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Reproductive Parameters and Changes in Blood Levels of Steroid Hormones and Minerals in the Hawksbill Turtle *Eretmochelys imbricata* in an Aquarium

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Abstract: The hawksbill turtle *Eretmochelys imbricata* is listed as "critically endangered" on the Red List of International Union for Conservation of Nature; it requires *in situ* and *ex situ* conservation. To establish an effective captive breeding technique for the hawksbill turtles, its reproductive parameters and behavior in captivity, and the physiological changes, including hormonal changes, that lead to egg laying must be understood. Here, we observed the mating behavior of male (n=5) and female (n=3) hawksbill turtles (housed in a tank) from 2003 to 2007. We regulated the tank water temperature and daylight hours to simulate their natural environment. We sampled their blood at regular intervals to measure plasma concentrations of minerals and sex hormones. One (No. Ei21) of the female turtles was reproductively more active than others. Further, males mounted it more frequently than others. Subsequently, the female (Ei21) laid eggs twice, on 10 May and 8 July 2003. Females including Ei21 showed annual changes in 17β-estradiol and progesterone (P4) levels, whereas males showed annual changes in testosterone levels. Plasma calcium (Ca) level peaked in only the egg laying turtle (Ei21); however, inorganic phosphorus did not show a sharp increase. In 2005, as in 2003, Ei21 underwent similar physiological changes. We conclude

that this phenomenon—of rapid rise in plasma P4, a hormone elevated after ovulation, followed immediately by a rise in plasma Ca level—is likely involved in the eggshell formation after ovulation. In future, we will further investigate these phenomena in hawksbill turtles.

Key words: Calcium metabolism; Hawksbill turtle; Progesterone; Reproductive behavior

Introduction

Of the current seven extant species of sea turtles in the world, three species lay eggs in Japan (Uchida and Nishiwaki, 1982). Among them, the hawksbill turtle, *Eretmochelys imbricata* (Linnaeus, 1766), has declined significantly due to impacts associated with human activities and is the less abundant than the green turtle, *Chelonia mydas*, and the loggerhead turtle, *Caretta caretta*. Therefore, in 1986, the hawksbill turtle was designated "critically endangered" on the Red List of the International Union for the Conservation of Nature (IUCN) (Mortimer and Donnelly, 2008). Research on breeding is necessary to protect endangered species (Kawazu et al., 2014, 2015); moreover, emphasis should be placed on establishing breeding techniques to advance further research. The breeding environments of endangered sea turtles, including temperature and daylight conditions, influence reproductive parameters such as clutch frequency and size and internesting interval. Providing an indoor breeding environment will greatly contribute to research on the reproductive physiology of sea turtles. The Port of Nagoya Public Aquarium (PNPA, Nagoya, Japan), which is equipped with well-stocked breeding tanks, is one aquarium that has successfully bred sea turtles (Kakizoe et al., 2013). In this study, we used PNPA's facilities to investigate the reproductive behavior and physiology of the hawksbill turtle.

Both male and female reptiles undergo physiological changes which help them lay eggs during the reproductive phase (Krohmer et al., 1987; Rostal et al., 2001; Sacchi et al., 2017; Zena et al., 2019). Increased concentration of the sex hormone testosterone (T) induces mating behavior in males (Sereau et al., 2010; Thompson et al., 2023). Meanwhile, changes in sex hormones, such as 17β-estradiol (E_2) and progesterone (P4), induce egg laying in females (Guillette et al., 1991; Rostal et al., 2001; Al-Habsi et al., 2006; Shankey and Cohen, 2024). Like in other vertebrates, E_2 stimulates synthesis of the calcium (Ca) binding protein vitellogenin in the liver of reptiles (Tinsley, 1985; Heck et al., 1997; Currylow et al., 2013). Additionally, in reptiles, E_2 elevates plasma Ca and inorganic phosphorus (iP) levels by bone resorption (Magliola, 1984; Sheehan et al., 1999). Taken together, Ca metabolism is closely associated with reptilian reproductive physiology.

To maintain a constant blood pH, turtles store divalent cations such as Ca and magnesium in their carapace (Warburton and Jackson, 1995; Jackson, 1997). The turtle embryos use Ca from the eggshell and egg yolk for skeletal and carapace development (Simkiss, 1962; Sahoo et al., 1998). Thus, Ca metabolism likely plays a more significant role in reproductive physiology of turtles than in other reptiles. However, the relationship between Ca metabolism and reproduction in turtles has not been fully studied. Unravelling these relationships could help improve breeding of endangered turtles.

Using PNPA's facilities, here, we aimed to establish a captive breeding method for the hawksbill turtle by examining their reproductive behavior in captivity while simultaneously examining the physiological changes (including hormones and minerals) that lead to egg laying.

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TABLE 1. Body size and mass of turtles used in the experiment

No.	Sex	Straight carapace length (cm)	Straight carapace width (cm)	Body mass (kg)	Measurement date
Ei15	female	76.4	54.2	65.4	2003/4/3
Ei21	female	73.0	48.6	65.4	2003/4/3
Ei27	female	70.3	49.8	48.5	2002/11/13
Ei16	male	78.8	59.6	82.3	2003/4/3
Ei17	male	76.2	51.6	58.0	2003/4/3
Ei20	male	72.6	54.2	54.9	2003/4/3
Ei25	male	72.4	49.5	53.3	2003/4/12
Ei26	male	73.5	47.4	46.5	2003/4/12

MATERIALS AND METHODS

Animals

Hawksbill turtles housed in the PNPA were studied here. Hawksbill turtle reproduction was studied using three female (tag-numbers: Ei15, Ei21, Ei27) and five male (tag-numbers: Ei16, Ei17, Ei20, Ei25, Ei26) turtles. Size and weight of these turtles is shown in Table 1.

