

Nesting ecology in the freshwater turtle *Podocnemis unifilis*: spatiotemporal patterns and inferred explanations

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Summary

1. Habitat selection has profound ecological and evolutionary consequences. For example, there may be strong selection for nest-site choice such that oviparous females, lacking parental care, may adaptively manipulate their offspring survival or phenotype. Alternatively, nesting decisions may be a passive by-product of other processes leading to similar nonrandom nesting patterns.

2. Here we examined the nesting ecology of the turtle *Podocnemis unifilis* at multiple spatiotemporal scales to determine whether randomness, adaptive nest-site selection or social facilitation best explained the observed population-level patterns. We addressed these alternative strategies by exploring how environmental and geographic variation in nest sites influences embryonic survival within and across four nesting beaches in three years.

3. We found nonrandom spatial and environmental patterns of nesting within beaches and years, consistent with both the adaptive nest-site selection and social facilitation hypotheses. However, nesting patterns were unpredictable among beaches and among years. Furthermore, environmental conditions at nest sites and offspring survival were not associated, and nests from the most gregarious nesting night clustered more tightly geographically and suffered lower predation than nests laid on other nights.

4. Together, our findings provide more extensive support for social facilitation as compared to the adaptive nest-site selection hypothesis. Our results suggest that selection for female nest-site choice in reptiles may be acting more strongly via offspring survival through nest clustering derived from conspecific cueing and less strongly via environmental cueing than previously anticipated.

5. Our findings underscore the importance of examining multiple sites during multiple seasons. This approach permits testing critical predictions about the consistency of population-level patterns across space and time that enable the distinction between models.

6. Our results support a shift with regards to the trait that is usually considered the target of selection for female nest-site choice in a way that exemplifies the classic dichotomy between selection *for* (survival of nests and perhaps of females) and selection *of* (offspring phenotype, such as sex).

Key-words: habitat selection, social facilitation, adaptive nest site choice, oviparous, fitness, evolution, adaptation, Pleurodiran reptile

Introduction

Habitat selection for nesting sites in oviparous animals may represent a trade-off among the risk of predation, availability of nearby food and microclimatic requirements, such that its genetic basis can be under opposing selection pressures

(e.g. Jaenike 1986). Because incubation conditions can greatly affect the phenotype and fitness of individuals and their offspring (e.g. Bull 1980; O'Steen 1998; Valenzuela 2001), strong selection should influence the ability of nesting females to discriminate between optimal and non-optimal sites, and to choose nest locations that are beneficial to their offspring (Resetarits 1996). Thus, the potential exists for oviparous females to adaptively manipulate the survival or phenotype of

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their offspring via nest-site choice. Alternatively, nesting decisions may be a passive by-product of other processes leading to similar nonrandom nesting patterns (e.g. Graves & Duvall 1995; Angilletta *et al.* 2009). One such alternative is social facilitation (Zajonc 1965), which explains imitating behaviour (e.g. Prokopy & Reynolds 1998). Under a social facilitation model, nesting females may choose oviposition sites by following other nesters instead of using environmental cues on their own.

To understand a species' nesting ecology and to infer the process responsible for the observed nesting patterns, one must evaluate which environmental cues might be used and how females identify them to select nesting sites (see e.g. Morjan & Valenzuela 2001; Brown & Shine 2004). Additionally, one must examine the predictions derived from alternative models. In birds, predator avoidance has received more attention compared to microclimatic variables (Lloyd & Martin 2004), while the converse is true for reptiles. For turtles, potential cues include soil temperature, soil moisture, soil composition, vegetation cover, slope, aspect (i.e. north and south facing slopes), climatic conditions (i.e. rainfall), distance to water or to vegetation, distance to low and high tide lines, and height above water (see e.g. Schwarzkopf & Brooks 1987; Janzen 1994; Doody *et al.* 2004).

In this study, we examine the spatial and environmental nesting patterns of the South American freshwater turtle, *Podocnemis unifilis* (Fig. 1), across multiple beaches and across multiple years, and explore the nesting strategies that best explain the observed female activity at the population level. For this, we examined observed nesting patterns relative to predictions from three models that serve as potential explanatory processes generating the population-level ecological patterns in turtles, namely the null random model, the widely considered model of adaptive nest site selection (NSS) and the under-studied alternative model of social facilitation (SF) as described below.



Fig. 1. Adult male of the South American freshwater turtle, *Podocnemis unifilis*. Photo by T. Escalona.

Material and methods

MODELS OF NESTING STRATEGIES

Using the data and analyses described below, we tested three potential explanations for the nesting patterns of *P. unifilis* (Fig. 2). First, we tested whether a random process could have generated the observed patterns. Second, we tested several population-level predictions derived from the most widely proposed explanation for nonrandom nesting patterns, namely adaptive nest-site selection by which females manipulate offspring phenotype and fitness via the selection of nesting sites with beneficial environmental characteristics. This model (NSS hereafter) requires several conditions to be met: (i) incubation conditions affect offspring phenotype and fitness, and (ii) can be predicted by environmental cues (iii) that females are able to identify (iv) and use for their nesting decisions (v) and for which they have genetically based preferences; thus, given that (vi) selection favours females that nest at optimal sites (adaptive peak), this process (vii) results in nonrandom nesting patterns at the population level (e.g. Bull *et al.* 1988; Jaenike & Holt 1991; Resetarits 1996; Roosenburg 1996; Kolbe & Janzen 2001; Doody *et al.* 2003a). Because selection increases the nest density around the optimum, NSS may generate nonrandom patterns that are consistent across space and time, and most importantly, this hypothesis predicts a positive correlation between the environmental conditions of the nesting sites and offspring fitness (Fig. 2; e.g. Resetarits 1996; Reinhold 1998; Wilson 1998; Clark & Shutler 1999; Valenzuela & Janzen 2001; Kamel & Mrosovsky 2005; Wahl *et al.* 2008).

