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The significance of predation in nest site selection of turtles: an experimental consideration of macro- and microhabitat preferences

Ricky-John Spencer and Michael B. Thompson

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Nest selection has important consequences for fitness in freshwater turtles, as nest predation is extremely high in most populations. However, nest predation has rarely been considered a major factor affecting nest site selection. Thermal qualities of a nest are often suggested as a factor influencing maternal choice of nest site because incubation temperature affects offspring performance and growth, as well as the sex ratio of the clutch in species with temperature dependent sex determination (TSD). Yet experimental manipulations of the nesting environment (habitat and threat) to test factors influencing maternal nest site selection in turtles are uncommon. In this paper, we show that nest site selection in an Australian turtle (*Emydura macquarii*) with genetically determined sex determination (GSD) exists at two spatial scales, with predation as the major factor affecting the location of a nest. Females prefer to nest in areas where nest predation is minimal but when the risk of direct predation is increased, they trade-off minimising nest predation with survival by locating their nests closer to shore. However, experimentally reducing cover demonstrated that females prefer to nest in open areas, which are more common closer to shore. Hence females are forgoing preferred microhabitat to locate their nests away from shore to minimise nest predation. Despite a preference for open nesting areas, females exhibit similar repeatability of microenvironment selection to a North American species with TSD. Repeatability of nesting overstory vegetation in the North American turtle, *Chrysemys picta*, suggests that females may exert some control over the thermal qualities of the nests; a key assumption in theoretical models of the microevolution and adaptive significance of TSD in reptiles. Incubation temperature is one factor that may affect microhabitat preferences in both turtles but other factors (e.g. predation and soil moisture) affected by microhabitat may be critical in the evolution of repeatability of overstory cover.

R.-J. Spencer, Department of Zoology and Genetics, Science II, Iowa State Univ., Ames, IA, 50011-3223, USA (rspencer@iastate.edu). – M. B. Thompson, School of Biological Sciences (A08), Univ. of Sydney, NSW 2006, Australia.

Nest-site selection can be defined as the placement of eggs by females at sites that differ from random sites within a delimited area (Wilson 1998). Selection on a female to disguise the nest from predators will be strong in most turtle populations as nest predation is one of the greatest sources of offspring mortality (Congdon et al. 1983, Thompson 1983, Iverson 1991, Brown and MacDonald 1995, Yerli et al. 1997, Burke et al. 1998, Escalona and Fa 1998). Nest site selection may occur at two spatial scales. Factors affecting nest site selection may occur at both the macrohabitat (Spencer 2002a) and microhabitat (Schwarzkopf and Brooks 1985, Janzen and Morjan 2001) levels (Fig. 1). Quite obviously, most studies have focused on non-random nesting with respect to microhabitat because of its effects on development (Bilinski et al. 2001), sex (Janzen and Morjan 2001) and survival (Wilson 1998, Kolbe and Janzen 2001), although females also respond to the threat of direct predation by locating their nests closer to shore (Spencer 2002a).

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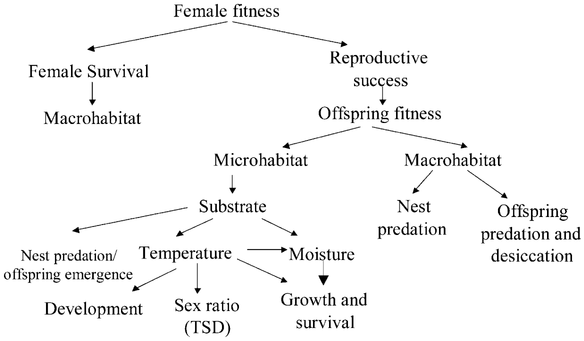

Fig. 1. Factors potentially affecting nest selection in freshwater turtles. Female fitness can be divided into survival and reproductive success. Female survival is predominantly affected by time on land and reproductive success relates to offspring fitness. Offspring fitness primarily depends on where the nest is constructed and is affected at the macro- and micro-habitat level through factors such as nest predation and incubation temperature.

