Asian Herpetological Research 2011, 2(1): 36-40
DOI: 10.3724/SP.J.1245.2011.00036

Nest-site Use by the Chinese Alligator (*Alligator sinensis*) in the Gaojingmiao Breeding Farm, Anhui, China

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Abstract Nest-site and nesting material used by the Chinese alligator (*Alligator sinensis*) was studied at the Gaojingmiao Breeding Farm, Langxi County, Anhui, China from May to September 2009. In this study, artificial nesting materials were placed in 43 potential nesting sites before the nesting season, 11 of which were used. Additionally, eight nests were built at natural sites without artificial nesting materials provided. Seven environmental variables were measured at each nest site: distance from water, height from water surface, sunlight duration, nearest bank slope, nest site slope, vegetation coverage and concealment. Statistical analyses indicated that concealment was significantly different between used and unused nest sites, with concealment being significantly correlated to the use of materials-placed sites. In comparing the nests at artificial vs. natural sites, only the nearest bank slope differed significantly. Further, principal component analysis of natural nests indicated that the duration of nest exposure to sunlight and vegetation coverage were more influential than the other factors studied.

Keywords Chinese alligator, nesting, artificial nest, natural nest, habitat use, reintroduction

1. Introduction

The Chinese alligator (*Alligator sinensis*) is a rare archosaurian reptile endemic to China and is one of the most endangered of the 23 extant crocodilian species (Jelden *et al.*, 2002). Wild Chinese alligator populations have shown a steady decline over the last century (Chen *et al.*, 1979; Ding *et al.*, 2004; Wu *et al.*, 2008). Releasing captive-bred Chinese alligators into wild field locations (i.e., reintroduction) is an effective way to repopulate wild communities (Jiang, 2004). An important aspect of reintroduction is to ensure the success of reproduction for individuals released into the wild. Nest site selection is a key component of nesting success, and thus directly affects the fitness of the reintroduced individuals in the populations. A well studied nesting period occurs between late June to early July (Chen *et al.*, 2003), where nests are built on the banks of ponds. Nests are usually made with plants, such as *Miscanthus sinensis*, *Cynodon dactylon*, and *Imperata cylindrical* var. *major* (Chen *et al.*, 2003).

Nesting ecology has been studied for several species of crocodilians (Joanen, 1969; Webb *et al.*, 1977; Metzen, 1977; Magnusson, 1978; Crawshaw and Schaller, 1980; Dietz and Hines, 1980; Wilkinson, 1983; Lutz, 1984; Hall and Kushlan, 1987; Frank, 1989). Previous studies of *A. sinensis* nesting ecology found that the weather in the area and moisture of the nesting material affected the nesting time and egg-laying time respectively (Xia and Jiang, 2005), the level of vegetation canopy and the distance to water also affected the selection of nesting sites (Zhang *et al.*, 2006), those sites with vegetation coverage of 63% to 88% were suitable for *A. sinensis* when nesting (Zhou, 2007). However, all the above studies were restricted to the captive-bred Chinese alligators, which may behave differently from the alligators in the wild. This study was conducted in an area near the reintroduction locality for *A. sinensis*, where the habitat is similar to that found in the wild. Using the data from environmental variables

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Received: 28 June 2010 Accepted: 4 March 2011

collected at the studied nest sites, we analyzed the factors that affected the nesting of *A. sinensis*, aiming to provide support for improving reintroduction habitats through better nest site selection for reintroduced individuals.

