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Research article

Nest relocation of Leatherback turtles (*Dermochelys coriacea*) decrease the rate of non-developed eggs

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Abstract

Female leatherback sea turtles (*Dermochelys coriacea*) often oviposit in locations with high risk of predation, human activity, flooding, or erosion; thus, influencing hatching rates. It has been hypothesized that the relocation of these nests would significantly increase hatching success. In this study, we measured various nest traits to determine whether nest relocation has any influence on hatching success. Ten parameters of relocated nests (n=8) and nonrelocated nests (n=8) were compared. These parameters included incubation period (days), number of hatched eggs, number of survival hatchlings, number of healthy hatchlings, number of hatchlings in critical care unit (CCU) box, number of stillborn hatchlings, number of non-hatched eggs, number of non-developed eggs, number of embryonic dead hatchlings, and number of eggs without yolks. Poisson distribution, a generalized linear model employing the log link function, was used to compare differences in the rate values of the parameters between relocated and nonrelocated groups. It was found that the rate of non-developed eggs in the relocated nests was significantly lower than in the nonrelocated nests ($P < 0.001$). In conclusion, nest relocation was not detrimental to hatching success and decrease rate of non-developed eggs. Based on these findings, nest relocation may be an effective conservation method for leatherback turtles.

Keywords: Hatch, Lute turtle, Marine turtle, Nest, Turtle egg

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INTRODUCTION

Thailand is home to 5 out of the 7 known species of sea turtles around the world, including leatherback turtles (*Dermochelys coriacea*), green turtles (*Chelonia mydas*), hawksbill sea turtles (*Eretmochelys imbricata*), olive ridley turtles (*Lepidochelys olivacea*), and loggerhead turtles (*Caretta caretta*). Importantly, leatherback sea turtles are the most widely distributed species of sea turtles and are considered one of the largest reptiles (1,000 kg). Their range includes all of the oceans in the world except for the Arctic Ocean (Reina et al., 2002). Generation length was estimated as 30 years (Wallace et al., 2013). *D. coriacea* is listed on CITES Appendix I and classified as a vulnerable species globally on the IUCN Red List of Threatened Species. However, the seven subpopulations vary widely in population and trends. Their numbers have declined due to threats such as fisheries bycatch, direct utilization of turtles or eggs for human use (i.e., consumption, commercial products), human-induced alteration of coastal environments, marine pollution and debris, and climate change (IUCN, 2019; Wallace et al., 2010, 2011).

The life cycle of the leatherback turtle begins with a female turtle laying eggs on a nesting beach. Throughout the nesting process, females stay close to the shore for 3-4 months and make repeated visits at 10-day intervals to set up eggs. The females set up 7 to 11 individual nests per season with 65-115 eggs per nest (Kamel and Mrosovsky, 2004; Tomillo et al., 2009). About 55-60 days after the female has laid eggs, hatchling turtles emerge from their nests, head to the sea, and follow ocean currents to pelagic nursery habitats (Hendrickson, 1980; Kamel and Mrosovsky, 2004). If a turtle finds an unsuitable site for her nest (e.g., illumination by flashlights or flooding into the chamber), she may return to the ocean without laying eggs (Hirth et al., 1993). *D. coriacea* strongly preferred to nest on sections of the beach where sediment composition was primarily sand as opposed to gravel or rocks and non-eroded (Campbell, 2019). Females will spend 3-4 years feeding to build up enough energy to nest again (Roe et al., 2013).

It is important to note that eggs and hatchlings are subject to a number of natural threats (e.g., beach erosion, storm and tidal inundation, native predators, root invasion) and other hazards (e.g., poaching, non-native predators and livestock, coastal development) (Eckert et al., 1999; Conrad et al., 2011). Only 85% of leatherback turtle eggs are viable (Tomillo et al., 2009). The average hatching success rate of leatherback turtle egg clutches (approximately 40 to 60%) is the lowest among all sea turtle species (Miller, 1997). Some studies reported that the low hatching success rate at Playa Grande, Costa Rica was not due to infertility in leatherback clutches, thus implicating other intrinsic factors (e.g. maternal identity, developmental factors, genetics), or extrinsic factors such as nest environmental conditions (Bell et al., 2004). Nest relocation, whether to a hatchery or another secure section of the beach, has been undertaken by many sea turtle monitoring programs to protect nests from threats and ensure successful incubation to hatching (Tiwari et al., 2011; Türkozan et al., 2007). The findings of Dutton et al. (2005) suggest that an increase in the size of the nesting population since 1991 was probably due to an aggressive program of beach protection and egg relocation. This program had actually been initiated more than 20 years ago. Beach protection and egg relocation campaigns provide a simple and effective conservation strategy for the Northern Caribbean nesting practices of the leatherback turtle population.

