Nature Conservation

Nature Conservation 58: 165–181 (2025)
DOI: 10.3897/natureconservation.58.143706

Research Article

Artificial breeding of the hawksbill turtle, *Eretmochelys imbricata*, in a captive facility in the Republic of Korea

Eunvit Cho[1,2], Dae-Yeon Moon[3], Il-Hun Kim[4], Donjin Han[1], Ki-Young Lee[2], Il-Kook Park[4]

1 *Aqua Planet Yeosu, Yeosu 59744, Republic of Korea*
2 *Department of Marine Biology, Kunsan National University, Gunsan 54150, Republic of Korea*
3 *Ocean & Fish Research, Busan 46262, Republic of Korea*
4 *Marine Biodiversity Division, National Marine Biodiversity Institute of Korea, Seocheon 33662, Republic of Korea*
Corresponding author: Il-Kook Park (ileveli2@naver.com)

Abstract

Captive breeding has been conducted across various regions to restore globally endangered sea turtle populations. However, understanding how turtles adapt to artificial breeding environments that differ considerably from their natural habitats remains underexplored. This study focused on the artificial breeding of two male and two female hawksbill turtles, *Eretmochelys imbricata*, in a controlled facility in the Republic of Korea, isolated from the outdoor environment and located far north of their natural nesting grounds. The main objectives were to document the entire breeding process while identifying limitations in the breeding methods and suggesting improvements. During the study period, the hawksbill turtles laid 864 eggs across 10 clutches, resulting in 83 hatchlings. The mean fertilization rate was 33%, whereas hatching success rates were 10% and 32% for the number of total laid eggs and fertile eggs, respectively. A female turtle laid eggs two-and-a-half years after post-mating, demonstrating that hawksbill turtles can utilize long-term sperm storage for laying eggs in subsequent breeding seasons without additional mating. Enhancing captive breeding methods is crucial for improving the efficiency of sea turtle population recovery and expanding our understanding of their ecological characteristics.

Key words: Captive breeding, growth rate, hatching success, population recovery, sperm storage

Academic editor: Md Mizanur Rahman
Received: 6 December 2024
Accepted: 3 March 2025
Published: 20 March 2025

ZooBank: https://zoobank.org/9DB1CC10-634A-4900-80C5-D2FDB6FF66C6

Citation: Cho E, Moon D-Y, Kim I-H, Han D, Lee K-Y, Park I-K (2025) Artificial breeding of the hawksbill turtle, *Eretmochelys imbricata*, in a captive facility in the Republic of Korea. Nature Conservation 58: 165–181. https://doi.org/10.3897/natureconservation.58.143706

Introduction

Given the global threats that sea turtles face, their sustainable breeding and reproduction are critical for their conservation and recovery. Persistent breeding and nesting disturbances in sea turtles include the loss of nesting ground because of coastal development (Zhang et al. 2023) and rising sea levels (Fuentes et al. 2011), feminization resulting from temperature-dependent sex determination linked to increased beach temperatures (Hays et al. 2023), and predation of eggs by invasive predators (Stokes et al. 2024). Multiple initiatives are being implemented to mitigate these issues and conserve sea turtle nesting, which include establishing coastal development mitigation policies (Lopez et al. 2015), providing shade on nesting beaches (Hill et al. 2015), and safeguarding against terrestrial predator depredation (O'Connor et al. 2017). As a result of these efforts, the nesting abundance of sea turtles has recovered in 12 of 17 regional management units (Mazaris et al. 2017).

In addition to coastal management, captive breeding plays a crucial role in preventing external threats, controlling breeding and hatching environments, and facilitating human involvement in recovering various sea turtle species. This approach significantly contributes to the recovery of sea turtle populations (Kobayashi 2012; Owens and Blanvillain 2013; Hart et al. 2014; Cho et al. 2022). Hatched sea turtles are sometimes released into the ocean immediately after hatching, while some hatchlings go through a head-starting process to return to the sea after they grow larger for about one year in captivity to increase their survivorship. Research confirms that artificially hatched sea turtles effectively adapt to natural habitats post-release, thereby aiding in the recovery of sea turtle populations (Bell et al. 2005; Fontaine and Shaver 2005; Kim et al. 2022).

The hawksbill turtle, *Eretmochelys imbricata*, is classified as "Critically Endangered" by the International Union for Conservation of Nature (IUCN), highlighting its status as one of the most threatened sea turtle species (Mortimer and Donnelly 2008). This species is the most tropical of all sea turtle species (Márquez 1990). Hence, in the northwestern Pacific, hawksbill turtle occurrences have been recorded even in southern Russia, while Ishigaki Island (24.39°N, 124.18°E), located in the southernmost area of Japan, is known as the northern limit of hawksbill turtle nesting (Shimizu et al. 2006; Okuyama et al. 2020). The Republic of Korea marks the northern limit of the distribution range of hawksbill turtles, with a small number of turtles sometimes observed near Jeju Island (Kim et al. 2017, 2020; Jang et al. 2024). Hawksbill turtles in Korean waters are supposed to have originated from Southeast Asia or Northern Australia, as no nesting sites have been observed on Korean coasts (Kim et al. 2020; Jang et al. 2024). In Japan, captive breeding facilities for hawksbill turtles are located near natural nesting grounds, allowing for successful breeding in outdoor enclosures (Shimizu et al. 2006; Kobayashi et al. 2006; Kobayashi 2012; Kawazu et al. 2018). In contrast to the method mainly used in Japan, the captive breeding of hawksbill turtles was performed in a fully enclosed facility devoid of outdoor environmental exposure in this study.

To the best of our knowledge, this study is the first attempt at captive breeding hawksbill turtles in a climate different from that of their natural nesting grounds. Here, we aimed to artificially breed hawksbill turtles in captivity and meticulously document the entire process, including adult rearing, mating, nesting, hatching, and first-year growth of hatchlings. Additionally, we aimed to identify the limitations of the breeding methods used in this study and propose areas for improvement. Disseminating our captive breeding methods and outcomes is anticipated to facilitate the successful restoration of sea turtle populations.

Methods

Breeding individuals and conditions

For the artificial breeding of hawksbill turtles, we used two males (H005 and H006) and two females (H011 and H019) at Aqua Planet Yeosu, Republic of Korea (34.7464°N, 127.7485°E). These four turtles were introduced for exhibition and research purposes from Underwater World Singapore (1.2581°N, 103.8114°E) on August 3, 2016. An International Endangered Species Import Permit (EK2016-00065) was obtained from the Yeongsan River Basin Environmental Office of the Republic of Korea. We assessed the physical characteristics of adult hawksbill turtles involved in artificial breeding by measuring the straight carapace length (SCL; 0.1 cm), straight carapace width (SCW; 0.1 cm), and body mass (BM; 0.1 kg). We measured SCL and SCW using Vernier calipers (The Mantax Blue, Haglof Inc., Långsele, Sweden) and measured BM using an electronic scale (NewFJ-500KLi, AND Inc., Seoul, Republic of Korea) (Table 1). Given that hawksbill turtles with SCLs greater than 60 cm are generally considered mature (Beggs et al. 2007; Avens et al. 2021), we judged all four individuals to be mature.

