* would require knowledge of the wet season diet, or a study of field energetics.

Another way of determining the importance of timing of reproduction is examining the correlates of lay date within years (Olsson and Shine, 1997b). In *C. insculpta* lay date was positively correlated with egg mass and egg width, and generally negatively correlated with clutch size after big wet seasons (Table 7.1). These associations were not evident after a small wet season (Table 7.1). Although more years are needed for confirmation, it appears that lay date is an important variable in determining the magnitude of success in energy accumulation for reproduction, evidenced by correlates in clutch characteristics.

<u>Nest site choice</u>. - What variables were turtles assessing when choosing a nest site? I was able to experimentally remove a constraint on nest site choice, demonstrating that (1) loose dry sand limits elevation of the nest site (Fig. 7.5), and that (2) turtles will nest at higher elevations if given the chance (Fig. 7.6). The latter assertion is supported by considerable flood mortality of lower elevation nests in 1996, when nesting began later (Fig. 7.8), and by qualitative observations of avoidance of the lowest elevation sites (see Fig. 7.4; unpubl. data). Quantitative data within beaches

indicated that turtles were nesting higher above and farther from water than mean available sites, at least on some beaches (Table 7.3). Although mean heights were similar between nest sites and available sites on other beaches, the average variance around mean available sites (3488) was approx. six times that of nest sites (567). This appears to reflect a ‘sandwich’ effect, whereby nest site choice is constrained by non-cohesive sand at higher elevations and apparently molded by natural selection through flood mortality at lower elevations (Fig. 7.5). The same argument applies to distance of the nest site from water; however, distance from water is likely an artifact of height above water, as turtles would often nest close to water when the beach was steep (see also Doody, 1995).

Annual variation in most nest site attributes, especially in height of the nest site, was unexpected. In theory, turtles should select nest sites with similar elevations among years if selection through flood mortality is operating on the lowest elevation sites. However, the magnitude of the upper limit on nest elevation (cohesive sand availability) could vary annually depending on weather patterns or among-beach variation in factors influencing the height of cohesive sand (e.g., substrate type, shading canopy). Weather patterns during the dry season in this region of northern Australia show very little annual variation (e.g., mean monthly rainfall amount for August = 1 mm). Therefore, I hypothesize that annual variation in nest site attributes reflects different constraints imposed by different beaches. The wet season resets beach location each year such that few individual beaches are available to turtles in consecutive years.

A few studies have demonstrated that turtles actively choose a nest site. For example, the turtle *Chrysemys picta* nested on steeper slopes with warmer aspects than random sites (Schwartzkopf and Brooks, 1987). Mud turtles selected cooler sites with more vegetation than random sites, with apparent consequences in embryonic survival (Wilson, 1996).

Embryonic survival and its determinants. - Embryonic survival was chiefly determined by lay date and nest height (Table 7.5, Table 7.6). Nests taken by predators were at higher elevations than surviving nests, and were laid 13 days later, on average. The main predator, the lizard *Varanus panoptes*, may be less likely to find eggs in nests at lower elevations farther from the vegetation line. I have no explanation why predation was higher in 1996 than in other years. However, this effect may be attributed to chance. If the main nest predator, the lizard *Varanus panoptes*, occupies a home range encompassing a major nesting beach, then all nests on that beach could be destroyed by the same patrolling lizard. Seven nests were destroyed by *V. panoptes* on a single beach in 1996.

In 1996, flood mortality occurred in 20 % of nests laid at low elevations late in the season (Fig. 7.8). This mortality was due to later timing of nesting in 1996: in 1997 and 1998 earlier nesting led to earlier hatching or emergence, and no nests were flooded. Timing of the first wet season flooding was similar among years, with river rises (> 1 m) beginning in mid- to late November. Historical river stage data also reveal low annual variation in timing of early wet season flooding over several decades (Chapter 6). Thus, late nesting will likely result in embryonic mortality of nests laid later at the lowest elevations.

Flooding is a common source of embryonic mortality in riverine turtle populations (Roze, 1964; Plummer, 1976; Doody, 1995). Although riverine turtles can seemingly respond through selection for the behavior of nesting at higher elevations, mortality occurs when (1) all available nest sites are flooded in extreme years (e.g., Plummer, 1976; Doody, 1995), or when (2) incubation extends into a wet season (present study, Alho and Padua, 1982; see also Webb et al., 1983; Webb and Smith, 1984 for data on crocodiles). Height of nest sites influenced embryonic mortality in the sea turtles *Dermochelys coriacea* and *Chelonia mydas* (Whitmore and Dutton, 1985). Nests of those species laid below the spring high tide line were more likely to flood during extreme high tide washovers. To this end *Malaclemys terrapin* nested during periods of high tide, possibly as an adaptation to reduce the possibility of nest flooding (Burger and Montevecchi, 1975).

If late nesting during 1996 was related to an energy accumulation limitation, then temporal placement of the reproductive season may reflect a left tail shaped by phenotypic plasticity (energy accumulation constraint), and a right tail maintained by natural selection via flood mortality of nests. Under this scenario, the onset of nesting would vary among years, while the right tail would likely represent a ‘hard line’, based on the average timing of flood mortality among years (Chapter 6). I am unaware of any previous studies on turtles demonstrating that timing of nesting or lay date influences embryonic mortality through nest flooding.

Determinants of nest temperatures and hatchling sex. - The distribution of nest temperatures in the our study was unimodal (Fig. 7.11) much like that found for the turtle *Chrysemys picta* (Schwartzkopf and Brooks, 1987), but different from the bimodal distribution found for two *Graptemys* species (Vogt and Bull, 1984). Bulmer and Bull (1982) predicted that a unimodal distribution would approach bimodality in a system with annual variation in nest temperatures and offspring sex ratios rarely are 1:1. I did find sex ratios of near 1:1 in two of three years, and this may explain the persistence of a unimodal distribution of nest temperatures in *C. insculpta*.

The pattern of sex determination as evidenced by data from natural nests was cooler temperatures producing males, intermediate temperatures (around 32 C) producing mixed sexes, and hotter temperatures producing females (Fig. 7.11). These data confirmed a type 1a pattern of TSD (Ewert and Nelson, 1991), as suggested previously for the species (Webb et al., 1986; Georges, 1992). The range of temperatures producing mixed sexes within a nest was about 1.5 °C (31.1-32.7 °C, CTE). In comparison to other species this pivotal temperature range is narrow but falls within ranges determined in the laboratory (Vogt and Flores-Villela, 1992; Viets et al., 1993; Lang and Andrews, 1994; Mrosovsky and Yntema, 1980).

This temporal pattern of sex production is attributable to a seasonal increase in nest temperatures (CTE's). In turn, the seasonal increase in nest temperatures corresponded with a seasonal increase in sand and air temperatures, which also decreases incubation period (Chapter 6).

Discriminant function analysis indicated that lay date was the most important determinant of hatchling sex (Table 7.5). Lay date explained 78 % of the variation in nest temperatures (CTE's, Fig. 7.7). Among years, a female-biased hatchling sex ratio in 1996 was attributable to later nesting (Fig. 7.9; Fig. 7.10). Hatchling sex ratios were near unity in years with earlier nesting (1997, 1998, Fig. 7.9; Fig. 7.10). Within years, mostly males were produced in early (first) clutches, whereas mainly females were produced in later (second) clutches. This was owed to a seasonal increase in air and sand temperatures during nesting and incubation (Fig. 7.10), which also decreases incubation period (Chapter 6). Seasonal trends in hatchling sex ratios are known in a few reptile species (Vogt and Bull, 1984; Webb and Smith, 1984; Mrosovsky et al., 1984, 1985; Mrosovsky, 1994; Harlow and Taylor, 2000), and are likely to be found for more species in future studies. At least one study had found no effect of lay date on hatchling sex ratios (Spotila et al., 1987). I agree with Shine (1999) that a seasonal pattern in sex production is likely to be common in tropical reptiles with TSD (i.e., in species with extended nesting seasons). However, such data are currently scarce for tropical TSD species (but see Webb and Shine, 1984).

Few studies have investigated the influence of nest site choice on hatchling sex ratios. The major spatial determinant of sex ratios in those studies was the extent of shading vegetation, although this was not quantified for individual nest sites (Bull and Vogt, 1979; Morreale et al., 1982; Vogt and Bull, 1984; Spotila et al., 1987). Hatchling sex ratios can also be influenced by nest site attributes such as aspect and solar exposure (Janzen, 1994). Conversely, Schwartzopf and Brooks (1987) found low correlation between nest site attributes and sex ratios in *Chrysemys picta*. In the present study nest site choice exerted an influence on hatchling sex ratios. Female-producing nests were constructed at higher elevations than male-producing nests (Table 7.5). For example, in several cases adjacent nests with similar lay dates but with different heights above water produced different sexes (Table 7.7). This usually occurred when nest temperatures cycled around the pivotal temperature during the middle portion of the nesting season. In contrast, nests laid early in the season produced mainly males, while nests laid late in the season produced mostly females, regardless of elevation (Fig. 7.9). Although no previous study has found an influence of nest site elevation on offspring sex ratios, temperatures in sea turtle (*Caretta caretta*) nests increased with an increase in distance from water (Foley et al., 2000), a likely artifact of nest elevation (Doody, 1995).

Burger (1976b) found that nest depth influenced embryonic survival; eggs failed to hatch from shallow depths of nests in sunny areas. Mud turtles, which dig shallow nests due to small body size, are apparently forced to nest in shaded microhabitats to ensure eggs don’t get too hot (Wilson, 1996). I found no influence of depth on sex ratios (Vogt and Bull, 1982) or on embryonic survival.

Fit of models for the evolution of TSD to *C. insculpta*. - Shine (1999) reviewed and clarified six ‘differential fitness’ models attempting to explain the evolution of TSD in reptiles. Each of these models proposes that TSD can enhance maternal fitness by enabling the embryo to develop as the sex best-suited to those incubation conditions. Each model posits links among incubation temperature, phenotype, and fitness. The models differ, however, in mechanisms generating phenotypic variance (Shine, 1999). For example, the mechanism for the ‘size-matching’ model is egg size, while that of the ‘nest philopatry’ model and ‘phenotype-matching’ model involves incubation temperatures. The model that best fits the *C. insculpta* system is the ‘time-matching’ model, whereby phenotypic variation is driven by sex ratio adjustment relative to date of hatching (Conover and Kynard, 1981; Conover, 1984; Shine, 1999).

In *C. insculpta*, males were produced early and females late, although a reduction in incubation period due to seasonal ramping of air temperatures, and embryonic aestivation (Chapter 6) partly compensates for this differential sex production in time. Two to three weeks separated the mean hatching or emergence dates of males and females. There may be a fitness advantage in producing males early in *C. insculpta*, as hypothesized for the lizard *Amphibolurus muricatus* (Harlow and Taylor, 2000). In the fish *Menidia menidia*, a size-induced increase in fitness is higher in females than in males, favouring the early production of females (Conover and Kynard, 1981; Conover, 1984). However, in both the fish and the lizard, maturation occurs in one season (Conover and Kynard, 1981; Harlow and Taylor, 2000), and thus, incubation temperatures may be linked to temperatures during growth

(Conover, 1984). Although maturation time in *C. insculpta* is unknown, long-lived animals such as turtles take a minimum of several years to mature (Gibbons, 1987; Iverson, 1991; Shine and Iverson, 1995). This means that (1) incubation temperatures cannot predict temperatures during growth and maturation, (2) a 2-3 week growth advantage is less likely to translate into adult fitness, and (3) establishing links among incubation temperature, phenotype, and fitness, relative to sex, will likely be difficult. I should also note that covariation between hatching date and nest temperatures is likely to be common in reptile species laying multiple clutches (Shine, 1999; Harlow and Taylor, 2000), and so differences in hatching dates between sexes is not necessarily evidence for the ‘time-matching’ model.

<u>Conclusions</u>. - Considerable annual variation timing of nesting and its consequences in embryonic survival and sex production both within and among years in *C. insculpta* highlights the importance of considering timing of reproduction in studies of reproductive tactics (Olsson and Shine, 1997b). I also suggest that attempts be made to link timing of nesting with environmental variation. Because selection has presumably already shaped the temporal placement of the nesting season, annual variation in weather patterns would be necessary to reveal how the tails of that distribution are maintained. In the present study I was fortunate to have 4 years of data following periods of rainfall patterns at both extremes. This variation gave us insights into how the reproductive biology of *C. insculpta* is shaped by the wet-dry tropics. My results combined with those of a companion study (Chapter 4) led me to hypothesize that the wet season is critical for reproduction in *C. insculpta,* and that big wet seasons are particularly beneficial.

The evolution of TSD introduces an additional problem, achieving balanced sex ratios, into the ecology of a species. Previous work indicates that understanding the reproductive biology in a system is crucial to finding the reason for why TSD evolved in that system (Conover, 1984; Roosenberg, 1996). Although our knowledge of the reproductive biology has not yet revealed why TSD occurs in *C. insculpta*, our research provides a framework for pursuing that question, and will no doubt help to reduce the number of competing explanations for an increasingly enigmatic phenomenon (Shine, 1999; Harlow and Taylor, 2000).

CHAPTER 8: CAN TURTLES PREDICT OFFSPRING SEX?

Can nesting turtles predict offspring sex?

(prepared for submission to Ecology)

J. S. Doody and A. Georges

INTRODUCTION

In the last two decades the number of animal species thought to possess environmental sex determination (ESD) has increased from a handful to over a hundred (see reviews in Ewert and Nelson, 1991; Viets et al., 1994; Lang and Andrews, 1994; Strussmann and Patino, 1995). Accordingly, during that period empirical evidence has chased theoretical efforts to understand why ESD evolves (Shine, 1999). Yet, at the turn of the century the evolutionary ecology of ESD is poorly understood. Recent work is encouraging, but putative reasons for its evolution remain controversial (reviewed in Shine, 1999).

In reptiles, the most popular adaptive explanations for the evolution of temperature-dependent sex determination (TSD) are the ‘differential fitness’ models (reviewed in Shine, 1999). These models hypothesize that if incubation temperatures differentially affect the fitness of male and female offspring, TSD can enhance maternal fitness by enabling the embryo to develop as the sex best-suited to those incubation conditions (Charnov and Bull, 1977). The models rest upon a central assumption that mothers have little or no control over offspring sex. An exception is a recent model for the evolution or maintenance of TSD in a turtle, whereby mothers apparently matched their maternal condition with nest site, thereby producing the ‘optimum sex’. In good years *Malaclemys terrapin* mothers deposited larger eggs into cooler female-producing sites, but in poor years smaller eggs were deposited in warmer male-producing sites (Roosenburg, 1996). Thus, according to Roosenburg (1996), mothers were able to predict or manipulate offspring sex through nest site choice. The recent discovery of TSD in a viviparous lizard further increases the likelihood that mothers could manipulate offspring sex (Robert and Thompson, 2001).

