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Effect of Retention Conditions and Duration on the Swim Frenzy of Hatchling Loggerhead Turtles

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Abstract.—**Retaining hatchling sea turtles following emergence may compromise their swim frenzy, a period of active swimming that is critical to their survival. To inform the best handling practices for sea turtles raised in hatcheries, we determined the interactive effects of retaining Loggerhead Turtle (*Caretta caretta*) hatchlings under different conditions (dark vs. light and air vs. water) and for different durations (24, 48, and 72 h) on swimming thrust measured at 24-h intervals during the swim frenzy period (72 h). The mean thrust of hatchlings placed in water immediately after emergence (control) was not significantly higher than the mean thrust of hatchlings retained in air (light or dark) for 24, 48, or 72 h. The mean thrust of hatchlings retained in water for 24, 48, and 72 h, however, was significantly lower than the mean thrust of hatchlings in the control treatment. This study indicates that the swim frenzy period of hatchlings can be delayed by retaining them in air for up to 72 h after emergence, such that hatchlings display uncompromised swimming following retention. Conversely, retaining hatchlings in water for the same duration of time can severely compromise their swimming performance following retention, which would put hatchlings at risk of predation upon entering the sea.**

Key Words.—*Caretta caretta*; conservation; hatchery management; Japan; swimming activity; threatened species

Introduction

Nest hatcheries represent a valuable tool in sea turtle population management in areas where threats to naturally incubating eggs become unsustainable, including excessive predation, poaching, and habitat degradation (Lutcavage et al. 1997; Mortimer 1999; Witherington and Witherington 2015). Hatcheries also provide opportunities for residents, students, and tourists to observe sea turtle conservation in action, facilitating education and enlightenment about the conservation of sea turtles (Shanker et al. 2003); however, there are costs associated with using nest hatcheries for management and education. Moving eggs into hatcheries can decrease hatching success when rotation and/or torsion of eggs kills fragile embryos (Limpus et al. 1979; Mortimer 1999) and removing eggs from the natural incubation environment can alter hatchling fitness and sex ratios (Lutcavage et al. 1997; Wyneken 2000). Additionally, the process of collecting and retaining hatchlings prior to release, a common practice in hatcheries, can also have detrimental effects on hatchling energy and behavior (Pilcher and Enderby 2001; van de Merwe et al. 2013).

In nature, sea turtle hatchlings take 2–5 d to hatch from their eggs and collectively dig their way out of the nest. Upon emergence, they crawl rapidly from the nest to the sea, where they enter a period of active swimming known as the swim frenzy, which transports them away from shore and into the open ocean (Davenport 1997; Pankaew and Milton 2018). During the beach crawl and swim frenzy, hatchlings are subject to predation, and it is assumed that the faster they transit from the nest to the open ocean, the greater their chances of survival (Witherington and Salmon 1992; Burgess et al. 2006; Whelan and Wyneken 2007; Ischer et al. 2009; Booth et al. 2013). At some hatcheries, hatchlings may be held in captivity for several days after they emerge from the nest before they are released onto the beach to provide tourist attractions, such as release programs on weekends (Hewavisenthi 1993; Shanker et al. 2003; Rajakaruna et al. 2013). Retaining hatchlings for these extended periods may compromise their crawling and swim frenzy performance, and prolonged holding may even inhibit the swim frenzy all together because this state generally only lasts for 1–2 d after emergence (Wyneken and Salmon 1992; Gyuris 1994; Wyneken 1997; Wyneken et al. 2008; Chung et al. 2009). In addition, during prolonged retention hatchlings may consume their initial energy stores (internal yolk) needed for offshore dispersal (Clusella Trullas et al. 2006; Jones et al. 2007). The holding conditions during retention may also exacerbate the problem. In some hatcheries, hatchlings are placed in tanks with seawater from the moment they are collected from the nests

(Mejías Balsalobre and Bride 2016), despite hatchery management guidelines (Mortimer 1999), which recommend that they should be kept out of water inside a damp cloth sack in a cool, dark, and quiet place.