Most studies on nest site selection have originated from North America and have primarily focused on species with temperature-dependent sex determination (TSD, Vogt and Bull 1984, Schwarzkopf and Brooks 1985, Bobyn and Brooks 1994, Davenport 1998, Bragg et al. 2000, Janzen and Morjan 2001). Sex ratio of a population with TSD is governed by the thermal sensitivity of embryonic sex determination and by maternal behavioural choice of thermal qualities of nest sites (Charnov 1982, Bull 1983, Janzen and Morjan 2001). Recently, the repeatability of nesting behaviour or particularly, nest site selection, in a species of turtle with temperature dependent sex determination (TSD) was used as evidence that females may exert some control over the thermal qualities of the nest by nesting non-randomly with respect to available overstory vegetation (Janzen and Morjan 2001). Maternal behavioural choice of the thermal regime of a potential nest site is a key assumption in theoretical models (Charnov 1982, Bull 1983) concerning the microevolution and adaptive significance of TSD in reptiles. Individual female *Chrysemys picta* consistently nest in different locations with similar quantities of overstory vegetation cover, which is a reasonable predictor of nest thermal environments and offspring sex ratios (Janzen 1994, Janzen and Morjan 2001). Repeatability is crucial because consistent individual differences are required for selection to act on the trait and it places an upper bound on its heritability; thus on the evolutionary response to selection (Janzen and Morjan 2001). While TSD can evolve independently, it cannot be described as a driving factor of nest site selection without first experimentally proving that thermal properties of a nest affect maternal selection. Even then the link between selection of the thermal properties of a nest and the manipulation of offspring sex ratio is inconclusive. Overstory vegetation may affect thermal environment and in turn the sex ratio of developing offspring in a species with TSD, but nesting substrate, which is also linked to overstory vegetation, also affects offspring performance and growth (Finkler 1995, Elphick and Shine 1998).

We tested the repeatability of nesting and microhabitat preferences in a species of turtle without TSD, where we can exclude manipulation of offspring sex ratio as a factor influencing maternal nest site selection. In this study, we show that an Australian freshwater turtle (*Emydura macquarii*), which exhibit genotypic sex determination, attempts to minimise nest predation at a macrohabitat level before exhibiting significant repeatability of microenvironment, despite preferring to nest in the open or areas of low vegetation.

Methods

Study animal and area

Emydura macquarii is a chelid turtle (suborder: Pleurodira) that is widespread, inhabiting the Murray-Darling drainage system, and with several forms distributed throughout eastern flowing rivers of coastal NSW and Qld (Cann 1998). Females grow to a carapace of over 300 mm and, in the Murray-Darling system, are primarily confined to permanent waters. They are omnivorous, consuming mainly filamentous algae, fish (usually the remains of European carp (*Cyprinus carpio*)) and insects (Spencer et al. 1998). Mating occurs from March to April (Cann 1998) and turtles over-winter in the water (Tasker 1991). Nesting occurs between late October and mid-December, and females generally produce only one clutch of 15–35 eggs per year (Chessman 1978, Spencer 2001). Offspring sex is determined genetically (Thompson 1983) and hatching occurs synchronously (Thompson 1989) to reduce the risk of predation (Spencer et al. 2001).

Five lagoons of 5–40 ha were studied in the upper Murray River near Albury-Wodonga (36°S, 46°E) in southeastern Australia. Much of the land between Hume weir and Lake Mulwala has been subject to large-scale vegetation clearing since settlement (Smith and Smith 1990) and is now mainly grassy plain. There is no mid-story and trees, predominately river red gum (*Eucalyptus camuldensis*), are sparse. Four of the lagoons are extensions of the River Murray near to Albury that only connect during large floods and hence each lagoon has distinct banks that restrict nesting to certain areas. The fifth lagoon is circular and relatively small (< 5 ha). Turtles can access any part of the bank and the lagoon is connected to Lake Mulwala by a small channel (< 3 m wide).