In order for oviparous reptilian mothers with TSD to predict or manipulate offspring sex I hypothesized that the following four physical and behavioral phenomena should hold: (1) a strong correlation must exist between assessable substrate temperatures during nest site choice and nest temperatures during the sex-determining period of development (thermosensitive period, hereafter TSP). This is based on the assumption that temperature is the sole mechanism in determining offspring sex (see Hewavisenthi and Parmenter, 2000). (2) Assuming that (1) is possible, mothers would need to be capable of correcting for temporal factors obscuring the predictable thermal characteristics of nest sites. This could be accomplished in two ways. By contracting nesting times mothers could assess the relative temperatures of alternate nest sites with some accuracy. A protracted distribution of nesting times could greatly reduce a mother’s ability to distinguish between, for example, a cooler nest site at a warmer time and a warmer nest site at a cooler time. Both sites may be 30 C upon her appraisal, but if she assesses them at different times they are likely to have very different thermal profiles, due to diel changes in substrate temperatures (Georges, 1992). Alternatively, mothers would need to be able to assess temporal changes in nest site temperatures. (3) Sufficient variation in thermal profiles *among* nest sites, relative to the breadth of temperatures producing both sexes (pivotal temperatures), would be necessary. For example, if most nests produced both sexes, then depth of the eggs would be the deciding factor determining sex, leaving little opportunity for nest site choice to produce one sex or the other. (4) Mothers would need access to nest sites spanning a range of thermal profiles in order to produce either offspring sex. To this end, home range size relative to the number and location of nesting beaches should be important.

Although support for these predictions would not ultimately demonstrate a mother's ability to manipulate offspring sex, their rejection would cast doubt on such maternal control. Currently, support for predictions (1) and (2) is lacking. No study has linked TSP temperatures with temperatures during nest site choice in reptiles. Although a few studies have quantified nesting times (e.g., reviewed in Pritchard and Trebbau, 1984), none have examined diel variation in nesting times within the context of assessing temperatures and subsequent sex determination. Prediction (3) seems to have general support in turtles, with many species producing a majority of unisexual nests (Ewert and Nelson, 1991). Prediction (4) has not been examined explicitly through movement studies. However, evidence of the spatial scope for controlling offspring sex is reflected in the production of either sex in the same general area (Vogt and Bull, 1984; Schwartzkopf and Brooks, 1987; Janzen, 1994). Roosenberg (1996) documented that *Malaclemys* mothers had the option of producing a particular offspring sex by nesting on male-producing or female-producing beaches. Studies on captive lizards with TSD have demonstrated that mothers select nest sites based on temperature (e.g., Bull et al., 1988; Bragg et al., 2000), but no other study on a TSD species has explicitly addressed the question of predicting offspring sex.

I tested these four predictions using *Carettochelys insculpta,* a beach nesting turtle with TSD. During a three-year field study I quantified nest site choice, nesting times, thermal characteristics of nests, hatchling sex ratios, and movements of nesting female *C. insculpta*. Preliminary analyses indicated that, like *M. terrapin*

(Roosenberg, 1996), *C. insculpta* deposited larger eggs in female-producing nest sites and smaller eggs in male-producing sites. I use my field data, both published and unpublished, in tandem with laboratory determination of the TSP, to assess the ability of *C. insculpta* to predict or manipulate offspring sex, a central problem in adaptive models for ESD in reptiles.

MATERIALS AND METHODS

To examine the four predictions underlying the ability to predict offspring sex, I needed the following data: temperatures at nesting, temperatures during the TSP, and predicted and observed hatchling sex ratios. To obtain sand temperatures at the actual diel time of nesting, I needed to know when the turtles nested. To determine nesting times I used remote camera systems employed on beaches near the water’s edge (Doody and Georges, 2000; Chapter 6). A turtle exiting the water to search for a nest site would cross an infra-red beam, triggering a camera mounted overhead. Daily searches for nests on these beaches, combined with examination of resulting photographs allowed determination of exact timing of nesting. From this timing data I could then determine air, water, and sand temperatures. Air and water temperatures were gleaned from a data logger employed in the study area.

Sand and nest temperatures were in 102 nests with either Datataker® DT500 multi-channel dataloggers or Stowaway® single-channel dataloggers. Temperatures were recorded at 15 min intervals by the Datataker dataloggers and at 1 h intervals using the Stowaway dataloggers. Typically, three temperature probes were fitted to each nest: one immediately below the deepest egg, one in the core of the nest, and one immediately above the shallowest egg. Three probes were used to facilitate a study modeling the relationship between fluctuating temperatures and hatchling sex (Georges et al., unpubl. data). When Stowaway dataloggers were used, often only core temperatures were recorded. The probes were fitted as soon as possible after discovery of the nest, usually within 1–2 days. The depth of each egg was measured before its removal and eggs were returned to their original positions and orientations after deployment of datalogger probes.

Sand temperatures at the time of nesting were estimated, because dataloggers were not employed into nests until 1-2 days after nesting. This was accomplished by backfilling temperature traces to the nesting date using temperature traces from other nests. Strong predictive relationships can be established between temperature traces from different nests. Values in the response trace can be predicted from values in a second predictor trace by (1) bringing the predictor trace into phase with the response trace with a translational shift, (2) decomposing both traces into a trend line and stationary time series, (3) developing predictive relationships between the two trend lines and the two stationary series using simple linear regression, (4) using these relationships to predict missing values in the response trend and stationary series, and (5) reconstructing the response trace, with missing values added. Validation using known data deleted from response traces indicated that thus approach was very accurate.

To obtain nest temperatures during the sex-determining period, I needed to know when the TSP occurred. The TSP for *C. insculpta* was experimentally determined to be the middle third of development (unpubl. data), matching other studies on turtles (Bull, 1987; Mrosovsky and Pieau, 1991). I then needed to demarcate the middle third of development on each nest temperature trace. This was done using a mathematical model that determines the cumulative proportion of development incurred by embryos each day (Georges et al., unpubl. data). The model also calculates the constant temperature equivalent (CTE, Georges, 1989; Georges et al., 1994) for each day from the fluctuating trace. This value is the only accurate predictor of offspring sex (Georges et al., 1994). Because it is not known precisely when sex is determined within the TSP (i.e., cumulatively or instantaneously), I averaged the daily CTE's throughout the TSP for each temperature trace (nest). Thus, I obtained one temperature for predicting hatchling sex, which could then be compared to the observed hatchling sexes for each nest.

Hatchling sex was determined in the field. When a nest contained eggs that were near-term, as estimated by knowledge of lay date and previous incubation data (Georges, unpubl. data), I removed the clutch and housed it in a makeshift field laboratory until hatching. In this way I could isolate eggs to facilitate determination of sex in relation to depth in the nest. In 1996 and 1997 I obtained hatching dates in the field laboratory. In 1998 I obtained actual emergence dates in the field using remote camera systems set up on the nests (Doody and Georges, 2000; Chapter 6). Hatchlings were measured, weighed, and killed with intercranial injection of pentobarbital or ethanol. In 1996 all hatchlings were sacrificed, thereby obtaining the sex of every hatchling, while in 1997-1998 I only killed 1-2 hatchlings from each nest to determine whether a nest contained all males, all females, or mixed sexes. For example, if the hatchling from a top egg in the nest was found to be a male, then all eggs in the nest were deemed to be males, because deeper eggs are cooler (Burger, 1976b; Wilhoft et al., 1983), and cooler *C. insculpta* eggs become males (Georges, 1992).

RESULTS

Sex differences in egg size. - Eggs producing female hatchling *C. insculpta* were generally larger than eggs producing males (Table 8.1). However, the difference is marginally significant, and when broken down by year, is not apparent in 1996 and insignificant in 1998 (Table 8.1). In addition, in some cases sample sizes are small when the data are treated separately for each year (Table 8.1).

Predicting TSP temperatures and offspring sex during nest site choice. - Table 8.2 lists the sand temperature during nest site choice, the beginning and end of the TSP, the mean daily CTE during the TSP, and predicted and observed hatchling sexes, for each nest. Nest temperatures during the thermosensitive period (TSP), expressed as constant temperature equivalents (CTE's), were significantly positively related to sand temperatures at the time of nesting in all three years (1996, $r^2 = 0.736$, $F_{1,27} = 72.64$, $p < 0.001$; 1997, $r^2 = 0.555$, $F_{1,27} = 32.47$, $p < 0.001$; 1998, $r^2 = 0.189$, $F_{1,43} = 9.82$, $p = 0.003$) (Fig. 8.1).

In determining the predictability of hatchling sex during nest site choice, I treated the data two ways. First, I included mixed-sex nests, meaning that many predictions were 'half correct'. For example, sand temperatures at nesting may have predicted a nest to produce all males when mixed sexes were actually produced. The proportion in this treatment is the number of correct predictions over the total number of possible correct. Resulting proportions were 85.7 % (1996), 60.7 % (1997), and 47.7 % (1998). In a second treatment of the data I excluded mixed-sex nests from the calculation, thereby forcing each prediction to be fully correct or fully incorrect. This proportion is defined as the number of correct

Table 8.1. Egg mass compared between eggs producing male and those producing female hatchling *C. insculpta*. Data are grand means, significance is based on single factor ANOVA, and sample sizes in parentheses represent number of clutches in sample.

Year	males	females	significance
All	34.7 ± 0.31 (43)	35.6 ± 0.29 (60)	$F_{1,102} = 4.08$, $p = 0.046$*
1996	33.7 ± 0.73 (9)	34.3 ± 0.53 (21)	$F_{1,29} = 0.41$, $p = 0.560$
1997	34.2 ± 0.64 (13)	36.4 ± 0.45 (14)	$F_{1,26} = 7.55$, $p = 0.011$*
1998	35.5 ± 0.35 (21)	36.3 ± 0.40 (25)	$F_{1,45} = 2.22$, $p = 0.143$

Table 8.2. Nests, their beaches, lay dates, developmental and thermal profiles, and hatchling sexes (predicted and actual). The TSP is the middle third of development, and was determined using a developmental model. Temperatures include the sand temperature at the nest site during nest site choice, and the mean daily CTE (constant temperature equivalent) during the TSP (thermosensitive period). Hatchling sexes are those predicted by the sand temperature at the nest site during nest site choice, those predicted by the mean daily CTE during the TSP, and the actual sex determined by histological examination. m = all male nest, f = all female nest, x = mixed sexes.

year	nest #	date laid	temp. at nesting	TSP start	TSP end	CTE (TSP)	predicted sex (nesting)	predicted sex (TSP)	actual sex
1996	3	21/08/96	26.5	4-Oct	20-Oct	31.41	m	m	m
1996	5	26/08/96	32.0	19-Sep	4-Oct	31.77	x	x	x
1996	6	28/08/96	32.1	23-Sep	11-Oct	31.34	x	m	x
1996	7	29/08/96	31.7	18-Sep	3-Oct	31.82	x	x	x
1996	8	29/08/96	32.4	20-Sep	6-Oct	31.77	x	x	x
1996	9	29/08/96	31.8	23-Sep	11-Oct	31.07	x	m	m
1996	10	29/08/96	32.3	25-Sep	13-Oct	31.60	x	x	x
1996	13	31/08/96	31.4	29-Sep	17-Oct	30.65	m	m	m
1996	14	31/08/96	33.0	19-Sep	6-Oct	32.31	f	x	x
1996	15	30/08/96	32.2	19-Sep	5-Oct	31.93	x	x	x
1996	18	31/08/96	35.1	15-Sep	30-Sep	33.44	f	f	x
1996	25	11/09/96	31.5	30-Sep	17-Oct	31.33	m	m	m
1996	31	24/09/96	34.8	7-Oct	20-Oct	33.71	f	f	f
1996	34	30/09/96	32.3	15-Oct	31-Oct	31.80	x	x	m
1996	37	3/10/96	33.3	27-Sep	14-Oct	31.12	f	m	m
1996	39	4/10/96	36.3	17-Oct	31-Oct	33.50	f	f	f
1996	41	4/10/96	37.0	17-Oct	31-Oct	33.91	f	f	f
1996	42	2/10/96	37.1	16-Oct	30-Oct	33.97	f	f	f
1996	44	30/09/96	36.2	15-Oct	30-Oct	33.89	f	f	f
1996	45	5/10/96	35.4	20-Oct	3-Nov	32.96	f	f	f
1996	47	5/10/96	36.9	19-Oct	3-Nov	34.11	f	f	f
1996	48	7/10/96	36.1	20-Oct	3-Nov	33.30	f	f	f
1996	49	5/10/96	36.3	19-Oct	2-Nov	34.05	f	f	f
1996	51	9/10/96	33.5	22-Oct	5-Nov	32.63	f	f	f

Table 8.2 Continued.