We ensured that the turtles were not traumatized by the PNPA veterinarians and staff during blood sampling. This study followed the ethical guidelines for animal exhibition and research issued by Japanese Association of Zoos and Aquariums, and was approved by the board of directors of the PNPA.

Experimental conditions

We observed the mating behavior of three females and five males housed together in a tank (Fig. 1). The tank is connected to a sandy beach, where the turtles can come ashore to lay eggs. The turtles were housed under conditions similar to natural environmental temperatures and daylight hours during all of experimental period (2002 to 2007). The tank water temperature was maintained to mimic the annual temperature variations as flows: (1) the low water temperature period from 5 January to 3 March 2003 was simulated by maintaining the tank water temperature at 22.0°C; and (2) the high water temperature period from 13 May to 10 June, and from 11 June to 9 August 2003 was simulated by maintaining that tank water temperature at 28.0 and 29.0°C, respectively. Further, the tank water temperature during the rest of the year was maintained to mimic the gradually decreasing natural temperature variations in the rest of 2003.

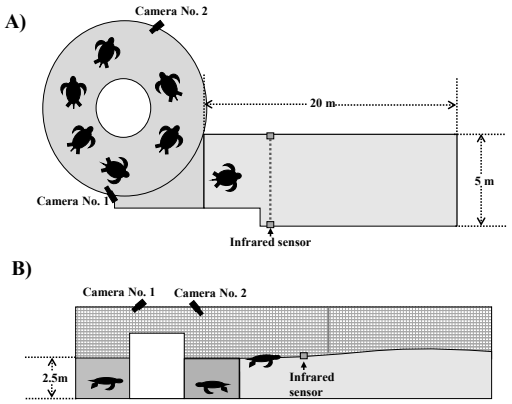

FIG. 1. Tank used in this study. (A) schematic representation; and (B) cross sectional views; The dark and light-gray areas indicate the tank and sandy beach, respectively. The tank is connected to the sandy beach, where the turtles can come ashore to lay eggs. Two high-sensitivity cameras were placed over the tank. Using these cameras, the behavior of turtles was recorded throughout the day (24 hours) using a time-lapse method at 12-second intervals. The coming ashore of turtles was observed using infrared sensors.

Two high-sensitivity cameras were installed in the tank at locations where they could cover certain areas from the top. The activity of turtles was recorded throughout the day (24 h)

through the entire period of the study, using the time-lapse method (where each frame was 12 sec long). This section of work was conducted for approximately nine months from 1 January to 5 October 2003. Subsequently, recordings of Camera No. 2 were played back at 12x speed, and their mating behavior was checked in detail and summarized in chronological order; the occurrences (start and termination times) of the mounting behavior and the tag-number of the individuals involved was recorded. During the other periods, only reproductive parameters were observed.

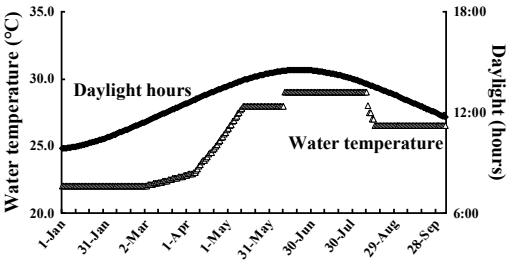

FIG. 2. Annual variations in water temperature and daylight hours in 2003. The turtles were maintained in the tank under conditions similar to natural environment temperatures and daylight hours during the entire experimental period.

Measurement of plasma hormones and minerals

Blood was sampled regularly once a month from the housed hawksbill turtles. Blood (10 ml) was collected from the jugular vein using a heparinized syringe (syringe with 18G needle, Terumo Corporation, Tokyo, Japan) to prevent clotting at around the same time of the day (between 13:00–15:00 h) every month. The blood plasma was immediately isolated using centrifugation (2,000 x g, 5 min) and stored frozen at –20°C. Subsequently, we measured plasma concentrations of hormones (E_2, P4, and T) and minerals (Ca and iP). The concentrations of E_2, P4, and T in the plasma samples were analyzed using chemiluminescent enzyme immunoassay kit by a vendor (Nagoya Rinsho Kensa Center, Nagoya, Japan). The detection limit of each hormone is 7.33 pg/ml for E_2, 0.1 ng/ml for P4 and 12.4 ng/ml for T, respectively. The plasma Ca and iP concentrations were measured using various kit (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) with Fuji Drychem-3000 (Kakizoe et al., 2013).