Macrohabitat

Nest predation

Nest predation rates around the four closed lagoons in Albury were determined between 1996 and 1998 (Spencer 2002a). Distance to shore was measured for each nest and total nest predation rates for each lagoon was calculated by dividing the number of destroyed nests by the total number of nests constructed (Spencer 2002a). Predation rates around each lagoon were obtained from nests where the female was observed during construction (i.e. excluding nests discovered subsequently destroyed or intact). From January 1997, foxes (*Vulpes vulpes*) were removed from around two Albury lagoons (Spencer 2002a). These lagoons were considered low risk areas because foxes are the major predator of nesting *E. macquarii* and their nests (Spencer 2002a). Two control lagoons, where foxes were not removed, were considered high risk areas. We have used the same data to determine the pattern of nest predation in relation to distance from shore, i.e. the number of nests destroyed divided by the total number of nests constructed in the following regions; 0–10 m, 11–20 m, 21–30 m, 31–40 m, 41–50 m and beyond 50 m from shore. Two-factor ANOVA was used to compare predation rates between high and low risk areas and distance from shore in 1997 and 1998. SNK multiple comparison was used to test for differences in predation rates in relation to distance from shore. Data were transformed (arcsin square root) if they failed a Levene Median test of equal variances. SYSTAT 9.0 (1999) was used for all statistical analyses.

Microhabitat

Microhabitat selection was experimentally tested around a lagoon at Lake Mulwala. Nesting is a discrete event that usually occurs during or after rainstorms in November each year (Cann 1998, Spencer 2002a). Nest predation rates are extremely high and the number of nests destroyed by predators were counted in January of each year to measure nesting activity around the Mulwala lagoon. Foxes are the major predators of nests and shell fragments usually remain after the nest is destroyed (Spencer 2002a). Some *Chelodina longicollis* nests may have been included in the analyses, however, they prefer smaller non-permanent water (Cann 1998) and densities are over 20 times lower than *E. macquarii* in October/November (Spencer 2001). Only nests where eggshell fragments could be identified were included in the analyses. The grass was mowed from 1 to 50 m from shore around half the lagoon (~ 250 m) in 1997 using a ROVER ride-on lawn mower. Grass was mowed on the 2–3 November 1997 and nesting began within 10 days of mowing. Searches for destroyed nests occurred by systematically walking around the lagoon – 10, 20, 30, 40 and 50 m from shore. Nests beyond 50 m were rare but any that were discovered during daily activity around the lagoon (e.g. trapping; Spencer 2002b) were also recorded.

The change in nesting activity (1996–1998) in the mowed area was compared against two (control) high risk areas in Albury. The Albury lagoons have distinct banks; hence nesting activity on either side was monitored each year. Each nest and 100 randomly selected points (total around each lagoon) within 50 m from shore were sampled for vegetation height every year. Vegetation height was measured at four points (N, S, E, W) within a half metre quadrat using a 1-m rule from the base of the vegetation to its highest point. Each year the vegetation height around the mowed lagoon was compared using a one-factor ANOVA. A two-factor ANOVA was used to compare vegetation height among each control site across years (1996–1998). We also tested the null hypothesis that no differences existed between microhabitat characteristics at nest and random sites at the two low risk Albury sites where foxes were removed and turtles nested further out from shore (Spencer 2002a). We compared the distributions of vegetation height and substrate type of each nest site to those measured at 100 random sites around each lagoon. We used ANOVA to determine if there were any differences in vegetation height between nest sites and random locations before and after predators were removed and within each site separately. The affect of vegetation height on nest predation in the high risk areas (i.e. non-removal lagoons, 1996–1998) was compared using t-tests (destroyed vs intact).