1996	52	8/10/96	33.9	21-Oct	3-Nov	33.51	f	f	f
1996	56	11/10/96	36.7	25-Oct	9-Nov	33.69	f	f	f
1996	58	10/10/96	34.5	23-Oct	6-Nov	33.39	f	f	f
1996	59	13/10/96	36.8	27-Oct	11-Nov	33.89	f	f	f
1997	2	21/07/97	25.1	26-Aug	27-Sep	31.36	m	m	x
1997	4	23/07/97	27.8	27-Aug	17-Sep	29.68	m	m	m
1997	5	25/07/97	28.0	29-Aug	18-Sep	30.16	m	m	x
1997	6	25/07/97	27.2	28-Aug	19-Sep	31.22	m	m	x
1997	9	24/07/97	28.8	18-Aug	5-Sep	31.29	m	m	x
1997	10	23/07/97	27.7	18-Aug	6-Sep	31.13	m	m	m
1997	11	23/07/97	28.8	15-Aug	6-Sep	32.21	m	x	x
1997	12	20/07/97	30.0	11-Aug	27-Aug	32.37	m	x	x
1997	15	30/07/97	31.1	22-Aug	7-Sep	31.91	m	x	m
1997	16	5/08/97	30.3	28-Aug	14-Sep	31.22	m	m	m
1997	17	6/08/97	30.6	27-Aug	14-Sep	31.60	m	x	m
1997	18	9/08/97	30.3	5-Sep	23-Sep	30.82	m	m	m
1997	25	29/08/97	29.1	18-Sep	6-Oct	31.42	m	m	f
1997	27	5/09/97	31.1	27-Sep	15-Oct	31.00	m	m	m
1997	29	3/09/97	32.7	20-Sep	4-Oct	32.55	f	f	f
1997	30	7/09/97	35.1	26-Sep	12-Oct	32.59	f	f	f
1997	31	9/09/97	35.9	25-Sep	13-Oct	33.51	f	f	f
1997	32	9/09/97	32.1	28-Sep	14-Oct	31.56	x	x	x
1997	34	10/09/97	32.7	1-Oct	19-Oct	31.77	f	x	f
1997	35	11/09/97	33.9	27-Sep	13-Oct	31.70	f	x	f
1997	38	12/09/97	31.5	29-Sep	16-Oct	31.90	x	x	f
1997	40	16/09/97	34.2	2-Oct	18-Oct	33.75	f	f	f
1997	41	16/09/97	32.0	7-Oct	25-Oct	32.74	x	f	f
1997	42	18/09/97	31.6	26-Oct	10-Nov	31.51	x	x	f
1997	43	18/09/97	33.8	6-Oct	23-Oct	33.56	f	f	f
1997	44	19/09/97	33.0	9-Oct	28-Oct	32.66	f	f	f
1997	45	21/09/97	35.4	10-Oct	25-Oct	34.67	f	f	f
1997	46	20/09/97	35.1	16-Oct	6-Nov	32.60	f	f	x
1998	2	12/07/98	29.5	31-Jul	19-Aug	31.40	m	m	x
1998	3	12/07/98	28.0	6-Aug	28-Aug	29.91	m	m	m
1998	4	14/07/98	29.1	16-Aug	9-Sep	29.68	m	m	m

Table 8.2 Continued.

1998	7	15/07/98	27.1	28-Aug	22-Sep	29.23	m	m	m
1998	9	18/07/98	31.3	19-Aug	13-Sep	30.12	m	m	m
1998	11	18/07/98	31.5	7-Aug	26-Aug	30.42	x	m	m
1998	13	19/07/98	31.4	10-Aug	4-Sep	28.50	m	m	m
1998	14	19/07/98	32.2	9-Aug	28-Aug	31.43	x	x	x
1998	15	19/07/98	32.6	9-Aug	30-Aug	30.69	f	m	m
1998	16	19/07/98	32.0	9-Aug	29-Aug	31.22	x	x	x
1998	17	19/07/98	32.6	8-Aug	27-Aug	31.42	f	m	m
1998	18	22/07/98	31.2	15-Aug	7-Sep	29.63	m	m	m
1998	21	22/07/98	30.2	20-Aug	12-Sep	30.67	m	m	m
1998	24	2/08/98	29.6	24-Aug	12-Sep	30.96	m	m	m
1998	25	8/08/98	28.7	17-Sep	5-Oct	31.51	m	x	f
1998	27	6/08/98	32.5	22-Aug	7-Sep	32.24	x	x	x
1998	29	2/08/98	27.0	30-Aug	19-Sep	31.94	m	x	m
1998	30	2/08/98	27.8	29-Aug	18-Sep	31.70	m	x	x
1998	31	2/08/98	28.0	28-Aug	17-Sep	31.19	m	m	x
1998	32	5/08/98	30.4	26-Aug	15-Sep	30.74	m	m	m
1998	33	21/08/98	30.3	16-Sep	5-Oct	30.99	m	m	x
1998	34	21/08/98	31.4	15-Sep	3-Oct	31.88	m	x	f
1998	35	22/08/98	30.8	18-Sep	6-Oct	30.97	m	m	f
1998	36	23/08/98	31.4	21-Sep	10-Oct	31.08	m	m	m
1998	37	24/08/98	32.3	13-Sep	29-Sep	31.93	x	x	m
1998	38	24/08/98	32.6	20-Sep	8-Oct	32.58	f	f	f
1998	39	24/08/98	30.8	21-Sep	7-Oct	31.96	m	x	f
1998	41	23/08/98	31.7	18-Sep	6-Oct	31.21	x	m	x
1998	43	21/08/98	31.3	12-Sep	29-Sep	31.81	m	x	x
1998	44	30/08/98	31.3	16-Sep	2-Oct	32.77	m	f	f
1998	47	31/08/98	34.6	14-Sep	1-Oct	32.84	f	f	f
1998	48	1/09/98	31.1	20-Sep	7-Oct	31.63	m	f	f
1998	49	1/09/98	31.2	21-Sep	8-Oct	32.24	m	x	f
1998	50	3/09/98	32.7	19-Sep	5-Oct	32.19	f	x	f
1998	51	2/09/98	29.7	20-Sep	7-Oct	32.43	m	x	f
1998	52	2/09/98	30.1	21-Sep	8-Oct	32.00	m	x	f
1998	53	3/09/98	32.2	19-Sep	7-Oct	33.06	x	f	f
1998	55	3/09/98	32.0	20-Sep	8-Oct	32.69	x	f	f

Table 8.2 Continued.

1998	56	4/09/98	32.7	19-Sep	4-Oct	32.95	f	f	f
1998	57	4/09/98	31.5	20-Sep	7-Oct	32.27	x	x	f
1998	58	5/09/98	30.4	25-Sep	11-Oct	32.29	m	x	f
1998	60	6/09/98	34.1	22-Sep	9-Oct	32.64	f	f	f
1998	64	9/09/98	30.9	29-Sep	14-Oct	32.20	m	x	f
1998	70	15/09/98	33.9	30-Sep	14-Oct	33.02	f	f	f

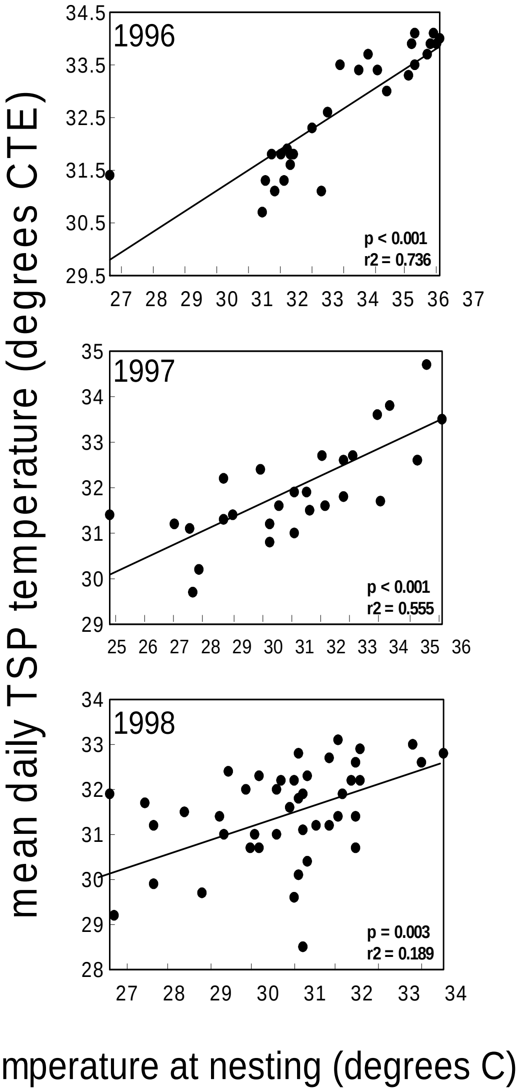
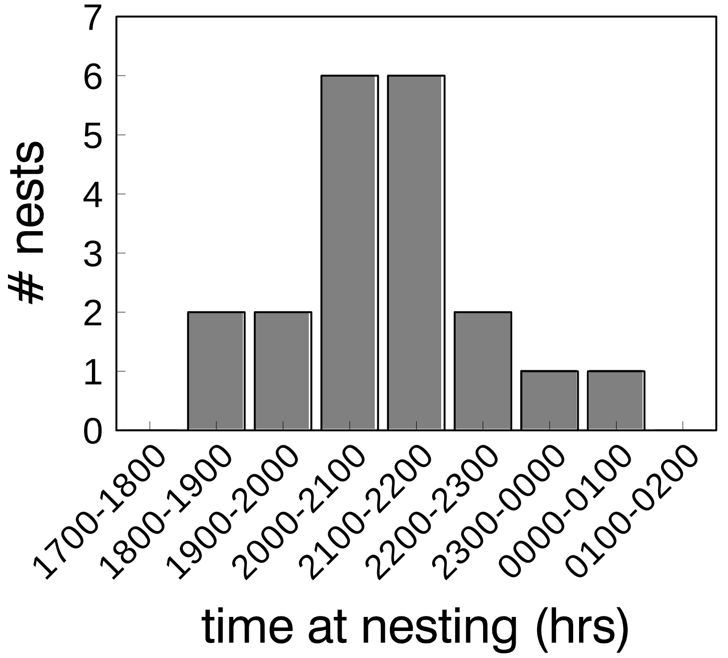


Fig. 8.1. Relationship between substrate temperatures of nest sites at nesting and the mean daily temperature during the thermosensitive period (TSP). The TSP temperatures were calculated as the mean of constant temperature equivalents (CTE's) for each day. CTE's were generated using a model that determined the proportion of development accrued each day in *C. insculpta* embryos. See text for explanation of how the CTE was calculated.



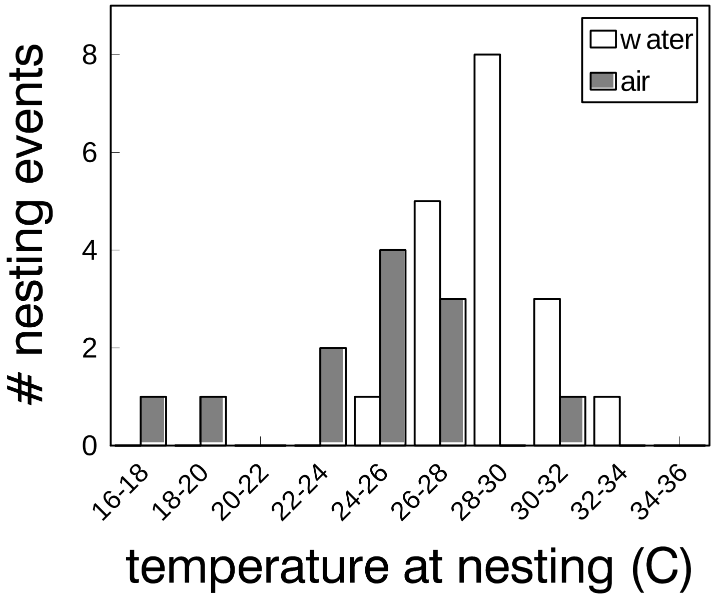


Fig. 8.2. Time of nesting events for 20 *C. insculpta* (top graph), and substrate temperatures at those times (bottom graph). Most times were obtained using remote camera systems employed on nesting beaches. A few times represented actual observations. Water and air temperatures were recorded by a data logger employed in the study area.

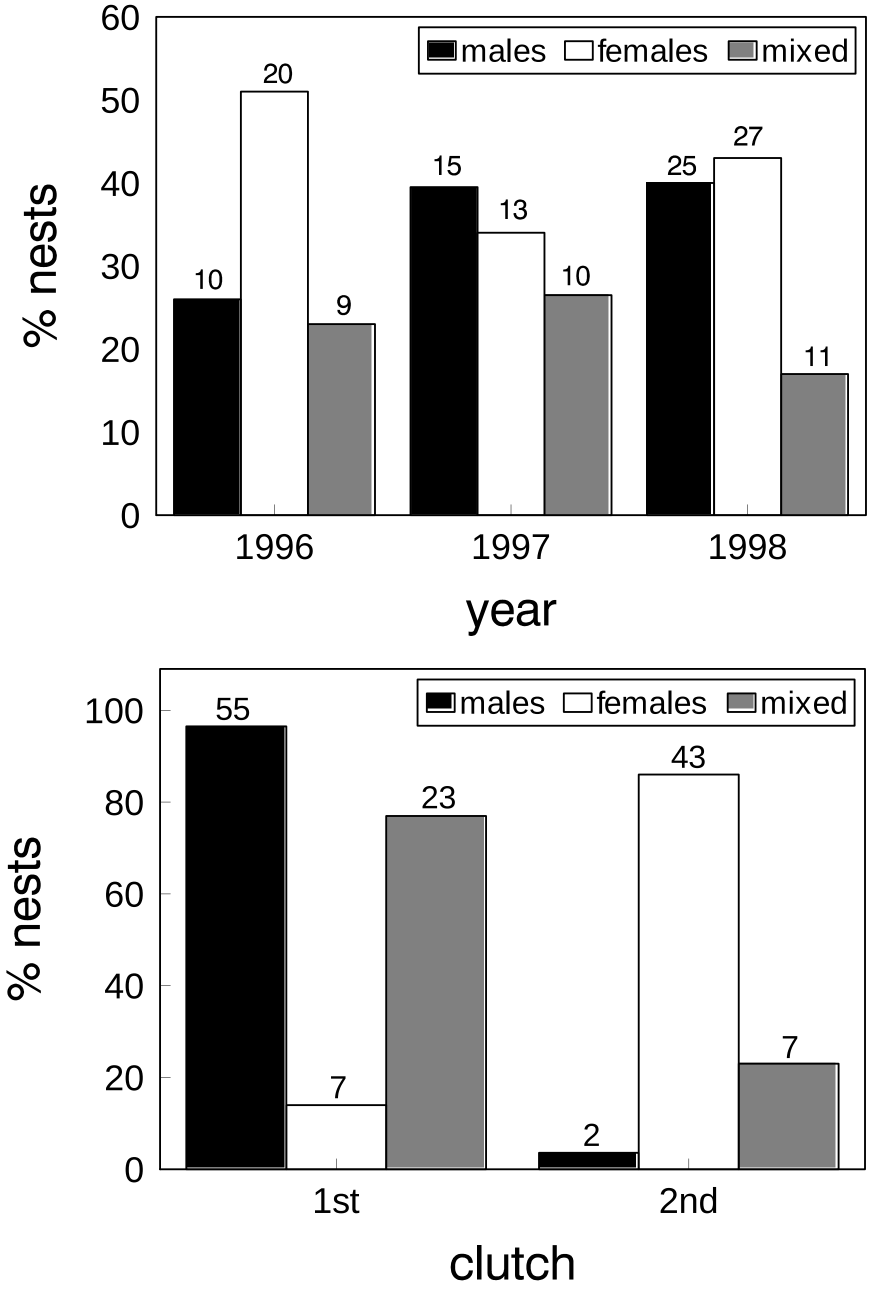


Fig. 8.3. Annual variation in hatchling sex ratios of *C. insculpta* nests during 1996-1998 (top graph). Mainly males are produced in the first clutch and mostly females produced in the second clutch (bottom graph). Samples sizes (number of nests) are presented above bars. Hatchling sex production among years in *C. insculpta*. Number of nests is indicated above bars.

