



Effect of noise on sand digging and emergence activities in green turtle (*Chelonia mydas*) hatchlings

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ABSTRACT

Anthropogenic noise affects animal behavior and physiology. However, relatively few studies have been conducted on the effects of noise on beach-associated animals. This study evaluated the effects of noise on sound emission, sand digging activity, and emergence from the sand surface by green turtle (*Chelonia mydas*) hatchlings. Acoustic recorders and infrared cameras were used to investigate the behavior of hatchlings in sand-filled chambers under three acoustic conditions: 1) a control treatment, which was silent throughout the experiment; 2) an environmental noise treatment, which exposed the hatchlings to white noise (WN) at a sound pressure level (SPL) approximately 13 dB higher than that of the control, which is similar to that of a natural beach; and 3) a loud noise treatment, which exposed them to WN at an SPL approximately 25 dB higher than that of the control. The WN ranged from 0 to 4 kHz with a repeating cycle of 30 min sound emission and 2 h 30 min silence. Sound emissions from green turtle hatchlings were detected; however, the amount of sound emissions was too small to discuss their ecological significance. The time to emergence and total number of digging bouts in the 24 h pre-emergence periods were not significantly different among treatments; however, the number of digging bouts was significantly higher during loud noise exposure. The response to loud noises may play a role in allowing hatchlings to escape from threats and/or coordinate synchronous behavior among multiple individuals. However, because the number of digging bouts decreased after loud noise exposure, prolonged exposure to loud noise may have detrimental effects on hatchlings, such as depletion of the energy reserves available to the hatchlings. Thus, anthropogenic noise on or around beaches and its effect on animals should be carefully considered.

1. Introduction

Anthropogenic noise may cause stress that can affect both the behavior and physiology of animals (Potvin, 2017; Kunc and Schmidt, 2019; Duarte et al., 2021). Therefore, masking auditory communication using vocalizing animals has become an important research area (Barber et al., 2010; Erbe et al., 2016; Grade and Sieving, 2016). In addition, the survival and reproduction of animals may be strongly affected by fleeing acoustically degraded habitats and compromised responses to signals from predators or prey (Potvin, 2017; Duarte et al., 2021). Furthermore, the effects of noise on underwater aquatic animals have recently attracted considerable research attention because sound propagates faster and farther under water (Duarte et al., 2021). In contrast, few studies have been conducted on beach animals that are potentially

affected by noises, such as noise caused by motorboats (Chan et al., 2010; Schlacher et al., 2016) and construction related to beach nourishment (Speybroeck et al., 2006).

Beach habitats are important for nesting and embryonic development in sea turtles. After eggs are deposited and have hatched in the sand, sea turtle hatchlings synchronously dig upwards and emerge on the sand surface (Rusli et al., 2016; Nishizawa et al., 2021). Synchrony in hatching and emergence from nests has been widely observed in Testudines (Doody, 2011; Spencer and Janzen, 2011). Thus, synchrony is potentially stimulated by environmental cues, such as temperature changes, vibrations (Doody et al., 2012), and sibling vocalizations (Riley et al., 2020; Lacroix et al., 2022). Sea turtle hatchlings are known to emit sounds that may stimulate synchronous behavior (Ferrara et al., 2014a, 2014b, 2019; Monteiro et al., 2019), although at least some of the sound

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emissions are considered byproducts of other processes such as hatchling movement (McKenna et al., 2019; Field et al., 2021). Nonetheless, the effects of the acoustic environment on sea turtle digging activity should be studied further because anecdotal observations indicate that bursts of hatchling activity are induced by jet noises (Balazs and Ross, 1974). We hypothesized that the acoustic environment of sea turtle hatchlings is important for their coordinated digging and emergence.

In this study, we evaluate the effects of white noise, a random mixture of sound waves with equal intensities, on sound emission, digging activity, and the emergence of sea turtle hatchlings on a sand surface. The auditory sensitivity of sea turtles indicates that acoustic stimuli are important cues in terrestrial environments (Piniak et al., 2016). Holtz et al. (2021) reported that the effect of anthropogenic noise on seafinding behavior after emergence from the nest was not significant, but its effect on underground activity remains unclear. We hypothesized that (1) the number and/or characteristics of sound emissions would change when they are masked by loud noise if sound emissions are important for communication; (2) digging would be activated by loud noise as a fleeing response or induced synchronous behavior; and (3) activated digging behavior would result in early emergence from the sand surface.