Given these concerns, several studies have investigated the effects of retaining sea turtle hatchlings for different durations following emergence. Pilcher and Enderby (2001) measured the swimming speed of Green Turtle (*Chelonia mydas*) hatchlings for 1 h following retention for 0–6 h and showed that the mean swimming speed progressively decreased (up to 12%) as retention time increased. Similarly, van de Merwe et al. (2013) found that the crawling speed of Green Turtle hatchlings progressively decreased following retention for 1, 3, and 6 h. In both studies, hatchlings were retained within nets in a hatchery under dark and dry conditions. Mejías Balsalobre and Bride (2016) measured the locomotory performance of Green Turtle hatchlings retained in water for up to 48 h and observed that crawling speed and swimming stroke rate decreased as retention duration increased. Further, Okuyama et al. (2009) studied the changes in offshore dispersal movements of Green Turtles and found that hatchlings retained in water for 7 d displayed lower migration velocity than hatchlings retained for only 1 d. These studies generally suggest that retention of hatchling for any duration after emergence could reduce the access of turtles to offshore developmental habitats and therefore reduce their chances of survival.

There are two important knowledge gaps stemming from the aforementioned studies. First, all studies were conducted on Green Turtles. While hatchling Loggerhead Turtles (*Caretta caretta*) show similar patterns of swimming activity during frenzy and post-frenzy periods as Green Turtles, there were small differences in the time spent swimming. Wyneken and Salmon (1992) found that nocturnal swimming activity of Green Turtle hatchling 6 d after the initiation of their swim frenzy was reduced to 13% of their original activity level, while Loggerhead Turtles ceased activity entirely. Such differences may reflect alternate survival strategies among these species that may also confer different responses to retention. Second, these studies tested the effect of retention duration, but not the effect of retention conditions. The conditions in which hatchlings are retained likely have an effect on their activity levels prior to release, which in turn may affect hatchling performance and their likelihood of survival.

In this study, we investigated the interactive effects of retaining hatchling Loggerhead Turtles under different conditions (dark vs. light and air vs. water) and for different durations (24, 48, and 72 h) on swimming performance during the swim frenzy and post-frenzy swim periods. We aimed to provide a basis for recommendations for the best way to handle hatchlings in cases where there was no option for release immediately after emergence from the nest. In this way, our results will contribute to sea turtle conservation by informing retention protocols at hatcheries worldwide.

Materials and Methods

Study site and egg collection.—We patrolled Kochi Beach (33°28′N, 133°30′E), central Kochi Prefecture, Shikoku Island, western Japan, in the early morning from early May to early August between 2016 and 2019. When we discovered the track of a nesting female, presumably left the previous night, we located the egg chamber and excavated the entire clutch to quantify clutch size (i.e., number of eggs in the nest). We then randomly selected 40–60 eggs to be used in our experiments and transferred the remaining eggs to a hatchery near the beach. We collected eggs from only one nest each day (Table 1). We placed the experimental eggs on a wet egg crate made of paper inside a plastic box (45 × 25 × 20 cm) and covered them with a wet towel. We then transported the eggs gently by car to the laboratory of the Usa Marine Biological Institute, Kochi University, approximately 7 km away from the beach. We transported eggs from the beach to the laboratory within approximately 1.5 h of discovery.

Egg incubation.—We maintained the eggs in separate groups (one group for each nest) in a digital incubator (Reptile 90 Pro, Rcom, Yangpyeong, South Korea) set at a constant temperature (29–30° C) and humidity (90%). Conditions in the incubator were dark, with a light-shielding film attached to the lid. We recorded the incubation temperature hourly using a temperature data logger (HOBO TidbiT v2, Onset, Bourne, Massachusetts, USA). Additionally, we frequently monitored the temperature in the incubator using an infrared radiation thermometer (FLIR TG165, FLIR system, Wilsonville, Oregon, USA) and examined the inside of the incubator using a monocular-type night vision scope (NVMT Spartan 2 × 24, YUKON Advanced Optics Worldwide, Vilnius, Lithuania) to avoid exposure to light.