RESULTS

Behavioral analysis

The tank water temperature was maintained in the low water temperature period from January to March at 22.0°C and then in the high water temperature period from May to June at 28.0°C and from June 11 to August 9 at 29.0°C (Fig. 2). By controlling the temperature, we were able to induce reproductive behavior as follows. The results are shown in Fig. 3. The female Ei15 was tracked 41 times and mounted 41 times during the period, with the male Ei17 as its primary partner. The seasonal peak of mating behavior was in late March under the low temperature period. In particular, mating behavior by the male Ei17 was observed 15 times in March and 18 times in April, with a maximum of nine times per day. Thereafter, male Ei17 showed a smaller peak of tracking and mounting activity in late May (less frequent than in late March). Although the number and duration of being mounted during the period was higher for Ei15 female than those for other females, every mating event did not lead to successful copulation. For instance, video footage showed the refusal behavior, in which the Ei15 female blocked the insertion of the male genitalia with her hind legs (refusal position).

The female Ei21 was mated the highest number of times during the study period, totaling 769 times among the three individuals. Ei26 was the most frequent male partner, with a total of 571 mating events, followed by Ei16 with 96 mating events and Ei25 with 75 mating events, while Ei17 and Ei20 were less frequent, with 16 and 11 mating events, respectively. The seasonal peak of mating behavior was observed at the beginning of May, with a total of 218 mating events in May, with a daily maximum of 32 mating events. Thereafter, the

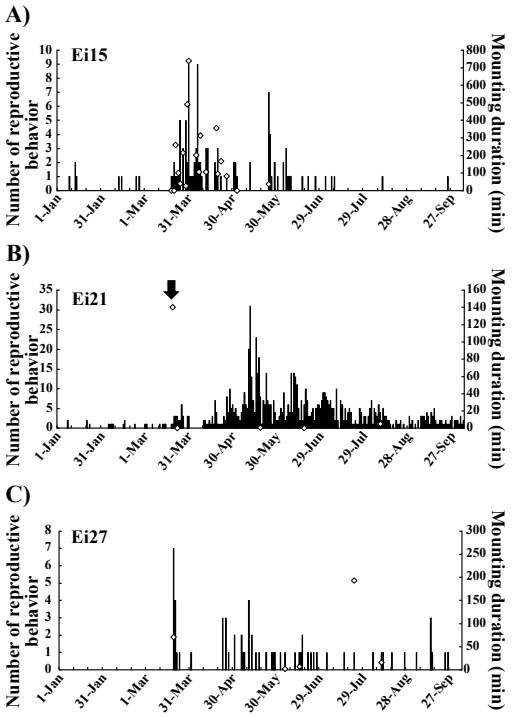

FIG. 3. Mating of three female hawksbill turtles (A: Ei15; B: Ei21; C: Ei27) in 2003. Video recorded by camera was used to count the number of mating behaviors of male and female hawksbill turtles. Mounting times are indicated by diamond shapes. Mounting in which mating was successful is indicated by arrow.

frequency declined slightly, but peaked regularly, about every other month. Although mating behavior was initiated often, mounting occurred only five times, with only one mounting activity between Ei21 female and Ei26 male, lasting for >30 min. This event occurred on 20 March 2003 under the low-temperature period with Ei26's mounting was of longer duration, lasting approximately 140 min, and genitalia insertion was visually confirmed. The most focused site of Ei21 being bitten by Ei26 was the "neck" (146 times).

The Ei27 female was observed being tracked at 55 times and mounted at five times during the study period. Some mountings lasted as long as 200 min. However, this individual was mated the least compared to the other two females.

Reproductive parameters of hawksbill turtles (Ei21) in the PNPA (2003–2007)

Table 2 shows the reproductive parameters of Ei21 from 2003 to 2007. In 2003, egg laying took place twice, with 59 days of the internesting interval. In the first nesting, 26 eggs were produced and did not hatch. However, in the next nesting, 90 eggs were produced, and the hatching success rate was 34.4%. In 2005, eggs were laid four times, with an internesting interval of 16 or 17 days. There were 112, 93, 154, and 154 eggs, and their hatching success rates were 0, 28, 29.9, and 22.1%, respectively. In 2007, eggs were laid twice, with an internesting interval of 28 days. There were 118 and 58 eggs laid, and their hatching success rates were 5.1 and 17.2%, respectively.

Changes in plasma T, E_2, and P4 levels

Male plasma T concentrations decreased from low to high water temperatures, then increased during the winter low water temperature period, peaking around March (Fig. 4).

The E_2 concentrations varied between the females. In 2002, E_2 concentrations increased, starting from the low water temperate period to the nesting season (Fig. 5). In 2004, the E_2 concentration began to increase in the Ei15 female at the beginning of the low temperature period, but remained low in the Ei21 and Ei27 females (Fig. 5).

The variation in P4 concentrations in the three females is shown in Fig. 6. All three individuals showed a change in P4 concentration from the low water temperature period to the end of the nesting season. In particular, a rapid surge-like increase in progesterone concentration (9 and 15 April 2003) was observed in Ei21, followed by egg laying on 10 May 2003. Thereafter, the second peak of plasma P4 in Ei21 was observed on June 14, which was followed by egg laying on 8 July 2003. The number of eggs laid on the 10 May and 8 July 2003 was 26 and 90, respectively.

