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Rock Installation Reduces Aggressive Behavior in Captive Hawksbill Turtles

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Abstract: Captive breeding programs are utilized worldwide for the recovery and conservation of endangered species, including the critically-endangered hawksbill turtle (*Eretmochelys imbricata*). However, aggression is an issue that is commonly reported among captive hawksbill turtles housed together. In the present study, we installed rocks in a captive environment to assess whether they can suppress aggressive behavior in captive hawksbill turtles. The behavior of immature female hawksbill turtles (n=6) was recorded over a 10 d period (between 0900 and 1700 h daily) in an experimental tank under the following conditions: (1) housed alone or paired with another individual in the (2) presence and (3) absence of rocks. Resting behavior was recorded as the total amount of time spent resting per day, while hiding and biting behaviors (i.e., aggressive behavior) were recorded as the total number of events observed per day. Serum corticosterone concentrations were measured at 0900 and 1700 h daily throughout the experimental period. The median number of hiding and biting events was significantly lower for paired individuals housed in the presence of rocks than that for those housed in the absence of rocks. Although no significant differences in serum corticosterone concentrations were observed among the experimental groups, paired individuals spent significantly more time resting in the presence of rocks. Our results suggest that underwater rock installations effectively reduce aggression in captive hawksbill turtles.

Key words: Bite; Captive husbandry; Hide; Rest; Serum corticosterone

Introduction

Hawksbill turtles (*Eretmochelys imbricata*) are distributed in tropical and subtropical coral reefs worldwide, and are listed as "critically endangered" on the International Union for Conservation of Nature (IUCN) Red List for Threatened Species (Mortimer and Donnelly, 2008). The active implementation of captive breeding programs offers a management tool for the recovery and conservation of this endangered species (Owens and Blanvillain, 2013). Such captive programs provide important insights that support the development of pioneering research approaches (Owens and Blanvillain, 2013). In addition, several techni-

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ques used for the captive husbandry of sea turtles (e.g., blood sampling and ultrasonographic diagnoses) have been utilized to study the reproduction and conservation of wild sea turtles (Owens and Blanvillain, 2013).

Captive sea turtles, including hawksbill turtles, exhibit aggressive behavior toward each other when reared together in the same tank; however, the triggers for aggression among turtles are poorly unknown (Higgins, 2003). Aggressive behavior, such as biting at the posterior carapace, front and rear flippers, and neck, can cause severe injuries (Higgins, 2003). Furthermore, skin lesions caused by biting may become infected with bacteria, thereby resulting in morbidity and mortality (Higgins, 2003). Additionally, the stress induced by aggression increases serum and plasma corticosterone concentrations in sea turtles (Jessop et al., 2002a, b; Milton and Lutz, 2003; Jessop et al., 2004; Jessop and Hamann, 2005; Hunt et al., 2012). An increase in the level of stress hormones (e.g., corticosterone) can have harmful physiological effects on turtles, including reduction in blood glucose levels and immune and salt gland functions (Milton and Lutz, 2003). The identification and investigation of such husbandry issues allows for further development of animal management and breeding programs to ensure that captive turtles are provided with a safe and healthy environment. In addition, such findings could advance our knowledge about the ecology of sea turtles.

Wild hawksbill turtles rest under coral reefs and ledges (van Dam and Diez, 1997; Houghton et al., 2003; Blumenthal et al., 2009; Okuyama et al., 2010; Walcott et al., 2014) and can utilize rocks for shelter during rest periods (Okuyama et al., 2010; Walcott et al., 2014). Thus, in captive hawksbill turtles, underwater rock installations may have an effect on different behaviors such as resting and aggression. This hypothesis, however, has not yet been quantitatively confirmed in captivity. In the present study, we investigated whether the use of rocks would reduce aggressive behavior among captive hawksbill turtles and provide a better captive environment for them. Behavioral observations (i.e., rest and aggression) or stress hormone levels (i.e., serum corticosterone concentration) were used to assess the responses of hawksbill turtles housed alone and paired with another individual in both the presence and absence of an underwater rock installation.

Table 1. Summary information on the hawksbill turtles used in the present study.