We defined hatching in the incubator as occurring when the head and one front flipper of an individual protruded from the eggshell (Godfrey and Mrosovsky 1997). As the artificially incubated hatchlings lacked the hatch-to-emergence process of natural nests, we retained the hatchlings in the incubator for a minimum of 96 h (4 d) to simulate the actual course of hatching and emergence from a nest. We determined the incubation period and hatching success of each group of experimental eggs (Table 1).

We defined emergence at the time of 2000 on the fourth day after hatching; this period is estimated to be

Table 1. Data on Loggerhead Turtle (*Caretta caretta*) nests used in our study and the distribution of hatchlings in different retention treatments (DA = under dark and in air, DW = under dark and in water, LA = under light and in air, and LW = under light and in water), with the number of hours of retention listed. The asterisk (*) means all hatchlings from nest 4 were used in an analysis of dehydration. The abbreviations IP = incubation period, Hatch. = hatchling, Aug. = August, Sept. = September, and Oct. = October.

Nest No.	Nest 1	Nest 2	Nest 3	Nest 4	Nest 5	Nest 6	Nest 7
Nesting date	20 July 2016	14 June 2017	19 June 2017	16 Aug. 2019	1 June 2019	10 July 2019	25 July 2019
Clutch size	121	110	153	135	122	135	137
No. eggs collected	60	60	60	60	55	40	40
Hatching date	20 Sept. 2016	11 Aug. 2017	17 Aug. 2017	10 Oct. 2019	27 July 2019	30 Aug. 2019	14 Sept. 2019
IP (days)	62	58	59	55	56	51	51
Hatch. success (%)	26.7	45.0	40.0	43.0	47.2	65.0	62.5
No. hatchlings							
LW	3	3	3		1	1	1
DW24	3	3			1	1	1
DW48		3	3		2	1	2
DW72			3		1	2	2
LA24	3	3			2	2	1
LA48		3	3		2	2	2
LA72		3			2	2	2
DA24	3	3			2	2	1
DA48		3	3		2	2	2
DA72			3	12*	2	2	2

enough time for the carapaces of hatchlings to straighten (Godfrey and Mrosovsky 1997). At emergence, we removed all hatchlings from the incubator and measured the straight carapace length (SCL; mm) and body mass (BM; g) under exposure to light (approximately 300–550 lux). We completed all measurements within 15 min and transferred hatchlings to the retention incubator or tank in the next room. Based on the BM of all hatchlings from each nest (16–27 individuals), we selected between 12–24 average-sized individuals and evenly distributed them between 4–10 experimental treatments (Table 1). We marked the carapace of each individual with a unique number using a white magic marker pen to identify them throughout each experiment.

Experimental treatments.—We exposed hatchlings to four retention conditions: (LW) under light and in water, (DW) under dark and in water, (LA) under light and in air, and (DA) under dark and in air. For light conditions, we exposed hatchlings to a 12 h/12 h light/dark cycle (on at 0700 and off at 1900) using overhead fluorescent lamps set 2 m above the tank water or incubator floor surface (approximately 300–550 lux). For dark conditions, we used blackout curtain to shut out all light. For water conditions, we held hatchlings in a plastic tank (up to three individuals per 70 × 40 × 30 cm tank) with seawater maintained at 28° C using a thermostat (model NX003, Gex Corporation, Higashi-Osaka, Japan) and heater (model SH 220, Gex Corporation). For air conditions, we kept hatchlings in an incubator (up to three individuals per 35 × 18 × 6 cm) maintained at 28° C and 90% humidity, with a transparent lid and a sand floor to make it possible to crawl.

We regarded LW as the control treatment. For the 12 hatchlings in the LW treatment, we performed the first swimming trial (see below) immediately after emergence at 2000, 1 h after switching to dark portion of the 12/12 photoperiod (1900), then again at the same time every 24-h interval for the next 144 h (Table 2). Because the experimental conditions in LW were the same as those used in the swimming experiments (in water and in light with a 12/12 photoperiod), it was unnecessary to have multiple groups of hatchlings that were retained in LW conditions for 24, 48, and 72 h. Instead, we performed swimming trials at three additional 24-h intervals after 72 h to compare the values with those from other experimental groups that took the same time after emergence (Table 2).