2. Materials and methods

2.1. Experimental set up

Green turtle eggs from six clutches were collected in August and September 2022, after incubated in situ for approximately 40 d from egg deposition at Omura Beach, Chichijima Island, Ogasawara, Japan, and then transferred to the Ogasawara Marine Center with permission from the Tokyo Metropolitan Government. When at least one of the eggs in the clutch was pipped, 60 eggs from each clutch were separated into three chambers (20 eggs each) made from black compost containers (diameter ~ 61 cm, height ~ 73 cm), which were then covered with a sound-proof sheet (940SS, Daiken Corporation) (Fig. 1).

Each chamber was separated into two compartments by using a glass plate at the midline. Beach sand was used to fill one of the compartments up to 50 cm, and 20 eggs were placed in the sand. The top of the eggs was 20 cm deep. An infrared camera (SecuSTATION SC-PX85, Sinec Inc.) was set up in the adjacent compartment, a speaker (SoundCore mini, Anker

Japan Co., Ltd.) was placed above the sand at the lid height, a microphone (AT9905, Audio-Technica Corporation) was placed at a depth of 10 cm into the sand, and a linear PCM recorder (TASCAM DR-5, Teac Corporation) was connected to the microphone and placed outside the chamber. The sound and camera were recorded until the hatchlings emerged on the sand surface.

One of the three chambers was used for loud noise treatment, one for environmental noise treatment, and one for control treatment. The speakers in the loud noise and environmental noise treatment chambers emitted white noise ranging from 0 to 4 kHz, covering the auditory range of green turtle hatchlings (up to 2 kHz; Rigway et al., 1969) with a repeating cycle of 30 min sound emission and 2 h 30 min silence. The control chamber remained silent throughout the experiment. The sound pressure level (SPL) for white noise in the environmental noise treatment was similar to that of waves on a beach (i.e., the SPL that hatchlings generally experience on natural beaches) and was approximately 13 dB higher than that of the control (Supplementary Fig. 1). The SPL for the loud noise treatment was approximately 25 dB higher than that for the control and masked sounds of 0–4 kHz (Supplementary Fig. 2). We evaluated the effects of noise by comparing the loud noise treatment with control and/or environmental treatments.

The body weight of the hatchlings (g) was determined generally in the next morning after all hatchlings had emerged. They were then released on the beach at night to complete their crawling into the ocean.

2.2. Data analysis

Hatchling emergence on the sand surface was determined using audio and video recordings and the time to emergence was calculated from the start of the experiment to emergence. We focused on sound emissions and digging bouts within 24 h before emergence when all hatchlings had finished pipping and exiting eggshells. Type I sounds in Ferrara et al. (2014a), harmonic and nonharmonic bands with frequency modulation (Supplementary Fig. 3), were counted as hatchling sound emissions from audio recordings. Hatchling digging bouts were defined as the synchronous digging activity of multiple hatchlings that resulted in wideband sound events (Nishizawa et al., 2021), and were counted using video and audio equipment. First, by using both video and acoustic data, we confirmed that wideband sound events acoustically defined by Nishizawa et al. (2021) (Supplementary Figs. 1 and 2) were generated by the synchronous digging activity of multiple hatchlings. Then, digging bouts were counted using either video or acoustic data (e.g., using acoustic data when the video recording was not clear, and video data when loud noise was exposed or when the audio recording was accidentally stopped).

