

## Reproductive Parameters of Captive Sea Turtles in Okinawa Churaumi Aquarium

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**ABSTRACT:** Okinawa Churaumi Aquarium, located on Okinawajima Island, Japan, has had a facility for sea turtle reproduction, rescue, and rehabilitation since 1994. The facility has an outdoor holding tank (16.8 m × 10.5 m × 2 m) with an open-water system and a sandy nesting area (115 m<sup>2</sup>). The water temperature of the tank ranges between 20 °C and 30 °C across a 12-month period, which is similar to the waters near Okinawajima Island. The turtles are fed at approximately 1-day intervals, with a diet that includes fish, squid, cabbage, and Chinese cabbage in quantities equivalent to 2% of their body weight. Loggerhead turtles, green turtles, hawksbill turtles, and a black turtle have produced 76, 100, 38, and 5 clutches in captivity since 1995, 1999, 2012, and 2017, respectively. For loggerhead turtles, the mean ± SD of the number of clutches per year, clutch size, and emergence rate was 3.9 ± 1.1 clutches (range: 2–6 clutches), 113.0 ± 19.7 eggs (57–146 eggs), and 10.4 ± 21.1% (0–89.9%), respectively. The corresponding values in green turtles were 4.8 ± 2.2 clutches (1–8 clutches), 91.9 ± 32.4 eggs (14–155 eggs), and 35.1 ± 30.3% (0–96.8%), and those in hawksbill turtles were 2.6 ± 1.2 clutches (1–5 clutches), 127.6 ± 41.9 eggs (46–194 eggs), and 12.9 ± 21.4% (0–69.6%). A black turtle produced 5 clutches, 45.2 ± 13.8 eggs (29–67 eggs), with an emergence rate of 12.1 ± 4.2% (7.3–13.8%). We suggest that the similarity of the environment at the facility to that in the wild may underlie the breeding success of these four sea turtle species. Further studies are required to extend our knowledge of the reproductive biology and ecology of sea turtles and improve the emergence rate.

### INTRODUCTION

The loggerhead (*Caretta caretta*), green (*Chelonia mydas*), black (*Chelonia agassizii*), hawksbill (*Eretmochelys imbricata*), olive ridley (*Lepidochelys olivacea*), and leatherback turtle (*Dermochelys coriacea*) are distributed around Okinawajima Island, Japan (Hirate and Kawazu, 2017a; 2017b; 2017c; Kino and Kawazu, 2014; Kino *et al.* 2015; Yoshikawa *et al.* 2016). These sea turtle species are listed as critically endangered in the International Union for Conservation of Nature (IUCN) Red List for threatened species due to the loss of their nesting grounds and their accidental capture in fishing gear (Wallace *et al.* 2011). The protection and restoration of sea turtle nesting grounds and improvement in fishing gear to reduce bycatch are clearly essential for the conservation of sea turtles (Wallace *et al.* 2011). In addition to conservation in the wild, captive breeding programs should also be actively implemented to assist with the recovery of these endangered species (Owens and Blanvillain, 2013).

Okinawa Churaumi Aquarium, located on Okinawajima Island, Japan, has had a facility for sea turtle reproduction, rescue, and rehabilitation since 1994. Loggerhead (Kawazu *et al.* 2015a; Kawazu *et al.* 2016; Teruya *et al.* 1997), green (Yanagisawa, 2012), hawksbill (Kawazu *et al.* 2015c), and black turtles (Kawazu *et al.* 2018) have already successfully bred at Okinawa Churaumi Aquarium since 1995, 1999, 2012, and 2017, respectively. The captive bred turtles are tagged and

head-started after 1 year of captive rearing to survey their migration. Such captive breeding programs provide important insights into developing pioneering research approaches (Owens and Blanvillain, 2013). For example, captive breeding studies have yielded biological reproductive information on sexual maturation (Kawazu *et al.* 2015b), mating function (Kawazu *et al.* 2014b);