For DW, LA, and DA, we retained different sets of 8–12 hatchlings under these specific conditions for 24, 48, and 72 h after emergence prior to initiating swimming trials. For these nine experimental treatments (DW24, DW48, DW72, LA24, LA48, LA72, DA24, DA48, and

Table 2. Time lapse flow of Loggerhead Turtle (*Caretta caretta*) hatching, emergence, retention, and swimming thrust trials. Each experiment is represented by one row. Acronyms for the conditions (Cond.) of the experiments are DA = under dark and in air, DW = under dark and in water, LA = under light and in air, LW = under light and in water, with the hours of retention listed. The abbreviation S = the start of swimming thrust measurements and the numbers in each row indicate the time lapse after the first swimming thrust measurement.

	Time Lapse after Emergence (h)						
Cond.	0	24	48	72	96	120	144
LW	S	24	48	72	96	120	144
DW24		S	24	48	72		
DW48			S	24	48	72	
DW72				S	24	48	72
LA24		S	24	48	72		
LA48			S	24	48	72	
LA72				S	24	48	72
DA24		S	24	48	72		
DA48			S	24	48	72	
DA72				S	24	48	72

DA72), we performed swimming trials at four 24-h intervals (0, 24, 48, and 72 h) following their respective retention period (24, 48, or 72 h). After each round of swimming trials, we kept hatchlings from each retention condition in separate plastic tanks (70 × 40 × 30 cm) filled with seawater to a depth of 20 cm under the above-mentioned LW condition until their next swimming trial. During retention periods and swimming trials, we did not feed the hatchlings. Following the completion of all experiments, we released all hatchlings off Tosa Bay using a boat.

Swimming trials.—To quantify swimming performance, we measured swimming thrust using a force data accumulation system using a Power Lab 8/35, Pod Expander FE305, Force Transducer MLTFO 50/ST (AD Instruments, New South Wales, Australia), programmed to record the force 40 times per second (Burgess et al. 2006; Saito et al. 2019). We connected eight force transducers to the system so that the swimming thrust of up to eight individuals could be measured at one time (Fig. 1). We calibrated each transducer by weighing the known mass sinker before each trial to calculate the exact force in millinewton (mN). A harness made from a hookless rig for Ayu (Sweetfish; *Plecoglossus altivelis*) fishing (Fig. 1) was fitted to the hatchlings and connected to the force transducer using PE line (BASS SUPER PE LINE 56lb#5, SUNLINE, Iwakuni, Japan), as it was more lightweight and less stretchable than nylon thread (Fig. 1). The total wet weight of the harness and connected PE line was < 1.5 g. We adjusted the string length so that the hatchling could swim freely without touching

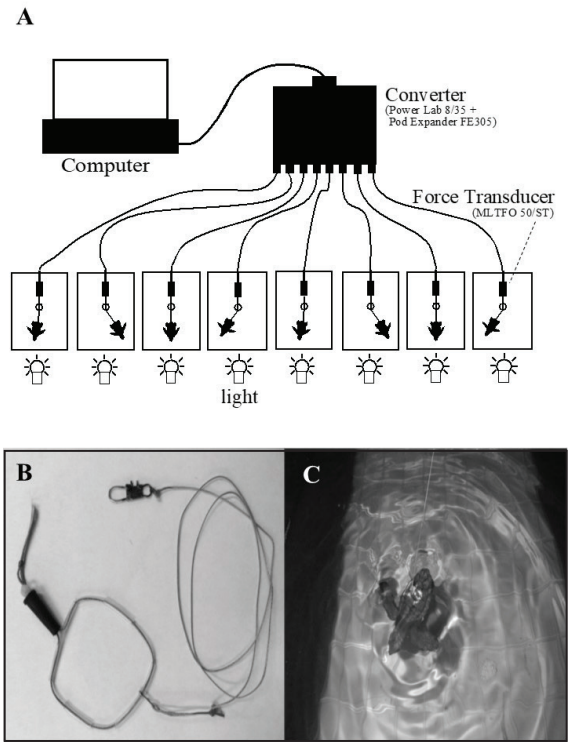

Figure 1. (A) Schematic diagram of experimental set-up to measure the swimming thrust in Loggerhead Turtle (*Caretta caretta*) hatchlings. (B) Harness made from a hookless rig. (C) Tethered Loggerhead Turtle hatchling with a harness. (Modified from Fujimoto et al. [2018] with the permission of Kuroshio Science, Kochi, Japan).

the walls or the bottom of the tank. The force of all directions was transferred to a vertical force.