In 2002, an increase in T concentrations in females was observed as the tank water temperature changed from that at the low water temperature period to that at the end of the

TABLE 2. Reproductive parameters of hawksbill turtle (Ei21) in the Port of Nagoya Public Aquarium (2003–2007)

Date of nesting	Order of nest within the same reproductive season	Internesting interval (days)	Eggs			Hatchlings			Hatching success rate (%)
			Number of egg	Axis (mm) mean±SD (range) n=10	Mass (g) mean±SD (range) n=30	Number of hatchling	SCL (mm) mean±SD (range)	BM (g) mean±SD (range)	
2003/5/10	1	—	26	36.2±1.7 (34.1–39.6)	22.5±0.9 (21.4–25.0)[a]	0	—	—	0
2003/7/8	2	59	90	33.7±0.7 (32.6–34.6)	20.8±1.2 (19.1–22.9)	31	39.1±1.2 (35.6–41.2)	11.6±0.9 (9.1–13.2)	34.4
2005/5/9	1	—	112	36.4±1.0 (34.1–38.3)	23.5±0.6 (21.6–24.8)	0	—	—	0
2005/5/26	2	17	93	35.4±0.6 (34.8–37.5)	23.2±0.8 (20.7–26.4)	26	39.8±1.4 (36.4–42.1)	13.0±1.0 (10.6–15.3)	28.0
2005/6/11	3	16	154	35.0±0.8 (33.9–38.3)	22.2±0.8 (16.5–24.1)	46	38.4±1.2 (36.1–41.0)	14.3±0.9 (12.7–16.3)	29.9
2005/6/27	4	16	154	33.3±1.1 (31.9–34.7)	20.7±1.3 (16.9–23.1)	34	37.3±1.8 (31.5–40.1)	12.6±1.3 (10.7–16.1)	22.1
2007/5/27	1	—	118	35.7±0.5 (34.0–37.5)	24.8±1.0 (22.5–27.5)	6	39.6±1.1 (38.1–40.3)	15.3±0.6 (14.6–16.2)	5.1
2007/6/24	2	28	58	35.5±0.4 (35.0–36.0)	25.1±0.8 (23.4–27.2)	10	40.8±0.8 (39.6–42.2)	15.2±0.9 (13.9–16.5)	17.2

[a]: n=26, SCL: Straight Carapace Length, BM: Body Mass.

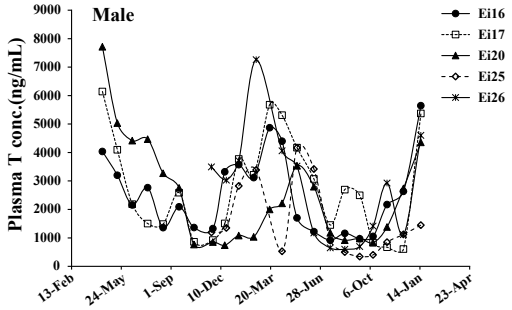

FIG. 4. Changes in plasma testosterone (T) concentration in the five male hawksbill turtles in 2002 to 2004.

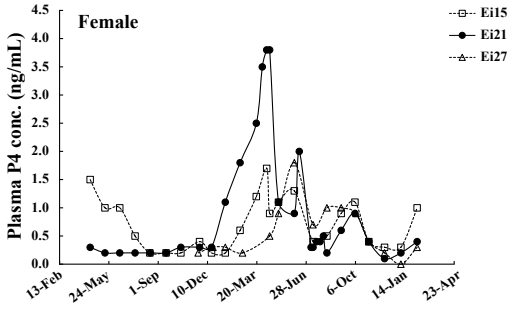

FIG. 6. Changes in plasma progesterone (P4) concentration in the three female hawksbill turtles in 2002 to 2004.

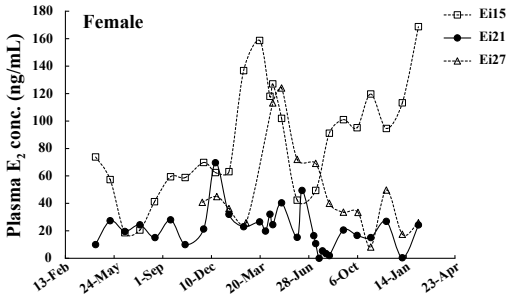

FIG. 5. Changes in plasma 17β-estradiol (E_2) concentration in the three female hawksbill turtles in 2002 to 2004.

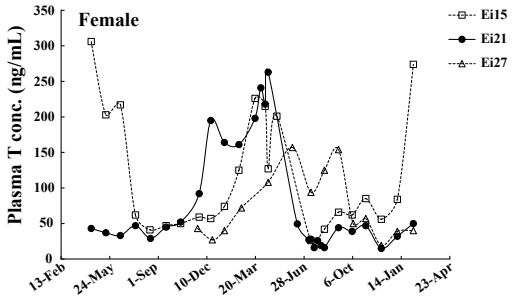

FIG. 7. Changes in plasma testosterone (T) concentration in the three female hawksbill turtles in 2002 to 2004.

nesting season (Fig. 7). In the winter of 2003, the concentrations of T in Ei15 female began to increase at low water temperatures but remained low in the Ei21 and Ei27 females (Fig. 7).

Noting the nesting in Ei21, changes in the plasma concentrations of T, P4, and E_2 are summarized in Fig. 8A. In addition, changes in plasma concentrations of T, P4 and E_2 from 2004 to 2005 are shown in Fig. 8B. In 2003, T, P4, and E_2 were highly variable and peaked at several times. In 2005, on the other hand, the major peaks of plasma T and P4 levels occurred only from April to May, when water temperature began to rise.