The second model that we examined was social facilitation or the enhancing effect that individuals can have on the behaviour of followers or imitators (Zajonc 1965; Prokopy & Reynolds 1998). Under this hypothesis (SF hereafter), nesting females choose oviposition sites by following other nesters instead of using environmental cues on their own. Such conspecific cueing causes females to nest in proximity to one another and thus, SF generates nonrandom population-level patterns similar to those from NSS, particularly within single sites or seasons. The critical difference, however, is that unlike NSS, spatial and environmental nesting patterns under SF may not only change over space and time, but more importantly, SF does not require a positive association between the environmental conditions of nesting sites and offspring fitness (Fig. 2) because the nest clumping that derives from conspecific cueing can occur irrespective of the particular spatial location or environmental characteristics of the nests. The SF model predicts that nests deposited during synchronous nesting episodes should cluster more tightly geographically than those from less gregarious or solitary episodes (while environmental clustering is directly predicted by NSS, geographic clustering is consistent but not required by NSS). Additionally, a separate, testable prediction is that if SF is adaptive, synchronous nesting should have a fitness advantage compared to less gregarious or solitary nesting. If SF confers a fitness advantage to offspring while in the nest, clutches from synchronous nesting nights should experience lower predation or higher hatching rates than those from less gregarious or solitary nesting episodes. By examining population level nesting patterns, we can distinguish among these alternatives by testing critical predictions about the consistency of such patterns across space and time that derive from these models.

STUDY SITE AND DATA COLLECTION

Data from 431 nests of *P. unifilis* were recorded from January to March, 1999–2001, on four nesting beaches in a pristine area of the

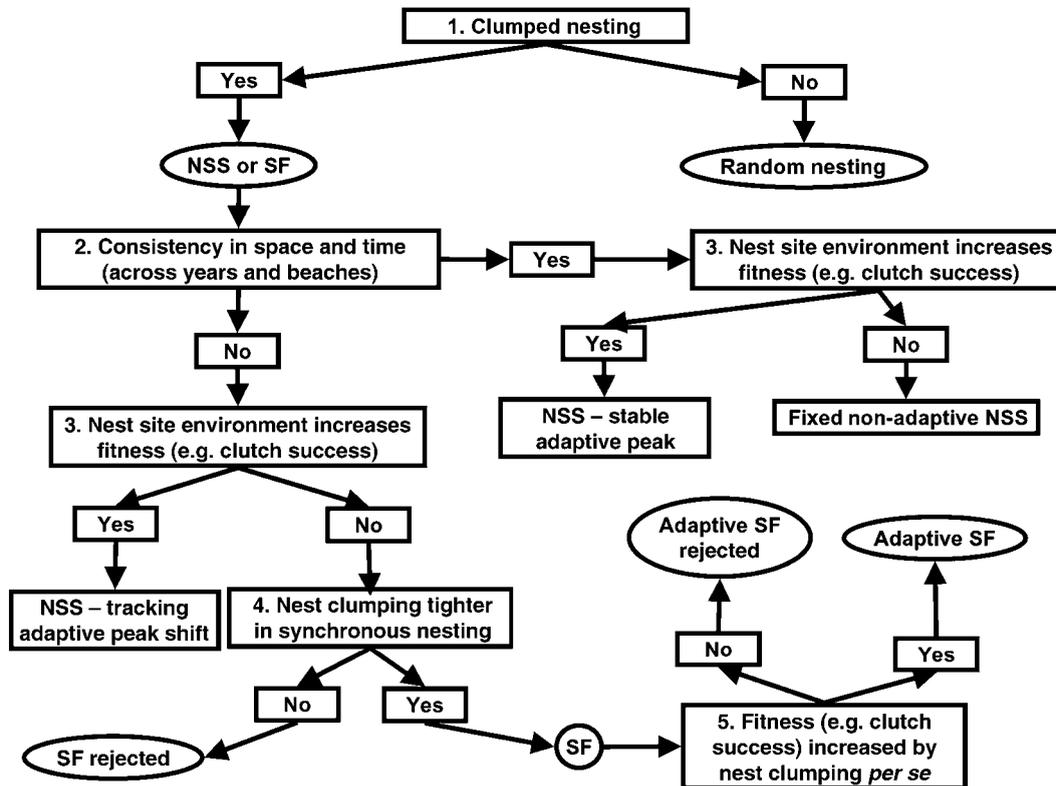


Fig. 2. Patterns predicted from random, adaptive nest-site-selection (NSS) and social facilitation (SF) as described in the text. Both NSS and SF can generate significant nest clustering in geographic and environmental space. However, while NSS requires a positive association between nest-site environment and fitness, plus additional consistency of the clumping pattern across years and sites under a stable adaptive peak scenario, nest clustering can vary in time and space under SF and fitness may be unrelated to nest-site environmental conditions. Additionally, SF makes testable predictions about the clumping patterns during the most synchronous vs. less gregarious or solitary nesting episodes. Finally, an adaptive SF model also makes testable predictions about fitness advantages experienced by offspring from nests laid during the most synchronous vs. less gregarious or solitary nesting events. Thus, direct tests at multiple spatial and temporal scales should be conducted to distinguish between these alternative models to explain nesting decisions made by oviparous females lacking parental care.

Orinoco basin of Venezuela (Fig. 3). Three beaches were located along a 13.1 km stretch on the Nichare River (El Saqueo, Caiman and Miguel), and one on the Tawadu River (Emilio). This choice of sites permitted the daily monitoring of the beaches by the field crew. Nesting forays initiated when the river levels dropped exposing the nesting grounds. Nests constructed each night were easily located the following morning by inspecting the beaches systematically along lengthwise transects 2 m apart throughout each beach, and following fresh female tracks which are highly visible. A small stick was placed on each nest site indicating the nest number, nesting date and its x and y coordinates based on a $5\text{ m} \times 5\text{ m}$ grid established in each beach.

