Reproductive ecology of the Beal's-eyed turtle (*Sacalia bealei*)

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The Beal's-eyed turtle (*Sacalia bealei*) is endemic to southeastern China and endangered due to poaching and habitat loss. Knowledge of *S. bealei* ecology is lacking and this study provides baseline information of its reproduction in a natural environment. We studied the reproductive ecology of *S. bealei* using X-ray, spool-and-line tracking, and direct observation. Six nesting females were successfully tracked and their nesting behaviors were documented in detail. Females produced only one clutch per year, with a mean clutch size of 2.2 eggs (range 1–3). The white, hard-shelled eggs were ellipsoidal with a mean length of 45.50 mm, a mean width of 23.20 mm, and mean weight of 14.8 g. The relative clutch mass (RCM) was 9.47%, while the relative egg mass (REM) was 4.60%. The mean incubation period was 94.7 days with a mean nest temperature of 25.08°C. Hatchlings had a mean weight of 9.7 g, carapace length of 40.1 mm, carapace width of 33.3 mm, carapace height of 17.4 mm, plastron length of 31.6 mm, and plastron width of 25.4 mm. The results of this study provide important information to formulate conservation plan and ex-situ breeding for this endangered species.

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ABSTRACT

The Beal's-eyed turtle (*Sacalia bealei*) is endemic to southeastern China and endangered due to poaching and habitat loss. Knowledge of *S. bealei* ecology is lacking and this study provides baseline information of its reproduction in a natural environment. We studied the reproductive ecology of *S. bealei* using X-ray, spool-and-line tracking, and direct observation. Six nesting females were successfully tracked and their nesting behaviors were documented in detail. Females produced only one clutch per year, with a mean clutch size of 2.2 eggs (range 1–3). The white, hard-shelled eggs were ellipsoidal with a mean length of 45.50 mm, a mean width of 23.20 mm, and mean weight of 14.8 g. The relative clutch mass (RCM) was 9.47%, while the relative egg mass (REM) was 4.60%. The mean incubation period was 94.7 days with a mean nest temperature of 25.08°C. Hatchlings had a mean weight of 9.7 g, carapace length of 40.1 mm, carapace width of 33.3 mm, carapace height of 17.4 mm, plastron length of 31.6 mm, and plastron width of 25.4 mm. The results of this study provide important information to formulate conservation plan and ex-situ breeding for this endangered species.

Subjects: Animal Behavior, Ecology, Turtle Conservation

Keywords: Beal's-eyed turtle, Reproduction, Incubation

INTRODUCTION

The reproductive biology of a species is an important component of its overall life history strategy (Gibbons, 1982). Thus, understanding reproductive ecology is important for turtle management and conservation (Tucker & Moll, 1997; Horne et al., 2003). Studies of turtle reproductive ecology have revealed important direct and indirect effects on fitness and demography (*Bobyn & Brooks, 1994; Weisrock & Janzen, 1999; Valenzuela, 2001; Spencer and Thompson, 2003; Janzen et al., 2007*). For example, research on the snapping turtle (*Chelydra serpentina*) found that initial egg mass and substrate moisture were positively correlated with hatchling mass, which could then affect hatchling survival (*Bobyn & Brooks, 1994*).

The Beal's Eyed Turtle (*Sacalia bealei*), endemic to southeastern China, is distributed in Guangxi, Guangdong, Fujian, Anhui, Guizhou, Jiangxi Provinces, and Hong Kong (*Shi et al., 2008*). It is listed as endangered on the IUCN Red List (*van Dijk et al., 2012*) and Appendix II of CITES. Poaching pressure on *S. bealei* is still strong, with the pet trade price increasing from 1500 RMB/kg in 2014 to 4200 RMB/kg in 2015 (*Hu et al., 2016*). Due to sustained illegal poaching and trade, *S. bealei* has become extremely rare in the field (*Shi et al., 2005; Gong et al., 2017*). Life history of this species is poorly understood, with only a few observations on diet and breeding in captivity (*Zhang et al., 1998*). Since 2014, we conducted field studies focusing on habitat selection, home rage, and reproductive ecology through methods of radio telemetry, spool-and-line tracking, and direct observation (*Hu, 2016*). The current study was part of this work and provides baseline information on the reproductive biology of *S. bealei* in a natural environment, and thus will contribute to the study and conservation of this species.