Changes in plasma Ca and iP levels

The changes in plasma Ca concentrations are shown in Fig. 9. Plasma Ca concentrations were almost constant throughout the year in males. In females, high plasma Ca concentrations were maintained from autumn until the end of the nesting season. Interestingly, the Ei21 female (she laid eggs) had markedly elevated plasma Ca concentrations on 15 January 2003. On the other hand, plasma iP levels in both male and female turtles were variable, but did not show periodicity (Fig. 10).

We focused on the spawning Ei21, and the changes in plasma Ca and iP concentrations are summarized in Fig. 11A. Changes in plasma Ca and iP concentrations from 2004 to 2005 are also shown in Fig. 11B. In 2003, plasma Ca concentrations had a pronounced peak, but no peak was observed in the iP. In 2005, on the other hand, plasma Ca concentra-

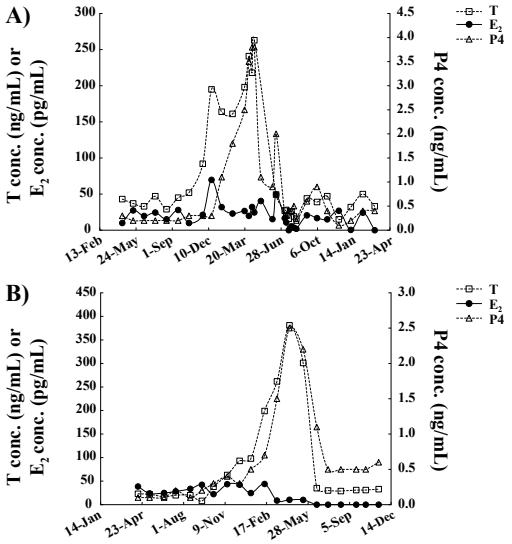

FIG. 8. Changes in the plasma concentrations of testosterone (T), progesterone (P4), and 17β-estradiol (E_2) in the spawning hawksbill turtle (Ei21) during 2002 to 2004 (A) and 2004 to 2005 (B).

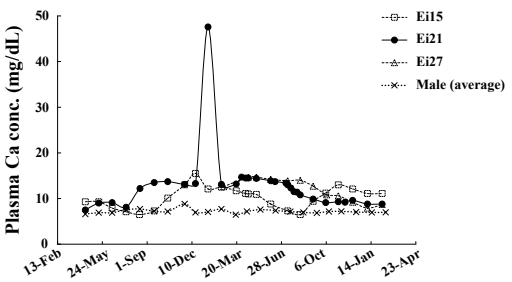

FIG. 9. Changes in plasma calcium (Ca) concentration in the three female and five male hawksbill turtles in 2002 to 2004. Male plasma Ca concentrations are shown as averages of five individuals in 2002 to 2004.

tions increased gradually and showed high values from October to February.

DISCUSSION

Here, we succeeded in the captive breeding of the endangered hawksbill turtle by mimicking natural environmental conditions (in terms

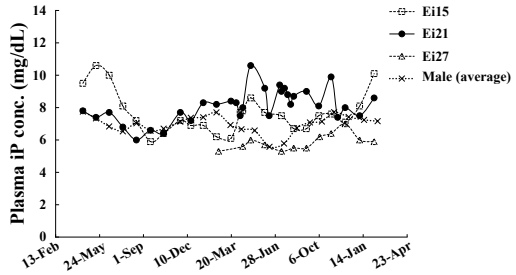

FIG. 10. Changes in plasma inorganic phosphorus (iP) concentration in the three female and five male hawksbill turtles in 2002 to 2004. Male plasma iP concentrations are shown as averages of five individuals in 2002 to 2004.

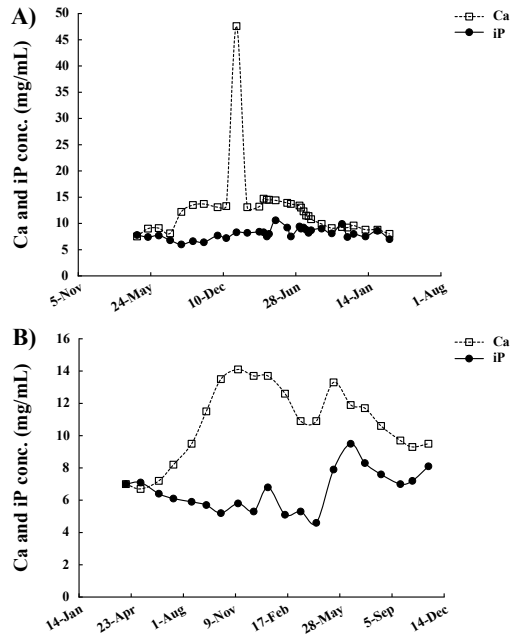

FIG. 11. Changes in the plasma calcium (Ca) and inorganic phosphorus (iP) concentrations in the spawning hawksbill turtle (Ei21) during 2002 to 2004 (A) and 2004 to 2005 (B).

of the water temperature and daylight hours) in an artificial rearing tank. Male plasma T levels peaked around March, encouraging male mating behavior, and mounting was observed. Mating with Ei26 male on 20 March 2003 allowed the Ei21 female to successfully lay

eggs twice, on 10 May and 8 July 2003, on the sandy beach. In 2005, egg laying also occurred four times, with internesting intervals of 16 or 17 days. In 2007, eggs were laid twice, with an internesting interval of 28 days. The internesting interval of nesting hawksbill turtles recorded in the wild was 11–28 days (Miller, 1997); thus, the internesting interval of our turtles is solidly within this range. Therefore, we can conclude that the experimental conditions created by us in terms of water temperature and daylight hours (regulated to mimic the natural environment) were suitable for the reproductive physiology of the hawksbill turtle.