The full data set included information on nest geographic location (xy coordinates); distance between nests, oviposition date and nine environmental variables considered to be important cues for female turtles to choose nesting sites based on previous studies (e.g. Schwarzkopf & Brooks 1987; Roosenburg 1996; Kolbe & Janzen 2002): (i) *percent vegetation cover* measured weekly as the proportion of cells containing vegetation in a 20-cell 1 m^2 grid; (ii) *leaf cover* was measured weekly as presence or absence (presence defined as areas of 100% leaf cover that contained a thick layer of leaf litter 20 cm deep or greater, mixed with sand; absence defined as sandy areas with very little or no leaf cover); (iii) *soil particle size* (classified by excavating a 20-cm deep section and using a grain size chart ranging from fine silt to very coarse grains); (iv) *water content* ($\%\text{m}^3\text{ m}^{-3}$) was

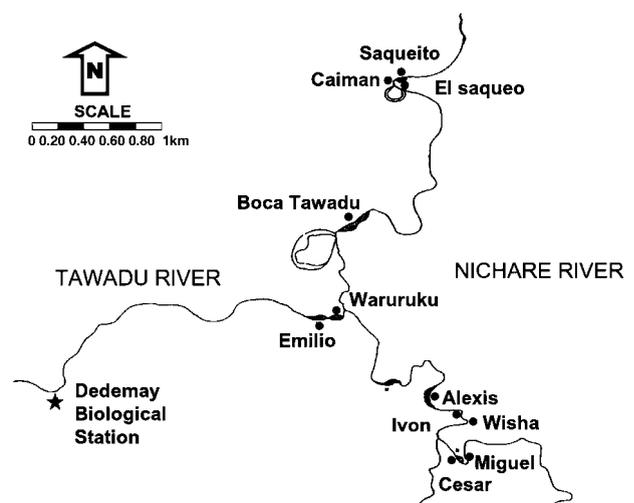


Fig. 3. Map of the study area (El Saqueo = $6^{\circ}23'66''\text{N}$, $64^{\circ}57'44''\text{W}$; Caiman = $6^{\circ}23'67''\text{N}$, $64^{\circ}57'56''\text{W}$; and Miguel = $6^{\circ}19'37''\text{N}$, $64^{\circ}57'28''\text{W}$) (Emilio = $6^{\circ}21'02''\text{N}$, $64^{\circ}58'48''\text{W}$). Only beaches that were monitored are shown, but additional beaches existed within the study area that were used negligibly or not at all by *Podocnemis unifilis* females during the study period.

measured by calculating the percent weight difference of soil samples after drying them at 105 °C for 48 h in 1999, and by changes in soil dielectric using a frequency domain sensor in 2000–2001; (v) *soil temperature* (± 0.1 °C) was measured every 2 h for 24 h at 15 cm depth (which represents the average depth to first and bottom eggs measured for > 360 nests) with digital pocket thermometers during 1999–2000 every 3 days, and daily with HoboR temperature data-loggers in 2001; (vi) *ground elevation* defined as the height (m) above water level, was measured every 2 m at each section of the diagonal of the beach; (vii) *slope* (θ) was calculated as the arcsine of the angle between grid points using their known ground elevation and linear distances; (viii) *distance to water* and (ix) *distance to vegetation* (m) was estimated from the coordinate points to the river's or the vegetation edge using ArcView 3.2a.

All environmental variables were estimated at nest sites and at the center of each grid point of the beach, with the exception of soil temperature and water content, which were measured at selected points along the beach, based on beach characteristics, and the spatial distribution of soil size, vegetation and leaf cover to obtain values representative of all possible microhabitats present. Surface grids and vector coverages for these environmental variables were generated using Inverse Distance Weighted (IDW) interpolation (ESRI, Inc., Redlands, CA), to provide estimates of environmental parameters over the entire beach. As both water and vegetation lines were known, the area of each beach was also estimated. Because all variables were measured at sites of clutches laid the night before (except for temperature which was measured repeatedly), these measurements represent the environmental conditions present at the time of nesting, which might have been used by females to select nesting sites. All environmental data were transformed to standard normal deviates prior to statistical analyses.

Here, we quantified fitness components during embryonic stages by measuring nest predation rate and hatching success to evaluate the effect of female nesting strategies on her reproductive success. Focusing on an early life stage that suffers the highest mortality is particularly important for long-lived taxa such as turtles (Myers *et al.* 2007). Predation rates and hatching success of nests from the most gregarious nesting night (i.e. the night with most nesting episodes) and for all other nights (which comprised less gregarious and solitary nesting episodes) were determined for the three beach-by-year combinations that did not experience significant flooding by the end of the nesting season (Miguel 2000, Caiman 2001, Emilio 2001). Additional data, including predation rates and hatching success were also obtained from seven other beaches within the same stretch of river (Fig. 3), and these additional data (10 available beach-by-year combinations: Wisha 2001, Alexis 2001, Boca Tawadu 2001, Cesar 2000 and 2001, Saqueito 2001, Waruruku 1999, 2000, and 2001, and Ivon 2000) were pooled with those from Miguel 2000, Caiman 2001, Emilio 2001 for a more comprehensive assessment of predation rate and hatching success.

Data were used to assess the following aspects of the nesting ecology as described below: (i) spatial and environmental clumping of nests *within* years and between beaches; (ii) consistency of spatial and environmental patterns *between* years and between beaches; (iii) correlation between nest-site environment and clutch success; and (iv) spatial clumping and survival of the most synchronous vs. less gregarious and solitary nests.