2. Methods

The study was conducted at the Chinese Alligator Gaojingmiao Breeding Farm (31°01′N, 119°12′E), which is located within the Gaojingmiao Forestry Farm in Langxi County, Anhui, China. This area (Figure 1) is surrounded by a 2.1 m-high iron fence and consists of 8 natural ponds. Each pond has a sluice to maintain a stable water level (depth of 1.5 m). The ponds are surrounded by vegetation, mainly composed of *Robinia pseudoacacia*, *Ligustrum lucidum*, *Pterocelis tatarinowii*, *Rosa multiflora*, *Rubus parvifolius* and *Pleioblastus amarus* (Zhou *et al*., 2004). The Farm has an annual mean temperature of 15.9 °C, and annual mean precipitation of 1,294 mm (Wu *et al*., 2008). 211 adult Chinese alligators, introduced from the Anhui Research Center for Chinese Alligator Reproduction (ARCCAR), were raised in the eight ponds of the Farm with a sex ratio of 3 females: 1 male and 0.024/m^2 population density. The snout-vent length (SVL) of the introduced Chinese alligators ranged between 64–79 cm and the age between 9–11 years.

In late May 2009, workers of ARCCAR placed supplemental (artificial) nesting materials at 43 possible nesting sites based on their experience. In early July, we watched pond banks daily at 12:00–14:00, when the Chinese alligators avioded sunlight by staying in the water (Zhang *et al*., 2006). Seven environmental variables were measured at each nest site: distance from water (m); height from water surface (m); sunlight duration (h, average time of sunlight in one day, recorded by authors from June to July); nearest bank slope (°); nest site slope (slope of nest bottom, measured by comparison of goniometer, and divided into four grades, 1: 0–5°; 2: 5–10°; 3: 10–15° and 4: 15–20°); vegetation coverage (percentage of vertical projection of plant above the nest to nest area, and divided into four grades by the percentage of vegetation coverage, 1: 0–25%; 2: 25%–50%; 3: 50%–75% and 4: 75%–100%); and concealment (divided into four grades by distance to passage and visible or not, 1: short distance and visible; 2: short distance and invisible; 3: long distance and visible and 4: long distance and invisible).

We used sine transformation for the data of the nearest bank slope. We did comparisons in two groups: used vs. unused sites and natural vs. artificial nests (i. e., used sites). As all the numerical data were normally distributed (tested by One-sample Kolmogorov-Smirnov Test), differences were compared by independent-samples *t*-test in each group. Categorical data were compared by Chi-square test in each group. Furthermore, the variables with significant differences in each group were tested by logistic regression to determine correlation. Finally, principal component analysis (PCA) was performed on the variables of natural nests, and we retained only those with eigenvalues greater than 1.0.

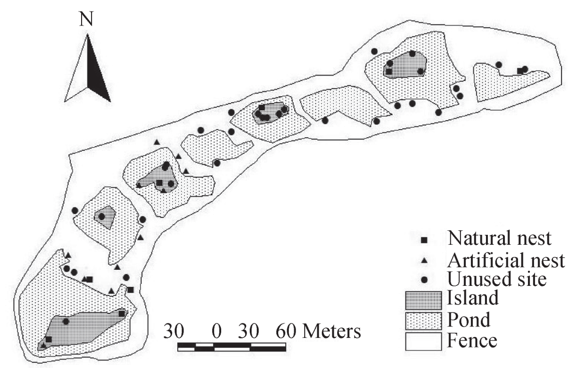

Figure 1 Map of the Gaojingmiao Breeding Farm of *Alligator sinensis* and the location of three types of nesting sites.

3. Results

Nests were constructed at 11 of the 43 sites with articifial materials (named as artificial nest) and 32 were unused (named as unused site) by Chinese alligators. Additionally, eight nests were built at sites without artificial nesting material (named as natural nest). All the sites were measured by GPS, and showed in Figure 1. The characteristics (mean±SD) of each type of nesting sites are shown in Table 1.

The differences in the variables were compared between used sites (artificial nests) and unused sites. Significant differences were not found in most of the variables except concealment (X^2 = 33.121, *df* = 3, *p* = 0.000; Table 1). Furthermore, the results of the logistic regression indicated that concealment was significantly correlated with the use of materials-placed sites (Wald = 8.784, *df* = 1, *p* = 0.003). Examination of the difference between artificial and natural nests within each variable indicated that only the nearest bank slope had significant difference (*p* = 0.13; Table 1).