For each swimming trial, we placed a tethered hatchling in an experimental plastic tank (70 × 40 × 30 cm) filled with seawater to a depth of 20 cm. We painted all sides of the tank black except for one side, where we placed a light (fluorescent lamp, 27 W, approximately 2,400–2,600 lux) to guide the hatchlings to swim in one direction. We kept the room temperature at 28° C using an air conditioner and maintained the water temperature of the experimental tank at 28° C using the thermostat and heater. We conducted swimming trials at 2000 in a dark room. We ran each experiment for 20 min, with the first 10 min regarded as acclimation time, and then we collected a total of 3 min of data during the following three 1-min intervals: 0–1 min, 5–6 min, and 9–10 min. We calculated the mean thrust (mN) by averaging all 7,200 sampling points during the three 1-min sampling periods.

Analysis of dehydration.—According to Bennett et al. (1986), hatchlings can become dehydrated when retained in air (light or dark). To quantify hatchling dehydration during retention, we held 12 hatchlings from nest 4 under DA conditions shortly after emergence

Table 3. Number, size, and swimming thrust (millinewtons; mN) of Loggerhead Turtle (*Caretta caretta*) hatchlings exposed to different retention conditions. Acronyms for the conditions (Cond.) of the experiments are DA = under dark and in air, DW = under dark and in water, LA = under light and in air, LW = under light and in water, with the hours of retention listed. Abbreviations are BM = body mass (g), and SCL = straight carapace length (mm). Data are shown as mean ± standard error.

		Size		Swimming thrust (mN)						
				Time lapse (h) after emergence						
Cond.	No. of hatchlings	SCL (mm)	BM (g)	0	24	48	72	96	120	144
LW	12	41.0 ± 0.4	15.0 ± 0.4	32.0 ± 2.7	11.5 ± 2.8	11.8 ± 2.3	9.9 ± 2.3	10.1 ± 2.7	14.3 ± 3.6	20.3 ± 2.9
DW24	9	41.1 ± 0.5	15.2 ± 0.6		15.5 ± 3.3	9.4 ± 2.9	11.3 ± 3.3	15.2 ± 3.7		
DW48	11	41.4 ± 0.5	14.8 ± 0.6			18.9 ± 3.3	10.9 ± 3.1	14.2 ± 2.7	8.1 ± 2.3	
DW72	8	41.5 ± 0.6	15.4 ± 0.4				17.6 ± 4.3	12.1 ± 4.1	16.5 ± 3.7	14.8 ± 3.4
LA24	11	41.4 ± 0.6	15.7 ± 0.5		31.4 ± 3.7	12.6 ± 3.0	15.3 ± 3.7	17.8 ± 3.1		
LA48	12	41.4 ± 0.5	15.2 ± 0.5			28.6 ± 3.1	6.3 ± 1.0	9.7 ± 2.1	9.7 ± 1.7	
LA72	9	41.7 ± 0.4	15.8 ± 0.6				28.2 ± 5.0	10.9 ± 3.7	12.5 ± 3.4	14.4 ± 3.8
DA24	11	40.7 ± 0.6	15.7 ± 0.6		33.3 ± 3.7	18.7 ± 3.9	12.3 ± 2.6	13.2 ± 3.2		
DA48	12	41.4 ± 0.5	15.3 ± 0.5			32.2 ± 3.0	13.7 ± 2.9	17.1 ± 2.5	18.5 ± 2.3	
DA72	9	42.4 ± 0.4	16.1 ± 0.5				37.9 ± 4.2	13.2 ± 2.9	12.7 ± 2.2	15.2 ± 3.0

and measured the initial BM. After retention for 24, 48, and 72 h, we measured BM of each hatchling and calculated the weight loss by comparing pre- and post-retention BMs.