DATA ANALYSIS

Statistical analyses were used to test the following predictions: Support for NSS would be found if nonrandom nesting patterns

were consistent across space and time, and a positive association between the environmental conditions of the chosen nesting sites and fitness was detected. Alternatively, females might have followed each other and nested in proximity (SF), also generating nonrandom nesting patterns within single sites or seasons. Notably, unlike NSS, nesting patterns under SF might have changed over space and time, and could lack a positive association between the environmental conditions of nesting sites and offspring fitness.

1A. Tests of spatial clumping within beaches and within years. For each beach, a first-order nearest-neighbour index of aggregation (R) was calculated for nests and used to assess nest distribution relative to a random ($R = 1$), a uniform ($R > 1$), or a clumped ($R < 1$) distribution (Clark & Evans 1954). Significance of R was determined using its associated z -score [$|z| = (r_E - r_A)/s_r$, where r_A = average observed distance, r_E = average expected distance and s_r = standard error of the expected distance (Shaw & Wheeler 1985)]. Additionally, we examined whether spatial patterns of nest sites changed throughout the season by assessing the correlation between oviposition date and the distance to the nearest nest at the time of oviposition; this analysis was performed using JMP 6.0 (SAS Institute 2005).

1B. Test of environmental clumping of nest sites within beaches and within years. The observed dispersion of nests in the environmental data space (D_{obs}) was estimated as the average Euclidean distances among nests (using the nine environmental variables). This measure incorporates the relative variation expressed across all variables and is equivalent to a multivariate disparity measure (Foote 1993). A set of random locations equal in number to the nest sites was generated in ArcView 3.2a, their associated environmental variables were determined and their dispersion in environmental space (D_{rand}) was compared to D_{obs} . This procedure was repeated 999 times and the proportion of random data sets (plus the observed data) with a smaller dispersion value than the observed dispersion among nest sites was treated as the significance of the observed data (i.e. were nests more similar than expected by chance).

2A. Test of spatial consistency of nest sites between years. A McNemar's test for the significance of changes was used, where the nesting data for each year and beach were converted to presence/absence data scored for each cell in the 5 m \times 5 m grid. Next, cells that changed between years were counted (e.g. absence to presence), and used to calculate a G-test of independence assessed against a null model from a chi-square distribution (see Sokal & Rohlf 1995: Box 17.16). G-tests were performed for all pairs of years for which data were available.

2B. Test of environmental consistency at nest sites between beaches and between years. Environmental consistency between beaches and between years was determined by comparing the main axis of environmental variation for each beach and year. First, a PCA of the environmental characteristics was run on nest sites to obtain the dominant eigenvector ($PC1_{obs}$) for each beach-by-year combination. A set of random locations equal in number to the nest sites was then generated as in 1B above for each beach-by-year combination, and $PC1_{rand}$ was determined. For relevant pairs of beach-by-years, the observed angle (correlation) between $PC1_{obs}$ vectors was calculated and compared to the angle between $PC1_{rand}$ for the same beach-by-year combination. This procedure was repeated 999 times and the proportion of random data sets (plus the observed data) with an angle between PC vectors smaller than the observed angle was treated as the significance of the observed data (i.e. were nests more similar in their environmental characteristics than expected by chance).

3. Test of association of nest environment with clutch success (hatching success or predation). Here, we calculated the correlation between the environmental characteristics of the nest locations and clutch success

Table 1. Summary data for all beach-by-year analyses. For each beach-by-year combination, the number of nests (N_{nests}) is shown, followed by (a): (1) spatial aggregation statistics: nearest neighbour aggregation index (Bragg *et al.* 2000), its z -score and significance (P); (2) nest crowding statistics: correlation of nearest nest distance and oviposition date (O_{date}) and its significance (P); (3) environmental aggregation statistics: average Euclidean distances between nests (disparity: D_{obs}), and its significance (P). (b): (4) For those beach-by-year combinations with available data, the association of nest environment with fitness (embryo survival or nest predation) is presented

Beach	N_{nests}	1: Spatial Aggregation			2: Nest Crowding		3: Environmental Aggregation		4: Association of Nest Environment with Fitness Variables					
		R_{agg}	z	P	O_{date}	P	D_{obs}	P	Nest Predation Statistic	df	P	Embryo Survival Statistic	df	P
Caiman 99	26	0.49	4.88	< 0.01	-0.43	0.03	355.66	0.001						
Caiman 00	73	0.62	6.14	< 0.01	-0.25	0.03	2127.83	0.01						
Caiman 01	35	0.53	5.33	< 0.01	-0.33	0.05	872.08	0.001	$\chi^2 = 9.08$	9	0.4298	$F = 1.63$	9, 25	0.158
Emilio 99	74	0.67	5.28	< 0.01	-0.3	0.01	2212.36	0.001						
Emilio 00	68	0.72	4.43	< 0.01	-0.41	0.01	1126.75	0.001						
Emilio 01	40	0.47	6.36	< 0.01	-0.43	0.01	904.35	0.001	$\chi^2 = 9.78$	8	0.2806	$F = 1.53$	8, 31	0.187
Miguel 99	58	0.7	4.31	< 0.01	-0.48	0.01	482.04	0.001						
Miguel 00	34	0.72	3.13	< 0.01	-0.49	0.01	252.87	0.001	$\chi^2 = 11.38$	7	0.1227			
Saqueo 99	8	0.48	2.79	< 0.01	-0.71	0.05	208.53	0.01						
Saqueo 00	15	0.59	3.03	< 0.01	-0.47	0.07	242.53	0.001						

Predation measured from multiple logistic regression [$Y = \text{predation (0/1)}$, $X = \text{environmental variables}$], and survival measured from multiple regression ($Y = \text{survival}$, $X = \text{environmental variables}$).

for each beach-by-year combination, using multiple logistic regression for nest predation, and multiple regression for embryonic survival (hatching success).