Adult turtles were separately housed in 15-t concrete water tanks (300 cm wide × 350 cm long × 145 cm deep; the same order hereafter), each completely isolated by concrete walls. Tank water conditions were maintained at a temperature of 25–26 °C, pH of 7.5–8, and salinity of 30‰. The photoperiod was regulated to provide light from 8:00 to 20:00 Korean time and darkness from 20:00 to the next day 08:00 the following day, consistently throughout the year. The conditions in all tanks were identical. We administered Japanese horse mackerel (*Trachurus japonicus*), Japanese flying squid (*Todarodes pacificus*), and whiteleg shrimp (*Litopenaeus vannamei*) biweekly at 3% of the turtle body mass, along with a nutritional supplement (MAZURI-5B48, Mazuri Co., Ltd., St. Louis, USA). Although adult hawksbill turtles are known to be omnivorous, they primarily feed on seaweeds and sponges (Meylan 1988; Clyde-Brockway et al. 2022). Nevertheless, we provided them with animal diets because of their pronounced preference for it and to mitigate substantial tank contamination by uneaten seaweeds.

Table 1. Morphometric measurements of the adult hawksbill turtles for artificial breeding.

ID	Sex	Straight carapace length (cm)	Straight carapace width (cm)	Body mass (kg)
H005	Male	83.0	66.1	78.1
H006	Male	67.6	46.1	60.6
H011	Female	82.2	54.1	90.0
H019	Female	80.8	59.5	87.4

Mating method

To determine suitable mating periods in relation to fertility, we conducted monthly blood tests on the turtles, following protocols by Kobayashi (2012) and Kawazu et al. (2015). First, we extracted 5 ml of blood from the jugular vein using a 5-ml syringe equipped with a 38 mm long and 20-gauge diameter needle once a month. We measured testosterone levels in males and triglyceride levels in females from the blood samples (Owens and Morris 1985; Kobayashi 2012). Testosterone, a representative male sex hormone, is secreted at high levels during the breeding season, while high triglyceride levels are directly correlated with the follicle size of sea turtles, serving as suitable parameters for determining mating periods (Hamann et al. 2002; Kawazu et al. 2015). Some studies have used the female sex hormone estradiol-17β to assess yolk development (Dobbs et al. 2007; Kobayashi 2012); however, in this study, the annual variation in estradiol-17β levels was minimal and inadequate for determining the optimal mating period.

Based on the blood test results, we considered males suitable for mating when their testosterone levels exceeded 20 ng/ml (Kobayashi 2012). Simultaneously, we conducted an additional ultrasound examination for females whose triglyceride levels were over 2,000 ng/ml using the Hitachi Aloka Noblus ultrasound machine (Hitachi, Tokyo, Japan) and a 5-1 MHz probe (Hitachi Aloka Medical Ltd., Tokyo, Japan). Based on the ultrasound results, we deemed females fit for mating when the largest yolk length exceeded 32 mm (Kakizoe et al. 2013). However, we mainly considered conditions of female hawksbill turtles rather than males for mating because males maintained testosterone levels above 20 ng/ml for most of the study period. Upon reaching the breeding condition, we placed one male and one female turtle together in a 115-t concrete water tank (7.5 m × 8.5 m × 2 m) designated for mating. Adjacent to this tank, an artificial sandy beach (3.0 m × 4.0 m × 1.3 m) composed of 1 mm quartz sand was established as an artificial nesting site with a ramp measuring 2 m wide, 1.5 m long, and inclined at 22° for landing (Fig. 1A, B). Turtle sampling and breeding were performed with permission from the Animal Experiment Ethics Committee of the National Marine Biodiversity Institute of Korea (MAB-23-02).

We defined a mating attempt as the act of a male climbing onto the back of a female and grabbing her with its fore and hind fins, whereas mating was

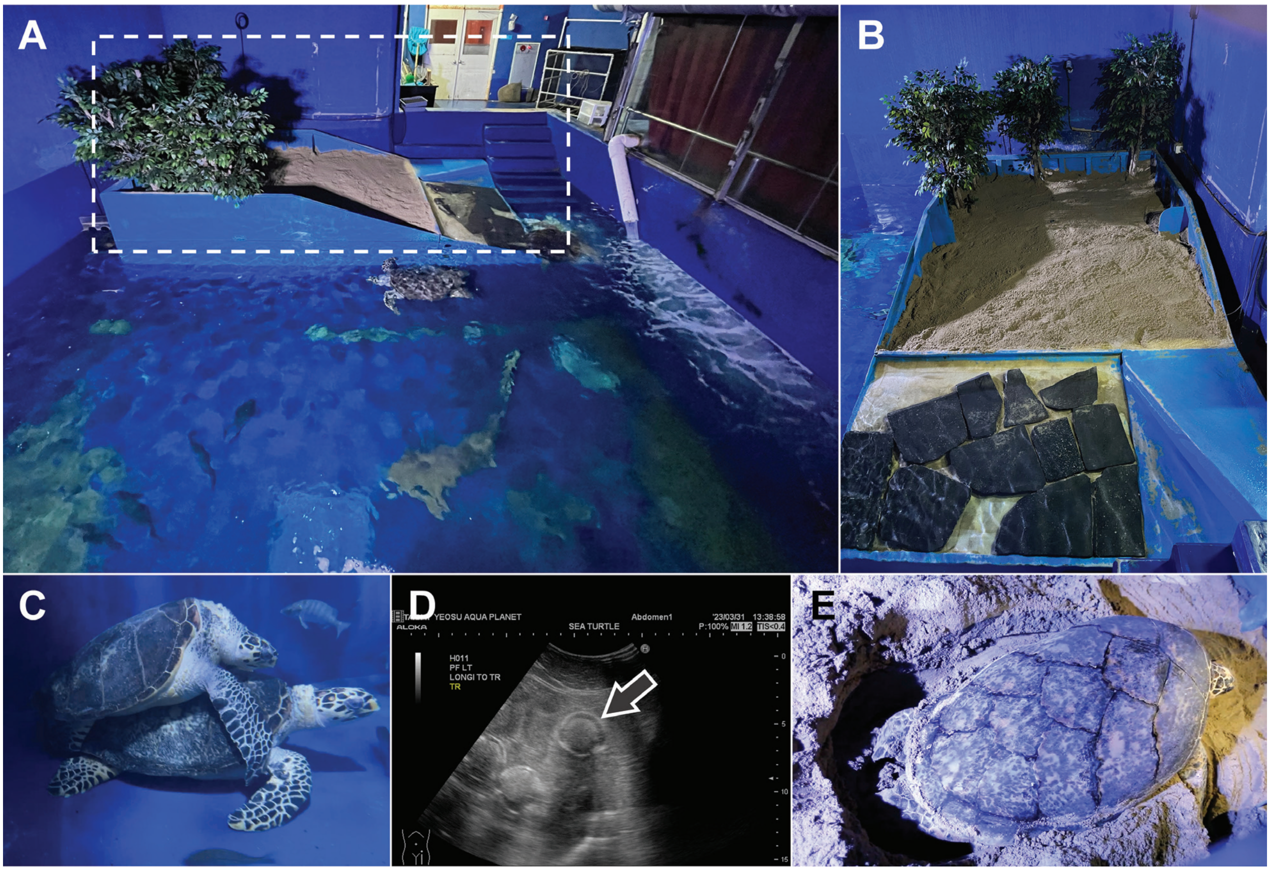

Figure 1. The mating tank with an artificial sandy beach and the nesting process of hawksbill turtles **A** side (dotted zone) and **B** front views of the artificial sandy beach **C** male and female hawksbill turtles mating in the tank, and **D** developed eggs, indicated by an arrow, inside the female, confirmed by ultrasound examination **E** gravid female lays eggs on the artificial sandy beach.