PCA on the environmental variables of natural nests extracted two principal components accounting for 72.1% of the variance (Table 2). The two principal components were extracted to calculate corresponding diagnostic vector for each variable (Table 3). The results indicated

Table 1 Characteristics of each type of nesting sites and probable differences in each comparison

Variable	Mean ± SD			Difference	
	Artificial nest (n=11)	Unused site (n=32)	Natural nest (n=8)	Artificial nest vs. unused site	Artificial vs. natural nest
Distance from water (m)	3.53±0.53	3.70±1.05	4.59±2.69	0.608[a]	0.218 [a]
Height from water surface (m)	1.08±0.35	1.10±0.27	1.07±0.27	0.905[a]	0.923 [a]
Sunlight duration (h)	7.00±3.25	8.34±1.93	5.00±3.58	0.106[a]	0.222 [a]
Nearest bank slope (°)	30.04±9.25	28.71±9.25	46.13±9.25	0.759[a]	0.013 [a]
Nest site slope	1.55±0.82	1.44±0.98	1.37±0.74	0.153[b]	0.944 [b]
Vegetation coverage	1.55±1.21	1.34±0.83	2.50±1.31	0.453[b]	0.059 [b]
Concealment	3.45±1.04	1.44±0.50	3.25±1.16	0.000[b]	0.097 [b]

[a]: Compared by the independent-samples *t*-test; [b]: Compared by the Chi-square test.

Table 2 Eigenvalues from principle components analysis of natural nests

	Principle component						
	1	2	3	4	5	6	7
Eigenvalue	3.847	1.200	0.837	0.738	0.293	0.086	0.001
Contribution (%)	54.952	17.149	11.951	10.536	4.180	1.222	0.010
Cumulative contribution (%)	54.952	72.100	84.051	94.587	98.768	99.990	100.000

Table 3 Environmental variables and associated PCA scores of natural nests

Environmental variable	Diagnostic vector	
	PC1	PC2
Distance from water (m)	0.507	–0.057
Height from water surface (m)	0.889	0.367
Sunlight duration (h)	0.946	–0.225
Nearest bank slope (°)	0.168	0.958
Nest site slope	–0.649	–0.134
Vegetation coverage	–0.930	0.190
Concealment	–0.768	0.202

that the greatest impact on PC1 (contribution = 54.9%) was sunlight duration (score = 0 .946) and the next was vegetation coverage (score = 0.930). The greatest impact on PC2 (contribution = 17.1%) was the nearest bank slope (score = 0.958).

4. Discussion

Artificial nests and unused sites only differed significantly in the variable concealment (Table 1). The logistic regression also indicated that whether a site with artificial materials was used was significantly correlated with concealment. By comparing the mean ± SD (Table 1), it can be found that the sites with artificial materials used by Chinese alligators occurred in more concealed habitat than unused sites. Furthermore, there was no significant difference between artificial and natural nests in concealment. Both artificial and natural nests were mostly located in the habitat with high concealment. Significant effect of concealment on nest-site seletion and nest success has often been found in studies of birds (Mark, 1997; David and Stanley, 2000). It is commonly assumed that species reduce the risk of predation by hiding their nests (Joan and Bridget, 1996; Frank *et al*., 1995). For the Chinese alligator, nest predation is the main cause of reproductive failure in the wild (Chen *et al*., 2003). The eggs of Chinese alligators laid in highly visible nests would suffer higher predation than concealed nests in the wild. Consequently, we suggest that habitats with high concealment of nests should take priority for reintroduction of Chinese alligators.