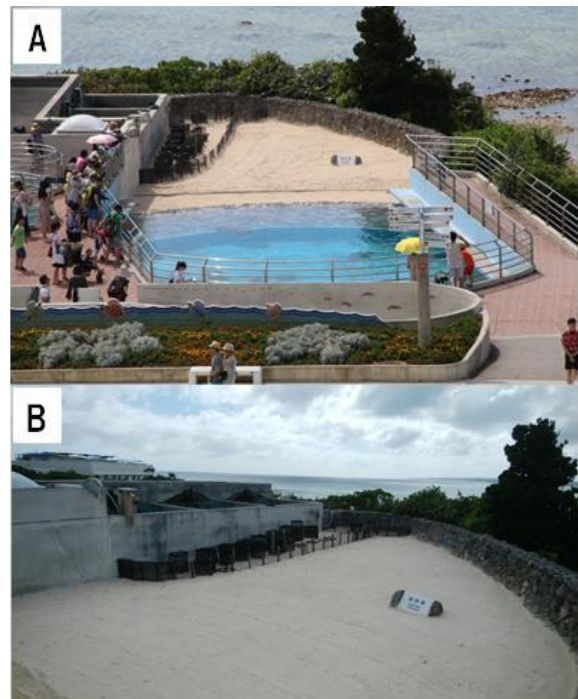


Fig. 1 Photographs of a sea turtle tank (A) and the artificial sandy beach (B) for nesting and hatching in Umigame-kan at the Okinawa Churaumi Aquarium.

2014c; Kawazu *et al.* 2015c; 2015d; Manire *et al.* 2008), and the reproductive cycle (e.g., spermatogenesis and vitellogenesis) of sea turtles (Kawazu *et al.* 2014b; Kawazu *et al.* 2015c). In addition, several techniques used for the captive husbandry of sea turtles (blood sampling and ultrasonographic diagnoses) have been used to study the reproduction and conservation of sea turtles in the wild (Owens and Blanvillain, 2013).

To develop captive breeding techniques, we recorded reproductive parameters, including the number of clutches per year, clutch size, inter-nesting interval, and emergence success rate, in captive loggerhead, green, hawksbill, and a black turtle at Okinawa Churaumi Aquarium during 1995–2017. Our purpose was to clarify the captive breeding technique by comparing between captive and wild reproductive parameters.

## MATERIALS AND METHODS

### Captive animals and holding tank

All sea turtles were rescued from around Okinawa Island, Japan and transferred to holding tanks at the Okinawa Churaumi Aquarium, Motobu-cho, Okinawa Prefecture, Japan during 1994 and 2017. The turtles used in the captive breeding program were maintained in an outdoor holding tank (16.8 × 10.5 × 2.0 m) that consisted of an open-water system with a sandy nesting ground (115 m<sup>2</sup>) (Fig. 1). The water temperature of the tank was measured daily for 1 year and ranged between 20 °C and 30 °C, similar to that of the sea surface around Okinawa Island. The turtles were fed a diet that included fish, squid, cabbage, and Chinese cabbage in quantities equivalent to 1–2% of their body weight, at 24-h intervals.

### Husbandry for breeding

During autumn-winter, vitellogenesis of the adult females was confirmed using blood metabolite measurements, including the triglyceride, total protein, and calcium, because these blood metabolite concentrations increase during winter-spring and are stored for follicular development (Kawazu *et al.* 2015a). One milliliter of blood was sampled from the jugular vein (either the left or right side of the neck) using a 70-mm 20-gauge needle (Terumo Inc., Japan) and a 10-mL syringe (Terumo Inc.) and then stored in heparin vacutainers (Fujifilm Inc., Japan), after which plasma was collected using a centrifugation (speed: 6000 rpm, time: 5 min). Plasma triglyceride, total protein, and calcium were measured from the plasma samples using a biochemistry autoanalyzer for animals (Fuji-drycem 7000 V; Fujifilm Inc., Japan).

During spring, the ovaries of the adult females

were observed to identify the presence of vitellogenic follicles and follicular development using ultrasonography with a 3.5-MHz probe (SSD-900, Aloka Inc., Japan) and a 5-MHz probe (Minidock P04341-04, Sonosite Inc., USA) at 1-year intervals. The turtles were placed in dorsal recumbency under a water depth of 200 mm, and the probes were positioned in the inguinal region of both the right and left rear flippers.

Although loggerhead, green, and black turtle females mated with males in the holding tank with a nesting ground, hawksbills females were unreceptive to males and prevented penile insertion into their cloaca by covering their cloaca with their rear flippers. Thus, hawksbill vitellogenic females were transferred separately from the holding tank to the pairing (mating) tank (5 × 5 × 1 m, indoor, open water system) with one male, after which we observed presence of calcified eggs using ultrasonography. Females were returned to the holding tank with a nesting ground. The hawksbill males were housed separately to the females throughout all periods, except during the mating procedures.