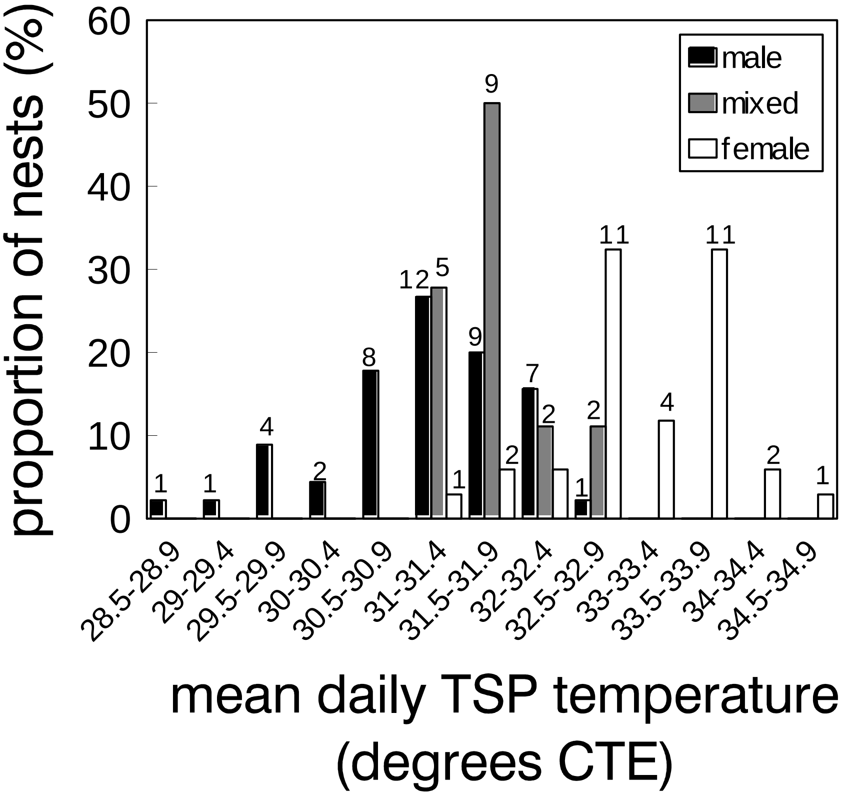


Fig. 8.4. Hatchling sex production in *C. insculpta*, as a function of the mean daily temperature during the thermosensitive period (TSP). Temperature data are constant temperature equivalents (CTE's – see text for explanation). The TSP was found to occur during the middle third of development in a concurrent laboratory experiment (unpubl. data).

predictions over the total possible. Resulting proportions were 95.8 % (1996), 94.4 % (1997), and 61.8 % (1998).

Nesting times and temperatures. - Nesting times were normally distributed (Fig. 8.2) and centered around 1900 hrs (ξ = 21:01 ± 86 min SD**,** N=20 turtles). Air and water temperatures during those nesting events are shown in Fig. 8.2. Air temperatures at nesting were lower than water temperatures (Fig. 8.2).

Among nest variation in temperatures and sex production. - Hatchling sex was determined in 140 nests (1996, N = 39; 1997, N = 38; 1998, N = 63). In total, there were 110 (79 %) unisexual nests and 30 (21 %) mixed-sex nests (Fig. 8.3). In a given year 17-26 % of nests contained both males and females. In all three years, mainly males were produced in the first clutch and mostly females were produced in the second clutch (Fig. 8.3).

The distribution of sexes produced in field nests as a function of mean daily temperature during the thermosensitive period, expressed as constant temperature equivalents, is given in Figure 8.4. Males were produced at cooler temperatures (ξ = 31.2 ± 0.92 °C SD; range = 28.5-32.8 °C, N = 45), mixed sexes resulted from intermediate temperatures (ξ = 31.8 ± 0.46 °C SD; range = 31.1-32.7 °C, N = 19), and females were produced at hotter temperatures (ξ = 33.1 ± 0.75 °C SD; range = 31.4-34.7, N = 35).

DISCUSSION

In our study population, females hatched from larger eggs than did males, on average (Table 8.1). These findings were similar to those of Roosenberg (1996), who found that in ‘good’ years the turtle *Malaclemys terrapin* laid larger eggs on female-producing (hotter) beaches, while in ‘poor’ years smaller eggs were laid on male-producing (cooler) beaches. Roosenberg (1996) interpreted this pattern as mothers exhibiting some control over which offspring sex is produced. However, in the present study the pattern of larger eggs producing females was a result of (1) larger but fewer eggs being produced in later (second) clutches (Table 4.2, Chapter 4), and (2) the production of mostly males in early (first) clutches and mainly females in late (second) clutches, due to a seasonal increase in air (and thus, nest) temperatures (Fig. 8.3). I do not know if these mechanisms would explain the pattern in *Malaclemys*, as Roosenberg (1996) does not include information on clutch number and any seasonal component of sex production.

The results of this study indicate that *C. insculpta* mothers would have difficulty predicting or manipulating offspring sex. This question was first proposed by Roosenberg (1996), who found evidence that in good years diamondback terrapin mothers deposited larger eggs in female-producing beaches, while in poor years smaller eggs were laid on male-producing beaches. Although *C. insculpta* eggs that became females were larger than those that became males, this was an artifact of two seasonal effects: (1) the influence of season on sex production whereby mainly males were produced in the year’s first clutch and mostly females were produced in the second clutch. (2) the seasonal changes in clutch and egg size whereby turtles laid a smaller clutch of larger eggs in the second nesting. Thus, clutch number (first vs. second) was the ultimate cause of the egg size differences between male and female clutches.

The recent finding of TSD in the viviparous lizard *Eulamprus tympanum* widened the possibility of sex production being under maternal control (Robert and

Thompson, 2001). This species could, in theory, control offspring sex through thermoregulation, the live-bearing counterpart for nest site choice in oviparous species. In oviparous species, I suggested four predictions necessary for mothers to predict offspring sex: (1) there must be a strong correlation between assessable temperatures during nest site choice and temperatures during the sex-determining period (TSP) (2) mothers would need a way of correcting for temporal changes in temperature, because such changes could obscure the predictability of thermal characteristics of nest sites. Here I suggested that nesting times should be canalized to reduce this problem (3) a wide range of thermal environments for nesting should be available to ensure the production of a fair proportion of unisexual nests (4) mothers would need to access beaches with differing thermal environments.

I found support for predictions (3) and (4). Around 75 % of nests in a year were unisexual, allowing the spatial scope for controlling offspring sex. In addition, home range size of females was large, covering several km (Chapter 4). Gravid females had an average of 5 beaches (with nests) within their home ranges (Chapter 4). Thus, a range of thermal environments were potentially available to nesting turtles. However, a strong seasonal component to sex production meant that mainly males were produced in the first clutch in a season, and mostly females were produced in the second clutch (Fig. 8.2). In particular, in the first third of the nesting season males dominated and in the last third almost all females were produced. Thus, a spatial scope for predicting or manipulating hatchling sex was only evident in the middle of the nesting season.

Was there a strong relationship between substrate temperatures at nesting and those during the TSP? A significant positive relationship existed in all three years (Fig. 8.4). However, only in 1996 was a large proportion of the variation in TSP temperatures explained by temperatures during nesting. In 1998 only 19 % of this variation was explained. The proportion of successful predictions of hatchling sex, based on temperatures at nesting was accordingly low. When considering mixed-sex nests (half-correct predictions = wrong predictions) predictability was around 50 % in 1997 and 1998. In 1996 predictability was high (86 %). If one excludes mixed-sex nests predictability is high in both 1996 and 1997, but less so in 1998. These data do not provide an overwhelming case against predicting hatchling sex, but are weak in support, particularly in light of the seasonal override in sex production.

Does the distribution of diel variation in nesting times allow for any accuracy in assessing nest site temperatures? Nesting times ranged widely, covering seven hours, and leading to a wide range of temperatures during nesting events (Fig. 8.1). This range of temperatures spanned the pivotal temperature separating the sexes (32 °C, unpubl. data). I hypothesize that such variation in temperature would lead to errors in the hypothetical case of mothers attempting to assess the nest site temperature. Consider two beaches, A and B, where A is a cooler beach. Soon after nightfall both beaches begin to cool. At this time beach A had reached its peak temperature of 30 °C. Three hours later, the warmer beach B cools to 30 °C. Both times were well within the nesting times of *C. insculpta*. Beach A was a male-producing beach and beach B was a female-producing beach. Because 30 °C is a male-producing temperature, our hypothetical turtle would predict the production of males at both sites. Her prediction for beach A would be correct, but not for beach B, a warm, female-producing beach. This oversimplified example leads us to conclude that the predictability of nest site temperatures for the production of a particular hatchling sex is obscured by a time-temperature relationship. It is conceivable that mothers may possess some yet-unknown mechanism for assessing this time-temperature relationship. Turtles may be able to incorporate the diel cooling of nesting beaches into their assessment of a nest site. However, this would likely require a complex physiological capability *ad hoc*.

Collectively, three lines of evidence lend support against *C. insculpta* mothers having the ability to predict offspring sex. First and foremost, a strong temporal influence on sex production results in early clutches producing mainly males and late clutches producing mostly females, regardless of spatial factors (nest site). Second, the varying and sometimes small amount of variation in TSP temperatures explained by temperatures at nesting, combined with erratic and sometimes low predictability of sex based on nesting temperatures, indicates a lack of support. Finally, due to the protracted distribution of nesting times, temperatures at nesting would possess a temporal component, obscuring the value of nest temperatures in predicting hatchling sex. Unless turtles have evolved a way to assess changes in temperature with time, predicting sex will be difficult.

Relatedly, a companion study on beach selection in *C. insculpta* indicated that turtles nested randomly with respect to thermal profiles of beaches (Chapter 6), further indicating that turtles were not manipulating hatchling sex.

Future attempts to answer the question of predicting offspring sex would benefit from studying animals in cold climates with a contracted nesting season. In those systems a strong temporal pattern of sex production will likely be absent, leaving more scope for manipulating offspring sex through nest site choice.

CHAPTER 9: SYNOPSIS

Offspring sex in TSD species under field conditions is determined by a wide range of influences, both proximal and distal. Temperature is well established as the proximal influence, but predictions are made difficult by the joint influence of mean and variability in temperature. The developmental window (thermosensitive period) in which temperature exerts its influence varies in extent in timing depending on the thermal trajectory of the nest. In species with extended nesting periods such as *C. insculpta*, timing of nesting in relation to seasonal trends in temperature will be influential, and there are a plethora of factors that can influence timing of nesting, including climate, rate of energy acquisition, and possibly thermal springs in *C. insculpta*. On a spatial scale, nesting beaches vary in temperature, based on aspect, and can thus influence offspring sex. Within nesting beaches, differences in mean and variability in temperature have implications for offspring sex through nest site choice. In the present study height of the nest site influenced sex ratios. The availability of moist sand constrained where nests could be laid, a result expected to apply to turtles nesting on beaches during dry periods. In the present study, an apparent low rate of energy acquisition, based on biennial reproduction, may have also influenced timing of nesting, which has flow-on consequences in offspring sex and embryonic survival. Timing of nesting and nest site choice (nest height) also worked in tandem to influence embryonic survival.

The present study contributed to our understanding of the complex integration of influences on offspring sex ratios and embryonic survival by answering the following questions:

- What measurable attributes of a nest site influence offspring sex?

Although sex ratios in reptiles with TSD are known to be influenced by nest site choice (Vogt and Bull, 1984; Janzen, 1994) and by lay date (Harlow and Taylor, 2000), the present study was the first to investigate the simultaneous effects of nest site choice and timing of nesting on both offspring sex and embryonic survival, finding that the height of nest sites interacted with lay date to dictate hatchling sex ratios and embryonic survival. Although I found no trade-offs between hatchling sex ratios and embryonic survival, clearly it is important that we simultaneously study variation in these response variables if we are to understand or hypothesize on their contribution to future generations.

- How might clutch parameters such as reproductive frequency effect hatchling sex ratios?

The unusual pattern of clutching twice every other year may have no consequences to offspring sex or embryonic survival, relative to the alternative scenario of laying one clutch each year. Conversely, laying one clutch each year would mean a contracted nesting season, and would likely lead to in increase in the influence of spatial factors on hatchling sex. Thus, nesting twice in a year leads to an extended nesting period, resulting in a strong temporal influence on hatchling sex, whereby mostly males were produced in the first clutch, and mainly females in the second clutch. This may have implications for adaptive scenarios for the evolution or maintenance of TSD, depending on whether hatching early favors males relative to females (Shine, 1999). Further study is needed on hatchling growth and survival – however, turtles are a challenging group to work on in this area, given their slow growth and longevity. The difficulty in tracking hatchlings in wet season conditions would exacerbate such an effort.

- How are sex ratios influenced by the availability of nesting areas with particular thermal profiles?

I studied the influence of spatial factors on two scales: among beaches and within beaches. Within beaches, height of the nest site, as constrained from above by loose sand, and as selected against from below by flood mortality, was the key factor. While nest flooding is common in riverine turtles, presumably shaping the height selected by nesting turtles (e.g., Roze, 1964; Doody, 1995), the finding of loose sand constraining nest site choice in this way was not previously known. This finding highlights the importance of understanding the physical environment when constructing hypotheses based on adaptive scenarios.

- Can turtles predict offspring sex?

A recent for the evolution or maintenance of TSD in turtles raises the notion that turtles may be able to predict offspring sex (Roosenberg, 1996), contrary to conventional theory (Bull, 1980). Several lines of evidence precipitated from the present study indicate a difficulty in mothers predicting or manipulating hatchling sex. First, a sweeping temporal effect, whereby seasonal increases in air temperatures dictated hatchling sex ratios, meant that spatial factors had less influence than expected. Mainly males were produced early, and mostly females were produced late. Although gravid turtles had access to several beaches with differing thermal profiles, due to their large home ranges, these turtles chose beaches randomly with respect to thermal characteristics. A strong correlation did exist between substrate temperatures at nest site choice and temperatures during the thermosensitive period, predictions of hatchling sex during nesting were only accurate some of the time. Finally, temporal factors obscuring the thermal predictability of a nest site meant that turtles could not have accurately discriminated between hot beaches at cooler times and cool beaches at warmer times, because nesting times were protracted. I recommend, however, that the question of predicting offspring sex be examined on a species with a narrow temporal window for nesting, thereby providing more opportunity for nest site choice to be tightly tied to offspring sex.