R version 4.1.3 (R Core Team, 2022) was used for statistical analyses. First, differences in the total numbers of hatchling sound emissions and digging bouts over 24 h among the treatments were analyzed using negative binomial generalized linear mixed modeling (GLMM) that incorporated the treatment as an explanatory variable and the clutch as a random variable. In the former analysis, sound emissions during loud noise exposure could not be counted because of masking and the duration of the analyzed periods (i.e., 24 h in the control and environmental noise treatments, and reduced noise exposure periods from 24 h in the loud noise treatments) was used as the offset term. In addition, to understand whether the number of digging bouts changed only during the noise exposure periods, the total numbers of digging bouts during quiet periods when noise was not emitted was compared among the treatments. Negative binomial GLMM was used in this analysis, and incorporated the duration of the quiet periods (i.e., 24 h in the control treatment, and reduced noise exposure periods from 24 h in the environmental noise and loud noise treatments) as the offset term. These analyses were performed using the `glmer.nb` function of the `lme4` package (Bates et al., 2015). Negative binomial GLMM was adopted because overdispersion was suggested in the Poisson GLMM. Because the number of hatchling sound emissions was small (see Results), further

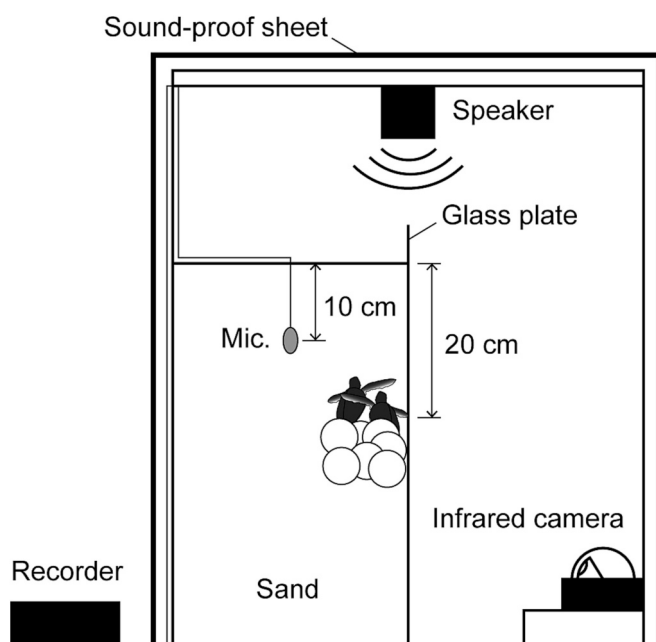


Fig. 1. Experimental setup for the present study.

analyses of hatchling sound emissions were not performed.

We then focused on 1 h 30 min periods that contained 30 min each of pre-noise exposure, noise exposure, and post-noise exposure to environmental and loud noises. The effects of the treatment (environmental noise and loud noise treatments), noise exposure (pre-noise exposure, noise exposure, and post-noise exposure), and their interactions on the number of digging bouts within 30 min were evaluated. Generalized additive mixed modeling (GAMM) was performed using the *mgcv* package (Wood, 2017), and it assumes a negative binomial distribution, considers the time before emergence as a smoothing variable, and the clutch as a random effect.

Finally, differences in time to emergence and body weight among treatments were tested using mixed linear modeling that incorporated the clutch as a random variable using the *lmer* function in the *lme4* package.

In the analyses above, clutches ($n = 6$ in general) were treated as replications and a random variable; however, the number of replications for the environmental and loud noise treatments was five because the recordings were missing owing to a mechanical problem. The *emmeans* package (Lenth, 2023) was used for post-hoc pairwise comparisons between treatments and noise exposure. The significance level was 0.05, but false discovery rate (FDR) adjusted p -values were used to represent statistically significant differences. Data are presented as mean \pm standard deviations.

3. Results

The number of hatchling sound emissions ranged from 0 to 8 (for 24 h), 0 to 4 (for 24 h), and 0 to 2 (for approximately 20 h) in the control, environmental noise, and loud noise treatments, respectively. There were no significant differences in the number of hatchling sound emissions among treatments ($\chi^2 = 0.46$, $df = 2$, $p = 0.793$).