defined as the insertion of the penis (Fig. 1C). If no mating attempts occurred during the one-week pairing period, the turtles were isolated for one week in their individual tanks and subsequently paired again for another week. If no mating attempts occurred during three consecutive pairing periods, we terminated pairing for that year. If mating or mating attempts were observed, we left the turtles in the mating tank for more than two weeks. The male was returned to its original tank two weeks after mating, while the female remained in the mating tank. Because eggshell formation occurred approximately one month after mating (Kobayashi et al. 2006), we conducted an ultrasound examination of the female 30 days post-mating. If the ultrasound results confirmed eggshell development, we retained the female in the mating tank; otherwise, we returned it to its original tank (Fig. 1D). The breeding cycle of female hawksbill turtles spans 2–3 years (Beggs et al. 2007; Santos et al. 2013). The cycles of the two females, H011 and H019, did not overlap; therefore, the females were not switched in the captive tanks within the same year. We monitored mating and egg-laying behavior using closed-circuit television (DC-T1642WRX, IDIS Ltd., Daejeon, Republic of Korea).

Artificial hatching

Upon confirming oviposition in the artificial nesting site (Fig. 1E), we carefully excavated the sand and manually removed the eggs to prevent any damage (Fig. 2A). Because of insufficient temperature and humidity control in the artificial nesting site and restricted egg-laying conditions, we transferred the eggs to incubators within 12 h post-laying to minimize the impact on hatching (Parmenter 1980). We counted the eggs and excluded small eggs with diameters less than 20 mm as undeveloped eggs, given that healthy egg sizes ranged from 34 to 38 cm (Hitchins et al. 2004; Hesni et al. 2016). The eggs were wrapped in sphagnum moss in an incubator (Rcom MARU 200, AutoElex Co., Ltd., Gimhae, Republic of Korea) and maintained at a constant temperature and humidity (Fig. 2B). The incubator temperature was set at 29 ± 1 °C with humidity at 95 ± 5%, given that the hatching success rate of the hawksbill turtle was optimal at an incubator temperature of 29.5 °C and a relative sand humidity of 100% (Flores-Aguirre et al. 2020, 2023). Also, eggs of the hawksbill turtle, which had characteristics of temperature-dependent sex determination, are known to develop with equal sexes at about 29 °C (Godfrey and Mrosovsky 1994).

We screened the condition of the eggs at two-week intervals and promptly discarded any rotten eggs to prevent contamination of the surrounding eggs. The fertilization status of an egg could be confirmed after approximately one month of development. Consequently, four weeks post-laying, during the second screening, we defined eggs with visible blood vessels as fertilized by illuminating them with an egg candler (Candler 200, AutoElex, Gimhae, Republic of Korea). In addition, we separated the unfertilized eggs, which were prone to rotting, from the confirmed fertilized eggs. We calculated the fertilization rate based on the proportion of the number of fertile eggs confirmed in the second screening and the number of total eggs laid. Although this method may underestimate actual fertilization rates by overlooking fertile eggs that rotted before

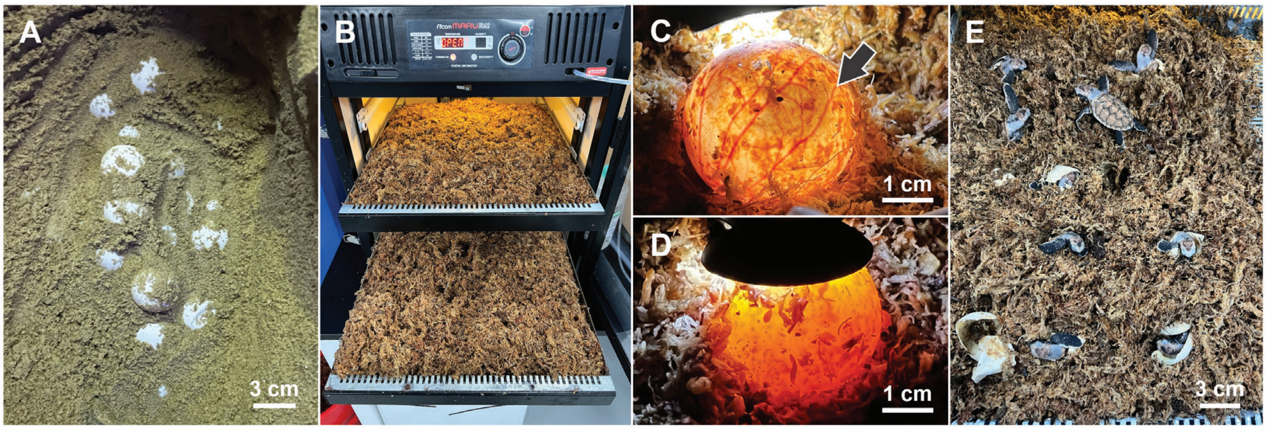

Figure 2. Management of artificially bred eggs of hawksbill turtles **A** eggs laid on an artificial sandy beach were dug out, wrapped in sphagnum moss, and managed in **B** an artificial incubator. Four weeks after laying eggs, the candling results indicate that **C** a fertilized egg displays veins, indicated by an arrow, whereas **D** an unfertilized egg lacks such features **E** hatchlings of the hawksbill turtle hatched in an incubator.

the second screening, we judged the omission rate to be minimal, given that unfertile eggs are much more likely to rot (Phillott and Parmenter 2001; Sarmiento-Ramirez et al. 2014). We also calculated the hatching success based on the number of hatched and fertile eggs confirmed during the second screening.

Growth pattern of hatchlings

Newly hatched hawksbill turtles were left in the incubator for 3–7 days to allow complete yolk absorption. Hatchlings with complete yolk absorption were transferred to an individual water tank (100 cm × 125 cm × 80 cm). The breeding environment was maintained similarly to that used for the adults, except for the tank size. Hatchlings were fed twice daily at 10:00 and 15:00. We administered a pre-starter feed with a particle size of 3 mm (New Deluxe No. 4, DaehanFeed Co., TD., Incheon, Republic of Korea) at 10:00 and 15% of turtle body mass of chopped Japanese horse mackerel and North Pacific krill (*Euphausia superba*) with a sea turtle-specific nutritional supplement (5B48; Mazuri, St. Louis, MO, USA) at 15:00.

The physical characteristics of each hatchling were recorded monthly. We only provided the one-year data because several hatchlings were released into the ocean or relocated to other aquariums after one year of hatching. We measured SCL and SCW using digital calipers (ISO-9000, Mitutoyo Corp., Tokyo, Japan) to an accuracy of 0.1 mm, whereas BM was measured using a digital scale (SW-1S, CAS, Yangju, Republic of Korea) to an accuracy of 0.1 g. To investigate the growth pattern of hatchling hawksbill turtles for one year, we conducted a second-order polynomial fit to SCL, SCW, and BM (Stimson et al. 1978).

$$Y = \beta_0 + \beta_1 X + \beta_2 X^2$$

Where Y is the dependent variable representing SCL, SCW, and BM in this study; X is the independent variable, indicating the number of days after turtle hatching; β_0, β_1, and β_2 are the least squares coefficients. We calculated the polynomial fits and created graphs using the *ggplot2* package (Wickham 2016) in R software version 4.3.3 (R Core Team 2024).