Statistical analysis.—After the confirmation of normality and homoscedasticity, we performed a one-way analysis of variance (ANOVA) for the objective variables mean thrust for swimming thrusts and BM for the dehydration test. For each retention condition (DW, LA, or DA), we compared mean thrusts between the first measurements during the trial of each retention period (24, 48, or 72 h) and the first measurements of LW. We tested for changes in BMs between each 24-h interval (0, 24, 48, and 72 h) using Fisher's Least Significant Difference method for subsequent multiple comparisons among the variables. We used Excel Statistics 2012 software package for Windows (Social Survey Research Information, Tokyo, Japan) and set the level of significance at $\alpha = 0.05$. Averages are shown as ± 1 standard error (SE).

Results

Comparison of swimming thrust.— Body size was not significantly different among hatchlings in different treatments (SCL: $F_{9,94} = 1.458$, $P = 0.194$, BM: $F_{9,94} = 1.004$, $P = 0.452$; Table 3). For hatchlings retained in the LW (control), LA24, LA48, LA72, DA24, DA48, and DA72 treatments, fluctuations in mean thrust were highest during the first swimming trial (range, 28–38 mN; Fig. 2, Table 3). For these treatments, the mean thrusts measured during all subsequent 24-h intervals (range, 6–20 mN) were lower than the mean thrusts measured during the first trial. Mean thrust of hatchlings placed in water immediately after emergence (LW; control) was not significantly different from the first swimming trial

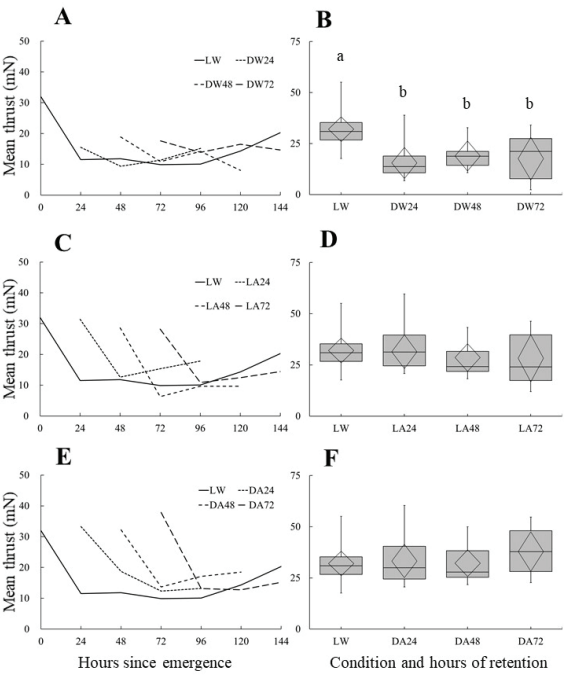

Figure 2. Fluctuations (A, C, E) and 0 h values (B, D, F) of mean swimming thrust for hatchling Loggerhead Turtles (*Caretta caretta*) following different retention conditions and durations. (A, B) Hatchlings retained in DW (under dark and in water) for 24, 48, and 72 h (DW24, DW48, and DW72) compared to hatchlings retained in LW (under light and in water). (C, D) Hatchlings retained in LA (under lights and in air) for 24, 48, and 72 h (LA24, LA48, and LA72) compared to hatchlings retained in LW. (E, F) Hatchlings retained in DA (under dark and in air) for 24, 48, and 72 h (DA24, DA48, and DA72) compared to hatchlings retained in LW. (B, D, F) The three horizontal lines of the box plots represent quartiles (25%, 50%, and 75% of the distribution) and the vertical lines represent the range. The rhombus is the mean value for each box plot. Difference in letters denotes statistically significant difference at $P < 0.05$ based on Fisher's LSD multiple comparison test following one-way ANOVA.