Kobayashi (2012) reported that plasma T levels in hawksbill turtle reared in a tank on Ishigakijima Island (Okinawa, Japan) increased between March–May. Consistently, we found that male plasma T peaked in March, which possibly induced the male mating behavior. Plasma T and E_2 concentrations in females showed similar changes because E_2 is metabolized and biosynthesized from T (Shankey and Cohen, 2024), like in other turtles such as yellow-blotched map turtle, *Graptemys flavimaculata* (Shelby et al., 2000) and common snapping turtle, *Chelydra serpentina* (Mahmoud and Licht, 1997). On the other hand, on 3 May 2003, plasma E_2 levels were elevated in the Ei21 female. The phenomenon of elevated plasma E_2 concentrations prior to mating has been observed in mature female wild loggerhead turtle (Wibbels et al., 1990) and in Kemp's ridley turtle, *Lepidochelys kempii* under captive conditions (Rostal, 2004), consistent with the results of this study. Furthermore, P4, a hormone that rises after ovulation, was elevated in the present study. To the best of our knowledge, our study is the first observation of the annual cycle of P4 in captive nesting hawksbill turtles. P4 elevation observed here was similar to that seen in other turtles such as the loggerhead turtles (Guillette et al., 1991) and the green turtles (Al-Habsi et al., 2006).

A unique feature of this study is that we also focused on Ca metabolism. E_2 causes bone resorption, and E_2 administration increases iP

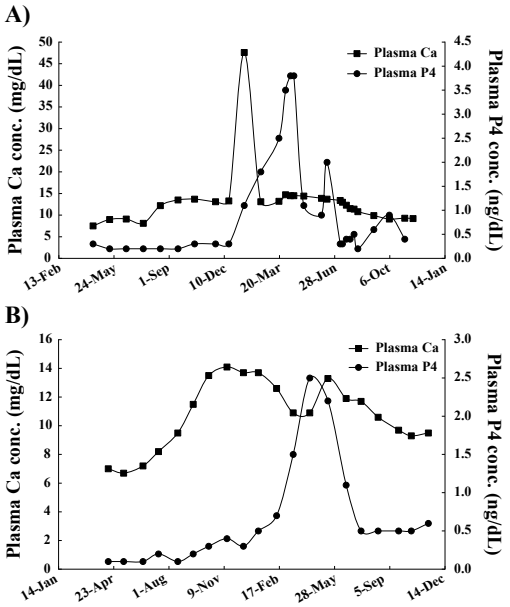

FIG. 12. Changes in plasma progesterone (P4) and calcium (Ca) concentration in the spawning hawksbill turtle (Ei21) during 2002 to 2004 (A) and 2004 to 2005 (B).

levels in the plasma along with Ca increase, in the three-toed box turtle, *Terrapene triunguis* (Magliola, 1984) and the red-eared slider, *Trachemys scripta elegans* (Sheehan et al., 1999). Here, the plasma Ca levels were markedly elevated in the nesting Ei21 female, but the iP levels were not. Thus, it is likely that the increase in plasma Ca concentration in Ei21 is not an E_2-induced effect. Moreover, Ca concentration increases in the uterus of female snapping turtles (*C. serpentina*) due to eggshell formation after ovulation (AlKindi et al., 2006). Indeed, we observed that subsequent to P4 rise, the plasma Ca concentration increased at the first time of egg laying in both 2003 and 2005 (Fig. 12). Particularly in 2003, we were able to detect a peak in plasma Ca levels. However, plasma Ca concentrations did not increase noticeably during the second P4 peak in 2003 (Fig. 12). The Ca concentration in the uterus may have lasted long, since there was no remarkable increase in plasma Ca before the second nesting. In 2005, the second Ca

peak (May to July) coincides with laying eggs. We surmise that Ca for the elevated plasma Ca levels may be sourced from other supply sources. We plan to investigate this hypothesis in more detail in the future.

The Ca metabolism related findings in this study are interesting. Therefore, we would like to focus on Ca metabolism in future studies, especially on the Ca-regulating hormone calcitonin. In fish, calcitonin is involved in reproductive physiology (Suzuki et al., 2004; Kuroda et al., 2023), and is elevated just before ovulation (Norberg et al., 1989). We have previously determined the structure of reptilian calcitonin and reported that its sequence is similar to that of fish (Suzuki et al., 1997). Further, we have developed an enzyme-linked immunosorbent assay (ELISA) to measure the plasma calcitonin level in fish (Suzuki et al., 2004; Kuroda et al., 2023). Preliminary experiments revealed that our ELISA can measure calcitonin in the blood of loggerhead turtle. In future, we would like to investigate the role of calcitonin in the reproductive physiology of the hawksbill turtles.