### Record of reproductive parameters

The turtles of all species laid eggs on the nesting ground attached to the holding tank approximately 1 month after mating with the males in the holding tank (loggerhead, green, and black females) and the pairing tank (hawksbill females). The date of nesting of each turtle was recorded; thus, the number of clutches per year and inter-nesting interval were calculated. After nesting, the clutch size was recorded as the number of normal eggs in each clutch (i.e., eggs containing both albumen and yolk: Miller, 1999). Moreover, emergence success rates (calculated as the ratio of the number of all hatchlings to all oviposited eggs) were recorded. These reproductive parameters for the four sea turtle species were presented as mean ± SD and compared with wild values in previous studies (Hirth, 1980; Marquez, 1990; Miller *et al.* 1997). Data on the reproductive parameters for black turtles were taken from Kawazu *et al.* (2018).

## RESULTS

### Loggerhead turtles

Three captive loggerhead turtles produced 76 clutches (9371 eggs) since 1995. The mean ± SD of the number of clutches per year, inter-nesting intervals, clutch size, and emergence rate were 3.9 ± 1.1 clutches (2–6 clutches, n = 19), 13.4 ± 2.3 days (9–19 days, n = 53), 113.0 ± 19.7 eggs (57–146 eggs, n = 84), and 10.4 ± 21.1% (0–89.9%, n = 81), respectively (Table 1). Comparison between captive

### Green turtles

Three captive green turtles produced 100 clutches (9906 eggs) since 1999. The mean  $\pm$  SD of the number of clutches per year, interesting intervals, clutch size, and emergence rate were  $4.8 \pm 2.2$  clutches (1–8 clutches,  $n = 18$ ),  $12.9 \pm 2.1$  days (10–19 days,  $n = 51$ ),  $91.9 \pm 32.4$  eggs (14–155 eggs,

$n = 72$ ), and  $35.1 \pm 30.3\%$  (0–96.8%,  $n = 71$ ), respectively (Table 1). Comparison between captive and wild reproductive parameters indicated that captive green turtles breed in captivity similarly to wild green turtles, with the exception of emergence success rate (Table 1).

Table 1. Reproductive parameters of captive sea turtles bred in Okinawa Churaumi Aquarium.

Turtles	Clutch frequency (Clutches/season)	Clutch size (egg)	Interesting interval (day)	Emergence success rate (%)
Loggerhead	Captive $3.9 \pm 1.1$ ( $n = 19, 2-6$ )	$113.0 \pm 19.7$ ( $n = 83, 57-146$ )	$13.4 \pm 2.3$ ( $n = 53, 9-19$ )	$10.4 \pm 21.1$ ( $n = 81, 0-89.9$ )
	Wild $3.5-4.5$ * <sup>1,2</sup>	$112-114$ * <sup>1,2</sup>	$13-17$ * <sup>1</sup>	$77.8$ * <sup>2</sup>
Green	Captive $4.8 \pm 2.2$ ( $n = 18, 1-8$ )	$91.9 \pm 32.4$ ( $n = 72, 14-155$ )	$12.9 \pm 2.1$ ( $n = 51, 10-19$ )	$35.1 \pm 30.3$ ( $n = 71, 0-96.8$ )
	Wild $1.8-4.5$ * <sup>1,2</sup>	$104-138$ * <sup>1,2</sup>	$10-17$ * <sup>1</sup>	$69-86$ * <sup>2</sup>
Hawksbill	Captive $2.6 \pm 1.2$ ( $n = 11, 1-5$ )	$127.6 \pm 41.9$ ( $n = 30, 46-194$ )	$16.2 \pm 4.1$ ( $n = 17, 13-28$ )	$12.9 \pm 21.4$ ( $n = 30, 0-69.6$ )
	Wild $1.8-4.0$ * <sup>1,2</sup>	$130-182$ * <sup>1,2</sup>	$11-28$ * <sup>1</sup>	$72-78$ * <sup>2</sup>
Black	Captive 5	$45.2 \pm 13.8$ ( $n = 5, 29-67$ )	$11.8 \pm 3.1$ ( $n = 4, 9-16$ )	$12.1 \pm 4.2$ ( $n = 3, 7.3-13.8$ )
	Wild $1.0-8.0$ * <sup>3</sup>	$38-152$ * <sup>3</sup>	$5-25$ * <sup>3</sup>	$40-70$ * <sup>3</sup>

\*<sup>1</sup> Miller *et al.* (1997), \*<sup>2</sup> Hirth (1980), \*<sup>3</sup> Marquez (1990)

Information on the reproductive parameters for black turtles was cited from Kawazu *et al.* (2018).