4A. Spatial clumping of the most synchronous vs. less gregarious and solitary nests. To examine whether females nesting in synchrony tended to nest in close geographic proximity to each other, we determined the night of maximal oviposition for each beach-by-year combination, and calculated both the maximal distance between nests and the average distance between nests for this and all other nights. The smaller of the two was determined for each beach-by-year combination, and the probability of the observed pattern across beaches was assessed using the binomial distribution with an expected frequency of 0.5 for each group (maximum oviposition night vs. all other nights).

4B. Survival of the most synchronous vs. less gregarious and solitary nests. To examine whether females nesting in synchrony experience a fitness advantage via increased offspring survival in the nest, we compared the proportion of nests preyed upon from the most gregarious night with that of nests across all other nights. A binomial test was used to assess this pattern across all beach-by-year combinations. A similar analysis was performed using hatching rate instead of predation rate to distinguish the effects on clutch success due to predation from other sources of egg mortality (e.g. failure in embryonic development, flooding).

Results

Nest sites were significantly spatially clumped within beaches and within years (R index: 0.42–0.72; Table 1a). Additionally, oviposition date was negatively correlated with the distance to the nearest nest at oviposition, indicating that nesting regions became more crowded as the season progressed (Table 1a; Fig. 4). We also found that nests from a single beach in a single year were significantly more similar to one another in their environmental characteristics relative to

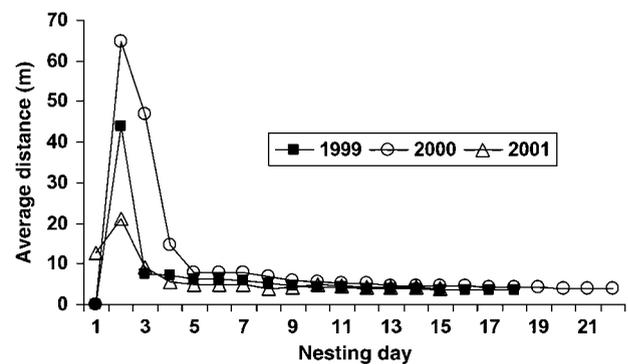


Fig. 4. Daily average distance to nearest nest on Emilio beach. Through the nesting season, the average distance to the nearest nest decreases, implying that nesting regions become more crowded as the nesting season progresses.

random locations from that same beach-by-year combination (Table 1a). Thus, from the within beach-within-year analyses, we found significant spatial and environmental clumping in nesting patterns, a result consistent with both the NSS and SF hypotheses.

In stark contrast, for all beaches, the spatial locations of nests changed significantly across years (Table 2a; Fig. 5). Additionally, the relative importance of environmental characteristics describing nesting patterns was not consistent across beaches or years (PCA loadings, Table 3). Some variables (e.g. temperature and height) had little influence on nesting across all beach-by-year combinations. Other variables, such as soil type and leaf cover, were extremely important for some

Table 2. (a) *Above the diagonal*, G values from the comparison of nest site locations between years using McNemar's test for the significance of changes. All values are compared to a chi-square value of 3.814 ($P = 0.05$), and all are significant at that level. (b) *Below the diagonal*, angle values are presented from a randomization test comparing PC1 vectors in environmental space for observed nests (between beach-by-year combinations) relative to those from random locations generated in each nesting beach. Shaded cells indicate significantly more similar environmental conditions than expected by chance, while white significant cells indicate more dissimilar environmental conditions than expected by chance. A Bonferroni correction for multiple comparisons within year or beach was applied to test for significance

Beach × Year	C1999	C2000	C2001	E1999	E2000	E2001	M1999	M2000	S1999	S2000
C1999	–	7.162	5.565							
C2000	7.55 NS	–	4.2							
C2001	19.13 NS	17.83 NS	–							
E1999	23.9***			–	6.467	5.436				
E2000		14.29**		12.53**	–	5.729				
E2001			87.01***	83.19***	87.88***	–				
M1999	88.06 NS			71.37 NS			–	5.016		
M2000		86.58 NS			87.59 NS		88.57***	–		
S1999	85.86 NS			83.3 NS			64.22† NS		–	4.339
S2000		88.47 NS			88.86‡ NS			76.02 NS	73.38*	–

* = 0.009, ** = 0.003, *** = 0.001.

† ≤ 0.017.

‡ = 0.012.

NS = not significant, C = Caiman, E = Emilio, M = Miguel, S = Saqueo.

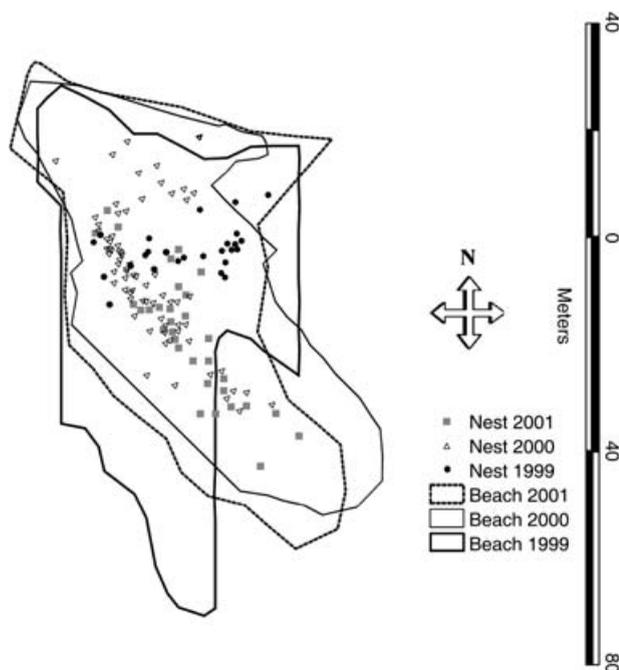


Fig. 5. An example of the spatial distribution of nests for each year in nesting beach Caiman. Analyses included only the area of the beach present during each year.

beach-by-year combinations, but not for others. Still other variables (e.g. humidity and distance to water) covaried positively within some beach-by-year combinations, but covaried negatively within other beach-by-year combinations. Furthermore, these qualitative observations were statistically corroborated. Generally, it was found that the environmental characteristics of nests across beaches and across years were inconsistent: 11 of 21 beach-by-year combinations were no different from what was expected by chance, 5 of 21 comparisons

were significantly more *different* (Table 2b) and only in three cases were they significantly more similar than expected by chance. Together, these results revealed limited spatial and environmental consistency across beaches or years, and thus, are not consistent with the NSS hypothesis.