METHODS

Study site

We conducted field research in Huboliao National Nature Reserve, Fujian Province, China (117° 12' 42"~117° 22'45" E; 24° 30' 05" ~ 24° 56' 20" N). The mean annual temperature in Huboliao is 21.1°C, with the lowest temperature in January (mean 10.9°C) and the highest in July (mean 26.7°C). The mean rainfall is 1733.5 mm, with a mean relative humidity of 81.4%. The major vegetation types in this reserve are evergreen broad-leaved forest, mixed forest, and bamboo forest (*Fan, 2001*).

Methods

In October 2014, we began a radio telemetry study to understand the habitat selection, daily rhythm, and home range of *S. bealei*. In total, 19 individuals, including 13 females and 6 males, were tracked using radio transmitters. In 2015, when turtles had completed their hibernation in late March, we captured them and brought females back to the field station for further inspection. We used portable X-ray radiography (BJI –UJ) to confirm the presence of oviductal eggs. If shell-eggs were found inside plastron, the radio-transmitter was removed and replaced with a plastic spool-and-line tracker. The spool-and-line tracker was 8 g total weight and contained 70 m fishing line. We designed the tracker and have successfully used to track daily turtle movement during reproduction period (Li, 2013; Yang, 2014). Ten of the 13 females were gravid, and took six morphological measurements of the individuals (weight, carapace length, carapace width, body height, plastron length, and plastron width) following methods in *Xiao et al.* (2014).

Every morning, we followed the fishing line of each of the 10 females to track its movement. The presence of fishing line on land indicated that the individual was attempting to nest. When we encountered such a situation, we took notes on the nesting and egg-laying behaviors, minimizing disturbance by observing using binoculars (8–10 m distance) during the day and headlamps with red light during the night. After the turtle laid eggs and returned back to the stream, we checked the nest, recording (1) nest chamber characters (*e.g.* size and nest materials), (2) clutch size and egg measurements (length, width, and weight), (2) distance crawled by the female before nesting, and (4) straight distance between the nest and the stream bank. Fertilized and unfertilized eggs were distinguished by the presence of a white spot on the eggshell. Relative egg mass (REM) (mean egg weight/body weight before oviposition) and relative clutch mass (RCM) (clutch weight/body weight before oviposition = REM x clutch size) were calculated for each gravid female as estimates of reproductive effort (*Wang et al., 2011*). To monitor the temperature and humidity of the nest during incubation, we buried a data logger (HOBO U23-001, Onset Computer Inc., USA) near the nest, at the same depth and using the same materials. To prevent predation, we placed a wire net above the nest.

We checked the nests at least twice a week during incubation. When hatchling turtles emerged from the nests, we measured their weight, carapace length, carapace width, plastron length, and body height. Hatchlings were taken back to the field station and released back to the stream when the yolk sac was completely absorbed. Data loggers were retrieved after hatchling emergence, and the data were downloaded by HOBOware Graphing & Analysis Software (Onset Computer Inc., USA).

Data analysis

Statistical analyses were performed in SPSS 19.0. Descriptive statistics were expressed by mean ± standard error. Significance level was set to 0.05. We used linear regression to investigate the relationship between female body size and clutch or egg size

RESULTS

Timing and behavior sequence of nesting

Among the ten gravid females, six individuals were successfully tracked, of which we also collected detailed nesting behavior from individuals. The remaining four individuals were lost because the fishing line broke. The nesting activities lasted from May 3–31. For the six females, we recorded 10 nesting activities—four failed attempts and six successful nesting. All observed nesting activities happened on rainy days and egg laying only happened at night. The behavior sequences of nesting could be categorized into five successive steps: 1) nest-site selection, 2) chamber excavation, 3) egg laying, 4) nest covering, and 5) returning to water.

During the nest-site selection, females emerged from water and made their way into the forest. Movement was not continuous, but instead occurred in stops and starts. During the pauses, individuals often elevated their head a few centimeters above the carapace and move side-to-side a few times, possibly as a vigilance behavior. While moving, the ground substrate did not impede their movement—they crawled along rocks, fallen wood, thick leaves, and gentle slopes. Often, females would attempt to dig nests 1–2 times (which we called “empty holes”) before they chose the final site.

After finding the right site, females excavated the nest chamber, alternating between the two hindlimbs. After completing excavation, females would use the rear portion of its body to hit the nest 4–6 times. The chamber excavation lasted 40–60 minutes.

After resting approximately 20 minutes, females would start laying eggs with hindlimbs straddling the chamber opening. To expel eggs, forelimbs and head would completely extend then simultaneously withdraw. Body tremors were obvious when the eggs were released into the nest. In total, egg laying lasted 8–15 minutes. No vigilance behaviors were observed during this sequence.