In addition to these major points, several notable findings emerged in the present study. These are united by three themes: the influence of the wet-dry tropics, the auteocological distinctiveness of *C. insculpta*, and the integration of features of the species biology, as they bear on hatchling sex ratios and embryonic survival.

The influence of the wet-dry tropics

Although difficult to directly test, the ecology of Daly River *C. insculpta* is apparently profoundly influenced by the wet-dry tropics. A key example is the evolution of embryonic aestivation in the species (Chapter 6). The present study examined the hypotheses of Webb et al. (1986) that embryonic aestivation occurred in nature in *C. insculpta*, and that it was an adaptive response to early wet season flooding. My findings indicate that indeed embryonic aestivation occurs in the species, and the exact timing of emergence is tightly associated with the onset of early wet season flooding, gleaned from historical river stage data. Further work is needed to uncover the ultimate mechanisms for the presence of aestivation (i.e., increased probability of hatchling survival). Another example of the influence of the wet-dry tropics is its apparent control over reproductive characteristics (Chapter 4). Although based on only 4 years of data, several reproductive traits aligned themselves with the magnitude of the previous wet season(s). For example, following ‘big’ wet seasons

C. insculpta nested earlier (up to 5 weeks), and produced larger clutches of larger eggs. There is evidence that this phenotypic plasticity may have important implications for fitness. Several studies on birds (reviewed in Drent and Daan, 1980) and at least one on reptiles (Olsson and Shine, 1997b) demonstrate that 'earlier is better' in terms of the influence of lay date on fitness. Larger eggs produce larger hatchlings which can also incur a fitness advantage (Janzen, 1994). If this is the case, why aren't larger eggs (hatchlings) produced early every year? The bird literature indicates that energy uptake is limited in poorer years, and from this I would predict that a 'small' wet season presents an energy uptake problem for *C. insculpta*. There were also 'follow on' effects of the wet-dry. For example, annual variation in timing of nesting (putatively due to the magnitude of the previous wet season(s)) exerted a marked influence on both embryonic survival and hatchling sex (Chapter 7). In nests laid later in the season temperatures during the thermosensitive period were hotter, resulting in more females. As a result, female-biased hatchling sex ratios were produced in years following big wet seasons, although variation in air temperatures during the dry season can obscure this effect. Similarly, later nests experienced lower embryonic survival, due to nest flooding following early wet season rains. Collectively, these findings indicate that the wet-dry tropics have played a major role in the evolution of the reproductive strategies of *C. insculpta*. Because the study spanned only 4 data years, however, further work is needed to confirm the importance of the magnitude of the wet season on clutch characteristics.

Autecological distinctiveness of *C. insculpta*

Several features of *C. insculpta* have proven to be unique in turtles, or nearly so, some of which were discovered in the present study. The best example is the finding that most Daly *C. insculpta* lay 2 clutches of eggs every other year (Chapter 4). Why produce two clutches every other year rather than one clutch each year? The answer is not presently known, but life history theory predicts that animals that skip years reproducing are energy-limited (Bull and Shine, 1979). This energy limitation, which may be the low available energy in the preferred dry season diet (aquatic vegetation) may also be responsible for a second unique finding in *C. insculpta*: the largest home range size of any known aquatic turtle species (Chapter 2). On average, female *C. insculpta* occupied > 8 km of river, with males using > 3 km. The present study was the first to directly test the influence of reproductive condition on sex differences in movements and activity in turtles. Another apparently unique feature of (Daly River) *C. insculpta* biology is their extensive use of thermal springs to elevate body temperatures (Chapter 3). Turtles spent many hours each day sitting on thermal springs for up to half the year, when river temperatures were lower than that of the springs. Although data were inconclusive, this thermal boost may result in earlier nesting, and probably allows the turtles to be active and feed for more months of the year. These findings, together with previous findings in *C. insculpta*, such as explosive hatching following immersion (Webb et al., 1986), highlight the uniqueness of this monotypic species. Thus, the persistence of the species should be considered a high priority, due not only to its restricted distribution and taxonomic position (Georges and Rose, 1993), but also due to its unique autecology.

Integration of aspects of the species biology

The main aim of the study was to establish how sex determination is accomplished in nature, or how the species' life history contributes to TSD in *C. insculpta*. To this end, I asked whether or not *C. insculpta* could predict offspring sex

(Chapter 8). This question is central to theory on why TSD might evolve from GSD (Charnov and Bull, 1977), and attempting to answer it requires knowledge of other aspects of the species biology. I made four predictions that must hold in order for mothers to predict or manipulate offspring sex. Results were mixed, but generally cast doubt on the ability of *C. insculpta* to predict hatchling sex. Gravid turtles had scope for producing a particular offspring sex by having large home ranges encompassing several nesting beaches with varying thermal profiles (Chapter 2, Chapter 8). However, turtles attempting to predict hatchling sex by assessing a particular nest site would meet with some difficulty (Chapter 8). Nesting times were protracted, reflecting a temporal component to nest site temperatures, obscuring the predictable thermal environment of nest sites (Chapter 8). And predictability of TSP temperatures (and thus, hatchling sex) from substrate temperatures at nesting was erratic and sometimes low (Chapter 8). A companion study corroborated this finding. In Chapter 5 I show that beaches are chosen randomly with respect to temperature. In Chapter 7 I demonstrate that height of the nest sites influences offspring sex, but that lay date has the overriding influence. Thus, sex is largely determined by lay date, regardless of where the eggs are laid, shedding more doubt on the ability of *C. insculpta* to control hatchling sex.

The present study revealed a complex picture of factors influencing offspring sex and embryonic survival (Fig. 9.1). The finding that embryonic survival is influenced by nest site choice (nest height) and lay date highlights the integration of different aspects of the species biology (Chapter 7). Wet season magnitude influences lay date and clutch attributes, and lay date effects hatchling sex ratios and embryonic survival. These findings should be important contextually in determining why TSD evolved in *C. insculpta* (the sister group to the Carettochelydidae, the Trionychidae,

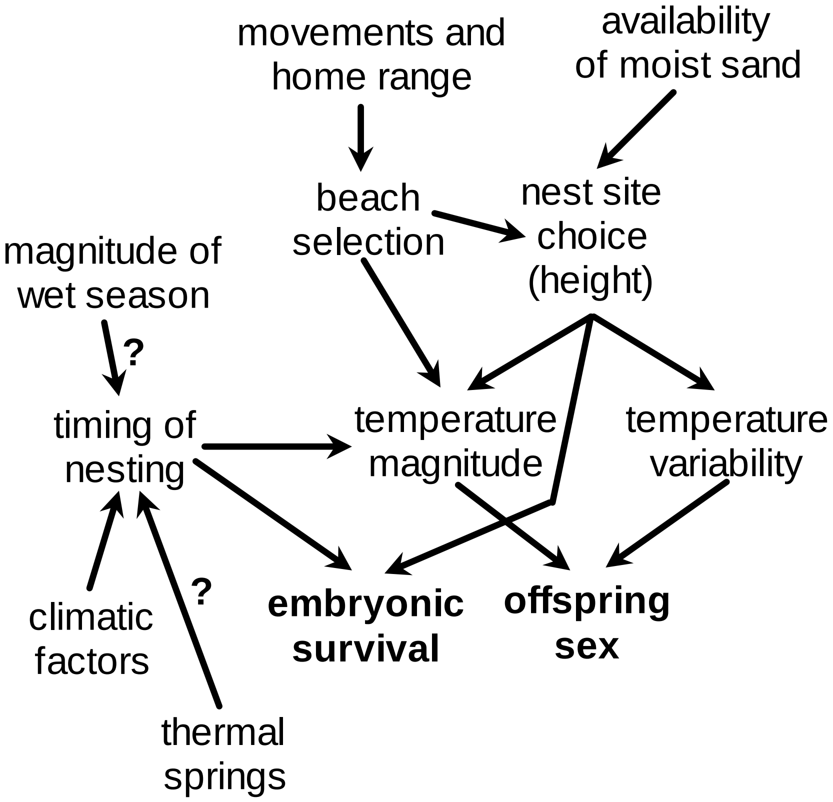


Figure. 9.1. Integration of factors determining hatchling sex and embryonic survival in *C. insculpta*.

possesses GSD). Shine (1999) reviewed adaptive explanations for why TSD has evolved in reptiles. The model that best fits *C. insculpta*, based on findings of the present study, is the ‘time-matching’ model (Shine, 1999), whereby lay date influences offspring sex (Chapter 7). However, I have no evidence that an advantage is conferred to males in hatching sooner than females, and the season-on-sex pattern present in *C. insculpta* is likely common in tropical TSD species with extended or prolonged nesting seasons.

REFERENCES

Abell, A. J. (1999). Variation in clutch size and offspring size relative to environmental conditions in the lizard *Sceloporus virgatus*. *J. Herpetol. 33:173-180.*

Alho, C. J. R., and Padua, L. F. M. (1982). Reproductive parameters and nesting behavior of the Amazon turtle *Podocnemis expansa* (Testudinata: Pelomedusidae) in Brazil. *Can. J. Zool. 60: 97–103.*

Allsteadt, J., and Lang, J. W. (1994). Incubation temperature affects body size and energy reserves of hatchling American alligators (*Alligator mississippiensis*). *Phys. Zool. 68:76-97.*

Anderson, P. K. (1958). The photic responses and water-approach behavior of hatchling turtles. *Copeia 1958: 211–215.*

Andersson, M. (1994). Sexual Selection. Princeton Univ. Press, Princeton, New Jersey.

Balazs, G. H. (1980). Synopsis of biological data on the green turtle in the Hawaiian Islands. National Oceanographic and Aeronautics Association Technical Memo, National Marine Fisheries Section, NOAA-TM-NMFS-SWFC-7.

Ballinger, R. E. (1977). Reproductive strategies: Food availability as a source of proximal variation in a lizard. *Ecology 58:628-635.*

Bartholomew, G. A., and Tucker, V. A. (1964). Size, body temperature, thermal conductance, oxygen consumption, and heart rate in Australian varanid lizards. *Physiol. Zool. 37:341-354.*

Beggs, K., Georges, A., Young, J., and West, P. 2000. Ageing the eggs and embryos of the pig-nosed turtle, *Carettochelys insculpta* (Chelonia:Carettochelydidae), from northern Australia. *Can. J. Zool. 78: 373–392.*

Benda, R. S., and Proffitt, M. A. (1973). Effects of thermal effluents on fish and invertebrates. In ‘Thermal Ecology’. (Eds. J. W. Gibbons and R. R. Sharitz). pp. 438-447. USAEC. Springfield, VA.

Bennett, A. F. (1982). The energetics of reptilian activity. In ‘Biology of the Reptilia, Vol. 13’. (Eds. C. Gans and F. Pough). pp 155-194. Academic Press, London, U.K.

Bernardo, J. (1996). Maternal effects in animal ecology. *Amer. Zool. 36:83-105.*

Bjorndal, K. A. (1981). The Consequences of Herbivory for the Life History Pattern of the Caribbean Green Turtle, Chelonia mydas, p. 111-116. *In:* Biology and Conservation of Sea Turtles. K. A. Bjorndal (ed.). Proc. of the World Conference on Sea Turtle Conservation, Washington D.C., 29-30 November, 1999. Smithsonian Institute Press, Washington D.C.

Blake, D.K. (1974). The rearing of crocodiles for commercial and conservation purposes in Rhodesia. *Rhodesian Science News 8: 315–324.*

Bodie, J. R., and Semlitsch, R. D. (2000). Spatial and temporal use of floodplain habitats by lentic and lotic species of turtles. *Oecologia 122:138-146.*

Bodie, J.R., Smith, K.R., and Burke, V.J. (1996). A comparison of diel nest temperature and nest site selection for two sympatric species of freshwater turtles. *Am. Midl. Nat. 136: 181–186.*

Bonnet, X., Naulleau, G., Shine, R., and Lourdais, O. (2001). Short-term vs. long-term effects of food intake on reproductive output in a viviparous snake, *Vipera aspis. Oikos 92:297-308.*

Boyer, D. R. (1965). Ecology of the basking habit in turtles. *Ecology 46:99-118.*

Bragg, W. K., Fawcett, J. D., Bragg, T. B., and Viets, B. E. (2000). Nest-site selection in two eublepharid gecko species with temperature-dependent sex determination and one with gentypic sex determination. *Biol. J. Linn. Soc. 69:319-332.*

Brown, G. P. and Brooks, R. J. (1991). Thermal and behavioral responses to feeding in free-ranging *Chelydra serpentina. J. Herpetol. 25:273-278.*

Brown, G. P. and Brooks, R. J. (1993). Sexual and seasonal differences in activity in a northern population of snapping turtles, *Chelydra serpentina. Herpetologica 49:311-318.*

Bull, J. J. (1980). Sex determination in reptiles. *Quart. Rev. Biol. 55:3-21.*

Bull, J. J. (1987). Temperature-sensitive periods of sex determination in a lizard: Comparisons with crocodiles and turtles. *J. Exp. Zool. 241:143-148.*

Bull, J. J. and Shine, R. (1979). Iteroparous animals that skip opportunities for reproduction. Amer. Nat. 114:296-303.

Bull, J. J. and Vogt, R. C. (1979). Temperature-dependent sex determination in turtles. *Science 206:1186-1188.*

Bull, J. J., Vogt, R. C., and McCoy, C. J. (1982). Sex determining temperatures in turtles: a geographic comparison. *Evolution 36:326-332.*

Bull, J. J., Gutske, W. H. N., and Bulmer, M. G. (1988). Nest choice in a captive lizard with temperature-dependent sex determination. *J. Evol. Biol. 2:177-184.*

Bulmer, M. G., and Bull, J. J. (1982). Models of polygenic sex determination and sex ratio control. *Ibid. 36:13-26.*

Burger, J. (1976a). Behavior of hatchling diamondback terrapins (*Malaclemys terrapin*) in the field. *Copeia 1976: 742–748.*

Burger, J. (1976b). Temperature relationships in nests of the northern diamondback terrapin, *Malaclemys terrapin terrapin. Herpetologica 32:412-418.*

Burger, J. and Montevecchi, W. A. (1975). Nest site selection in the terrapin *Malaclemys terrapin. Copeia 1975:113-119.*

Burke, R. L. (1993). Adaptive value of sex determination mode and hatchling sex-ratio bias in reptiles. *Copeia 1993:854-859.*

Bury, R. B. (1972). Habits and home range of the pacific pond turtle, *Clemmys marmorata*, in a stream community. Ph.D. Dissertation. Univ. Calif. Berkeley, 146 pp.