Results

Breeding and hatching

During the study period, one male (H005) and two females successfully mated, whereas the male H006 did not mate. H011 mated in July 2019 (the exact date was not recorded), and on January 21, 2023, whereas H019 mated on July 18, 2018. For 2020 and 2022, we refrained from pairing females with males because of their underdeveloped yolks. Both males and females that mated once showed no further interest in mating in the same year, and mating did not occur more than twice per year (Fig. 3).

Female H011 laid eggs twice in 2019 and five times in 2023, whereas female H019 laid eggs once in 2018 and three times in 2021 (Table 2). On January 12, 2021, having not mated since 2018, H019 laid eggs in the water within its original tank, devoid of the artificial sandy beach. Despite confirming 15 eggs underwater, these were excluded from the results because of the potential for fish in the tank to consume the other eggs. After observing the eggs laid in the water, H019 was transferred to the mating tank, featuring an artificial sandy beach, without introducing males. In 2018, H019 laid eggs twice on the artificial sand beach.

Females laid a total of 864 eggs across 10 clutches, excluding underwater laying, yielding a mean of 86.4 ± 35.1 standard deviation (unless otherwise specified), and 216.0 ± 169.6 per year was laid per clutch (Table 2). In cases where females laid eggs multiple times a year, the mean interval between laying dates was 28.2 ± 12.6 days, ranging from 15 to 49 days (n = 6). The number of fertile eggs was 319, with a mean fertilization rate of 32.7 ± 28.9%. The mean egg diameter was 34.8 ± 0.5 mm, and the mean mass was 26.1 ± 0.5 g.

A total of 83 hawksbill turtles hatched, with a mean of 10.4 ± 8.6 turtles per clutch. The mean incubation period was 57.9 ± 2.9, ranging from 53 to 61 days. The mean hatching success rates were 9.9 ± 10.5% and 32.3 ± 19.2% for the total number of eggs and fertile eggs, respectively.

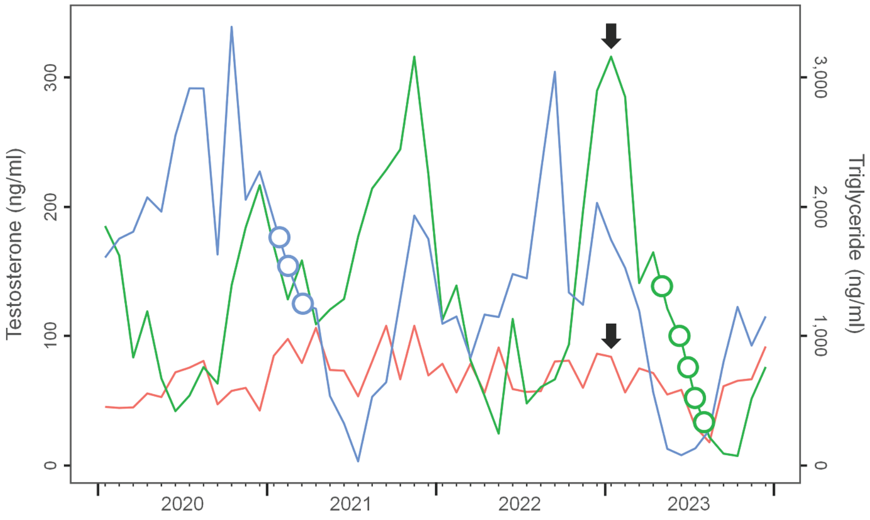

Figure 3. Change in testosterone and triglyceride levels of hawksbill turtles. The testosterone value matches the red line (male H005), while the triglyceride value matches the green (female H011) and blue (female H019) lines. Arrows and circles indicate mating and laying dates, respectively. The records for the initial years of the study, 2018 and 2019, were poorly documented and, as a result, were not included.

Table 2. Breeding information of two female hawksbill turtles laid eggs and first-year mortality of the hatchlings. The data of eggs laid in water by H019 on Jan 1, 2021 was not included.

No.	Laying date	Number of eggs laid	Fertile eggs (Fertilization rate)	Incubation period (d)	Number of Hatchlings	Hatching success (%)	One-year mortality
H011							
1	6 Sep 2019	68	37 (54.4%)	55	14	37.8	1 (7.1%)
2	7 Oct 2019	48	19 (39.6%)	58	6	31.6	3 (50.0%)
3	22 Apr 2023	71	0 (0%)	–	–	–	–
4	28 May 2023	116	2 (1.7%)	61	1	50.0	0 (0%)
5	14 Jun 2023	118	27 (22.9%)	58	4	14.8	0 (0%)
6	29 Jun 2023	101	39 (38.6%)	57	12	30.8	2 (16.7%)
7	21 Jul 2023	63	30 (47.6%)	53	20	66.7	4 (20.0%)
Mean		83.6 ± 27.8	22.0 ± 15.8%	57.0 ± 2.8	9.5 ± 7.1	38.6 ± 17.9	
H019							
1	28 Sep 2018	157	145 (92.4%)	60	24	16.6	13 (54.2%)
2	12 Jan 2021	–	–	–	–	–	–
3	1 Feb 2021	68	20 (29.4%)	61	2	10.0	0 (0%)
4	3 Mar 2021	54	0 (0%)	–	–	–	–
Mean		68.3 ± 55.9	55.0 ± 78.6%	60.5 ± 0.7	13.0 ± 15.6	21.1 ± 4.6	

Growth rate

Newborn hawksbill turtles had a mean SCL of 37.6 ± 1.5 mm, mean SCW of 27.8 ± 1.5 mm, and mean BM of 14.7 ± 1.2 g. After one year, 23 hatchlings died, and 60 survived. Veterinarians performed autopsies on the dead turtles, but the cause of death could not be confirmed. One-year hatchlings had a mean SCL of 215.6 ± 40.3 mm, mean SCW of 168.1 ± 36.6 mm, and mean BM of 1,295.5 ± 543.6 g. The growth trends of hawksbill turtles throughout the year after hatching were confirmed, with all coefficients being significant ($Ps < 0.05$; Table 3, Fig. 4).

$$\text{SCL (mm)} = 32.45 + 0.45d\ (\text{day}) + 1.96\text{e-}04 \times d^2$$

$$\text{SCW (mm)} = 26.20 + 0.32d + 2.19\text{e-}04 \times d^2$$

$$\text{BM (g)} = 54.92 - 1.22d + 1.27\text{e-}02 \times d^2$$

Discussion

Fertilization rate and hatching success

Eight clutches of hawksbill turtle eggs were incubated, yielding hatching success rates of 10% of total eggs and 32% of fertilized eggs. These rates are notably lower than the reported wild-hatching success rates, which range from 69% to 86% (Moncada et al. 1999; Richardson et al. 1999; Hesni et al. 2016; Flores-Aguirre et al. 2023; See et al. 2024). Previous studies have indicated that the hatching success of long-term captive hawksbill turtles is lower than that of wild individuals by less than 22% (Kobayashi 2012; Kawazu et al. 2018). Low hatching success rates in captive sea turtles have