Nest covering began immediately after laying the last egg. Soil and leaves were packed down using alternate, backward movements of the hindlimbs, followed by a bobbing motion of the body that further compressed the soil and leaves into the nest chamber. Nest covering lasted 40–70 minutes.

After covering the nest, females immediately returned to water. While returning to the water, similar behaviors to those when choosing a nest-site were exhibited, such as movement in stops and starts, and crawling along shelters like rocks and fallen wood.

Distance of nests from the stream

Straight distance from nest to the stream bank was 8.55 ± 1.23 m (n=6), while straight distance from nest to the sites where they emerged from water and returned to water, was 11.47 ± 1.31 m and 16.55 ± 2.93 m (n=6), respectively. Females crawled a distance of 42.67±7.18 m (n=6) to choose a nesting site.(see Table 1).

Characteristics of nests and eggs

Nests were well camouflaged by leaves and soil, making it difficult to find nests without the help of fishing line. The shape of nests was hemispherical, with a diameter of 70–100 mm and depth of 55–70 mm. Due to the relatively shallow depth, eggs were often half-buried by soil then covered by leaves. Clutch size was 2.2 ± 0.3 (range 1–3, n=6). The white, hard-shell eggs were ellipsoidal, and weighed 14.8 ± 0.8 g, with a length of 45.5 ± 1.0 mm and width of 23.2 ± 0.5 mm (n=13). A negative, linear correlation was found between mean egg length and female plastron width (Pearson correlation coefficient= 0.934, R^2 = 0.934, P <0.05), and a positive, linear correlation found between mean egg width and female carapace height (Pearson correlation coefficient = 0.847, R^2 = 0.718, P < 0.05). The mean RCM was 9.47±1.01% (5.14–12.21%, n=6) and the mean REM 4.60 ± 0.44% (2.98–6.11%, n=6) (see Table 2).

Incubation period and temperature

Among the 13 eggs inside six nests, 10 were fertilized (76.9%, 33.3%-100% per nest) and 7 successfully hatched (70%, 0-100% per nest). The incubation period was 85–108 days (mean = 94.7 ± 2.5 days; n=7) and the temperature range during incubation was 17.51–29.64 °C (mean = 25.08 ± 0.13 °C; n=7). (see Table 3).

Hatchling turtles

Of the seven hatchings, two from the same clutch died two days after hatching. Their deaths were probably due to ant infestation, as we found ants on their body when the nest was opened. The remaining five hatchlings were kept in the laboratory and their yolk sac was absorbed, after which we released them to the stream.

The measurements of the hatchlings are found in Table 3. Hatchlings had soft, reddish-brown carapaces with curled margins that gradually flattened after several days, orange plastron and skin, and bright yellow stripes on their neck. Four bright-green eye-spots were obvious on the head, with the two spots on the same side linked together. A black dot could be seen in every eye-spot.

DISCUSSION

The process of selecting a nesting site is important to females in an immediate sense because they are more vulnerable to terrestrial predators at this time (*Spencer, 2002*). Therefore, many turtle species spend less than three hours out of water during the nesting process, especially for those species nesting mainly on sandy beaches and banks (*Doody et al., 2009; Booth, 2010*). However, we found *S. bealei* could spend 4 – 10 hours out of water when nesting. We believe this is mainly due to the environment, where heavy forest and shrubs are present and offer protection from predators. In addition, small body size and cryptic coloration (dark-brown carapace) might also contribute their safety.

Females constructed shallow, bowl-shaped nests covered by soil and leaves. This is similar to two other forest species in Asia, *Platysternon megacephalum* (*Sung et al., 2014*) and *Cuora mouhotii* (*Wang et al., 2011*), but different from the flask-shaped, deeper nests of *Trachemys scripta elegans* (Li, 2013), *Carettochelys insculpta* (*Doody et al., 2009*), and *Phrynops tuberosus* (*Rodrigues & Silva, 2014*) that all nest in open areas. Nests that are flask-shaped, compared to bowl-shaped, are advantageous in that they hold more eggs and daily temperature fluctuations inside the nest are reduced (*Booth, 2006; Booth, 2010*). Sea turtles and larger freshwater turtle species tend to construct such flask-shaped nests are more commonly constructed by sea turtles and larger freshwater species as these turtles have stronger and longer hindlimbs (*Parris et al., 2002; Corra-H et al., 2010; Booth, 2010*). *Sacalia bealei* may be constrained to constructing bowl-shaped nests due to their relatively small body size. Additionally, temperatures may not fluctuate greatly in *S. bealei* nests as nest sites are often inside heavy forest where temperatures are moderated by shade.