Bustard, H. R. (1967). Mechanism of nocturnal emergence from the nest in green turtle hatchlings. *Nature 214: 317.*

Bustard, H. R. (1972). Sea turtles. Their natural history and conservation. Collins, London.

Butler, B. O., and Graham, T. E. (1995). Early post-emergent behavior and habitat selection in hatchling Blanding's turtles, *Emydoidea blandingii*, in Massachusetts. *Chelonian Conserv. Biol. 1: 187–196.*

Cagle, F. R. (1950). The life history of the slider turtle, *Pseudemys scripta troosti* (Holbrook). *Ecol. Monogr. 20:31-54.*

Carr, A. (1984). So excellent a fishe: a natural history of sea turtles. Charles Scribner's Sons, New York.

Carr. A., and Hirth, H. (1961). Social facilitation in green turtle siblings. *Anim. Behav. 9: 68–70.*

Carr, A., and Ogren, L. (1959). The ecology and migrations of sea turtles, 3: *Dermochelys* in Costa Rica. *Amer. Mus. Novitates (1959): 1–29.*

Carr, A., and Ogren, L. (1960). The ecology and migrations of sea turtles, 4: The green turtle in the Caribbean Sea. *Bull. Amer. Mus. Nat. Hist. 121: 5–48.*

Carter, S. L., Haas, C. A., and Mitchell, J. C. (2000). Movements and activity of bog turtles (*Clemmys muhlenbergii*) in southwestern Virginia. *J. Herpetol. 34:75-80.*

Charland, M. B. and Gregory, P. T. (1990). The influence of female reproductive status on thermoregulation in a viviparous snake, *Crotalus viridis*. *Copeia 1990:1089-1098.*

Charnier, M. (1966). Action de la temperature sur la sex ratio chez l'embryon d'*Agama agama* (Agamidae, Lacretilian). *C. R. Soc. Biol., 160:620-622.*

Charnov, E. L. and Bull, J. J. (1977). When is sex environmentally determined? *Nature 266:828-830.*

Chessman, B.C. (1987). Atmospheric and aquatic basking of the Australian freshwater turtle, *Emydura macquarii. Herpetologica 43:301-306.*

Christens, E. (1990). Nest emergence lag of loggerhead sea turtles. *J. Herpetol. 24: 400–402.*

Christens, E. and Bider, J. R. (1987). Nesting activity and hatching success of the painted turtle (*Chrysemys picta marginata*) in southwestern Quebec. *Herpetologica 43:55-65.*

Christian, K. and Green, B. (1994). Seasonal energetics and water turnover of the frillneck lizard, *Chlamydosaurus kingii*, in the wet-dry tropics of Australia. *Herpetologica 50:274-281.*

Christian, K. A., Corbett, L. K., Green, B., and Weavers, B. W. (1995). Seasonal activity and energetics of two species of varanid lizards in tropical Australia. *Oecologia 103:349-357.*

Christian, K., Bedford, G., Green, B., Griffiths, A., Newgrain, K., and Shultz, T. (1999a). Physiological ecology of a tropical dragon, *Lophognathus temporalis. Austr. J. Ecol. 24:171-181.*

Christian, K. A., Bedford, G. S., and Shultz, T. J. (1999a). Energetic consequences of metabolic depression in tropical and temperate-zone lizards. *Austr. J. Zool. 47:133-141.*

Christy, E. J., Farlow, J. O., Bourque, J. E., and Gibbons, J. W. (1973). Enhanced growth and increased body size of turtles living in thermal and post-thermal aquatic systems. In 'Thermal Ecology'. (Eds. J. W. Gibbons and R. R. Sharitz). pp. 277-284. USAEC. Springfield, VA.

Clark, P. J., Ewert, M. A., and Nelson, C. E. (2001). Physical apertures as constraints on eggs size and shape in the common musk turtle, *Sternotherus odoratus*. *Funct. Ecol. 15:70-77.*

Congdon, J. D. and Gatten, Jr., R. E. (1989). Movements and energetics of nesting *Chrysemys picta*. *Herpetologica 45:94-100.*

Congdon, J. D., Breitenbach, G. L., van Loben Sels, R. C., and Tinkle, D. W. (1987). Reproduction and nesting ecology of snapping turtles *(Chelydra serpentina*) in southeastern Michigan. *Herpetologica 43:39-54.*

Congdon, J.D., Tinkle, D.W., Breitenbach, G.L., and van Loben Sels, R.C. (1983). Nesting ecology and hatching success in the turtle *Emydoidea blandingi*. *Herpetologica, 39: 417–429.*

Congdon, J.D., Nagle, R.D., Dunham, A.D., Beck, C.W., Kinney, O.M., and Yeomans, S.R. (1999). The relationship of body size to survivorship of hatchling snapping turtles (*Chelydra serpentina*): an evaluation of the "bigger is better" hypothesis. *Oecologia 121:224-235.*

Conover, D. O. (1984). Adaptive significance of temperature-dependent sex determination in a fish. *Amer. Natur. 123:297-313.*

Conover, D. O., and Kynard, B. E. (1981). Environmental sex determination: interaction of temperature and genotype in a fish. *Science 213:577-579.*

Craig, M. J. (1992). Radio-telemetry and tagging study of movements, activity cycles, and habitat utilization in Cagle's map turtle, *Graptemys caglei*. Unpublished M.S. Thesis , West Texas State Univ., Canyon.

Crawford, K. M., Spotila, J. R., and Standora, E. A. (1983). Operative environmental temperatures and basking behavior of the turtle *Pseudemys scripta. Ecology 64:989-999.*

Cree, A., Cockrem, J. F., and Guilette, Jr., L. J. (1992). Reproductive cycles of male and female tuatara (*Sphenodon punctatus*) on Stephens Islands, New Zealand. *J. Zool. (London) 226:199-217.*

deCandolle, A.P. (1855). Geographique Botanique Raisonee. Masson, Paris.

DeMarco, V. G. (1989). Annual variation in the seasonal shift in egg size and clutch size in *Sceloporus woodi. Oecologia 80:525-532.*

DePari, J.A. (1996). Overwintering in the nest by hatchling painted turtles, *Chrysemys picta*, in northern New Jersey. *Chelon. Conserv. Biol. 2: 5–12.*

deSouza, R.R., and R. C. Vogt. (1994). Incubation temperature influences sex and hatchling size in the neotropical turtle *Podocnemis unifilis*. *J. Herpetol. 28:453-464.*

Diamond, A.W. (1976). Breeding biology and conservation of hawksbill turtles, *Eretmochelys imbricata* L., on Cousin Island, Seychelles. *Biol. Conserv. 9: 199–215.*

Dodd, C. K., Jr. (1989). Secondary sex ratio variation among populations of the flattened musk turtle, *Sternotherus depressus. Copeia 1989:1041-1045.*

Doody, J. S. (1995). A comparative nesting study of two syntopic species of softshell turtles (*Apalone mutica* and *Apalone spinifera*) in southeastern Louisiana. Unpubl. M.s. Thesis, Southeastern Louisiana University, Hammond.

Doody, J. S. (2000). The territory's intriguing turtles. Austr. Geogr. 58:22-23.

Doody, J. S., and Georges, A. (2000). A novel technique for gathering nesting and emergence phenology data in turtles. *Herpetol. Rev. 31:220-222.*

Doroff, A. M. and Keith, L. B. (1990). Demography and ecology of an ornate box turtle (*Terrapene ornata*) population in south-central Wisconsin. *Copeia 1990:387-399.*

Drent, R. H. and Daan, S. (1980). The prudent parent: energetic adjustment in avian breeding. *Ardea 68:225-252.*

Dunham, A. E. (1981). Populations in a fluctuating environment: The comparative population biology of *Sceloporus merriami* and *Urosaurus ornatus*. Misc. Publ. Mus. Zool. Univ. Mich. 158:1-62.

Ehrenfeld, D.W. 1979. Behaviour associated with nesting. In 'Turtles: perspectives and research'. (Eds. M. Harless and H. Morlock). pp. 417–434. John Wiley and Sons, New York.

Elgar, M. A., and Heaphy, L. J. (1989). Covariation between clutch size, egg weight and shape: comparative evidence for chelonians. *J. Zool. 219:137-152.*

Ernst, C. H. (1970). Home range of the spotted turtle, *Clemmys guttata* (Schneider). *Copeia 1970:391-393.*

Ernst, C. H. (1986). Environmental temperatures and activities in the wood turtle, *Clemmys insculpta. J. Herpetol. 20:22-29.*

Ernst, C. H, Lovich, J. E., and Barbour, R. W. (1994). Turtles of the United States and Canada. Smithsonian Institution Press, Washington D.C.

Ewert, M.A. 1985. Embryology of turtles. In 'Biology of the Reptilia. Vol. 14'. (Eds. C. Gans, F. Billett and P.F.A. Maderson). pp. 75–267. John Wiley and Sons, New York.

Ewert, M. A., and Nelson, C. E. (1991). Sex determination in turtles: Diverse patterns and possible adaptive values. *J. Exp. Zoology 270:3-15.*

Ferguson, G. W., Brown, K. L., and DeMarco, V. G. (1982). Selective basis for the evolution of variable egg and hatchling size in some iguanid lizards. *Herpetologica 38:178-188.*

Ferguson, G. W., Snell, H. L., and Landwer, A. J. (1990). Proximate control of variation of clutch, egg, and body size in a west-Texas population of *Uta stansburiana stejnegeri* (Sauria: Iguanidae). *Herpetologica 46:227-238*.

Fisher, R. A. 1930. The Genetical Theory of Natural Selection. Oxford University Press, Oxford, England.

Fitch, H. S. (1981). Sexual size difference in reptiles. *Misc. Publ. Mus. Nat. Hist., Univ. Kansas 70:1-72.*

Foley, A. M., Peck, S. A., Harman, G. R., and Richardson, L. W. (2000). Loggerhead turtle (*Caretta caretta*) nesting habitat on low-relief mangrove islands in southwest Florida and consequences to hatchling sex ratios. *Herpetologica 56:433-445.*

Ford, N. B. and Seigel, R. A. (1989). Phenotypic plasticity in reproductive traits: evidence from a viviparous snake. *Ecology 70:1768-1774.*

Forsman, A. and Shine, R. (1995). Sexual size dimorphism in relation to frequency of reproduction in turtles (Testudines: Emydidae). *Copeia 1995:727-729.*

Gatten, R. E., Jr. (1974). Effect of nutritional status on the preferred body temperature of the turtles *Pseudemys scripta* and *Terrepene ornata*. *Copeia 1974:912-917.*

Georges, A. (1989). Female turtles from hot nests: is it duration of incubation or proportion of development at high temperatures that matters? *Oecologia 81:323-328.*

Georges, A. (1992). Thermal characteristics and sex determination in field nests of the pig-nosed turtle, *Carettochelys insculpta* (Chelonia: Carettochelydidae), from northern Australia. *Austr. J. Zool. 40:511-521.*

Georges, A., and Kennett, R. (1993). Dry season distribution and ecology of *Carettochelys insculpta* (Chelonia: Carettochelydidae) Kakadu National Park, northern Australia. *Austr. Wildl. Res. 16:323-335*.

Georges, A. and Rose, M. (1993). Conservation biology of the pig-nosed turtle, *Carettochelys insculpta*. *Chelon. Conserv. Biol. 1:3-12*.

Georges, A. and Wombey, J. C. (1993). Family Carettochelydidae. In ‘Fauna of Australia, Vol. 2A, Amphibia and Reptilia’. (Eds. C. J. Galsby , G. J. B. Ross, and P. L. Beesley). pp. 153-156. Australian Government Publishing Service: Canberra.

Georges, A., Limpus, C., and Stoutjesdijk, C. (1994). Hatchling sex in the marine turtle *Caretta caretta* is determined by proportion of development at a temperature, not daily duration of exposure. *J. Exp. Zool. 270:432-444.*

Georges, A., Rose, M., and Doody, J. S. (2001). *Carettochelys insculpta,* the pig-nosed turtle (Ramsay 1886). In ‘The conservation biology of freshwater turtles’. (Eds. P. C. H. Pritchard and A. G. J. Rhodin). Chelonian Research Monographs. IUCN Publications, Gland, Switzerland.

Georges, A., Choquenot, D., Coventry, A. J., and Wellings, P. (1989). A note on *Carettochelys insculpta* (Testudinata: Carettochelydidae) from northern Australia. *Northern Territory Nat. 11:8-11*.

Gibbons, J. W. (1986). Movement patterns among turtle populations: Applicability to management of the desert tortoise. *Herpetologica 42:104-113*.

Gibbons, J. W. (1987). Why do turtles live so long? *Bioscience 37:262-269*.

Gibbons, J.W., and Nelson, D.H. (1978). The evolutionary significance of delayed emergence from the nest by hatchling turtles. *Evolution 32: 297–303*.

Gibbons, J. W., Greene, J. L., and Congdon, J. D. (1990). Temporal and spatial movement patterns of sliders and other turtles. In ‘Life History and Ecology of the Slider Turtle’. (Ed. J. W. Gibbons). pp. 201-215. Smithsonian Institution Press, Washington D. C.

Gordon, D. M. and MacCulloch, R. D. (1980). An investigation of the ecology of the map turtle, *Graptemys geographica* (Le Seur), in the northern part of its range. *Can. J. Zool. 58:2210-2219*.

Gregory, P. T. (1982). Reptilian Hibernation. In ‘Biology of the Reptilia, Vol. 13’. (Eds. C. Gans and F. H. Pough). pp 53-154. Academic Press, London.

Gregory, P. T. and Skebo, K. M. (1998). Trade-offs between reproductive traits and the influence of food intake during pregnancy in the garter snake, *Thamnophis elegans*. *Amer. Nat. 151:477-486*.

Gregory, P. T., Macartney, J. M., and Larsen, K. W. (1987). Spatial Patterns and Movements. In ‘Snakes: Ecology and Evolutionary Biology’. (Eds. R. A. Seigel, S. S. Novak, and J. T. Collins). pp. 396-421. MacMillan, New York.