Here, plasma hormone levels were altered in male and female hawksbill turtles under captive conditions, leading to successful mating, and nesting. Further, the understanding of the reproductive biology of hawksbill turtles has improved due to data accumulating from other recent studies. Head-starting—in which hatchlings are raised in captivity for a certain period of time (few days to several years) before being released into the wild—has been attempted for green turtles, Kemp's ridley sea turtles, and hawksbill turtles, as a means of restoring sea turtle stocks (Sato and Madriasau, 2001; Fontaine and Shaver, 2005; Jualaong et al., 2021). We would like to continue our basic research for head-starting to increase the population of endangered sea turtles, including the hawksbill turtle, which are listed on the IUCN Red List.

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Literature Cited

Al-Habsi, A. A., AlKindi, A. Y. A., Mahmoud, I. Y., Owens, D. W., Khan, T., and Al-Abri, A. 2006. Plasma hormone levels in the green turtles *Chelonia mydas* during peak period of nesting at Ras Al-Hadd-Oman. *Journal of Endocrinology* 191: 9–14.

AlKindi, A. Y. A., Mahmoud, I. Y., Woller, M. J., and Plude, J. L. 2006. Oviductal morphology in relation to hormonal levels in the snapping turtle, *Chelydra serpentina*. *Tissue and Cell* 38: 19–33.

Currylow, A. F., Tift, M. S., Meyer, J. L., Crocker, D. E., and Williams, R. N. 2013. Seasonal variations in plasma vitellogenin and sex steroids in male and female eastern box turtles, *Terrapene carolina carolina*. *General and Comparative Endocrinology* 180: 48–55.

Fontaine, C. and Shaver, D. 2005. Head-starting the Kemp's ridley sea turtle, *Lepidochelys kempii*, at the NMFS Galveston laboratory, 1978–1992: a review. *Chelonian Conservation and Biology* 4: 838–845.

Guillette, L. J. Jr, Bjorndal, K. A., Bolten, A. B., Gross, T. S., Palmer, B. D., Witherington, B. E., and Matter, J. M. 1991. Plasma estradiol-17β, progesterone, prostaglandin F, and prostaglandin E_2 concentrations during natural oviposition in the loggerhead turtle (*Caretta caretta*). *General and Comparative Endocrinology* 82: 121–130.

Heck, J., MacKenzie, D. S., Rostal, D., Medler, K., and Owens, D. 1997. Estrogen induction of plasma vitellogenin in the Kemp's ridley sea turtle (*Lepidochelys kempi*). *General and Comparative Endocrinology* 107: 280–288.

Jackson, D. C. 1997. Lactate accumulation in the shell of the turtle *Chrysemys picta bellii* during anoxia at 3°C and 10°C. *Journal of Experimental Biology* 200: 2295–2300.

Jualaong, S., Kanghae, H., Thongprajukaew, K., Saekhow, S., Amartiratana, N., and Sotong, P. 2021. Optimal feeding frequency for captive hawksbill sea turtle (*Eretmochelys imbri-*

cata). *Animals* 11: 1252.

Kakizoe, Y., Sakaoka, K., Akune, Y., Kanou, Y., Saito, T., and Uchida, I. 2013. Change of plasma chemistry values in captive breeding loggerhead turtles (*Caretta caretta*). *ISRN Zoology*: Article ID 514576.

Kawazu, I., Kino, M., Maeda, K., and Teruya, H. 2015. Age and body size of captive hawksbill turtles at the onset of follicular development. *Zoo Biology* 34: 178–182.

Kawazu, I., Kino, M., Maeda, K., Yamaguchi, Y., and Sawamukai, Y. 2014. Induction of oviposition by the administration of oxytocin in hawksbill turtles. *Zoological Science* 31: 831–835.

Kobayashi, M. 2012. Ecological, behavioral, and physiological evidence of the hawksbill turtle reproduction in captivity. *Bulletin of Fisheries Research Agency* 36: 107–142.

Krohmer, R. W., Grassman, M., and Crews, D. 1987. Annual reproductive cycle in the male red-sided garter snake, *Thamnophis sirtalis parietalis*: field and laboratory studies. *General and Comparative Endocrinology* 68: 64–75.

Kuroda, K., Hatano, K., Kawamura, R., Fukushima, A., Sasayama, Y., Tabuchi, Y., Furusawa, Y., Ikegame, M., Hattori, A., Hirayama, J., Fukuda, T., Uekura, S., Matsubara, H., Kawago, U., Sekiguchi, T., Srivastav, A. K., and Suzuki, N. 2023. Possible involvement of Calcitonin I and II in calcium metabolism of the female reproductive physiology of goldfish (*Carassius auratus*). *International Aquatic Research* 15: 15–26.

Magliola, L. 1984. The effects of estrogen on skeletal calcium metabolism and on plasma parameters of vitellogenesis in the male, three-toed box turtle (*Terrapene carolina triunguis*). *General and Comparative Endocrinology* 54: 162–170.

Mahmoud, I. Y. and Licht, P. 1997. Seasonal changes in gonadal activity and the effects of stress on reproductive hormones in the common snapping turtle, *Chelydra serpentina*. *General and Comparative Endocrinology* 107: 359–372.

Miller, J. D. 1997. Reproduction in Sea Turtles. p. 51–81. *In*: P. L. Lutz and J. A. Musick (eds.), *The Biology of Sea Turtles*. CRC Press, Boca Raton.