No association of the nest environment with clutch success (hatching success or predation) was found in any beach-by-year combination (Table 1b), counter to the NSS model, but consistent with the SF model. Predation rates (0–50%) and hatching rates (40–92%) varied from beach to beach and from year to year. Overall hatchling emergence success varied from 39% to 100%. Nest predators in the study area included *Tupinambis nigropunctatus* (common tegu lizard, diurnal), *Buteogallus urubitinga* (great black-hawk, diurnal) and *Felis pardalis* (ocelot, nocturnal) (Escalona 2003). Other causes accounted for 19–87% of nest mortality and included flooding and natural embryonic failure (Escalona 2003).

When testing for potential fitness benefits of nesting decisions, we found that for 9 out of 10 beaches, the maximal distance between nests and the average distance between nests on nights with the most gregarious nesting were smaller than what was observed on all other nights (binomial probability; $P = 0.009766$). Additionally, overall predation rates were lower on nests laid during nights with the most gregarious nesting (9 of 90 = 10%), relative to nights with fewer nesting episodes (64 of 394 = 16%). When compared across beaches, 11 of 13 beach-by-year combinations exhibited this pattern, implying a significant decrease in predation rates for clutches laid during the most gregarious nesting episodes (binomial probability; $P = 0.009521$). Hatching success did not differ statistically for nests oviposited during the most synchronous nesting nights (71%) relative to other nights (67%) (binomial probability; $P = 0.2185$) although higher values were observed for 5 out of 8 beach-by-year combinations. Hatching success of nests suffering predation was 12%, while that of nests that did not suffer

Table 3. Values of the PC1 loadings for each environmental variable examined at the nesting sites of *Podocnemis unifilis* for each beach-by-year combination

Beach × Year	Hum	Temp	Soil Type	Distance Water	Distance Veg	Leaf Cover	Veg Cover	Height	Slope
C1999	0-00000	0-00000	-0-00011	0-00286	0-03793	-0-99639	-0-07535	0-00074	0-00912
C2000	0-00000	0-00000	-0-00093	0-09781	0-04276	0-99042	0-08072	-0-00270	-0-03399
C2001	0-18898	0-01246	-0-00019	-0-01335	0-13892	0-95847	-0-08283	-0-01023	-0-13795
E1999	-0-39865	0-00690	0-00725	-0-06529	0-03420	-0-91257	-0-05164	-0-00200	-0-00895
E2000	0-20562	0-00009	-0-01445	0-03829	-0-05260	0-97547	0-00000	-0-00275	-0-04158
E2001	0-16490	0-00559	-0-00482	0-01704	-0-02938	0-00000	0-98552	-0-00137	-0-01861
M1999	-0-63604	0-02599	0-03034	-0-53118	0-45685	0-00000	-0-26231	-0-01639	-0-18417
M2000	0-57244	-0-01101	-0-00640	-0-69858	-0-12557	0-00000	0-00000	-0-04358	-0-40799
S1999	0-14123	-0-01476	-0-31702	0-12324	-0-06560	0-00000	0-92708	-0-00152	-0-01924
S2000	-0-04238	0-00591	0-95958	0-25594	0-09716	0-00000	0-00000	0-00427	0-04905

C = Caiman, E = Emilio, M = Miguel, S = Saqueo.

predation was 77%. Thus, the reduction in hatching success was largely attributable to indirect effects of predation (e.g. microclimate changes associated with nest disturbance by predators may have lowered hatching success of uneaten eggs).

Discussion

Understanding the proximate and ultimate mechanisms and consequences of nesting decisions made by oviparous animals is a longstanding question in evolutionary ecology (Bernardo 1996; Resetarits 1996). Here, we examined the nesting patterns of the turtle *P. unifilis*, an oviparous reptile lacking parental care, and tested whether the observed population-level nesting patterns can be explained by randomness, NSS or SF. We explored how environmental and geographic variation in nesting sites at several spatiotemporal scales (within and across four nesting beaches over 3 years) impact offspring survival while in the nest and therefore maternal strategies.

Nests were significantly clumped in space and had more similar environmental characteristics than expected by chance in all beaches and in all years. Additionally, the environmental characteristics of the nest sites at the time of oviposition did not change through the nesting season, but nesting areas became more crowded. Although consistent with NSS, rejecting a random nesting pattern at any particular single site or season falls short of enabling strong inferences about the existence of adaptive NSS as it leaves untested some critical predictions about the consistency of population-level patterns across space and time that derive from this model (Fig. 2). For instance, we found no repeatability of the spatial or environmental predictors of nest-sites across years (within beach) or across beaches (within years). These results are critical because this unpredictable variation in nesting patterns among sites and seasons counters expectations if maternal NSS is adaptive under a model of stable environmental conditions (Resetarits 1996; Fig. 2).