Repeatability of microhabitat

Nesting around the four Albury lagoons was monitored in 1996, 1997 and 1998. Foxes were continually removed from two lagoons after the first nesting season (from January 1997 to December 1998; Spencer 2002a). Repeatability of overstory vegetation was tested using only females observed nesting on two or more occasions around the non-predator removal sites. Only females that were first observed nesting in 1996 around the two removal lagoons and subsequently in either 1997 or 1998 were used to test for the affect of predation risk on repeatability. Sites were searched for nesting females during and after the first major rain or storm activity in November (Spencer 2001). Females were marked with a unique combination of notches to the marginal scutes (Thompson 1982) as they left the nesting area after completing the nesting process in 1996. During 1997 and 1998, most nesting females were marked from a large mark-recapture program conducted in the lagoons over the three years (Spencer 2001). We measured the height of the vegetation and distance to nearest tree of each nest constructed. No females were caught nesting more than once per year. Overstory vegetation is the best predictor of the thermal properties of a nest (Janzen 1994, Janzen and Morjan 2001) and vegetation height surrounding a nest was used to predict relative nest temperatures in this study because there is no mid-story vegetation and less than two trees (> 25 m from the nearest nest) are present in any nesting area. We calculated the repeatability of nest site selection from a one-way ANOVA with individual as the main effect. We calculated the observational components of variance from the mean squares and the harmonic mean of the number of nesting events (Lessells and Boag 1987, Brodie and Russell 1999), and estimated repeatability (R) using a repeatability equation from Brodie and Russell (1999).

Results

Macrohabitat

Mean distance of nests (including nests of females observed during construction and all other nests found consequently) increased over 10 m after foxes were reduced (Spencer 2002a). Over 70% of nests (including only nests of females observed during construction) were located between 0 and 20 m from shore in low risk areas in 1996 (before fox removal) (Fig. 2a), however only 40% of nests were constructed in the same region after foxes were reduced in 1997 and 1998 (Fig. 2a). Between 60% and 80% of nests were located 0–20 m in high risk areas over the same period (Fig. 2b). Predation rates were significantly lower in low predation risk areas in 1997 ($F_{1,19} = 115$, $p < 0.001$) and 1998 ($F_{1,19} = 40$, $p < 0.001$). Nest predation was also affected by distance to shore in both years (1997: $F_{4,19} = 15.1$, $p < 0.001$; 1998: $F_{4,19} = 10.8$, $p = 0.001$). No significant interaction between risk and distance from shore was detected in either year. Rates of nest predation were greatest close to shore but declined consistently to a minimum at 21–30 m from shore in high and low risk areas in 1997 and 1998 (Fig. 3). Nest predation rates were significantly higher close to shore (0–10 m) than further out (> 11 m, $p < 0.05$), except in 1998 when predation rates were similar between 0–10 m and 11–20 m. Nest predation rates between 21 and 30 m from shore were significantly lower than areas closer to shore in both years and all sites (high and low predation risk, $p < 0.01$). Predation rates remained similar to 21–30 m from shore beyond 30 m, except in 1998 when they were significantly higher at 41–50 m from shore ($p < 0.05$). Despite successful reduction in fox numbers (Spencer 2002a), the majority of nests were destroyed by foxes in both high and low risk areas throughout the study. Birds (e.g. magpies (*Gymnorhina tibicen*) and water rats (*Hydromys chrysogaster*) were other predators of nests (Spencer 2002a). Vegetation height did not affect rates of nest predation in either of the high-risk areas.