for hatchlings retained in LA for 24, 48, or 72 h ($F_{3,40}$ = 1.411, P = 0.269) or DA for 24, 48, or 72 h ($F_{3,40}$ = 0.592, P = 0.624). For hatchlings retained in the DW24, DW48, and DW72 treatments, mean thrusts measured during the first swimming trial (range, 15–19 mN), as well as all subsequent 24-h intervals (range, 8–17 mN), were comparable to the swimming thrust measured after the first trial in the other treatments (range, 6–20 mN). Mean thrust of hatchlings placed in water immediately after emergence (LW; control) was significantly higher than the first swimming trial for hatchlings retained in DW for 24, 48, and 72 h ($F_{3,35}$ = 5.686, P = 0.003).

Analysis of dehydration.—The mean BM of the hatchlings from nest 4 (n = 12) was 16.4 ± 0.3 g shortly after emergence. After 24, 48, and 72 h of retention in DA conditions, the mean BM of these same hatchlings were 16.9 ± 0.3 g, 16.5 ± 0.2 g, and 16.2 ± 0.3 g, respectively. Hatchlings retained in DA with 90% humidity for up to 72 h did not show significant loss in BM ($F_{3,41}$ = 0.754, P = 0.525), suggesting that no dehydration had occurred.

Discussion

Comparison of swimming performance.—The mean swimming thrust of hatchlings retained in air (light or dark) for up to 72 h were not significantly lower than hatchlings placed in water immediately after emergence (LW; control). In each series of swimming trials for LW, LA and DA, hatchlings displayed their highest swimming performance during their first trial either directly after emergence (LW) or following periods of retention in air (LA, DA), then displayed lower performance during all subsequent trials over the next 72 h. This behavior is likely representative of the swimming performance exhibited during an initial swim frenzy period within the first 24 h upon reaching the sea followed by a post-frenzy period of decreased swimming activity once hatchlings reach safer waters offshore (Salmon and Wyneken 1987). These results suggest that the onset of the swim frenzy phase is flexible. Because the period between hatching/emergence and hyperactive crawling and swimming varies naturally in response to nest microhabitat conditions (e.g., depth, degree of compactness, water content) (Godfrey and Mrosovsky 1997), retaining hatchlings in air may simply delay this frenzy period without effecting hatchling performance. From a management perspective, our results suggest that it is possible to retain hatchlings for up to 72 h in air (light or dark) without detrimentally affecting the swim frenzy phase that is critical to hatchling survival.

These results are somewhat different than previous studies on Green Turtles. Pilcher and Enderby (2001) and van de Merwe (2013) found that locomotory performance (swimming speed and crawling speed, respectively) significantly decreased after 6 h of retention in a hatchery (dry and dark conditions); however, our study concluded that swimming performance of Loggerhead hatchlings did not significantly decrease even after 72 h of retention in air (light or dark). Regarding this difference, in our study, the longer retention time from 24 to 72 h exceeds the time tested by Pilcher and Enderby (2001). In this study, the authors hypothesized that the swimming speed was attenuated several hours after emergence because of the fatigue of constantly moving after emergence. We confirmed that the hatchlings rested during retention in our study, however, and it seemed possible for them to recover their physical strength afterward.

Contrary to our results for hatchlings retained in air, hatchlings retained in water for as little as 24 h failed to display swimming performance that was indicative of the swim frenzy behavior. Hatchlings retained in DW24, DW48, and DW72 displayed lower swimming performance during all trials following retention, comparable to the lower post-swim frenzy performance of the other treatments. These results are consistent with Mejías Balsalobre and Bride (2016) and Okuyama et al. (2009), in which the locomotory performance and dispersal movements of Green Turtle hatchings were diminished following retention in water. Wyneken (1997) found that the swim frenzy occurs shortly after hatchlings enter the seawater. Hatchlings in our study therefore likely entered their swim frenzy period shortly afterward being placed in water, which then did not occur again throughout retention and measurement periods. These results suggest that when hatchlings are retained in water, the swim frenzy period is spent in confinement and may be subsequently unattainable following their release into the sea. Hatchlings retained under these conditions are therefore more likely to not reach offshore developmental habitats and/or fall prey to nearshore predators. These results indicate that sea turtles raised in hatcheries for either management or education should never be retained in water, which is consistent with established hatchery management guidelines (Mortimer 1999).