NSS could still be operating under changing optima, albeit in a more circuitous manner. That is, we observed that

females nested at sites with nonrandom environmental characteristics but the chosen characteristics differed depending on the beach at any particular year, or on the year at any particular beach. For such conditions to be optimal and nesting to be adaptive, it would be necessary that the adaptive peak changed over space and time (years) and was tracked by females (Fig. 2). However, contrary to this scenario, we found no association between the environmental conditions of the chosen nest sites and offspring fitness while in the nest (nest predation and embryo survival) as required by NSS. Thus, we found no evidence of adaptive NSS in *P. unifilis*, even under the case of changing environmental optima. This result may not be surprising given that while theoretically possible, a near instantaneous behavioural response by females to varying optimal environmental conditions would be unlikely (Orians & Wittenberger 1991). Second, the assumption of a shifting adaptive peak at the scale of the detected changes is countered by the observation that factors typically associated with fitness have optimal values that are consistent within populations [e.g. developmental and hatching rates increase with temperature and the extreme values that arrest development change little for a given species (Rhen & Lang 2004)].

Given that nest site choice in *P. unifilis* appears inconsistent with NSS, why do spatial and environmental patterns within beaches and years exist at all? SF is a possible explanation and we tested several of its predictions directly. First, under SF, nests deposited during the most synchronous nesting episodes should cluster more tightly in geographic or environmental space than those from less gregarious or solitary nesting (Fig. 2). Second, if SF confers a fitness advantage to offspring while they are in the nest, clutches from the most synchronous nesting should experience lower predation or higher hatching rates than those from less gregarious or solitary nesting episodes (Fig. 2). Our data supported these predictions, and were also consistent with the theoretical expectation that nesting decisions ought to depend on proximate discrimination of direct and easily detectable binomial variables (e.g. presence/absence of predators or of nesting conspecifics as in SF) more often than on continuous variables (Resetarits 1996).

Social effects on nesting have been documented in multiple taxa (e.g. Burger & Gochfeld 1990; Katvala & Kaitala 2003), including turtles (Hendrickson 1958). Extreme examples of social facilitation are the massive ('arribada') nesting in sea turtles (e.g. Caut *et al.* 2006b) and colonial or communal nesting in reptiles and birds (e.g. Burger & Gochfeld 1990; Graves & Duvall 1995). Other turtles also nest in groups of numerous females per night at single sites (e.g. Doody *et al.* 2003b; Feinberg & Burke 2003). Thus, the existence of SF in *P. unifilis* would not be unexpected given that it displays synchronous nesting (Soini 1994). Ground nuzzling by nesting females (Morjan & Valenzuela 2001; Doody *et al.* 2003b) may be a mechanism used by nesting females to assess the movements and nesting decisions of other females in addition to, or instead of, visual or auditory cues. Incidentally, *P. unifilis* nesting is more frequent on nights with moonlight, suggesting that females may use visual cues (Escalona 2003). It should be noted that as with most social nesting turtles (e.g. Vanzolini 1967; Plotkin *et al.* 1997), gregarious nesting is not a fixed behaviour in *P. unifilis*. Although SF has not received as much attention as an explanation for nesting patterns in turtles the potential exists for its presence in a wider array of species and warrants direct testing (Graves & Duvall 1995; Angilletta *et al.* 2009).

We found evidence that social nesting in *P. unifilis* was adaptive. Nesting synchrony may reduce female or nest predation (Robinson & Bider 1988; Eckrich & Owens 1995; but see Doody *et al.* 2003b) via a 'safety in numbers' strategy that would be effective irrespective of the particular spatial position or environmental characteristics of the clusters of nests. Thus, at the population level, nest clustering via SF is not expected to be consistent across space and time. Interestingly, the highest frequency of nest destruction in *P. unifilis* occurred less than a week after oviposition (Escalona 2003). Nest predators in the study area included ocelots, lizards and birds of prey (Escalona 2003). No data were available to test a dilution effect that SF might have on the predation of hatchlings (e.g. Doody *et al.* 2003a for eggs; Gochfeld 1982 for nestlings); however, the positive effect on offspring survival that we found indicates that female reproductive success was enhanced by SF. SF may reduce other costs incurred by females during their nesting forays (Congdon & Gatten 1989) by enabling the selection of sites used by conspecifics. If present in *P. unifilis*, any such benefit for maternal survival will add to the advantages of SF detected here for embryonic survival. Finally, we did not detect any disadvantage to nest clumping, such as nest destruction or increased parasitism, as reported in other studies (e.g. Caut *et al.* 2006a; Blouin-Demers *et al.* 2004).

In addition, we tested the possibility that nests might be more clumped during gregarious events if optimal nesting sites are spatially auto-correlated, by examining patterns of spatial autocorrelation through correlograms on all environmental variables on all beaches (data not shown). From these analyses, we found significant spatial autocorrelation of environmental variables, but only at the smallest distances (e.g. typically under 5 m). This finding is not surprising, as locations in near proximity on the beach should have similar

environmental characteristics (e.g. slope, temperature, etc.). Interestingly however, these patterns were not consistent as the spatially autocorrelated variables were not always the same across beaches. Further, our comparisons of environmental variables across beaches and years using PCA found that the most important environmental variables for nesting differed among beaches and across years. Taken together, these findings indicate a slight autocorrelation of environmental variables, but the spatiotemporal inconsistency does not provide a clear pattern concordant with females cueing on environmental parameters at nest sites.

If chooser females make adaptive nesting decisions based on environmental cueing (which are then followed by copiers), a positive association between environmental variables of the chosen nest sites and fitness would still be expected, but was not observed in our study. Importantly, SF does not require leaders because group movements in animals can occur in the absence of leaders [e.g. animal navigation (Codling *et al.* 2007; Ward *et al.* 2008), fish schooling (Ward *et al.* 2008)]. Additionally, if the environmental characteristics chosen were adaptive via a phenotypic effect that confers longer term fitness, such selection will be operating secondarily after the initial selection on early survival of offspring.