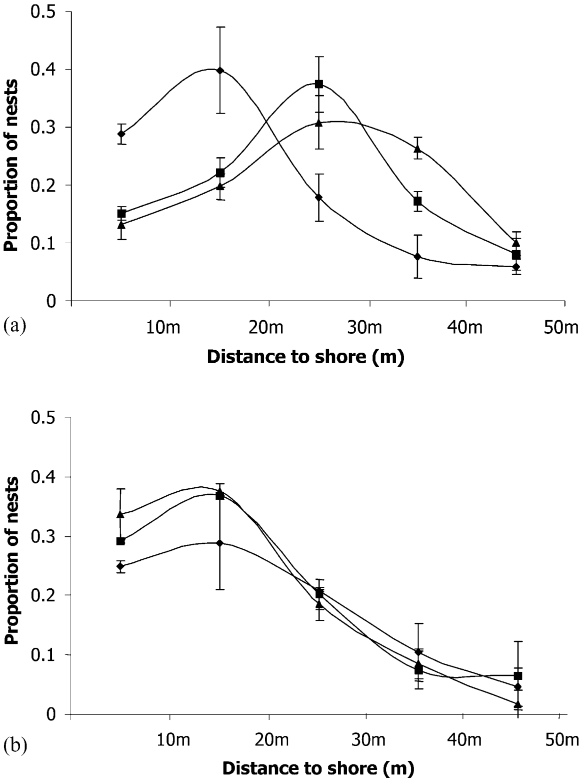

Fig. 2. Proportion of total nests observed during construction (from Spencer 2002a) divided into 10m intervals from shore. High (a) and low (b) risk areas in 1996 (diamond), 1997 (square) and 1998 (triangle). A combined total (two sites per treatment) of 54 and 49 females in 1996, 79 and 99 females in 1997, and 69 and 91 females in 1998 were observed nesting in low and high risk sites, respectively.

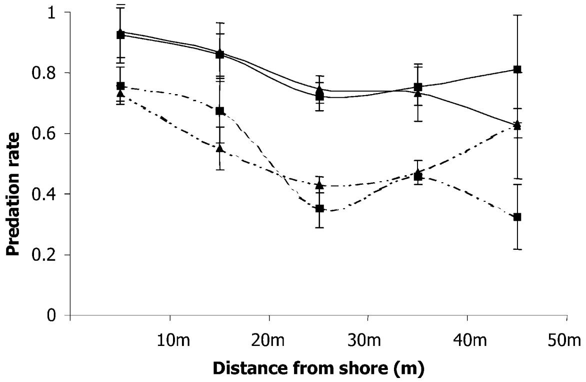

Fig. 3. Nest predation rates (± S.D.) in relation to distance from shore from high (solid) and low (dashed) risk areas in 1997 (diamond) and 1998 (square).

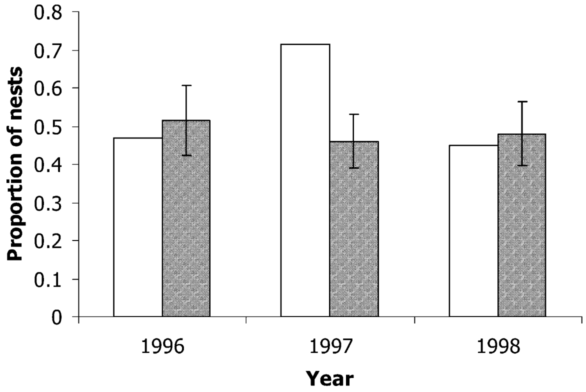

Fig. 4. Nesting activity in mowed vs control areas from 1996 to 1998. Mowing took place around half a lagoon (open) in 1997. Two control lagoons (grey) represents nesting activity from a randomly chosen bank of each lagoon (mean ± S.D.).

Microhabitat

Female turtles preferred to nest in areas that were mowed, because there was a 25% increase in nesting activity after mowing with no corresponding increase in activity in the control sites (Fig. 4). Total nesting activity remained similar over the three years with 166, 172 and 145 nests discovered around the mowed site from 1996–1998, respectively.

After mowing, vegetation height was significantly lower than the year previous to and after mowing ($F_{2,154} = 149$, $p < 0.01$). Vegetation heights were similar in the two control lagoons in all years, but vegetation height decreased in both sites in 1997 ($F_{2,339} = 53.0$, $p < 0.001$) because of low rainfall (Spencer 2001), returning to 1996 levels in 1998 (Fig. 5). Height of vegetation in the central areas in 1997 did not fall to that of the mowed site (Fig. 5) and it resulted in no significant increase in nesting activity (Fig. 4).

Habitat close to shore was generally open with little vegetation at each site, compared to a soil substrate of medium vegetation height (~ 12 cm) beyond 20 m. The overall relationship between vegetation height, nesting substrate and distance from water was significant ($F_{2,97} = 76.7$, $p < 0.001$; $R^2 = 0.61$). Habitat preferences after foxes were removed from an area did not differ despite turtles nesting on average further from the water (Spencer 2002a). A disproportionate number of turtles constructed nests in open areas with little or no vegetation (Site 1: $F_{1,133} = 6.1$, $p = 0.01$; Site 2: $F_{1,150} = 9.7$, $p = 0.002$). Turtles preferred similar areas with little vegetation (Site 1: $F_{1,160} = 9.1$, $p = 0.003$; Site 2: $F_{1,151} = 8.5$, $p = 0.004$) in 1997, despite nesting further away from water where these habitats are less common.