Mortimer, J. A. and Donnelly, M. (IUCN SSC Marine Turtle Specialist Group). 2008. *Eretmochelys imbricata*. The IUCN Red List of Threatened Species 2008: e.T8005A12881238. https://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T8005A12881238.en (accessed 25 October 2024)

Norberg, B., Björnsson, B. Th., Brown, C. L., Wichardt, U.-P., Deftos, L. J., and Haux, C. 1989. Changes in plasma vitellogenin, sex steroids, calcitonin, and thyroid hormones related to sexual maturation in female brown trout (*Salmo trutta*). *General and Comparative Endocrinology* 75: 316–326.

Rostal, D. C. 2004. Seasonal reproductive biology of the Kemp's ridley sea turtle (*Lepidochelys kempii*): comparison of captive and wild population. *Chelonian Conservation and Biology* 4: 786–798.

Rostal, D. C., Grumbles, J. S., Palmer, K. S., Lance, V. A., Spotila, J. R., and Paladino, F. V. 2001. Changes in gonadal and adrenal steroid levels in the leatherback sea turtle (*Dermochelys coriacea*) during the nesting cycle. *General and Comparative Endocrinology* 122: 139–147.

Sacchi, R., Scali, S., Mangiacotti, M., Sannolo, M., Zuffi, M. A. L., Pupin, F., Gentilli, A., and Bonnet, X. 2017. Seasonal variations of plasma testosterone among colour-morph common wall lizards (*Podarcis muralis*). *General and Comparative Endocrinology* 240: 114–120.

Sahoo, G., Sahoo, R. K., and Mohanty-Hejmadi, P. 1998. Calcium metabolism in olive ridley turtle eggs during embryonic development. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology* 121: 91–97.

Sato, F. and Madriasau, B. B. 2001. Preliminary report on natural reproduction of hawksbill sea turtle in Palau. *Marine Turtle Newsletter* 55: 12–14.

Sereau, M., Lagarde, F., Bonnet, X., El Mouden, El H., Slimani, T., Dubroca, L., Trouvé, C., Dano, S., and Lacroix, A. 2010. Does testosterone influence activity budget in the male Greek tortoise (*Testudo graeca graeca*)? *General and Comparative Endocrinology* 167: 181–189.

Shankey, N. T. and Cohen, R. E. 2024. Neural control of reproduction in reptiles. *Journal of*

Experimental Zoology Part A: Ecological and Integrative Physiology 341: 307–321.

Sheehan, D. M., Willingham, E., Gaylor, D., Bergeron, J. M., and Crews, D. 1999. No threshold dose for estradiol-induced sex reversal of turtle embryos: how little is too much? *Environmental Health Perspectives* 107: 155–159.

Shelby, J. A., Mendonça, M. T., Horne, B. D., and Seigel, R. A. 2000. Seasonal variation in reproductive steroids of male and female yellow-blotched map turtles, *Graptemys flavimaculata*. *General and Comparative Endocrinology* 119: 43–51.

Simkiss, K. 1962. The sources of calcium for the ossification of the embryos of the giant leathery turtle. *Comparative Biochemistry and Physiology* 7: 71–79.

Suzuki, N., Eguchi, C., Hirai, T., and Sasayama, Y. 1997. Nucleotide sequences of reptile calcitonins: their high homology to chicken calcitonin. *Zoological Science* 14: 833–836.

Suzuki, N., Yamamoto, K., Sasayama, Y., Suzuki, T., Kurokawa, T., Kambegawa, A., Srivastav, A. K., Hayashi, S., and Kikuyama, S. 2004. Possible direct induction by estrogen of calcitonin secretion from ultimobranchial cells in the goldfish. *General and Comparative Endocrinology* 138: 121–127.

Thompson, D. M., Ligon, D. B., Edmond, B. S., Spangler, S. J., Fillmore, B. M., Graves, K. G., and Lovern, M. 2023. Reproductive cycles of alligator snapping turtles (*Macrochelys temminckii*). *General and Comparative Endocrinology* 341: 114310.

Tinsley, D. 1985. A comparison of plasma levels of phosphoprotein, total protein and total calcium as indirect indices of exogenous vitellogenesis in the Crucian carp, *Carassius carassius* (L.). *Comparative Biochemistry and Physiology Part B: Biochemical and Molecular Biology* 80: 913–916.

Uchida, I. and Nishiwaki, M. 1982. Sea turtles in the waters adjacent to Japan. p. 317–319. *In*: K. Bjorndal (ed.), *Biology and Conservation of Sea Turtles*. Smithsonian Institution Press, Washington D. C.

Warburton, S. J. and Jackson, D. C. 1995. Turtle (*Chrysemys picta bellii*) shell mineral content is altered by exposure to prolonged anoxia. *Physiological Zoology* 68: 783–798.

Wibbels, T., Owens, D. W., Limpus, C. J., Reed, P. C., and Amoss, M. S. 1990. Seasonal changes in serum gonadal steroids associated with migration, mating, and nesting in the loggerhead sea turtle (*Caretta caretta*). *General and Comparative Endocrinology* 79: 154–164.

Zena, L. A., Dillon, D., Hunt, K. E., Navas, C. A., Bícego, K. C., and Buck, C. L. 2019. Seasonal changes in plasma concentrations of the thyroid, glucocorticoid and reproductive hormones in the tegu lizard *Salvator merianae*. *General and Comparative Endocrinology* 273: 134–143.

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