Turtle body size has been shown to influence reproductive potential in female turtles (*Valenzuela, 2001*), as the area of the pelvic girdle is correlated with female size and may constrain the size and number of eggs an individual can oviposit (*Bowden et al., 2004*). Though considered a small-size turtle species, *S. bealei* produces larger eggs (length 45.5 mm, width 23.2 mm, weight 14.8 g) than some larger freshwater species, such as *T. s. elegans* (egg length 35.4 mm, width 22.1 mm, weight 10.36 g, *Tucker & Janzen, 1998*). However, *S. bealei* produces smaller clutches (average 2.2 eggs) of larger eggs, while *T. s. elegans* produces larger clutches (average 12.5 eggs) of smaller eggs. Consequently, the RCM of *S. bealei* (5.14–12.21%) was similar to other freshwater turtle species: *C. mouhotii* (6.9–14.6%, *Wang et al., 2011*), *C. flavomarginata* (2.3–9.2%, *Chen & Lue, 1999*) and *T. scripta* (3–17%, *Ernst & Lovich 2009*). Therefore, the total volume for eggs is relatively similar, but different species take different approaches to eggs—many small or few big. No correlations were found in our study between female body size and clutch size or egg size, however this may be due to small sample size.

The incubation period of *S. bealei* (94.7 ± 2.5 days at mean 25.1°C) was relatively longer when compared to *T. s. elegans* (62.25 days at mean 27.4 °C, *Yang, 2014*) and Magdalena River Turtle (*Podocnemis lewyana*) (59.1 days at mean 32.8 °C, *Corra-H et al., 2010*). This is likely due to the nest conditions—*S. bealei* nests in cooler, shaded forests, while the other two species nest in open areas with higher nest temperatures.

Both fertilization and hatching rate of *S. bealei* (76.9% and 70%, respectively) in our study were higher than *S. quadriocellata* in captivity (22.6% and 47.4%, respectively; *He et al., 2010*) and *T. scripta elegans* in the field in Hainan Province (33.7% and 36.7%, respectively; *Li, 2013*). Hatching rate for *S. bealei* may have been aided by caging of the nests. Captive stress and lack of male individuals could cause the low fertilization rate of female turtles in captivity or wild (*Cadi et al., 2004; He et al., 2010*). Based on the high fertilization and hatching rate of *S. bealei*, we believe the population in our research area is healthy, with low stress levels and a proper sex ratio.

Ant predation probably caused the death of two hatchling turtles in our study. This occurrence is commonly reported in many other turtle species (*Parris et al., 2002; Ferreira Júnior et al., 2011; Buhlmann & Offman, 2001; Correa-H et al., 2010; Yang, 2014; Erickson & Baccaro, 2016*). In some cases, invertebrates including ants, flies, and beetles could infest more than 50% nests (*Baran et al., 2001*). No evidence of vertebrate nest-predators was found in our study, probably because of the nest protection we constructed.

CONCLUSIONS

Like other turtle species in China, *S. bealei* is facing critical crisis due to heavy poaching.

During our work, we found more than 30 illegal turtle traps in the streams. Huge demand in cities is a major driver of turtle poaching in reserves (*Gong et al., 2017*). Even just a few years ago, species in the genus *Sacalia*, (*S. bealei* and *S. quadriocellata*) were not considered valuable as they emit a musk odor. However, the rarity of other turtle species has made *Sacalia* species more popular (*Linh et al., 2016*), and price in local markets has sharply increased to 4200 RMB/kg (*Hu et al., 2016*).

Poaching, along with low fecundity and habitat encroachment, threaten the survival of *S. bealei* across its range. Low fecundity (only one clutch with 1–3 eggs per year) and long incubation period imply that *S. bealei* has a low intrinsic rate of population increase. Thus, the survival of populations should be more difficult under high poaching pressure. Habitat encroachment also threatens the survival of *S. bealei*. *S. bealei* inhabits medium elevation of 276–389 m, which overlaps with banana and grapefruit plantations (*Hu et al., 2016*). We believe that effective conservation measures should include eliminating poaching and preserving native forest and stream habitats. Meanwhile, no successful captive breeding of *S. bealei* has been reported and we hope the information yielded from our study could improve methods for its captive breeding and ex-situ conservation.

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ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

There are no competing interests

Author Contributions

- Liu Lin, Qingru Hu and Haitao Shi conceived and designed the experiments, performed the fieldwork, analyzed the data, contributed materials/analysis tools, and wrote the paper.
- Jonathan J. Fong analyzed the data, wrote, and reviewed the drafts of the paper.
- Jiangbo Yang, Zhongdong Chen and Feiyu Zhou conceived and designed the experiments, performed the fieldwork, and contributed materials/analysis tools.
- Jichao Wang and Fanrong Xiao contributed materials/analysis tools and reviewed drafts of the paper.