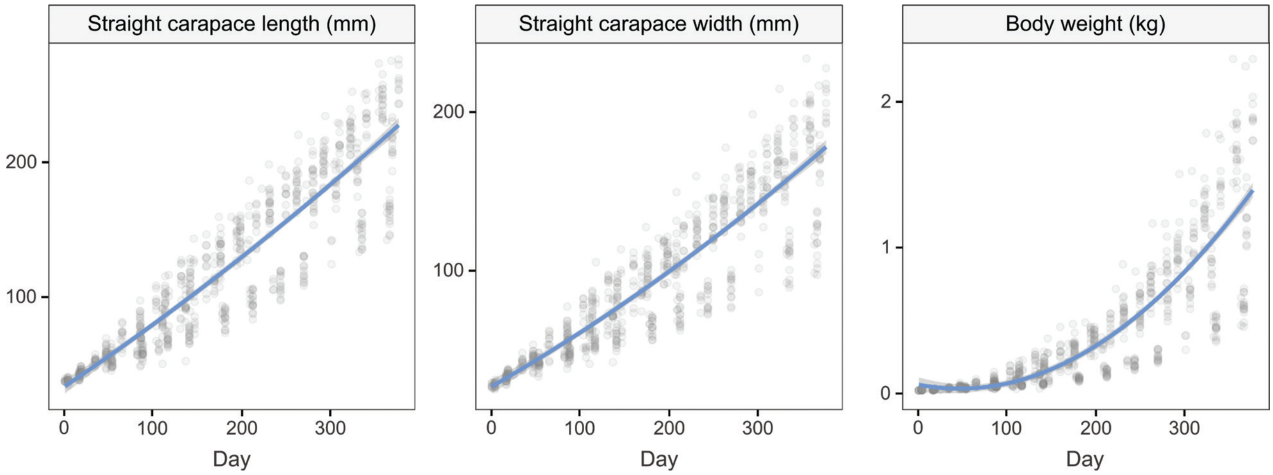

Figure 4. Growth trends of hatchling hawksbill turtles over one year.

Table 3. Coefficient values of the growth rate curve of hawksbill turtles. SE = Standard error.

Value	Straight carapace length			Straight carapace width			Body mass		
	β_0	β_1	β_2	β_0	β_1	β_2	β_0	β_1	β_2
Mean	32.45	0.45	1.96e-04	26.20	0.32	2.19e-04	54.92	-1.22	0.01
SE	2.78	0.03	8.76e-05	2.30	0.03	7.18e-05	26.15	0.32	8.22e-04
t-value	11.67	13.03	2.24	11.40	11.48	3.05	2.10	-3.79	15.48
p-value	< 0.001	< 0.001	0.026	< 0.001	< 0.001	0.002	0.036	< 0.001	< 0.001
R^2	0.838			0.816			0.755		

also been reported in green turtles, *Chelonia mydas* (Cho et al. 2022), and Kemp's Ridley turtles, *Lepidochelys kempii* (Shaver and Rubio 2008). However, the hatching success rates of eggs laid on natural sandy beaches but incubated in artificial facilities, as well as recently rescued hawksbill turtles in artificial captivity, have been confirmed to exceed 90% (Shimizu et al. 2006; Hart et al. 2014). In addition, long-term captive-bred Kemp's Ridley turtles showed high hatching success rates similar to those of wild populations after 10 years of release (Simon et al. 1975).

The low fertilization rate in this study could be attributed to the restricted breeding conditions. We inferred two potential causes. First, administering animal-based prey likely did not provide sufficient nutrients for reproductive hormone production or egg development. The quantity and variety of food sea turtles consume greatly influence their hormone levels (Moon et al. 1999; Sözbilen and Kaska 2018). Adult hawksbill turtles primarily consume seaweed and sponges (Meylan 1988). However, we administered animal feed because of the limited accessibility of animal prey and to mitigate the contamination caused by residual seaweed in the breeding tank. Some research suggested that the nutritional differences between captive and wild turtles, for example, the lack of essential fatty acids in the diet of captive females, could affect egg viability (Craven et al. 2008; Owens and Blanvillain 2013). This implies that maintaining captive sea turtles under conditions similar to those of the wild as much as possible could improve breeding success, as suggested by previous research (Kawazu et al. 2018). Second, the year-round breeding conditions for hawksbill turtles remained constant. In their natural habitat, females experience a short estrus period characterized by elevated levels of appetite-suppressing protein (leptin) and hunger-stimulating peptide (ghrelin), leading to reduced feeding (Goldberg et al. 2013; Kawazu et al. 2015). Throughout the study period, females consumed a consistent amount and type of food. Moreover, their estradiol levels remained similar throughout the year, making them unsuitable as indicators for mating readiness. Static breeding environments may not be suitable for intensive hormonal changes. The participation of only one male in mating is unlikely to be responsible for the low fertilization rate. Hawksbill turtles exhibit multiple paternity; nonetheless, more than 90% of females mate with only one male per season before laying eggs (Phillips et al. 2013; González-Garza et al. 2015). It is, therefore, unlikely that mating with a single male is responsible for the low fertilization rate.

To address the low hatching success rate of fertile eggs observed in this study, it is necessary to consider several factors that may have contributed to this outcome. Given the low fertilization rate and restricted environment, unhealthy egg yolks might have been fertilized in the present study. Additionally, it should not be overlooked that the incubation process might have contributed to low hatching success, although the incubation method used in this study was based on previous studies that demonstrated high hatching success rates (Shimizu et al. 2006; Hart et al. 2014; Flores-Aguirre et al. 2020, 2023). In natural nests, failure to hatch has been primarily attributed to early-stage embryos (71%) and late-stage embryos (18%), accounting for nearly 90% of unsuccessful hatching (Mau et al. 2024). In this study, the temperature of the artificial sandy beach was not controlled, so initial external factors may have had a negative effect on successful egg development. Additionally, considering that the early developmental stage has the greatest impact on hatching success, minimizing external disturbances and maintaining temperature and humidity during the transfer from a sandy beach to an artificial incubator and during periodic inspection could be critical factors to enhance hatching success.

Unexpected breeding

Among the 11 clutches observed, underwater egg-laying was confirmed once. In previous studies, captive hawksbill turtles exhibited underwater egg-laying behavior, even in tanks equipped with sandy beaches (Kobayashi et al. 2006; Kobayashi 2012). Additionally, underwater egg-laying by a wild hawksbill turtle has been reported (Beyneto and Delcroix 2005). This phenomenon occurs when sea turtles face difficulties locating appropriate nesting sites or when the interval distances between subsequent nesting sites are excessively long (Beyneto and Delcroix 2005; Kobayashi 2012). H019 initially laid eggs underwater in a tank without a sandy beach; however, after being relocated to a tank with sandy beaches, it subsequently laid all eggs on the beach. Therefore, H019 may have unintentionally laid eggs underwater because of its inability to access a sandy beach. Although limited in this research facility, breeding turtles in tanks connected to a sandy beach could effectively cope with unexpected spawning management.