Turtle	Sex	Age (years old)	Straight carapace length (cm)	Body mass (kg)
T1	Female	14	67.7	34.3
T2	Female	14	68.4	35.4
T3	Female	14	69.0	34.2
T4	Female	14	67.6	34.6
T5	Female	14	66.5	27.2
T6	Female	14	65.6	29.8

Materials and Methods

Study animals

We studied six immature female hawksbill turtles (Table 1) that were hatched in captivity in 1994 and maintained in captivity at the Okinawa Churaumi Aquarium, Motobu, Okinawa Prefecture, Japan. Prior to the experiments, the turtles were housed in cylindrical holding tanks (0.75×1.50 m, depth×diameter). The water temperature of the tanks ranged from 20 to 30°C throughout the 12-month study period, i.e., it was maintained at a thermal range similar to that experienced by hawksbill turtles under natural conditions (sea surface temperature) around Okinawa Island (26°30 N, 127°00 E). The turtles were identified as females after this study based on observed follicular development (Kawazu et al., 2015). The turtles were fed once daily; their diet included fish, shrimp, and squid, at 0.5–2% of their body mass.

Experimental conditions

We conducted experiments between August

and October 2008. We measured serum corticosterone concentration and behavioral parameters (resting and aggression) of the six hawksbill turtles under following housing conditions: 1) housed alone (hereafter referred to as 'single'), 2) paired with another individual in the absence of rocks (hereafter referred to as 'pairing without rocks'), and 3) paired with another individual in the presence of rocks installed in the experimental tank (hereafter referred to as 'pairing with rocks'). The rock installation consisted of two rocks (limerock, 0.5 m in diameter) placed on the floor of the experimental tank. The turtles were separately transferred from their individual holding tanks to two outdoor experimental tanks (1.5×2.5 ×0.9 m, length×width×depth, open water system) for a 10 d period, after which they were returned to their respective holding tanks. The six turtles were paired according to similarities in straight carapace length and body mass, as follows: T1 vs. T2, T3 vs. T4, and T5 vs. T6 (Table 1). In August, the 'single' experiment was performed on each turtle (T1–6) using two experiment tanks, and 'pairing without and with rocks' (T1 vs. T2, T3 vs. T4, and T5 vs. T6) were done in September and October. The same turtles were used in the experiments with at least 20 d intervals between consecutive observations. Maintenance, animal handling, and all procedures associated with this study were conducted in accordance with the ethical guidelines of the Japanese Association of Zoos and Aquariums (JAZA).

Behavioral recording and analysis

The behavioral observations were conducted between 0900 and 1700 h each day during the 10 d experimental periods. Turtles were monitored using a digital video camera (DCR-HC96, Sony, Japan), which was positioned at the upper part of the tank. Video recordings were stored on an external hard drive (LC-13C1, Sharp, Japan). Three behavioral categories were defined and recorded; resting behavior (inactivity for 1 s or longer on the bottom of the tank) was recorded as the total amount of time spent resting per day, and hiding (head or a part of the body under another turtle) and biting (biting a part of the body of another turtle) behaviors were recorded as the total number of events observed per day. During all experiments, all the turtles were identified based on their carapace patterns and the behavioral events of each individual were separately recorded. Additionally, observation and analysis were performed by the same person.

Blood sampling and serum corticosterone measurements

The turtles were removed from the experiment tanks at 0900 and 1700 h daily throughout the experimental period and kept in a prone position. Blood samples (5 mL) were collected from either the right or left jugular vein using 70 mm 20-gauge needles and 10 mL syringes, and the turtles were returned to the experiment tanks. Blood samples were stored in 10 mL vacutainer tubes (VP-P100K, Terumo, Japan). Serum samples were obtained from the blood samples by centrifugation at 3,500 rpm for 20 min. Additionally, the blood samples were shared with veterinarians and aquarium staff of the Okinawa Churashima Foundation to assess the health status of turtles. All turtles were determined to be healthy throughout the study period.

Serum corticosterone concentrations were measured using a time-resolved fluorometric immunoassay and the DELFIA System (PerkinElmer, USA). Microtiter plates (Thermo Fisher Scientific Inc., US) were coated with 200 μL of corticosterone-3CMO-BSA (0.5 μg/mL in 0.1 M sodium carbonate buffer, pH 9.5; Cosmo-Bio, Japan). Steroid hormones were extracted from the serum samples using the ether extraction method with 50 μL of corticosterone standards (100, 20, 4, 0.8, 0.16, and 0.032 ng/mL). Thereafter, the extracted samples were incubated with 50 μL of rabbit anti-corticosterone-3-CMO-BSA antiserum (1:50,000; Cosmo Bio) in a 96-well plate coated with antigen for 2 h at room temperature. After washing, 100 μL europium-labeled secondary antibody (DELFIA Eu-N0-labeled anti-rabbit antibody, 1:5,000; PerkinElmer) was added and the plates were incubated for 1 h at room temperature. After washing, 100 µL of enhancement solution was added. Finally, fluorescence was measured after 5 min using a time-resolved fluorometer (DELFIA Fluorometer; PerkinElmer).