Griffiths, A. D. and Christian, K. A. (1996). Diet and habitat use of frillneck lizards in a seasonal tropical environment. *Oecologia 106:39-48.*

Gutske, W. H. N. and Paukstis, G. L. (1983). Influence of the hydric environment on sex differentitation of turtles. *J. Exp. Zool. 226:467-469.*

Gyuris, E. (1993). Factors that control the emergence of green turtle hatchlings from the nest. *Wildl. Res. 20: 345–353.*

Gyuris, E. (1994). The rate of predation by fishes on hatchlings of the green turtle (*Chelonia mydas*). *Coral Reefs 13: 137–144.*

Hammer, D.A. (1969). Parameters of a marsh snapping turtle population, LaCreek Refuge, South Dakota. *J. Wildl. Manag. 33: 995–1005.*

Hammond, K. A., Spotila, J. R., and Standora, E. A. (1988). Basking behavior of the turtle *Pseudemys scripta*: Effects of digestive state, acclimation temperature, sex, and season. *Physiol. Zool. 61:69-77.*

Harcourt, R. G., Hindell, M. A., Bell, D. G., and Waas, J. R. (2000). Three-dimensional dive profiles of free-ranging Weddell seals. *Polar Biol. 23:479-487.*

Harlow, P. S. and Taylor, J. E. (2000). Reproductive ecology of the jacky dragon (*Amphibolurus muricatus*): an agamid lizard with temperature-dependent sex determination. *Austral Ecol. 25:640-652.*

Hart, D. R. (1983). Dietary and habitat shift with size of red-eared turtles (*Pseudemys scripta*) in a southern Louisiana population. *Herpetologica 39:285-290.*

Hays, G. C., Speakman, J. R., and Hayes, J. P. (1992). The pattern of emergence by loggerhead turtle (*Caretta caretta*) hatchlings on Cephalonia, Greece. *Herpetologica, 48: 396–401.*

Heaphy, J. L. (1990). The ecology of the pig-nosed turtle, *Carettochelys insculpta*, in northern Australia. Unpubl. PhD thesis, University of New South Wales, Sydney.

Heath, M. E., and McGinnis, S. M. (1980). Body temperature and heat transfer in the green sea turtle *Chelonia mydas*. *Copeia, 1980: 767–773*.

Hendrickson, J. R. (1958). The green sea turtle, *Chelonia mydas* (Linn.) in Malaya and Sarawak. *Proc. Zool. Soc. Lond. 130: 455–535*.

Hewavisenthis, S. and Parmenter, C. J. (2000). Hydric environment and sex determination in the flatback turtle (*Natator depressus* Garman) (Chelonia:Cheloniidae). *Austr. J. Zool. 48:653-659*.

Hughes, D. A., and Richard, J. D. (1974). The nesting of the Pacific Ridley turtle *Lepidochelys olivacea* on Playa Nancite, Costa Rica. *Mar. Biol. 24: 97–107*.

Iverson, J. B. (1991). Life history and demography of the yellow mud turtle, *Kinosternon flavescens*. *Herpetologica 47:373-395*.

Iverson, J. B. (1991). Patterns of survivorship in turtles. *Can. J. Zool. 69:385-391*.

Iverson, J. B., Balgooyen, C. P., Byrd, K. K., and Lydan, K. K. (1993). Latitudinal variation in egg and clutch size in turtles. *Can. J. Zool. 71:2448-2461*.

Iverson, J. B., Higgins, H., Sirulnik, A., and Griffiths, C. (1997). Local and geographic variation in the reproductive biology of the snapping turtle (*Chelydra serpentina*). *Herpetologica 53:96-117*.

Jackson, D. C. (1971). The effect of temperature on ventilation in the turtle, *Pseudemys scripta elegans*. *Resp. Physiol. 12:131-140*.

James, C. D. and Shine, R. (1985). The seasonal timing of reproduction: a tropical-temperate comparison in Australian lizards. *Oecologia 67:464-474*.

James, C. D. and Whitford, W. G. (1994). An experimental study of phenotypic plasticity in the clutch size of a lizard. *Oikos 70:49-56*.

Janzen, F. J. (1994). Vegetational cover predicts the sex ratio of hatchling turtles in natural nests. *Ecology 75:1593-1599*.

Joanen, T. and McNease, L.L. (1989). Ecology and physiology of nesting and early development of the American alligator. *Amer. Zool. 29:987-998*.

Johnson, S. A. and Ehrhart, L. M. (1994). Reproductive ecology of the Florida green turtle: clutch frequency. *J. Herpetol. 30:407-410*.

Jones, R. L. (1996). Home range and seasonal movements of the turtle *Graptemys flavimaculata*. *J. Herpetol. 30:376-385*.

Judd, F. W. and Ross, R. K. (1978). Year-to-year variation in clutch size of island and mainland populations of *Holbrookia propinqua* (Reptilia, Lacertilia, Iguanidae). *J. Herpetol. 12:203-207*.

Kepenis, V. and McManus, J. J. (1974). Bioenergetics of young painted turtles, *Chrysemys picta*. *Comp. Biochem. Physiol. 48A:309-317*.

King, J. M., Kuchling, G., and Bradshaw, S. D. (1998). Thermal environment, behavior, and body condition of wild *Pseudemydura umbrina* (Testudines: Chelidae) during late winter and early spring. *Herpetologica 54:103-112*.

Kramer, M. (1995). Home range of the Florida red-bellied turtle (*Pseudemys nelsoni*) in a Florida spring run. *Copeia 1995:883-890*.

Krawchuk, M. A. and Brooks, R. J. (1998). Basking behavior as a measure of reproductive cost and energy allocation in the painted turtle, *Chrysemys picta*. *Herpetologica 54:112-121*.

Kuchling, G. (1993). Possible biennial ovarian cycle of the freshwater turtle *Erymnochelys madagascariensis*. *J. Herpetol. 27:470-472*.

Kuchling, G. (1999). Reproductive Biology of the Chelonia. Springer, Berlin.

Kuchling, G. and Bradshaw, S. D. (1993). Ovarian cycle and egg production of the Western Swamp Tortoise *Pseudemydura umbrina* (Testudines: Chelidae) in the wild and in captivity. *J. Zool. (London) 229:405-419*.

Landa, K. (1992). Adaptive seasonal variation in grasshopper offspring size. *Evolution 46:1553-1558*.

Lang, J. W. and Andrews, H. V. (1994). Temperature-dependent sex determination in crocodilians. *J. Exp. Zool. 270:28-44*.

Lefevre, K. and Brooks, R. J. (1995). Effects of body size on basking behavior in a northern population of the painted turtle, *Chrysemys picta*. *Herpetologica 51:217-224*.

Legler, J. M. (1960). A simple and inexpensive device for trapping aquatic turtles. *Utah Acad. Sci. Proc. 37:63-66*.

Limpus, C. J. (1980). Observations on the hawksbill turtle (*Eretmochelys imbricata*) nesting along the Great Barrier Reef. *Herpetologica, 36: 265–271*.

Limpus, C. J., and Reed, P. C. (1985). The green turtle, *Chelonia mydas*, in Queensland: a preliminary description of the population structure in a coral reef feeding ground. In ‘Biology of Australasian Frogs and Reptiles’. (Eds. G. Grigg, R. Shine, and H. Ehmann). pp. 47-52. Royal Zool. Soc., New South Wales.

Limpus, C. J. and Nicholls, N. (1988). The southern oscillation regulates the annual numbers of green turtles (*Chelonia mydas*) breeding around northern Australia. *Austr. Wildl. Res. 15:157-161*.

Lindeman, P. V. (2000). Evolution of the relative width of the head and alveolar surfaces in map turtles (Testudines: Emydidae: *Graptemys*). *Biol. J. Linn.Soc. 549-576*.

Lue, K.- Y. and Chen, T.- H. (1999). Activity, movement patterns, and home range of the yellow-margined box turtle (*Cuora flavomarginata*) in northern Taiwan. *J. Herpetol. 33:590-600*.

MacCulloch, R. D. and Secoy, D. M. (1983). Movement in a river population of *Chrysemys picta bellii* in southern Saskatchewan. *J. Herpetol. 17:283-285*.

Mace, G. M., Harvey, P. H., and Clutton-Brock, T. H. (1983). Vertebrate home-range size and energetic requirements. In 'The Ecology of Animal Movement'. (Eds. I. R. Swingland and P. J. Greenwood). pp. 33-53. Clarendon Press, Oxford.

Madsen, T. and Shine, R. (1996). Seasonal migration of predators and prey: pythons and rats in tropical Australia. *Ecology 77:149-156*.

Maloney, J. E., Darian-Smith, C., Takahashi, Y., and Limpus, C. J. (1990). The environment for development of the embryonic loggerhead turtle (*Caretta caretta*) in Queensland. *Copeia, 1990: 378–387*.

Manning, B., and Grigg, G. C. (1997). Basking is not of thermoregulatory significance in the "basking" freshwater turtle *Emydura signata*. *Copeia 1997:579-584*.

Maynard-Smith, J. (1978). The ecology of sex. In 'Behavioural ecology: an evolutionary approach'. (Eds. J. R. Krebs and N. B. Davies). pp. 159-179. Sinaur Associates Inc., Sunderland, MA.

Miller, J. D. (1997). Reproduction in sea turtles. In 'The Biology of Sea Turtles'. (Eds. P. L. Lutz and J. A. Musick). pp. 51-81. CRC Press, Boca Raton.

Miller, J. D., and Limpus, C. J. (1981). Incubation period and sexual differetiation in the green turtle *Chelonia mydas* L. In: Proceedings of the Melbourne Herpetological Symposium. C. B. Banks and A. A. Martin, eds. pp. 66-73.

Moll, E. O., and Legler, J. M. (1971). The life history of the slider turtle, *Pseudemys scripta* (Schoepff), in Panama. *Bull. Los Angeles Mus. Nat. Hist. 11:1-102.*

Moll, E. O. (1979). Reproductive cycles and adaptations. In 'Turtles: Perspectives and Research'. (Eds. M. Harless and H. Morlock). pp. 305-331. John Wiley, New York.

Morreale, S. J., Ruiz, G. J., Spotila, J. R., and Standora, E. A. (1982). Temperature-dependent sex determination: current practices threaten conservation of sea turtles. *Science 216:1245-1247.*

Morreale, S. J., Gibbons, J. W., and Congdon, J. D. (1984). Significance of activity and movement in the yellow-bellied slider turtle (*Pseudemys scripta*). *Can. J. Zool 62:1038-1042.*

Mortimer, J. A. and Carr, A. (1987). Reproduction and migrations of the Ascension Island green turtle (*Chelonia mydas*). *Copeia 1987:103-113.*

Mosquiera Manso, J. M. (1960). Las tortugas del Orinoco. Segunda edicion, corr. Y aument. Ed. Citania, Buenos Aires.

Mrosovsky, N. (1968). Nocturnal emergence of hatchling sea turtles: control by thermal inhibition of activity. *Nature, 220: 1338–1339.*

Mrosovsky, N. (1980). Thermal biology of sea turtles. *Amer. Zool. 20: 531–547.*

Mrosvosky, N. (1988). Pivotal temperatures for loggerhead turtles (*Caretta caretta*) from northern and southern nesting beaches. *Can. J. Zool. 66:661-669.*

Mrosovsky, N. and Pieau, C. (1991). Transitional range of temperature, pivotal temperatures and thermosensitive stages for sex determination in reptiles. *Amphibia-Reptilia 12:169-179.*

Mrosvosky, N. and Yntema, C. L. (1980). Temperature dependence of sexual differentiation in sea turtles: implications for conservation practices. *Biol. Conserv. 18:271-280.*

Mrosovsky, N., Hopkins-Murphy, S. R., and Richardson, J. I. (1984). Sex ratio of sea turtles: seasonal changes. *Science 225:739-741.*

Mrosovsky, N., Dutton, P. H., and Whitmore, C. P. (1984). Sex ratios of two species of sea turtle nesting in Suriname. *Can. J. Zool. 62:2227-2239.*

Muller, J. F. (1921). Notes on the habits of the soft-shell turtle *Amyda mutica*. *Amer. Midl. Natur. 7: 180–184.*

Neill, W. T. and Allen, E. R. (1954). Algae on turtles, some additional considerations. *Ecology 35:581-584.*

Nieuwolt-Dacanay, P. M. (1997). Reproduction in the western box turtle, *Terrapene ornata luteola*. *Copeia 1997:819-826.*

NT Water Resources. (1999). Daly River levels. Dorisvale Crossing (Station no. G8140067). Office of Lands, Planning and Environment, Darwin.

Nussbaum, R. A. (1981). Seasonal shifts in clutch size and egg size in the side-blotched lizard, *Uta stansburiana* Baird and Girard. *Oecologia 49:8-13.*

Obbard, M. E. and Brooks, R. J. (1980). Nesting migrations of the common snapping turtle *Chelydra serpentina*. *Herpetologica 36:158-162.*

Obbard, M. E. and Brooks, R. J. (1981). A radio-telemetry and mark-recapture study of activity in the common snapping turtle, *Chelydra serpentina*. *Copeia 1981:630-637.*

O’Hara, J. (1980). Thermal influences on the swimming speed of loggerhead turtle hatchlings. *Copeia, 1980: 773–780.*

Olsson, M. M. and Shine, R. (1997a). The limits to reproductive output: offspring size versus number in the sand lizard (*Lacerta agilis*). *Amer. Natur. 149:179-188.*

Olsson, M. M. and Shine, R. (1997b). The seasonal timing of oviposition in sand lizards (*Lacerta agilis*): why earlier clutches are better. *J. Evol. Biol. 10:369-381*.

Packard, G. C., and Packard, M. J. (1988). The physiological ecology of reptilian eggs and embryos. In ‘Biology of the Reptilia, vol. 16’. (Eds. C. Gans and R. B. Huey). pp. 523-605. Alan R. Liss, New York.

Packard, G. C., Packard, M. J., and Birchard, G. F. (1989). Sexual differentiation and hatching success by painted turtles in different thermal and hydric environments. *Herpetologica 45:385-392*.

Packard, G. C., Packard, M. J., and Benigan, L. (1991). Sexual differentiation, growth, and hatching success by embryonic painted turtles incubated in wet and dry environments at fluctuating temperatures. *Herpetologica 47:125-132*.

Packard, G. C., Paukstis, G. L., Boardman, T. J., and Gutske, W. H. N. (1985). Daily and seasonal variation in hydric conditions and temperature inside nests of common snapping turtles (*Chelydra serpentina*). *Can. J. Zool. 63: 2422–2429*.

Palmer-Allen, M., Beynon, F., and Georges, A. (1991). Hatchling sex ratios are independent of temperature in field nests of the long-necked turtle, *Chelodina longicollis* (Testudinata: Chelidae). *Wildl. Res. 18: 225–231*.