After being transferred to a tank with a sandy beach, H019 laid 116 eggs in two clutches, of which 20 were confirmed fertilized, resulting in only two hatchlings. Despite the low fertilization rate and hatching success, it is notable that the most recent mating date for H019 occurred 909 days before the nesting date. As H019 had not mated with a male since July 2018, the eggs would have been fertilized with sperm stored for approximately two-and-a-half years. Most female sea turtles use stored sperm from mating within the same year to fertilize their eggs during that breeding season (González-Garza et al. 2015). Hawksbill turtles have been reported to store sperm for up to 75 days (Joseph and Shaw 2011; Phillips et al. 2013); however, sperm storage periods of up to 2 years have been estimated (Phillips et al. 2014). Therefore, this observation represents the longest recorded sperm storage period for a hawksbill turtle. Long-term sperm storage by females can result in diminished quantity or functionality, thereby leading to reduced fertilization rates and hatching success. Nevertheless, this suggests that female hawksbill turtles, capable of storing sperm for more than two-and-a-half years, can lay eggs in subsequent breeding seasons without additional mating, consistent with their nesting cycles of two to three years (Richardson et al. 1999; Phillips et al. 2014).

Mating suitability test

Mating and reproduction were successful when pairing males with high testosterone levels and females with high triglyceride levels and developed yolk sacs. This reaffirms the usefulness of these factors in confirming the reproductive cycle of sea turtles. However, the triglyceride test, which is highly correlated with yolk development and convenient to test (Hamann et al. 2002; Kawazu et al. 2015), cannot fully determine the reproductive period of females. The blood test failed to detect a high triglyceride level in H019, which laid eggs in January 2021. However, a subsequent ultrasound examination confirmed a yolk diameter of 32.7 mm immediately after egg laying. This indicates that the triglyceride test has limitations in detecting exceptional circumstances, such as mid-winter breeding, in contrast to confirming yolk size. Therefore, to more accurately assess the reproductive period of female hawksbill turtles, we recommend confirming yolk size through periodic ultrasound examinations in addition to the triglyceride level test.

During the study period, two females laid eggs consistently over 9 of the 11 months. Despite regional differences, hawksbill turtles lay eggs from April to September when sea and beach temperatures are high (Beggs et al. 2007; Santos et al. 2013; Kawazu et al. 2015; Okuyama et al. 2020). The hawksbill turtles, kept under stable tank conditions and photoperiods throughout the year, were unlikely to sense seasonal changes, resulting in a lack of intensive egg-laying during certain periods. Providing individuals with diverse and variable environmental conditions, such as temperature fluctuations and sufficient food variety, is practically limited in captivity. However, managing the photoperiod in a breeding facility is relatively straightforward. The photoperiod influences sex hormone patterns and reproductive cycling in sea turtles (Owens and Morris 1985); therefore, adjusting the photoperiod in the breeding facility according to sunrise and sunset times in the Republic of Korea may aid in stabilizing the hormonal cycles of the individuals.

Hatchling growth

The egg sizes, incubation periods, and physical characteristics of the hatchlings observed in this study were similar to those observed in natural nests (Márquez 1990; Pilcher and Ali 1999; Kobayashi 2012). Hatchlings showed nearly a 6-fold increase in SVL and SCW and approximately an 88-fold increase in BM after one year. Over one year, hatchlings exhibited similar growth rates in SVL and SCW, whereas BM experienced a rapid increase at approximately 100 days. This study is expected to contribute to understanding the growth rate and ecology of hatchling hawksbill turtles, as existing information on their natural growth rate remains limited. However, prey consumption primarily affects the growth rate of hawksbill turtles (Diez and van Dam 2002); therefore, it is important to interpret the observation that hatchlings in captive settings, where food is consistently provided, may demonstrate higher growth rates than individuals in their natural habitat with caution (Cho et al. 2022).

Conclusions

This study focused on aiding the recovery of the globally endangered hawksbill turtle population through artificial breeding in a fully controlled facility isolated from natural environments. The breeding method presented in this study demonstrated certain limitations, including low fertilization rates and hatching success. Nevertheless, improving artificial breeding methods that consider the reproductive ecology of hawksbill turtles and the captive breeding techniques presented in this study could enhance the effectiveness of sea turtle population recovery. This comprehensive approach to organizing and sharing propagation methods can extend beyond specific regions or species, potentially contributing to the recovery of various sea turtle species globally. Furthermore, we elucidated the ecological characteristics of hawksbill turtles, such as extended sperm storage periods and growth rates of early hatchlings, which are challenging to investigate in wild populations. Although the findings from captive breeding may differ from those in natural habitats, integrating knowledge from the findings on both captive breeding and wild individuals will improve our understanding of their ecological characteristics and aid in developing more effective conservation initiatives.

Acknowledgments

We thank Gyuseok Shim for the assistance with managing and measuring turtles.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This work was supported by grants from the National Marine Biodiversity Institute of Korea (2025E00300), funded by the Ministry of Oceans and Fisheries.

Author contributions

Eunvit Cho: Formal analysis, investigation, data curation, writing-original draft preparation, visualization; Dae Yeon Moon: Methodology, validation, writing-review and editing; Il-Hun Kim: Conceptualization, Funding acquisition, writing-review and editing; Dongjin Han: Investigation, resources, writing-review and editing; Ki-Young Lee: Validation, writing-review and editing; Il-Kook Park: Conceptualization, methodology, software, data curation, writing-original draft preparation, supervision, project administration.

Author ORCIDs

Il-Hun Kim https://orcid.org/0000-0003-0022-8219
Ki-Young Lee https://orcid.org/0000-0003-4023-2765
Il-Kook Park https://orcid.org/0000-0002-2274-4639

Data availability

All of the data that support the findings of this study are available in the main text.

References

Avens L, Ramirez MD, Goshe LR, Clark JM, Meylan AB, Teas W, Shaver DJ, Godfrey MH, Howell L (2021) Hawksbill sea turtle life-stage durations, somatic growth patterns, and age at maturation. Endangered Species Research 45: 127–145. https://doi.org/10.3354/esr01123

Beggs JA, Horrocks JA, Krueger BH (2007) Increase in hawksbill sea turtle *Eretmochelys imbricata* nesting in Barbados, West Indies. Endangered Species Research 3: 159–168. https://doi.org/10.3354/esr003159

Bell CD, Parsons J, Austin TJ, Broderick AC, Ebanks-Petrie G, Godley BJ (2005) Some of them came home: the Cayman Turtle Farm headstarting project for the green turtle *Chelonia mydas*. Oryx 39: 137–148. https://doi.org/10.1017/S0030605305000372

Beyneto S, Delcroix E (2005) Underwater oviposition by a hawksbill turtle in Guadeloupe, French West Indies. Marine Turtle Newsletter 107: 14.