In Thailand, Phang-nga and Phuket are two important nesting grounds during leatherback sea turtle nesting season (i.e., November through February). The current population is less than 1% of that from 60 years ago. The reported nesting rate falls to about 1.5 nests per year in the last decade (Department of Marine and Coastal Resources, 2020). Nest relocation, therefore, has been one of the conservation techniques. However, there are limited studies on the impact of nest relocation on the hatching success in leatherback turtles (Furler, 2005; García–Grajales et al. 2019; Sieg et al., 2011). The hypothesis of this study is that relocated nests would improve hatching success by increasing hatching rate and survival hatchings; and decreasing unhealthy hatchings, non-developed eggs, embryonic dead hatchlings, and eggs without yolks.

MATERIALS AND METHODS

Sample and nest relocation

In this retrospective study during October 2020 – Febuary 2021, a total of 16 nests of leatherback turtles were included. The study was conducted as part of continuing management strategy on nest relocation of Phuket Marine Biological Center, Department of Marine and Coastal Resources situated in Phuket, Thailand. The nests were divided into two groups: relocated nests (n=8) and nonrelocated nests (n=8). Female turtles came to lay their eggs on 8 specific beaches (Figure 1). Nesting females were identified individuals by measuring the lengths of their body and paddle-like forelimb from the footprints on the ground.

After egg laying, both relocated and nonrelocated nests were marked off with stakes and a sign to keep the area protected so the eggs are safe during the incubation period. HOBO® MX2301 Temperature and Relative Humidity Data Logger was buried inside the nests and sand beside the nests at the same depth. The measurements were done daily through the incubation period. All the nests were monitored until hatching using a closed-circuit camera by the Monitoring Center of Leatherback Turtles, Department of Marine and Coastal Resources.

The relocated nests were nests that were in unsuitable locations i.e., area with the possibility of a flood, coastal erosion, proximity to tree roots, or in order to be in located in an area that could be better managed and monitored by conservation experts. The nest relocation method suggested by Eckert et al. (1999) and Phillott and Godfrey (2020) was used. Briefly, these methods include moving eggs within 24 hours after laying. While nests were relocated from their original site, they were kept within the same beach. The distance of the new nests was less than one kilometer from the original nests. The nests were relocated by the same group of staff that have had experience in nest relocation of sea turtles. The original nests were carefully dug down to top eggs. A foam box with a layer of sand at the bottom was used to transfer eggs to the new nests. The eggs from the original nests were taken immediately, one at a time and without rotating them, to the new nests. The nest depth (to the bottom of the clutch) of the original nests was measured and the nest depth of the relocated nest was the same as for the original nest. Each egg was placed carefully and orderly into the new nest. The number of laid eggs were counted. When all the eggs had been placed in their new nest, they were covered with sand in order to protect them from predators and to incubate them. The incubation period was recorded.

Data collection and statistical analysis

At the date of hatching, eggs of relocated and nonrelocated nests were assessed by a veterinarian. Gross necropsies were performed on stillborn hatchlings. Non-hatched eggs were investigated by size measurement, egg candling, and microscopic examination. The 10 parameters for comparing the nests suggested by Bell (2004) and Irvine et al. (2015) were included as follows:

1. Incubation period (days): the number of days between egg-laying and the time at which the majority of hatchlings emerged from the nest.
2. Number of hatched eggs: the number of eggs that hatchlings were out of egg.
3. Number of survival hatchlings: the number of hatchlings that were alive.
4. Number of healthy hatchlings: the number of survival hatchlings that were healthy (i.e., start moving around and becoming active).
5. Number of hatchlings in critical care unit (CCU) box: the number of survival hatchlings that were unhealthy (e.g., less movement, not active, bent body, or having remaining yolk) and were taken care in a unit box until healthy. In the CCU box, a group of hatchlings was kept in a box with a layer of sand. Temperature was controlled to be not more than 31^{o}C. Hatchlings were hydrated by water spray to lubricate eyes and mucous membranes.
6. Number of stillborn hatchlings: the number of hatchlings that died soon after breaking out of egg.
7. Number of non-hatched eggs: the number of eggs that hatchlings did not out of egg.
8. Number of non-developed eggs: the number of non-hatched eggs that had a yolk but no embryo.
9. Number of embryonic dead hatchlings: the number of non-hatched eggs that had a dead embryo.
10. Number of eggs without yolks or shelled albumin globs (SAGs): the number of non-hatched eggs that were clear and yolkless.