Statistical analysis

The Wilcoxon rank-sum test was used to determine the median of the total number of hiding and biting events observed per day in turtles housed in pairs in the presence and absence of rocks. We used a Steel-Dwass test to determine significant differences in the median of serum corticosterone concentrations and the total amount of time spent resting per day. The Wilcoxon signed-ranks test was used to determine significant differences in the median serum corticosterone concentrations measured daily at 0900 and 1700 h in turtles housed under the different experimental conditions (i.e., single and pairing without and with rocks).

Results

Behavioral observations

The median number of hiding and biting events was 59.5 (n=60) and 24.0 (n=60) times per day, respectively, in the 'paring without rocks' experiments and 11.0 (n=60) and 6.0 (n=60) times per day in the 'paring with rocks' experiments, respectively (Fig. 1). The number of hiding and biting events observed in individuals housed in pairs in the presence of rocks was lower than that for individuals housed in pairs in the absence of rocks ($p<0.05$, Wilcoxon signed-ranks test).

The median of the total amount of time spent resting in the 'single', 'pairing without rocks', and 'pairing with rocks' experiments was 0.52 (n=60), 0.99 (n=60), and 1.87 (n=60) h, respectively (Fig. 2). The total amount of time spent resting differed among all the experiments ($p<0.05$, Steel-Dwass test).

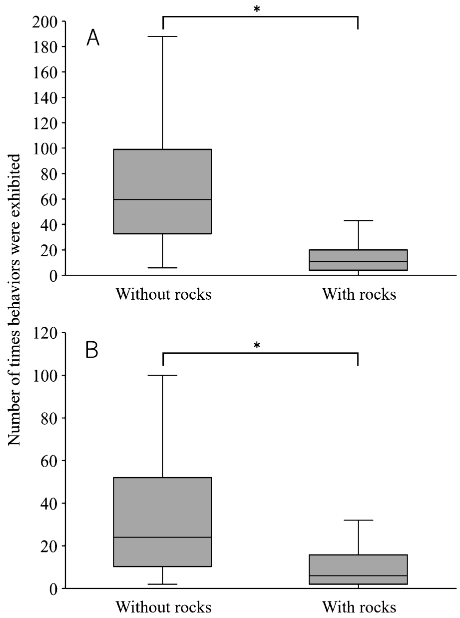

Fig. 1. Total number of times captive hawksbill turtles were observed exhibiting hiding (A) and biting (B) behavior when housed in pairs in the presence and absence of rocks ('pairing with rocks' and 'pairing without rocks', respectively). Whiskers represent the range, boxes represent the inter-quartile range (25–75%), and lines represent the medians. Asterisks represent significant differences among the medians of the total number of hiding and biting events exhibited ($p<0.05$).

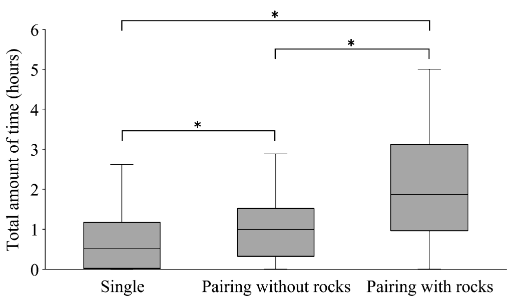

Fig. 2. Total amount of time captive hawksbill turtles spent resting when housed alone ('single') or in pairs in the presence ('pairing with') and absence ('pairing without') of rocks. Whiskers represent the range, boxes represent the inter-quartile range (25–75%), and lines represent the medians. Asterisks represent significant differences among the medians of the total amount of time spent resting ($p<0.05$).

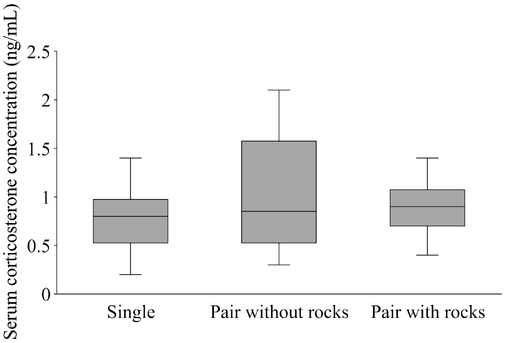

Fig. 3. Serum corticosterone concentrations in captive hawksbill turtles housed alone ('single') or in pairs in the presence ('pairing with') and absence ('pairing without') of rocks. Whiskers represent the range, boxes represent the inter-quartile range (25–75%), and lines represent the medians. Asterisks represent significant differences among the medians of the serum corticosterone concentrations ($p<0.05$).