Cho E, Kim IH, Han DJ, Im J, Cho IY, Lee KY, Moon DY (2022) Analysis of the rate of post-hatching growth of the first artificially bred green turtle *Chelonia mydas* juveniles in Korea. Korean Journal of Fisheries and Aquatic Science 55: 917–926. https://doi.org/10.5657/KFAS.2022.0917

Clyde-Brockway CE, Heidemeyer M, Paladino FV, Flaherty EA (2022) Diet and foraging niche flexibility in green and hawksbill turtles. Marine Biology 169: 108. https://doi.org/10.1007/s00227-022-04092-1

Craven KS, Parsons J, Taylor SA, Belcher CN, Owens DW (2008) The influence of diet on fatty acids in the egg yolk of green sea turtles, Chelonia mydas. Journal of Comparative Physiology B 178: 495–500. https://doi.org/10.1007/s00360-007-0242-8

Diez CE, van Dam RP (2002) Habitat effect on hawksbill turtle growth rates on feeding grounds at Mona and Monito Islands, Puerto Rico. Marine Ecology Progress Series 234: 301–309. https://doi.org/10.3354/meps234301

Dobbs KA, Miller JD, Owens DW, Landry AM (2007) Serum testosterone and estradiol-17*β* levels during the peak period in a year-round hawksbill turtle *Eretmochelys imbricata*

L. nesting population. Pacific Conservation Biology 13: 151–157. https://doi.org/10.1071/PC070151

Flores-Aguirre CD, Díaz-Hernández V, Salgado Ugarte IH, Sosa Caballero LE, Méndez de la Cruz FR (2020) Feminization tendency of hawksbill turtles (*Eretmochelys imbricata*) in the western Yucatán Peninsula, Mexico. Amphibian & Reptile Conservation 14: 190–202.

Flores-Aguirre CD, Díaz-Hernández V, Moreno DA, de la Cruz FRM (2023) Effect of moisture, temperature, and maternal influence on the hatching, phenotype, and performance of hawksbill turtles *Eretmochelys imbricata*. Endangered Species Research 50: 217–234. https://doi.org/10.3354/esr01229

Fontaine C, Shaver DJ (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.

Fuentes MMPB, Limpus CJ, Hamann M (2011) Vulnerability of sea turtle nesting grounds to climate change. Global Change Biology 17: 140–153. https://doi.org/10.1111/j.1365-2486.2010.02192.x

Godfrey MH, Mrosovsky N (1994) Simple method of estimating mean incubation temperatures on sea turtle beaches. Copeia 1994: 808–811. https://doi.org/10.2307/1447199

Goldberg DW, Leitão SAT, Godfrey MH, Lopez GG, Santos AJB, Neves FA, Garcia de Souza EP, Moura AS, da Cunha Bastos J, da Cunha Bastos VLF (2013) Ghrelin and leptin modulate the feeding behaviour of the hawksbill turtle *Eretmochelys imbricata* during nesting season. Conservation Physiology 1: cot016. https://doi.org/10.1093/conphys/cot016

González-Garza BI, Stow A, Sánchez-Teyer LF, Zapata-Pérez O (2015) Genetic variation, multiple paternity, and measures of reproductive success in the critically endangered hawksbill turtle (*Eretmochelys imbricata*). Ecology and Evolution 5: 5758–5769. https://doi.org/10.1002/ece3.1844

Hamann M, Limipus CJ, Whittier JM (2002) Patterns of lipid storage and mobilisation in the female green turtle (*Chelonia mydas*). Journal of Comparative Physiology B 172: 485–493. https://doi.org/10.1007/s00360-002-0271-2

Hart CE, Ley-QuiÑonez C, Maldonado-Gasca A, Zavala-Norzagaray A, Abreu-Grobois FA (2014) Nesting characteristics of olive ridley turtles (*Lepidochelys olivacea*) on El Naranjo Beach, Nayarit, Mexico. Herpetological Conservation and Biology 9: 524–534.

Hays GC, Laloë JO, Lee PL, Schofield G (2023) Evidence of adult male scarcity associated with female-skewed offspring sex ratios in sea turtles. Current Biology 33: R14–R15. https://doi.org/10.1016/j.cub.2022.11.035

Hesni MA, Tabib M, Ramaki AH (2016) Nesting ecology and reproductive biology of the Hawksbill Turtle, *Eretmochelys imbricata*, at Kish Island, Persian Gulf. Journal of the Marine Biological Association of the United Kingdom 96: 1373–1378. https://doi.org/10.1017/S0025315415001125

Hill JE, Paladino FV, Spotila JR, Santidrián Tomillo P (2015) Shading and watering as a tool to mitigate the impacts of climate change in sea turtle nests. PLoS ONE 10: e0129528. https://doi.org/10.1371/journal.pone.0129528

Hitchins PM, Bourquin O, Hitchins S, Piper SE (2004) Biometric data on hawksbill turtles (*Eretmochelys imbricata*) nesting at Cousine Island, Seychelles. Journal of Zoology 264: 371–381. https://doi.org/10.1017/S0952836904005850

Jang SJ, Jo K, Jang S, Nishizawa H, Kim M, Balazs G, Im J, Suk HY, Kim BY, Kim T (2024) Connectivity between sea turtles off Jeju Island on the Korean Peninsula, and other populations in the western Pacific. Frontiers in Marine Science 11: 1281897. https://doi.org/10.3389/fmars.2024.1281897

Joseph J, Shaw PW (2011) Multiple paternity in egg clutches of hawksbill turtles (*Eretmochelys imbricata*). Conservation Genetics 12: 601–605. https://doi.org/10.1007/s10592-010-0168-7

Kakizoe Y, Sakaoka K, Akune Y, Kanou Y, Saito T, Uchida I (2013) Change of plasma chemistry values in captive breeding loggerhead turtles (*Caretta caretta*). International Scholarly Research Notices 2013: 514576. https://doi.org/10.1155/2013/514576

Kawazu I, Kino M, Yanagisawa M, Maeda K, Nakada K, Yamaguchi Y, Sawamukai Y (2015) Signals of vitellogenesis and estrus in female hawksbill turtles. Zoological Science 32: 114–118. https://doi.org/10.2108/zs140212

Kawazu I, Fukada S, Maeda K, Maeda K, Kino M, Omata M, Makabe M, Kobuchi T (2018) Reproductive parameters of captive sea turtles in Okinawa Churaumi Aquarium. In Proceedings of 10th International Aquarium Conference Fukushima. Fukushima, Japan, 74–79.

Kim IH, Moon DY, Cho IY, Kim MS, An YR, Han D, Han WM, Han DJ, Park D (2017) Occurrence of sea turtles in the Korean waters and the morphological characteristics of two major species. Korean Journal of Fisheries and Aquatic Sciences 50: 311–318. https://doi.org/10.5657/KFAS.2017.0311

Kim IH, Yi CH, Han DJ, Park D, Park J, Cho IY, Kim MS (2020) First record of the hawksbill turtle (*Eretmochelys imbricata*, Reptilia: Testudines: Cheloniidae) from South Korea. Journal of Asia-Pacific Biodiversity 13: 151–155. https://doi.org/10.1016/j.japb.2020.02.006

Kim IH, Park IK, Han DJ, Kim MS, Park D, Moon DY, Cho IY, Im JE, Park J, An YR (2022) Movement patterns of juvenile loggerhead turtles (*Caretta caretta* L. 1758) and green turtles (*Chelonia mydas* L. 1758) hatched in captivity and released in the Korean waters. Animals 12: 2157. https://doi.org/10.3390/ani12162157

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

Kobayashi M, Shimizu T, Yoseda K (2006) Mating and nesting behavior of hawksbill turtle (*Eretmochelys imbricata*) in captivity. Proceedings of the 3rd International Symposium on SEASTAR2000 and Asian Bio-logging Science, 13–15.