The number of digging bouts within the 24 h pre-emergence period was 116.8 ± 29.1 (control), 122.6 ± 30.8 (environmental noise treatment), and 146.5 ± 34.5 (loud noise treatment) (Fig. 2). There were no significant differences in the total number of digging bouts ($\chi^2 = 3.82$, $df = 2$, $p = 0.148$); however, there were significant differences in the number of digging bouts during quiet periods ($\chi^2 = 16.94$, $df = 2$, $p = 0.0002$) among the treatments. The number of digging bouts during

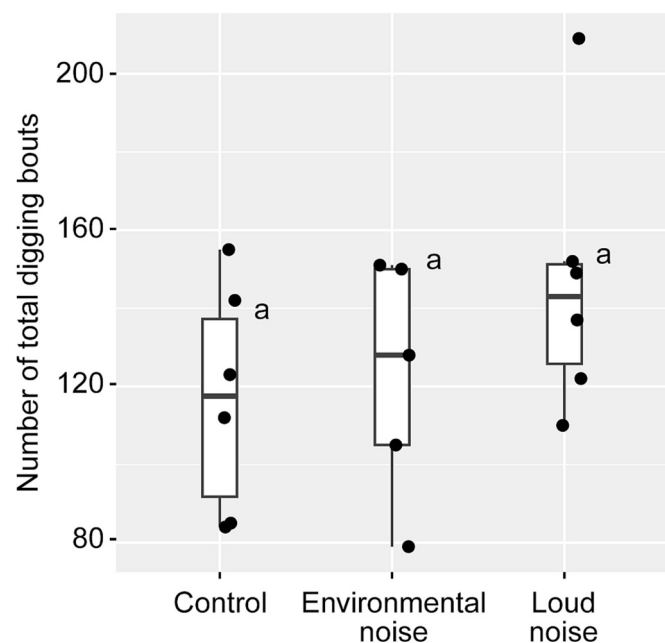


Fig. 2. Total number of digging bouts in 24 h before emergence. Same letters above the boxes indicate statistically non-significant differences.

quiet periods was significantly lower in the loud noise treatment than that in the control ($p < 0.0001$) and environmental noise ($p < 0.0001$) treatments (Fig. 3).

Whereas the number of digging bouts increased as the emergence point became closer ($\chi^2 = 65.83$, $edf = 3.91$, $p < 0.0001$; Fig. 4A), GAMM indicated that digging bouts increased significantly in the loud noise treatment during the noise period ($p < 0.0001$; Fig. 4B). The number of digging bouts in the post-noise period in the loud noise treatment was significantly lower than in the pre-noise and noise periods ($p = 0.0108$ and $p < 0.0001$, respectively), and in all periods in the environmental noise treatment ($p < 0.0001$; Fig. 4B).

The time to emergence was not significantly different among treatments (164.0 ± 20.3 h, 163.9 ± 17.1 h, and 148.0 ± 16.1 h for control, environmental noise, and loud noise treatments, respectively; $\chi^2 = 5.31$, $df = 2$, $p = 0.070$). In addition, there were no significant differences in the hatchling body weights among treatments (23.6 ± 2.0 g, 23.1 ± 1.5 g, 23.7 ± 2.0 g for control, environmental noise, and loud noise treatments, respectively; $\chi^2 = 3.41$, $df = 2$, $p = 0.182$).

4. Discussion

One of the threats posed by anthropogenic noise to vocalizing animals is masking auditory communication (Barber et al., 2010; Erbe et al., 2016; Grade and Sieving, 2016). Sea turtle hatchlings emit sounds (Ferrara et al., 2014a, 2014b, 2019; McKenna et al., 2019; Monteiro et al., 2019), and harmonic and non-harmonic bands with frequency modulation (Type I sound in Ferrara et al., 2014a) were detected in the present study. However, the number of hatchling sound emissions was not significantly different among the treatments and was extremely small compared with the number of digging bouts. Therefore, it is not clear from the results of the present study whether hatchling activities were stimulated by sound emissions, which supports the idea that hatchling sound emissions are not vocalizations for communication (McKenna et al., 2019; Field et al., 2021).