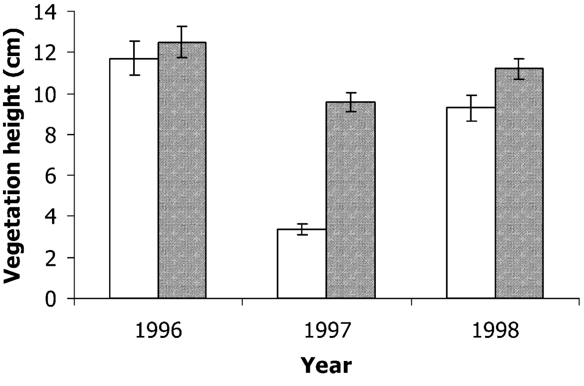

Fig. 5. Mean vegetation height (± S.E.) of mowed (open) and control areas (grey) from 1996 to 1998. The area was mowed prior to nesting in November 1997.

Repeatability

A total of 94 females were observed nesting on two or more occasions around all lagoons. We detected a significant repeatability of overstory vegetation cover around nests at oviposition in areas where foxes had not been removed ($R = 0.73$, $N = 65$). Nest site fidelity was low, as 60% of females observed nesting on two or more occasions emerged at different access points and nested in different areas from the previous occasion.

Around the predator removal lagoons, repeatability of overstory vegetation cover was also strong ($R = 0.68$, $N = 29$), despite females nesting an average 16.3 ± 5.7 m S.D. further away from water.

Discussion

Predation is a major factor driving nest site selection in *E. macquarii*. At the macrohabitat level, nest site selection by female *E. macquarii* is affected by the presence of foxes because females consistently nest 10–15 m closer to shore in high-risk areas (Fig. 2; Spencer 2002a). There are potentially greater risks of mortality nesting at 30 m from shore than at 15 m considering foxes harass and kill females while they are nesting (Spencer 2002a). However, females that nest further from shore may increase offspring fitness and nest survival. If offspring mortality is a function of distance from shore (or elevation), the curve would be broadly U-shaped because offspring mortality generally increases closer to shore due to flooding (Milton et al. 1994, Standing et al. 1999), nest predation (Horrocks and Scott 1991) and inappropriate nesting substrates (Spencer 2002a). Similarly if a female nests too far from shore, her risk of direct predation increases, as does offspring mortality because hatchlings must spend more time traversing over land to the water (Wood and Bjorndal 2000). There is some area in any system where total offspring mortality is minimised (Fig. 6). Nest predation in the present study reflects the total offspring mortality curve (Fig. 6) because predators destroy 90% of eggs. Nest predation rates are increased close to shore and are lowest 21–30 m from water;

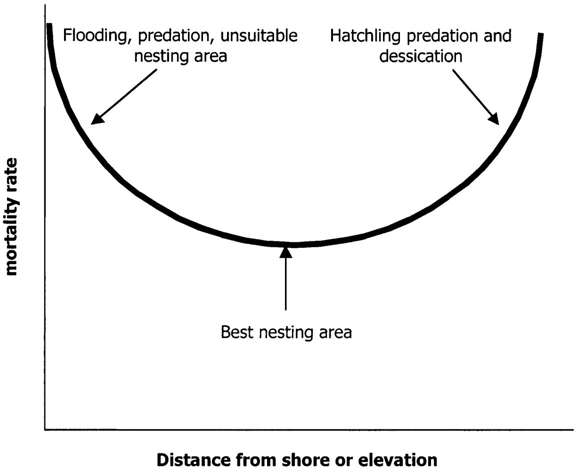

Fig. 6. A theoretical model of offspring mortality in relation to distance to shore or elevation. Total mortality curve is the addition of all sources of offspring mortality during the incubation process. Mortality is high close to shore because of increased nest predation or risk of flooding. Mortality is also increased far from shore because the risk of predation or dehydration to hatchlings increases the more time that they spend traversing over land.

where the majority of females are nesting in low risk areas. Yet by nesting further from shore, the availability of preferred open nesting areas is less, suggesting that nest site selection in *E. macquarii* possibly exists at two spatial scales. Female *E. macquarii* forgo preferred microhabitats closer to shore to minimise nest predation and thus a trade-off exists between minimising the risk of direct predation and minimising nest predation in high-risk areas. The mowing experiment provides further evidence of nesting occurring at two spatial scales, where females may select an area to emerge from the water on the basis of macro-habitat characteristics.