Lopez GG, Saliés EDC, Lara PH, Tognin F, Marcovaldi MA, Serafini TZ (2015) Coastal development at sea turtles nesting ground: Efforts to establish a tool for supporting conservation and coastal management in northeastern Brazil. Ocean & Coastal Management 116: 270–276. https://doi.org/10.1016/j.ocecoaman.2015.07.027

Márquez MR (1990) FAO species catalogue, Vol.11 sea turtles of the world. An annotated and illustrated catalogue of sea turtle species known to date. FAO, Rome.

Mau A, Rodríguez PS, Picknell A, Tepedino A, Capaldo D, Fenton H, Pemberton L, Stewart KM, Dennis MM (2024) In-nest mortality and pathology of hawksbill sea turtle (*Eretmochelys imbricata*) embryos and hatchlings in St. Kitts and Nevis. Journal of Comparative Pathology 209: 13–21. https://doi.org/10.1016/j.jcpa.2024.01.004

Mazaris AD, Schofield G, Gkazinou C, Almpanidou V, Hays GC (2017) Global sea turtle conservation successes. Science Advance 3: e1600730. https://doi.org/10.1126/sciadv.1600730

Meylan A (1988) Spongivory in hawksbill turtles: a diet of glass. Science 239: 393–395. https://doi.org/10.1126/science.239.4838.393

Moncada F, Carrillo E, Saenz A, Nodarse G (1999) Reproduction and nesting of the hawksbill turtle, *Eretmochelys imbricata*, in the Cuban Archipelago. Chelonian Conservation and Biology 3: 257–263.

Moon DY, Owens DW, MacKenzie DS (1999) The effects of fasting and increased feeding on plasma thyroid hormones, glucose, and total protein in sea turtles. Zoological Science 16: 579–586. https://doi.org/10.2108/zsj.16.579

Mortimer JA, Donnelly M (2008) *Eretmochelys imbricata*. The IUCN Red List of Threatened Species 2008: e.T8005A12881238.

O'Connor JM, Limpus CJ, Hofmeister KM, Allen BL, Burnett SE (2017) Anti-predator meshing may provide greater protection for sea turtle nests than predator removal. PLoS ONE 12: e0171831. https://doi.org/10.1371/journal.pone.0171831

Okuyama J, Ishii H, Tanizaki S, Suzuki T, Abe O, Nishizawa H, Yano A, Tsujimura M, Ishigaki T, Ishigaki T, Kobayashi M, Yanagida H (2020) Quarter-century (1993–2018) nesting trends in the peripheral populations of three sea turtle species at Ishigakijima Island, Japan. Chelonian Conservation and Biology 19: 101–110. https://doi.org/10.2744/CCB-1428.1

Owens DW, Blanvillain B (2013) Captive reproduction of sea turtles: An important success story. Proceedings of the international symposium on Reproduction of Marine Life, Birth of New Life, 23–40.

Owens DW, Morris YA (1985) The comparative endocrinology of sea turtles. Copeia 1985: 723–735. https://doi.org/10.2307/1444766

Parmenter CJ (1980) Incubation of the eggs of the green sea turtle, Chelonia mydas, in Torres Strait, Australia: the effect of movement on hatchability. Wildlife Research 7: 487–491. https://doi.org/10.1071/WR9800487

Phillips KP, Jorgensen TH, Jolliffe KG, Jolliffe SM, Henwood J, Richardson DS (2013) Reconstructing paternal genotypes to infer patterns of sperm storage and sexual selection in the hawksbill turtle. Molecular Ecology 22: 2301–2312. https://doi.org/10.1111/mec.12235

Phillips KP, Jorgensen TH, Jolliffe KG, Richardson DS (2014) Potential inter-season sperm storage by a female hawksbill turtle. Marine Turtle Newsletter 140: 13–14.

Phillott AD, Parmenter CJ (2001) The distribution of failed eggs in sea turtle eggs and the appearance of fungi in artificial nests of green (*Chelonia mydas*) and loggerhead (*Caretta caretta*) sea turtle eggs at Heron Island, Australia. Australian Journal of Zoology 49: 713–718. https://doi.org/10.1071/ZO00051

Pilcher NJ, Ali L (1999) Reproductive biology of the hawksbill turtles, *Eretmochelys imbricata*, in Sabah Malaysia. Chelonian Conservation and Biology 3: 330–336. https://doi.org/10.1017/S0025315415001125

R Core Team (2024) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.

Richardson JI, Bell R, Richardson TH (1999) Population ecology and demographic implications drawn from an 11-year study of nesting hawksbill turtles, *Eretmochelys imbricata*, at Jumby Bay, Long Island, Antigua, West Indies. Chelonian Conservation and Biology 3: 244–250.

Santos AJB, Bellini C, Vieira DHG, Neto LD, Corso G (2013) Northeast Brazil shows highest hawksbill turtle nesting density in the South Atlantic. Endangered Species Research 21: 25–32. https://doi.org/10.3354/esr00505

Sarmiento-Ramirez JM, Abella-Perez E, Phillott AD, Sim J, Van West P, Martin MP, Marco A, Dieguez-Uribeondo J (2014) Global distribution of two fungal pathogens threatening endangered sea turtles. PLoS ONE 9: e85853. https://doi.org/10.1371/journal.pone.0085853

See KW, Latip NSA, Hashim ZH (2024) First report of the egg fertility of the hawksbill turtle (*Eretmochelys imbricata*) (Testudines: Cheloniidae) in Melaka, Malaysia. Journal of Asia-Pacific Biodiversity 17: 451–456. https://doi.org/10.1016/j.japb.2024.02.001

Shaver DJ, Rubio C (2008) Post-nesting movement of wild and head-started Kemp's ridley sea turtles *Lepidochelys kempii* in the Gulf of Mexico. Endangered Species Research 4: 43–55. https://doi.org/10.3354/esr00061

Shimizu T, Asami K, Yamamoto K, Dan S, Yoseda K (2006) Successful spontaneous nesting of the hawksbill turtle (*Eretmochelys imbricata*) at Yaeyama Station, National Center for Stock Enhancement, Japan. Proceedings of the 2nd International Symposium on SEASTAR2000 and Asian Bio-logging Science, 69–74.

Simon MH, Ulrich GF, Parkes AS (1975) The green sea turtle (*Chelonia mydas*): mating nesting, and hatching on a farm. Journal of Zoology 177: 411–423. https://doi.org/10.1111/j.1469-7998.1975.tb02242.x

Sözbilen D, Kaska Y (2018) Biochemical blood parameters and hormone levels of foraging, nesting, and injured loggerhead sea turtles (*Caretta caretta*) in Turkey. Turkish Journal of Zoology 42: 287–296. https://doi.org/10.3906/zoo-1801-19

Stimson JA, Carmines EG, Zeller RA (1978) Interpreting polynomial regression. Sociological Methods & Research 6: 515–524. https://doi.org/10.1177/004912417800600405

Stokes HJ, Esteban N, Hays GC (2024) Predation of sea turtle eggs by rats and crabs. Marine Biology 171: 17. https://doi.org/10.1007/s00227-023-04327-9

Wickham H (2016) ggplot2: Elegant graphics for data analysis. Springer, New York, USA. https://doi.org/10.1007/978-3-319-24277-4_9

Zhang T, Lin L, Li M, Kong L, Wang J, Shi HT (2023) Investigation on beach debris on the historical nesting grounds of green turtles (*Chelonia mydas*) in Hainan Island, South China. Heliyon 9: e13400. https://doi.org/10.1016/j.heliyon.2023.e13400