Considerations for retention of hatchlings.—Mortimer (1999) suggested that hatchery management guidelines should state that hatchlings should not be kept in water before release. Indeed, the results of this study support this important guideline. When hatchlings from artificial hatcheries or incubators must be temporarily stored, it is better to keep them in humid air to prevent the initiation of the swim frenzy phase and limit dehydration.

The Loggerhead hatchlings were reported to lose 12% of their BM based on the maximum at emergence (Bennett et al. 1986). The hatchlings of Olive Ridley

Turtles (*Lepidochelys olivacea*) experienced significant body mass loss as well; 12% of initial BM in 4 d between pipping and emerging at the sand surface (Clusella Trullas et al. 2006). In the present study, retaining Loggerhead hatchlings for 72 h in air showed no significant reduction in BM. In air with 90% humidity, it was concluded that no dehydration occurred for at least 72 h after emergence.

Another consideration for retaining sea turtle hatchlings is their limited energy stores, where the animals must rely on stored egg yolk until they reach the nursery areas of the sea and begin to feed. Retention for several days may deplete these yolk stores. Clusella Trullas et al. (2006) indicated that the resting hatchlings of Olive Ridley Turtles had a metabolic rate of 1.95 kilojoule (kJ) d^{-1}, which is about 20% compared to the value of 9.88 kJ d^{-1} while digging out the sand. Although we have no idea what ratio of egg yolk energy consumption to water loss occurred after 72 h of retention, it seems that the hatchlings from the incubator, which had not experienced emergence, had little energy consumption or water loss. Even if they are temporarily dehydrated, given the results of the swimming performance tests, it does not seem to adversely affect their swimming performance.

Nevertheless, they would consume energy for basic metabolism during the maintenance period. Leatherback Turtles (*Dermochelys coriacea*) emerged with 75–90 kJ of energy in the residual yolk for growth and activity, whereas Olive Ridley Turtles emerged with 45 kJ (Jones et al. 2007). These energies are stored for their dispersal migration from the shore. Clusella Trullas et al. (2006) estimated that, if hatchlings of Olive Ridley Turtles swim at frenzy levels, they can rely on the energy from yolk reserves for only 3–6 d once they reach the ocean.

Any retention may therefore reduce the possibility that hatchlings reach the open ocean. As the previous studies suggested (Mortimer 1999; Wyneken 2000; Pilcher and Enderby 2001; van de Merwe et al. 2013; Mejías Balsalobre and Bride 2016), the best way to treat hatchlings is not to retain them for any period, but instead to release them to the sea immediately after they emerge from their nest to facilitate natural migration and increase their survival. If, however, for any reason hatchlings cannot be released immediately after emergence, our results provided a basis for doing so without significantly affecting their swim frenzy period and hopefully their likelihood of survival.

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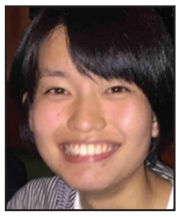

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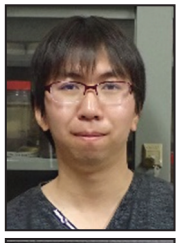
Yosuke Kobayashi was a graduate student at the Usa Marine Biological Institute, Kochi University, Tosa, Japan. He was engaged in conservation for Loggerhead Turtles mainly on Kochi Beach, and in research on the biology and ecology of Loggerhead Turtles as a core member of the laboratory. He studied the state of the environment at the Loggerhead Turtle nesting beaches in Kochi Prefecture. (Photographed by Tomomi Saito).



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Yoshinori Kumazawa was a leader of Haruno no Shizen wo Mamoru Kai, the local citizen association that was engaged in conservation activities for Loggerhead Turtles, mainly on Kochi Beach in Kochi-shi and Tosa-shi, central Kochi Prefecture. (Photographed by Tomomi Saito).

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