In contrast to sound emissions, the present study clearly showed that hatchling digging activity increased during exposure to loud noises. One possible explanation is an escape response. Escaping from loud noises is a widely observed behavior in aquatic (Pichegru et al., 2017; Duarte et al., 2021; Stasso et al., 2023) and terrestrial animals (Ware et al.,

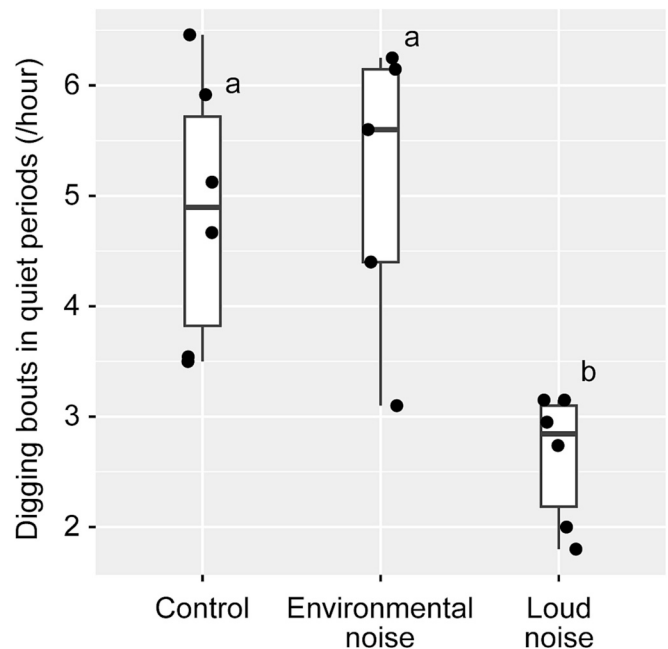


Fig. 3. Number of digging bouts during quiet periods (numbers/h). Different letters above the boxes indicate statistically significant differences.

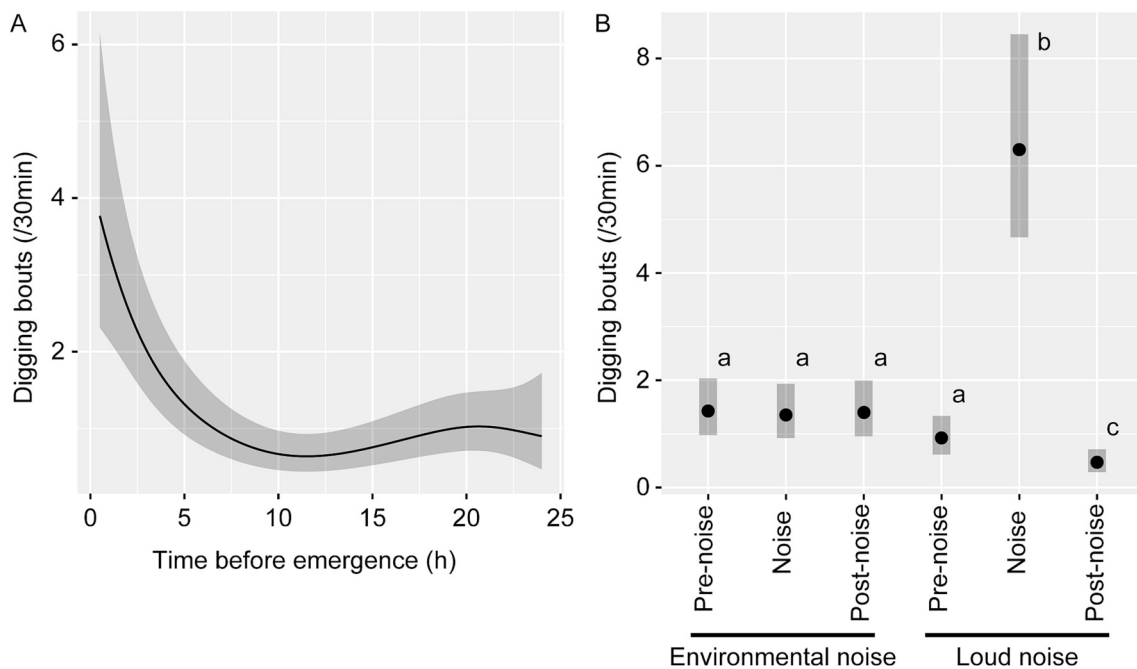


Fig. 4. (A) Effect of time before emergence on the number of digging bouts. The shaded area surrounding the curve indicates a 95% confidence interval. (B) Effect of treatments and noise exposure on the number of digging bouts. Black circles indicate point estimates and shaded bars indicate 95% confidence intervals. Different letters above the bars indicate statistically significant differences.