To maximise reproductive success, a female turtle must choose a microhabitat that provides appropriate incubation conditions and is disguised from predators because simply placing eggs at random 20–30 m from shore will not result in lower nest predation rates. However the selective forces driving microhabitat selection have rarely been experimentally tested. Incubation temperature is commonly proposed as a driving factor of nest site selection (Vogt and Bull 1984, Schwarzkopf and Brooks 1985, Bobyn and Brooks 1994, Davenport 1998, Bragg et al. 2000, Janzen and Morjan 2001). *Emydura macquarii* prefer to nest in open areas with little or no vegetation, which influences thermal conditions experienced by developing embryos of many turtles (Bull and Vogt 1979, Wilhoft et al. 1983, Janzen 1994). Field incubation environments can affect offspring sex in many species, as well as affect future growth and survivorship of hatchlings exiting the nest cavity (Burger 1976, Leshem and Dmi'el 1986, Cagle et al. 1993, Wilson 1998). Low incubation temperatures reduce hatching success, post hatching survival and hatchling growth in snapping turtles (*Chelydra serpentina*) (Bobyn and Brooks 1994). Indeed, incubation conditions (i.e. low temperatures) may be the most important factor affecting the northern distribution of snapping turtles in Canada (Bobyn and Brooks 1994). Similarly, Murray River populations of *E. macquarii* are at their southern most distribution in Australia (high latitude) and females may construct nests in relatively hot open microhabitats to minimise incubation period and maximise hatchling growth prior to winter.

Emydura macquarii is a medium–large freshwater turtle that matures around 12 years and can produce more than 35 eggs per clutch (Spencer 2002b). Other North American species of comparable size prefer open nest sites (e.g. *Chrysemys*; Schwarzkopf and Brooks 1987, *Apalone*; Plummer et al. 1994 and *Chelydra*; Petokas and Alexander 1980), whereas smaller striped mud turtles (*Kinosternum baurii*) prefer to nest close to vegetation and avoid open areas (Wilson 1998). Larger turtles prefer to nest in open areas because they have the capacity to dig relatively deeper nests than small turtles (Ehrenfeld 1979, Wilson 1998). Nest depth is correlated with nest temperature (Burger 1976, Packard and Packard 1988, Thompson 1988, Congdon and Gibbons 1990) and larger turtles place their nests in microhabitats that have little surrounding vegetative cover, so that the eggs can reach the appropriate incubation temperature for complete embryonic development (Congdon et al. 1987, Butler and Hull 1996).

Moisture content is also affected by overstory vegetation (Janzen and Morjan 2001). However, moisture content is unlikely to influence nest site selection in *E. macquarii* because they have rigid-shelled eggs (Thompson 1988) that prevents the loss or uptake of large amounts of water from the environment (Packard et al. 1982). Also, hatching success and hatchling growth in this type of egg appears unaffected by differing levels of soil hydration (Packard et al. 1979). Overstory vegetation height is also associated with substrate type, which can affect nest predation and incubation conditions. Predation rates are similar in open and densely vegetated nests, despite lower nest predation rates in river sand compared to soil (Spencer 2002a). River sand constitutes a relatively small proportion of the total nesting substrate and does not occur around all lagoons. Only nests that survive the first week are likely to complete incubation regardless of surrounding vegetation, because of the ability of foxes to detect nests declines (Spencer 2002a).