In the second group comparison, only the nearest bank slope was significantly different between natural and artificial nests. Comparing the mean ± SD of the nea-rest bank slope in the three types of nesting sites (Table 1), we found that Chinese alligators preferred to use the sites on the bank where the slopes ranged between 30–46°. Crawl marks were not found on the banks that were too steep during our observation, as the banks were too steep for Chinese alligator to climb up. On the other hand, if they chose a nesting site near a bank with very small slope, they would extend the distance of the nest from water to avoid submergence during the rainy season. In that case, the danger to the eggs would increase when females were in water. The nests of the American alligator (*Alligstor mississippiensis*) are influenced by the hydrologic conditions in an area (Laura and Frank, 2000). Moreover, water level was the most important factor affecting their nest-site selection (Joanen and McNease, 1989). Chinese alligators are also considered to have this ability to predict probably water level changes (Gu and Zhang, 1983).

Vegetation coverage was considered the most important variable affecting the nesting of the Chinese alligator in previous studies (Zhang *et al*., 2003; Xia and Jiang, 2005;

Zhou, 2007), but sunlight duration was not measured. However, in this study, sunlight duration has the greatest scores in PC1. So it was considered to be the most principal variable affecting the nest-site choice in natural habitat. Crocodiles do not hatch their eggs, and the nest temperature is mainly kept by sunshine (Magnusson *et al*., 1985; Chen *et al*., 1979). Too much sunlight can cause the nest temperature to be too high and thus can lead to hatching failure (Wang *et al*., 2000). Similarly, too little sunlight would result in low temperature for successful incubation. Habitat providing the correct level of sunlight duration could be chosen by the Chinese alligators for nesting. Comparing the mean±SD of sunlight duration in the three types of nesting sites (Table 1), we found that the Chinese alligators intentionally kept their nesting temperature at lower level. Nesting temperature is an important determinant of embryonic developmental rate (Grahame *et al*., 1983) and it is proportional to the successful rate (Wang *et al*., 2000). Additionally, nest temperature is also proportional to incubation time in a certain range (Wang *et al*., 2000). Consequently, the lower nest temperature maintained by the Chinese alligator in the Gaojingmiao Breeding Farm would reduce the successful rate of embryonic development and prolong the incubation time. Further, in the wild, longer incubation times would increase the danger of eggs from enemies. This may be one of the reasons why the Chinese alligators choose habitats with short sunlight duration for nesting regardless of those disadvantages. Studies on the American alligator indicated that sex determination was influenced by egg incubation temperature (Ferguson and Joanen, 1982). The same conclusions were confirmed with the Chinese alligator (Chen *et al*., 2003). As for the Chinese alligators, more females are produced in lower temperature nests. The sex ratio of the Chinese alligators in the study area was 1 M : 3 F. But the sex ratio of 1 M : 5 F might be more preferable in the wild as this ratio was found in both the wild Chinese and American alligators (Chen *et al*., 2003). That means that the females in this area were less in number compared to those in the wild. Thus, Chinese alligators may try to adjust the sex ratio by choice of nest sites. A study on the American alligator also found that nest-site selection was related to the sex ratio of its population (Ferguson and Joanen, 1983).

Nesting position significantly influenced the egg volume and weight, as well as hatching success and reproductive success (Grahame, 1983). Whether the habitat is fit for the Chinese alligator nesting is an important judgment for reintroduction. This study area adjoined the reintroduction area of the Chinese alligator, and its habitat was similar to the wild to a large extent.

Based on this study, the concealment, nearest bank slope and sunlight duration were considered to be important factors affecting the nest-site use of the Chinese alligators in the wild. As the Chinese alligators would build their nests at natural site in the wild, the data from the natural nests were considered. We think that the optimal nesting site for the Chinese alligators in a reintroduction area would be described as habitat with high concealment, sunlight duration of 5 h and nearest bank slope of 46°.

Acknowledgements This work was financially supported by the National Natural Science Foundation of China (NSFC, No. 30770312), the Excellent Creative Research Team of Animal Biology in Anhui Normal University and the Key Laboratory of Biotic Environment and Ecological Safety in Anhui Province. We are grateful to the assistance of Anhui Research Center for Chinese Alligator Reproduction in this study. We would like to thank Dr. Travis GLENN for his editing, and Yufang GAO, Lingling CHEN and Mian YANG for their help in the study.

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