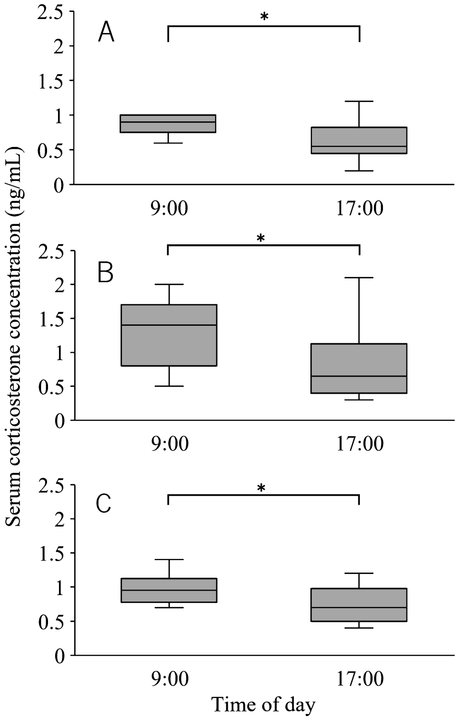

Fig. 4. Serum corticosterone concentrations in hawksbill turtles measured daily at 09:00 and 17:00 when housed alone (A), housed in pairs in the absence of rocks (B), or housed in pairs in the presence of rocks (C). Whiskers represent the range, boxes represent the inter-quartile range (25–75%), and lines represent the medians. Asterisks represent significant differences among the medians of the serum corticosterone concentrations ($p<0.05$).

Serum corticosterone concentration

The median serum corticosterone concentrations in the 'single', 'pairing without rocks', and 'pairing with rocks' experiments were 0.8 (n=120), 0.85 (n=120), and 0.9 (n=120) ng/mL and did not differ among the experiments ($p>0.05$, Steel-Dwass test) (Fig. 3). However, the median serum corticosterone concentration at 0900 h were significantly higher than that at 1700 h in all experiments ('single': 0.9 [n=60] vs 0.55 [n=60] ng/mL, 'pairing without rocks': 1.9 [n=60] vs 0.65 [n=60] ng/mL, and 'pairing with rocks': 0.95 [n=60] vs 0.7 [n=60] ng/mL, $p<0.05$, Wilcoxon signed-ranks test) (Fig. 4).

Discussion

This study is the first to report a reduction of aggressive behavior in captive hawksbill turtles. We found that the presence of rocks in the captive environment effectively reduced aggressive behavior in captive hawksbill turtles. The findings of this study can be used to enhance the captive management of hawksbill turtles.

The aggressive behavior of hawksbill turtles may be associated with the opportunities to encounter other turtles. The decrease in the number of hiding and biting events (Fig. 1) and the increase in the time spent resting (Fig. 2) in the presence of rocks indicate a reduction in interaction with other individuals. Wild hawksbill turtles occasionally rest under coral reefs and ledges (van Dam and Diez, 1997; Houghton et al., 2003; Blumenthal et al., 2009; Okuyama et al., 2010; Walcott et al., 2014), suggesting that they prefer a relatively narrow resting space. The results of the present study also support this hypothesis as the total amount of time that the turtles spent resting under rock installation was the longest among the experimental conditions (Fig. 2). In other words, the rock installations created narrow spaces in the tank, which lead to an increase in the time that the turtles spent resting. Consequently, this can decrease the opportunities of them encountering other turtles. Moreover, the turtles subjected in the present study displayed a behavior of hiding under other turtles; this may be attributed to the possibility that they tried to substitute other individuals for rocks. Thus, the presence of the rocks can be useful in preventing or overcoming aggressive behaviors in captive hawksbill turtles, such as hiding and biting.

Corticosterone levels in reptiles, including sea turtles, typically increase in response to stressors in the environment (Jessop et al., 2002a, b; Milton and Lutz, 2003; Jessop et al., 2004; Jessop and Hamann, 2005; Hunt et al., 2012). However, no significant differences were observed in serum corticosterone levels among the three experimental groups in the current study. This finding suggests that the presence and actions of other turtles, including hiding and biting behaviors, in the present study are unlikely to induce an increase in the corticosterone levels. In green turtles (*Chelonia mydas*), the mean plasma corticosterone concentrations increased significantly to approximately 5–22 ng/mL in response to 8 h of capture stress (Jessop et al., 2002b; Jessop and Hamann, 2005). These values are similar to those recorded for hawksbill turtles, i.e., approximately 8–12 ng/mL at 5 h after capture (Jessop et al., 2004). The mean corticosterone levels of cold-stunned juvenile Kemp's ridley turtles (*Lepidochelys kempi*) were found to be 39.3 ng/mL (Hunt et al., 2012). The physiological values reported in these previous studies were substantially higher than the corticosterone levels (<1 ng/mL) measured in turtles under all three experimental conditions in the present study (Fig. 3). Indeed, no trauma, including external and internal bleeding, was incurred from biting behavior in captive turtles during this study.