Animal Ethics

This work was approved by Animal Research Ethics Committee of Hainan Provincial Education Center for Ecology and Environment, Hainan Normal University (HNECEE-2014-001).

Field Study Permissions

The study was approved by Administration and Services Centre of Nanjing County, Fujian Province, and conducted under Licenses ID 20141203NJ0173.

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Table 1(on next page)

Table1 Distance of nests from the stream

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Turtle ID	Straight Distance from nest to stream bank (m)	Straight Distance from nest to site emerging from water (m)	Straight Distance from nest to site returning to water (m)	Whole crawling distance during nest selection (m)
13	7.00	9.00	13.00	30.50
14	7.80	11.50	18.00	38.00
15	11.00	13.00	17.00	67.00
17	13.00	17.00	20.50	47.50
18	8.00	10.00	26.00	55.00
20	4.50	8.30	4.80	18.00
Mean±SE	8.55±1.23	11.47±1.31	16.55±2.93	42.67±7.18

Table 2(on next page)

Table 2 Characters of nesting female S. bealei and their fecundity

1 Table 2 Characters of nesting female *S. bealei* and their fecundity

Turtle ID	Body Weight (g)	Carapace Length (mm)	Carapace Width (mm)	Plastron Length (mm)	Plastron Width (mm)	Body Height (mm)	Clutch size	No. of Fertilized Eggs	Egg length (mm)	Egg width (mm)	Egg weight (g)	RCM (%)	REM (%)
13	340	133.9	94.6	121.2	78.6	50.3	3	2	42.1	24	14	11.47	3.82
									46	23.6	12		
									46.7	24.5	13		
14	378	143	96.8	126.9	77.3	53.9	2	2	49.4	25	18.7	9.76	4.89
									49.5	24.9	18.2		
15	298	128.3	93.3	117.8	77.3	57.1	2	2	49.5	24.9	18.2	12.21	6.11
									46.5	26.1	18.2		
17	290	132.8	92	115.1	76.5	52.1	1	1	49	23.3	14.9	5.14	5.14
18	342	140.6	91.8	124.1	79.8	49.4	2	1	45.6	20	15.6	9.27	4.65
									45.9	20	16.1		
20	373	142.8	103.8	131.5	84.4	50.4	3	2	41.1	22.7	11.8	8.95	2.98
									42.5	21.8	11.5		

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									38.1	21.4	10.1		
11	370	138.5	93.2	121.8	80	52	/	/	/	/	/	/	/
12	341	156	95.8	123.5	82.5	51.4	/	/	/	/	/	/	/
16	299	133.1	92.8	120.1	76.7	48.7	/	/	/	/	/	/	/
19	265	131.2	90.3	115.3	74.8	47.2	/	/	/	/	/	/	/
Mean±SE	329.6±11.8	136.3±1.5	94.4±1.1	121.7±1.5	78.8±0.9	51.3±0.8	2.2±0.3	1.7±0.2	45.5±1.0	23.2±0.5	14.8±0.8	9.47±1.01	4.60±0.44

Table 3 (on next page)

Table 3 Information on hatchling.

Table 3 Information on hatchling

Hatchling ID	Female ID	Egg-laying date	Hatching Date	Incubation Period (Day)	Mean incubation temperature (°C)	Body Weight (g)	Carapace Length (mm)	Carapace Width (mm)	Body Height (mm)	Plastron Length (mm)	Plastron Width (mm)
1	13	19-May	19-Aug	92	25.17	8.0	39.0	31.9	20.3	29.9	24.9
2	13	19-May	24-Aug	97	25.22	8.0	37.6	25.7	15.9	29.3	22.1
3	14	20-May	22-Aug	94	25.09	10.0	40.5	38.0	17.5	31.9	27.3
4	14	20-May	24-Aug	96	25.11	11.0	40.1	33.5	17.4	31.3	24.0
5	15	31-May	24-Aug	85	25.32	10.0	39.9	35.4	17.0	32.6	27.2
6	15	31-May	30-Aug	91	25.29	12.0	42.1	36.7	16.0	33.5	27.8
7	18	03-May	19-Aug	108	24.33	9.0	41.5	32.1	17.5	32.6	24.3
			Mean±SE	94.7±2.5	25.08±0.12	9.7±0.5	40.1±0.5	33.3±1.4	17.4±0.5	31.6±0.5	25.4±0.7

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