Parmenter, R. R. (1980). Effect of food availability and water temperature on the feeding ecology of pond sliders (*Chrysemys s. scripta*). *Copeia 1980:503-514.*

Parmenter, R. R. (1981). Digestive turnover rates in freshwater turtles: The influence of temperature and body size. Comp. *Biochem. Physiol. 70:235-238.*

Paukstis, G. L., Gutske, W. H. N., and Packard, G. C. (1984). Effect of substrate water potential and fluctuating temperatures on sex ratios of hatchling painted turtles (*Chrysemys picta*). Can. J. Zool. 62:1491-1494.

Peters, A., Verhoeven, K.J.F., and Strijbosch, H. (1994). Hatching and emergence in the Turkish Mediterranean loggerhead turtle, *Caretta caretta*: Natural causes for egg and hatchling failure. *Herpetologica, 50: 369–373.*

Plummer, M. V. (1976). Some aspects of nesting success in the turtle *Trionyx muticus. Herpetologica 32:353-359.*

Plummer, M. V. (1977). Activity, habitat, and population structure in the turtle, *Trionyx muticus. Copeia 1977:431-440.*

Plummer, M. V., and Shirer H. W. (1975). Movement patterns in a river population of the softshell turtle, *Trionyx muticus. Occ. Pap. Mus. Nat. Hist. Kansas 43:1-26.*

Plummer, M. V. and Farrar, D. B. (1981). Sexual dietary differences in a population of *Trionyx muticus. J. Herpetol. 15:175-179.*

Plummer, M. V., Mills, N. E., and Allen, S. L. (1997). Activity, habitat, and movement patterns of softshell turtles (*Trionyx spiniferus*) in a small stream. *Chelon. Conserv. Biol. 2:514-520.*

Pluto, T. G., and Bellis, E. D. (1988). Seasonal and annual movements of riverine map turtles, *Graptemys geographica. J. Herpetol. 22:152-158.*

Pritchard, P.C.H., and Trebbau, P. (1984). The Turtles of Venezuela. Society for the Study of Amphibians and Reptiles.

Reibish, J. (1902). Uber den einfluss der temperatur auf die entwicklung von fischeiern. Wiss. *Meeresuntersuch, 2:213–231.*

Robert, K. A., and Thompson, M. B. (2001). Viviparous lizard selects sex of embryos. *Nature 412:698-699.*

Rohr, D. H. (1997). Demographic and life-history variation in two proximate populations of a viviparous skink separated by a steep altitudinal gradient. *J. Anim. Ecol. 66:567-578.*

Roosenberg, W. M. (1996). Maternal condition and nest site choice: An alternative for the maintenance of environmental sex determination? *Amer. Zool. 36:157-168.*

Roosenberg, W. M. and Dunham, A. E. (1997). Allocation of reproductive output: Egg-and clutch-size variation in the diamondback terrapin. *Copeia 1997:290-297.*

Ross, D. A., and Anderson, R. K. (1990). Habitat use, movements, and nesting of *Emydoidea blandingi* in central Wisconsin. *J. Herpetol. 24:6-12.*

Rowe, J. W. (1994). Reproductive variation and the egg size-clutch size trade-off within and among populations of painted turtles (*Chrysemys picta bellii*). *Oecologia 99:35-44.*

Rowe, J. W., and Moll, E. O. (1991). A radiotelemetric study of activity and movements of the Blanding’s turtle (*Emydoidea blandingi*) in northeastern Illinois. *J. Herpetol. 25:178-185.*

Rowe, L., Ludwig, D., and Schluter D. (1994). Time, condition, and the seasonal decline of avian clutch size. *Amer. Natur. 143:698-722.*

Roze, J. A. (1964). Pilgrim of the river. Life cycle of the Oronico River has many unusual features. *Natur. Hist. 73:34-41.*

Schubauer, J. P., Gibbons, J. W., and Spotila, J. R. (1990). Home range and movement patterns of slider turtles inhabiting Par Pond. In ‘Life History and Ecology of the Slider Turtle’. (Ed. J. W. Gibbons). pp. 223-232. Smithsonian Institution Press, Washington D. C.

Schwartzkopf, L. (1993). Costs of reproduction in water skinks. *Ecology 74:1970-1981.*

Schwartzkopf, L., and Brooks, R. J. (1987). Nest-site selection and offspring sex ratio in painted turtles, *Chrysemys picta. Copeia 1987:53-61.*Schwartzkopf, L., and

Shine, R. (1991). Thermal biology of reproduction in viviparous skinks, *Eulamprus tympanum*: Why do gravid females bask more? *Oecologia 88:562-569.*

Shine, R. (1980). “Costs” of reproduction in reptiles. *Oecologia 46:92-100.*

Shine, R. (1988). Constraints on reproductive investment: a comparison between aquatic and terrestrial snakes. *Evolution 42:17-27.*

Shine, R. (1992). Relative clutch mass and body shape in lizards and snakes: is reproductive investment constrained or optimised? *Evolution 46:828-833.*

Shine, R. (1999). Hot sex in the underground: Why is sex determined by nest temperature in so many reptiles? *Trends Ecol. Evol. 14:186-189.*

Shine, R., and Iverson, J. B. (1995). Patterns of survival, growth, and maturation in turtles. *Oikos 72:343-348.*

Shine, R., and Madsen T. (1997). Prey abundance and predator reproduction: rats and pythons on a tropical Australian floodplain. *Ecology 78:1078-1086.*

Shine R., and Harlow, P. S. (1996). Maternal manipulation of offspring phenotypes via nest-site selection in an oviparous lizard. *Ecology 77:1808-1817.*

Smith, C. C. and Fretwell, S. D. (1974). The optimal balance between size and number of offspring. *Am. Natur. 108:499-506.*

Smith, G. R., Ballinger, R. E., and Rose, B. R. (1995). Reproduction in *Sceloporus virgatus* from the Chiricahua Mountains of southeastern Arizona with emphasis on annual variation. *Herpetologica 51:342-349.*

Spencer, R., Thompson, M. B., and Hume, I. D. (1998). The diet and digestive energetics of an Australian short-necked turtle, *Emydura macquarii*. *Comp. Bioch. Physiol. A, 121:341-349.*

Spotila, J. R., Standora, E. A., Morreale, S. J., and Ruiz, G. J. (1987). Temperature dependent sex determination in the green turtle (*Chelonia mydas*): effects on the sex ratio on a natural nesting beach. *Herpetologica 43:74-81.*

Stancyk, S.E. (1982). Non-human predators of sea turtles and their control. In 'Biology and conservation of sea turtles'. (Ed. K.A. Bjorndal). pp. 139–152. Smithsonian Institution Press, Washington, D.C.

Standing, K. L., Herman, T. B., and Morrison, I.. (1999). Nesting ecology of blanding's turtle (*Emydoidea blandingii*) in Nova Scotia, the northeastern limit of the species' range. *Can J. Zool. 77:1609-1614.*

Standora, E. A. (1982). A telemetric study of the thermoregulatory behavior and climate-space of free-ranging yellow-bellied turtles, *Pseudemys scripta.* Unpubl. Ph.D. diss. Univ. of Georgia, Athens.

Strussmann, C. A. and Patino, R.. (1995). Temperature manipulation of sex differentiation in fish. Proc. of the Fifth Intl. Symp. on Reproductive Physiology of Fish. Austin, Texas, USA, 2-8 July.

Studier, E. H., Studier, A. L., Essy, A. J., and Dapson, R. W. (1977). Thermal sensitivity and activation energy of intrinsic intestinal motility in small vertebrates. *J. Thermal Biol. 2:101-105.*

Swingland, I. R., and Coe, M. (1978). The natural regulation of giant tortoise populations on Aldabra Atoll. Reproduction. *J. Zool. Lond. 186:285-309.*

Swingland, I. R., and Lessells, C. M. (1978). The natural regulation of giant tortoise populations on Aldabra Atoll. Movement polymorphism, reproductive success and mortality. *J. Anim. Ecol. 48:639-654.*

Swingland, I. R., and Greenwood, P. J. (1983). The Ecology of Animal Movement. Clarendon Press, Oxford.

Taylor, J. A., and Tulloch, D. (1985). Rainfall in the wet-dry tropics: extreme events at Darwin and similarities between years during the period 1870-1983 inclusive. *Austr. J. Ecol. 10:281-295.*

Thomas, R. B., Vogrin, N., and Altig, R. (1999). Sexual and seasonal differences in behavior of *Trachemys scripta* (Testudines: Emydidae). *J. Herpetol. 33:511-515.*

Thompson, M.B. (1988). Nest temperatures in the pleurodiran turtle, *Emydura macquarii*. *Copeia 1988: 996–1000.*

Trauth, S. E. (1983). Nesting habitat and reproductive characteristics of the lizard *Cnemidophorus sexlineatus* (Lacertilia: Teiidae). *Amer. Midl. Natur. 109:289-299.*

Trivers, R. L. (1972). In 'Sexual selection and the descent of man'. (Ed. B. Campbell). pp. 136-179. Aldine-Athestton Inc., Chicago, IL.

Tuberville, T. D., Gibbons, J. W., and Greene, J. L. (1996). Invasion of new aquatic habitats by male freshwater turtles. *Copeia 1996:713-715.*

Tucker, A. D. (2000). Cumulative effects of dams and weirs on freshwater turtles: Fitzroy, Burnett, and Mary River Catchments. Qld. Parks and Wildlife Service report. 250 pp.

Tucker, J. K. (1997). Natural history notes on nesting, nests, and hatchling emergence in the red-eared slider turtle, *Trachemys scripta elegans*, in west–central Illinois. *Ill. Natur. Hist. Surv. 140: 1–13.*

Tucker, J. K. (2000). Annual variation in hatchling size in the red-eared slider turtle *(Trachemys scripta elegans). Herpetologica 56:8-13.*

Turner, F. B., Medica, P. A., and Lyons, C. L. (1984). Reproduction and survival in the desert tortoise (*Scaptochelys agassizii*) in Ivanpah Valley. *Copeia 1984:811-820.*

Turner, F. B., Hayden, P., Burge, B. L., and Roberson, J. B. (1986). Egg production by the desert tortoise (*Gopherus agassizii*) in California. *Herpetologica 42:93-104.*

Van Noordwijk, A. J., and de Jong, G. (1986). Acquisition and allocation of resources: their influences on variation in life history tactics. *Amer. Natur. 128:137-142.*

Viets, B. E., Tousignant, A., Ewert, M. A., Nelson, C. E., and Crews, D. (1993). Temperature-dependent sex determination in the leopard gecko, *Eublepharis macularius. J. Exp. Zool. 265:679-683.*

Viets, B. E., Ewert, M. A., Talent, L. G., and Nelson, C. E. (1994). Sex-determining mechanisms in squamate reptiles. *J. Exp. Zool. 270:45-56.*

Vitt, L. J. (1982). Reproductive tactics of *Ameiva ameiva* (Lacertilia:Teiidae) in a seasonally fluctuating tropical habitat. *Can. J. Zool. 60:3113-3120.*

Vitt, L. J. and Congdon, J. D. (1978). Body shape, reproductive effort, and relative clutch mass in lizards: resolution of a paradox. *Amer. Natur. 112:595-608.*

Vitt, L. J., van Loben Sels, R. C., and Ohmart, R. D. (1978). Lizard reproduction: annual variation in environmental correlates in the iguanid lizard *Urosaurus graciosus*. *Herpetologica 34:241-253.*

Vogt, R. C. and Bull, J. J. (1982). Temperature-controlled sex-determination in turtles: ecological and behavioral aspects. *Herpetologica 38:156-164.*

Vogt, R. C., and Bull, J. J. (1984). Ecology of hatchling sex ratio in map turtles.

Ecology 65:582-587.

Vogt, R. C. and Flores-Villela, O. (1984). Effects of incubation temperature on sex determination in a community of neotropical freshwater turtles in southern Mexico. *Herpetologica 48:265-270.*

Weatherhead, P. J., Brown, G. P., Prosser, M. R., and Kissner, K. J. (1999). Factors affecting neonate size variation in northern water snakes, *Nerodia sipedon. J. Herpetol. 33:577-589.*

Webb, G. J. W., and Smith, A. M. A. (1984). Sex ratio and survivorship in the Australian freshwater crocodile *Crocodylus johnstoni. Symp. Zool. Soc. Lond. 52:319-355.*

Webb, G. J. W., Buckworth, R., and Manolis, S. C. (1983). *Crocodylus johnstoni* in the McKinlay River, N.T. VI. Nesting biology. *Austr. Wildl. Res. 10:607-637.*

Webb, G. J. W., Choquenot, D., and Whitehead, P. J. (1986). Nests, eggs, and embryonic development of *Carettochelys insculpta* (Chelonia: Carettochelydidae) from northern Australia. *J. Zool. Lond. 1B: 521–550.*

Weisrock, D. W., and Janzen, F. J. (1999). Thermal and fitness-related consequences of nest location in painted turtles (*Chrysemys picta*). *Funct. Ecology 13:94-101.*

Welsh, M. (1999). Resource partitioning among the freshwater turtles of the Daly River, Northern Territory. Unpubl. Honours Thesis, University of Canberra, Canberra, Australia.

Whitmore, C. P., and Dutton, P. H. (1985). Infertility, embryonic mortality and nest-site selection in leatherback and green sea turtles in Suriname. *Biol. Conserv. 34:251-272.*

Whittow, G. C., and Balazs, G. H. (1982). Basking behavior of the Hawaiian green turtle (*Chelonia mydas). Pac. Sci. 36:129-139.*

Wilhoft, D.C., Hotaling, E., and Banks, P. (1983). Effects of temperature on sex determination in embryos of the snapping turtle, *Chelydra serpentina. J. Herpetol. 17: 38–42.*

Wilson, D. S. (1998). Nest-site selection: Microhabitat variation and its effects on the survival of turtle embryos. *Ecology 79:1884-1892.*

Witherington, B.E., Bjorndal, K.A., and McCabe, C.M. (1990). Temporal pattern of nocturnal emergence of loggerhead turtle hatchlings from natural nests. *Copeia 1990: 1165–1168.*

Witherington, B.E., and Salmon, M. (1992). Predation on loggerhead turtle hatchlings after entering the sea. *J. Herpetol. 26: 226–228.*

Wolda, H., and Kreulen, D. A. (1973). Ecology of some experimental populations of the landsnail *Cepaea nemoralis* (L.). II. Production and survival of eggs and juveniles. *Netherl. J. Zool. 23:168-188.*