Typical diurnal corticosterone fluctuations were observed in captive hawksbill turtles under all experimental conditions. This result was consistent with previous findings in captive juvenile green turtles, wherein plasma corticosterone levels were substantially elevated during the day (0600 to 1500 h) and declined to basal levels at night (Jessop et al., 2002b). These results suggest that the captive turtles were not physiologically affected by the experimental conditions of the present study, thus indicating the reliability of our behavioral findings.

Acknowledgments

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Literature Cited

Blumenthal, J. M., Austin, T. J., Bothwell, J. B., Broderick, A. C., Ebanks-Petrie, G., Olynik, J. R., Orr, M. F., Solomon, J. L., Witt, M. J., and Godley, B. J. 2009. Diving behavior and movements of juvenile hawksbill turtles *Eretmochelys imbricata* on a Caribbean coral reef. *Coral Reefs* 28: 55–65.

Higgins, B. M. 2003. Sea turtle husbandry. p. 411–440. *In*: P. L. Lutz, J. A. Musick, and J. Wyneken (eds.), *The Biology of Sea Turtles Volume II*. CRC Press, Boca Raton.

Houghton, D. R., Callow, M. J., and Hays, G. C. 2003. Habitat utilization by juvenile hawksbill turtles (*Eretmochelys imbricata*, Linnaeus, 1766) around a shallow water coral reef. *Journal of Natural History* 37: 1269–1280.

Hunt, K. E., Innis, C., and Rolland, R. M. 2012. Corticosterone and thyroxine in cold-stunned Kemp's ridley sea turtles (*Lepidochelys kempii*). *Journal of Zoo and Wildlife Medicine* 43: 479–493.

Jessop, T. S. and Hamann, M. 2005. Interplay between age class, sex and stress response in green turtles (*Chelonia mydas*). *Australian Journal of Zoology* 53: 131–136.

Jessop, T. S., Knapp, R., Whittier, J. M., and

Limpus, C. J. 2002a. Dynamic endocrine responses to stress: Evidence for energetic constraints and status dependence in breeding male green turtles. *General and Comparative Endocrinology* 126: 59–67.

Jessop, T. S., Limpus, C. J., and Whittier, J. M. 2002b. Nocturnal activity in the green sea turtle alters daily profiles of melatonin and corticosterone. *Hormone Behavior* 41: 357–365.

Jessop, T. S., Sumner, J. M., Limpus, C. J., and Whittier, J. M. 2004. Interplay between plasma hormone profiles, sex and body condition in immature hawksbill turtles (*Eretmochelys imbricata*) subjected to a capture stress protocol. *Comparative Biochemistry and Physiology Part A* 137: 197–204.

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.

Milton, S. L. and Lutz, P. L. 2003. Physiological and genetic responses to environmental stress. p. 163–197. *In*: P. L. Lutz, J. A. Musick, and J. Wyneken (eds.), *The Biology of Sea Turtles Volume II*. CRC Press, Boca Raton.

Mortimer, J. A. and Donnelly, M. 2008. Marine Turtle Specialist Group 2008 IUCN Red List Status Assessment, Hawksbill Turtle (*Eretmochelys imbricata*). http://www.iucnredlist.org/attachments/639. pdf (accessed 17, October, 2021)

Okuyama, J., Shimizu, T., Abe, O., Yoseda, K., and Arai, N. 2010. Wild versus head-started hawksbill turtles *Eretmochelys imbricata*: Post-release behavior and feeding adaptions. *Endangered Species Research* 10: 181–190.

Owens, D. W. and Blanvillain, B. 2013. Captive reproduction of sea turtles: An important success story. p. 23–40. *In*: K. Sato (ed.), *Proceedings of the International Symposium on Reproduction of Marine Life, Birth of New Life! Investigating the Mysteries of Reproduction*. Okinawa Churashima Foundation, Motobu.

van Dam, R. P. and Diez, C. E. 1997. Diving behavior of immature hawksbill turtles (*Eretmochelys imbricata*) in a Caribbean reef habitat. *Coral Reefs* 16: 133–138.

Walcott, J., Eckert, S., Oxenford, H. A., and Horrocks, J. A. 2014. Use of a towed camera system to investigate benthic habitat use by inter-nesting female hawksbill sea turtles. *Endangered Species Research* 24: 159–170.

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