Females repeatedly nest in similar microhabitats, despite the strong preference for open nest locations. Together with preferences for open nest sites, repeatability for overstory vegetation demonstrates that *Emydura macquarii*, a freshwater turtle with genetically determined sex determination (GSD) from Australia, has similar microhabitat preferences to *C. picta*, a freshwater turtle with TSD from North America

(Schwarzkopf and Brooks 1987, Janzen and Morjan 2001). Repeatability of overstory vegetation might be a response of female *C. picta* to the thermal environment of the nest and that this meets a crucial assumption of theoretical models concerning the microevolution and adaptive significance of TSD in reptiles (Janzen and Morjan 2001). Models (Charnov 1982, Bull 1983) predict that after TSD is established, the microevolution of sex ratio is governed by selection on, and heritable variation in, two key factors: (1) thermal sensitivity of embryonic sex determination and (2) maternal behavioural choice of the thermal qualities of nest sites. Obviously offspring sex does not influence of microhabitat selection in *E. macquarii* and, while the same nesting microhabitat preferences may have evolved independently, both *C. picta* and *E. macquarii* are similar turtles in many respects. Both species are of similar size, occur in high densities and have similar corresponding wide distributions across North America and Australia, respectively (Cann 1998, Ernst et al. 1994). Both species prefer slow moving water such as lagoons and ponds and are omnivorous (Ernst et al. 1994, Spencer et al. 1998). Life history strategies are also extremely similar, as both *E. macquarii* and *C. picta* have relatively high rates of nest predation, a long juvenile stage and high adult survivorship (Iverson and Smith 1993, Mitchell 1988). Thus, similar forces driving nest site selection may occur in both species. Regardless of the mechanisms behind repeatability of overstory in *C. picta*, be it predation, thermal environment or soil moisture, TSD may evolve adaptively because females nest non-randomly with respect to overstory vegetation and thermal environment (Janzen and Morjan 2001).

Why has TSD evolved in *C. picta* but not in *E. macquarii*? Of course phylogenetic constraints may restrict the evolution of TSD, as none of the Chelidae studied to date have TSD (Cann 1998). However, this question relates to the adaptive significance of TSD and despite it being a key demographic parameter, the evolutionary dynamics of TSD has rarely been clarified (Conover and Heins 1987). While it is impossible to know when TSD evolved in *C. picta*, it is easier to discuss how TSD may benefit *C. picta* over *E. macquarii* under current demographic and environmental conditions. An immediate impact of TSD may occur within the nest. Post hatching physical performance is a fitness trait correlated with sex and incubation temperature (Ciofi and Swingland 1997). Male and female snapping turtles (*Chelydra serpentina*) from nests where incubation temperatures produce one sex have higher first year survival than individuals from intermediate temperatures (Janzen 1995). *Chrysemys picta* overwinter within the nest (Weisrock and Janzen 1999) and TSD would be advantageous if sex differences occurred in overwinter survivorship. Initial differences in incubation temperature establishes offspring sex in *C. picta*. However, differences in nest temperature occur throughout the entire incubation and overwintering period (summer to spring, Janzen, unpubl.). Hence, energetic requirements of incubation and overwintering may differ between nests and sexes. *Emydura macquarii* do not overwinter within nests (Spencer 2001) and water temperatures are relatively stable and rarely fall below 10°C, hence sex differences in survivorship are unlikely. TSD may have evolved as an adaptive response to incubation conditions rather than a facultative response by females, thus playing little role in nest site selection. In another study on nest site selection by *C. picta*, females selected nests to maximise egg survival rather than to influence offspring sex ratio (Schwarzkopf and Brooks 1987).

Many studies have assumed that non-random nesting of species with TSD is selection by a female based on the thermal regime of the nest (Vogt and Bull 1984, Schwarzkopf and Brooks 1985, Bobyn and Brooks 1994, Davenport 1998, Bragg et al. 2000, Janzen and Morjan 2001). However, we have shown that nest site selection may occur at two spatial scales and that predation risk is a major factor driving nest site selection in an Australian freshwater turtle. Despite predation being a major factor driving nesting, *E. macquarii* has similar repeatability for microhabitat and non-random nesting with respect to open nests to a similar North American species with TSD. Hence, while selection of nest site based on thermal properties is well established in many turtles, the influence of offspring sex on nesting remains unknown.

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