### Hawksbill turtles

Two captive hawksbill turtles produced 38 clutches (3828 eggs) since 2012. The mean  $\pm$  SD of the number of clutches per year, interesting intervals, clutch size, and emergence rate were  $2.6 \pm 1.2$  clutches (1–5 clutches,  $n = 11$ ),  $16.2 \pm 4.1$  days (13–28 days,  $n = 17$ ),  $127.6 \pm 41.9$  eggs (46–194 eggs,  $n = 30$ ), and  $12.9 \pm 21.4\%$  (0–69.6%,  $n = 30$ ), respectively (Table 1). Comparison between captive and wild reproductive parameters indicated that captive hawksbill turtles breed in captivity similarly to wild hawksbill turtles, with the exception of emergence success rate (Table 1).

### Black turtle

One captive black turtle produced 5 clutches (226 eggs) in 2017. The mean  $\pm$  SD of the number of clutches per year, interesting intervals, clutch size, egg size, and emergence rate were 5 clutches,  $11.8 \pm 3.1$  days (9–16 days,  $n = 4$ ),  $45.2 \pm 13.8$  eggs (29–67 eggs,  $n = 5$ ), and  $23.6 \pm 13.8\%$  (0–33.3%,  $n = 2$ ), respectively (Table 1). Comparison between captive and wild reproductive parameters indicated that the captive black turtle bred in captivity similarly to wild black turtles, with the exception of

emergence success rate (Table 1). All results for the black turtle have already been reported by Kawazu *et al.* (2018).

## DISCUSSION

This study demonstrated that four sea turtle species successfully bred at Okinawa Churaumi Aquarium, in which light and temperature conditions are maintained similar to natural conditions around Okinawajima Island. To the best of our knowledge, Okinawa Churaumi Aquarium has had the most reproductive success for sea turtle species of the world's aquariums and facilities to date. These data for reproductive success provide information of reproductive parameters in captive sea turtles.

A low hatching rate in captive sea turtles is common in green (Wood and Wood, 1980), hawksbill (Kobayashi *et al.* 2010), and Kemp's ridley turtles (Wood and Wood, 1988). Similarly, in Okinawa Churaumi Aquarium, the emergence success rate in the four captive sea turtles was low (11.2% for loggerhead turtles, 33.0% for green turtles, 22.3% for hawksbill turtles, and 23.6% for

black turtles). In Okinawa Churaumi Aquarium, the emergence success rate of loggerhead turtle nests that were rescued and transferred to artificial sandy beaches from the wild were high (69%), and similar to those of wild turtles (Kawazu personal com.). Therefore, we suggest that the low emergence success rate could be caused by the egg condition during the egg formation process (i.e., vitellogenesis, mating, and fertility).

Wood and Wood (1980) reported that the hatching rate of captive green turtles improves with the length of time that the female is mounted. If females are mounted for 100–199 min, the hatching success is 30% or less, but this increases if females are mounted for more than 400 min (Wood and Wood, 1980). During mating, ejaculated sperms progress into oviducts, and are stored in the upper portion of each oviduct where they fertilize ovulated eggs (Gist and Jones, 1989; Owens, 1980). Thus, we believe that fertility is affected by the dosage of semen injection. Polyspermic fertilization is a characteristic of some birds and reptiles with large eggs (Mizushima *et al.* 2014). Further study is required to confirm this hypothesis, which is needed to establish artificial insemination techniques in sea turtles. Semen collection (Kawazu *et al.* 2014b; Kawazu *et al.* 2015d), ovulation induction (Kawazu *et al.* 2014c), and oviposition induction techniques (Kawazu *et al.* 2014a) have already been developed in sea turtles. However, some problems regarding further development and success of the technique for artificial insemination of sea turtles also need to be solved.

The low emergence success rate might possibly be affected by the condition of the female turtle in captivity. The triglycerides and proteins ingested by females are utilized for follicular development (Kawazu *et al.* 2015c; 2016). Craven *et al.* (2008) reported that yolk fatty acids profiles were different between captive and wild green turtles and were influenced by the diet of the female. Thus, egg condition, involving fertility and embryogenesis, might be affected by diet.

Souza *et al.* (2018) reported that hatching success in loggerhead and green turtles is affected by egg copper (Cu) and zinc (Zn) concentration. Also, the low hatching rate of leatherback turtles might be caused by a lack of selenium in females (Perrault *et al.* 2011). In avian eggs, which have the same consistency of calcium, albumen, and yolk as sea turtles, the increase in egg selenium concentration via diet are associated with better antioxidant protection during embryonic development and post-hatching, which leads to high hatching rate and low motility post-hatching (Surai, 2002). Also, Surai (2002) reported that selenium has a sustaining effect on sperm function in male

avians, so that a lack of selenium might explain low fertility rates. Further studies are required to assess the relationship between diet, including the contents of fatty acids and trace elements, and fertility and hatching rate, which will contribute to the conservation of wild sea turtles and improve our knowledge of reproductive biology.

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