2015) despite inter-specific or inter-individual differences in responses. For sea turtle hatchlings near the sand surface, promoting emergence from the nest in response to potential noise by approaching or digging predators, such as wild boars and monitor lizards (Sasai et al., 2016; Rusli et al., 2020), may help improve survivability.

The observed response may also play an important role in coordinating the synchronous behavior of multiple individuals. The digging activity by sea turtle hatchlings in sand is synchronously coordinated by multiple individuals (Balazs and Ross, 1974; Rusli et al., 2016; Nishizawa et al., 2021). However, it is unclear how this synchrony is coordinated, although one potential cue is a physical stimulus or vibrations produced by the actions of a neighboring individual (Doody et al., 2012). In addition to physical stimuli, acoustic stimuli, such as sounds caused by digging and sand collapse, may also play important roles in inducing activity. In fact, bursts in hatchling activity have been shown to be associated with jet noise, although it is unclear whether noise or related ground vibrations induced hatchling activity (Balazs and Ross, 1974).

The results showed a clear increase in hatchling digging activity during exposure to loud noises, whereas hatchling digging activity decreased during the post-loud noise period. The activity level was lower than during the pre-noise exposure period and for all periods in the environmental noise treatment. Increased hatchling activity during loud noise exposure may have produced a larger amount of lactate, which would have required longer resting periods (Dial, 1987). This potential explanation for the differences in digging activity was also supported by the significant decrease in digging bouts during quiet periods in the loud noise treatment; however, no significant difference in total digging bouts in the 24 h pre-emergence period resulted in no significant differences in hatchling body weights and time to emergence.

An acoustic cycle of 30 min sound emission and 2 h 30 min silence was used in the present study to avoid acclimation to noise exposure for ethical reasons. No significant differences were observed in time to emergence or body weight among the treatments. However, prolonged exposure to noise may have detrimental effects on hatchlings. Falsely induced digging bouts may deplete energy reserves in the residual yolk, which are mostly used during nest escape (Clusella Trullas et al., 2006;

Rusli et al., 2016). This depletion could result in hatchlings becoming vulnerable to starvation during the subsequent off-shore migration phase (Booth, 2017).

The effect of underwater noise on sea turtles has become a major concern and has been investigated previously (Samuel et al., 2005; Nelms et al., 2016). The general disturbance of nesting sea turtles caused by anthropogenic noise is also a major concern (Wilson and Tisdell, 2001). The present study indicates that anthropogenic noises on or around beaches may alter the behavior of hatchling sea turtles. White noise, which contains a wide range of frequencies, was used in this study; therefore, the specific acoustic signals that induce digging activities should be studied further. However, anthropogenic noises in or around beaches, such as that produced by motorboats (Chan et al., 2010; Schlacher et al., 2016) and beach nourishment (Speybroeck et al., 2006), should be carefully considered.

5. Conclusion

This study detected sound emissions produced by green turtle hatchlings; however, the amount of hatchling sound emissions was too small to discuss their ecological significance. In contrast, the loud noise treatment increased the number of digging bouts exhibited by green turtle hatchlings. Responses to loud noises may play a role in their ability to escape predators and other threats, and/or coordinate the synchronous behavior of multiple individuals. However, there is a concern that prolonged exposure to loud anthropogenic noise may deplete the energy reserves available to hatchlings. Anthropogenic noise in or around beaches and its effects on animals should be further investigated.

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CRediT authorship contribution statement

Yuya Maeda: Conceptualization, Investigation, Formal analysis, Writing – review & editing. **Hideaki Nishizawa:** Conceptualization, Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Satomi Kondo:** Investigation, Resources, Writing – review & editing. **Tomoatsu Ijichi:** Investigation, Writing – review & editing. **Kotaro Ichikawa:** Conceptualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Dataset and R script are available in the supplementary materials.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jembe.2023.151974>.

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