We considered alternatives to NSS or SF that may generate the observed nonrandom nesting patterns. For instance, multiple beaches were available for *P. unifilis* within the stretch of both rivers, some of which were used heavily for nesting and were monitored in this study and some which were used negligibly or not at all despite having conditions similar to those utilized. Furthermore, some suitable areas were devoid of nests [suitable areas were determined as the regions occupied or not that had statistically similar environmental characteristics to those found at nesting sites (Escalona 2003)]. Thus, the presence of unused beaches within the study area and of unused areas within nesting beaches, plus the lack of nest destruction by other females as mentioned above, indicates no limitation of nesting space that would force females to cluster nests in a few areas (e.g. Graves & Duvall 1995; Doody *et al.* 2003b). Additionally, although some beaches changed somewhat in shape or size from year to year (e.g. Fig. 4), changes were negligible in the suitable nesting areas within beaches. Moreover, the variables that might have been affected the most by beach size or shape, namely nest height above water and distance to water, either had little importance in describing nesting patterns (i.e. height), or covaried positively in some beach-by-year combinations, negatively in others or had little influence in others (i.e. distance to water; Table 3). Thus, the inconsistency of nesting decisions across years was not caused by beach changes during the study period. Finally, even though our study encompassed a larger spatiotemporal scale than is typically used in studies of NSS, we might not have examined a long enough window of time to detect evidence for NSS. However, this hypothesis cannot explain why our findings were consistent with a potentially adaptive SF model at the scale used. Alternatively, the changing nesting patterns across

multiple beaches and years may reflect plastic behavioural responses to local environmental conditions (Wecker 1963; Goldberg *et al.* 2006; Asbury & Adolph 2007), which will prevent evolutionary change across generations, even in the presence of selection for particular nesting choices due to the effect that nest-sites may have on offspring fitness or sex (Brown & Shine 2007).

Our findings also raise interesting questions beyond the nesting ecology of *P. unifilis*. For instance, our results suggest that selection for female nest-site choice in reptiles may act more strongly via offspring survival through nest clustering derived from conspecific cueing, and more weakly via environmental cueing. Since environmental cueing is the alternative most often invoked in models of maternal manipulation of offspring phenotype, particularly in reptiles (e.g. Shine & Harlow 1996; Janzen & Morjan 2001), our data opens the possibility that in systems where conspecific cueing occurs, offspring phenotype could be the passive by-product of nesting decisions that are shaped by earlier selection on offspring survival, even though fitness advantages may also accrue from such phenotypes. Consistently, most adaptive explanations of nesting timing and location in birds are attributed to increasing nest survival (e.g. decreasing predation risk) rather than to direct phenotypic manipulation (e.g. Forstmeier & Weiss 2004) even though incubation conditions may have significant phenotypic effects in the offspring (e.g. Lindstrom 1999). Therefore, the drivers of reptilian and avian nesting decisions may be more similar than previously anticipated. If true, this would imply that nest site choice in sauropsids could exemplify the classic dichotomy between selection *for* (survival of nests and perhaps of females) and selection *of* (offspring phenotype, such as sex) (Sober 1984). Further research should test this prediction.

Thus, our study cautions about reporting evidence for the evolution of NSS for phenotypic manipulation on the basis of data from a single location or year as such evidence would be equivocal. Strong evidence of NSS in the wild requires testing nonrandom nesting patterns, along with testing for consistency in space and time (e.g. Schwarzkopf & Brooks 1987; Valenzuela & Janzen 2001; Doody *et al.* 2003a; Angilletta *et al.* 2009), and for consistency of the phenotypic and fitness consequences of nesting decisions in natural populations (e.g. Kamel & Mrosovsky 2005). Such fitness effects may be further pronounced in species with environmental sex determination due to the added effects of nest-site choice on sex ratio evolution (Bull *et al.* 1988). Most importantly, alternative processes that generate similar nonrandom nesting patterns should also be considered.

Our findings are concordant with other recent studies. For instance, tests of nest site philopatry and the evolution of temperature-dependent sex determination (TSD) in *Chrysemys picta* found that nesting choices were inconsistent with adaptive NSS in the framework of the TSD evolution (Reinhold 1998) and that phenotypic consequences of nesting decisions as required by NSS (Janzen & Morjan 2001) are not repeatable in the same population (Valenzuela & Janzen 2001) and thus, are not likely the main target of selection. Likewise, NSS

for sex ratio manipulation was ruled out in *Carettochelys insculpta* (Doody *et al.* 2003a). Further, nest site choice may evolve much slower than embryonic thermal sensitivity (Morjan 2003) such that theoretically, NSS is not always expected to be adaptive for sex ratio manipulation.

We suggest that further attention should be paid to the potential of conspecific cueing in other systems. Experimental manipulations that provide direct test for SF (e.g. Plummer 1980; Radder & Shine 2007) may be precluded from many turtles for logistical reasons, as is the case for *P. unifilis*, but they may be possible in others chelonians (e.g. Wilson 1998). Nonetheless, indirect tests such as those used in this study remain valuable and necessary.

Acknowledgements

Field work was enabled by invaluable help from Cesar Escalona, Maria Edilia Varela, Miguel Estaba (and his family), Felix Daza (and his family), Conrad Vispo and Claudia Knab-Vispo, Ivonne Monge and Andres Rosenchein. This study was partially funded by grants from the Wildlife Conservation Society, Sustainable Aquatic Resources Center, Saint Louis Zoo, Jersey Zoo, Organization of Tropical Studies, International Center for Tropical Ecology, and the Graduate School-Dissertation Fellowship of the University of Missouri, St. Louis (all to T.E.); and NSF grants DEB-0446758 to DCA and IOS-0743284 to N.V. We are indebted to W.M. Roosenburg and S. Doody for their critical comments.

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Received 18 July 2008; accepted 25 February 2009

Handling Editor: Michael Angilletta