Descriptive data were reported as mean ± standard deviation (SD), range, and a boxplot. The parameters were calculated based on count data adjusted by total laid eggs for each group (relocation or non-relocation). The total laid eggs or population size was defined as offset for the statistical analysis.

Since response variables were included in the data according to Poisson distribution, a generalized linear model in conjunction with the log link function was conducted to compare differences in the rate values of all parameters between relocated and nonrelocated groups. The statistical form of the Poisson model (Dobson and Barnett, 2018) was as follows:

$$\log(y/pop)=\alpha+\beta_1 x_1$$

where y = response variable, α= intercept, β_1= regression coefficient, and x_1= group (1 = A and 2 = B) and pop = total laid eggs (offset)

The *glm* function with Poisson distribution established from R version 4.1.0 was used to perform the analysis. The level of statistical significance was set to $\alpha = 0.05$.

RESULTS

A total of 16 nests of leatherback turtles from 3 known and 1 unknown nesting females were included in this study. Data of temperature and humidity was not complete in every nests so they were not shown in this study. Averages of variables obtained from relocated and nonrelocated groups were as follows: (a) Mean incubation period was 57.06 ± 2.62 (range, 54–64) days; (b) Mean total laid eggs was 112.60 ± 29.66 (range, 36–151); and (c) Hatching success rate was 48.10 ± 26.62 on average (range, 0–76%). Descriptive statistics of incubation period, total laid eggs, and hatching parameters between relocated and nonrelocated groups are shown in Figure 2 and Table 1. Mean incubation period was similar between groups. The relocated nests had higher total laid eggs, number of hatched eggs, number of embryonic dead hatchlings and number of eggs without yolks, but lesser number of non-developed eggs. Only the rate of non-developed eggs in the relocated nests was significantly lower than in the nonrelocated nests ($P < 0.001$) (Table 2).

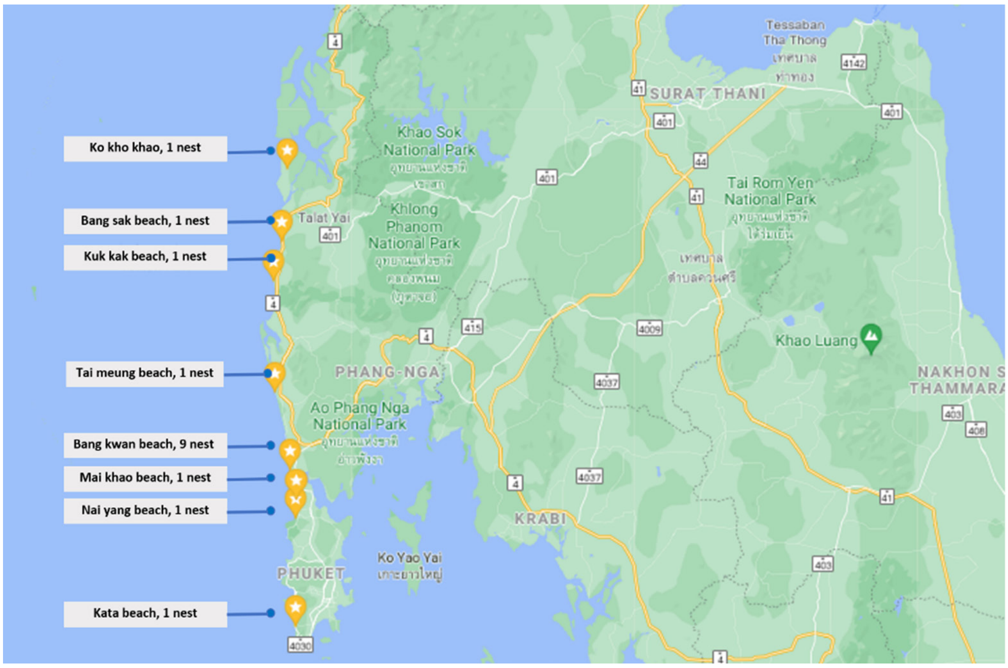

Figure 1 Location of 16 nests of leatherback turtles in Phuket and Phang-nga.

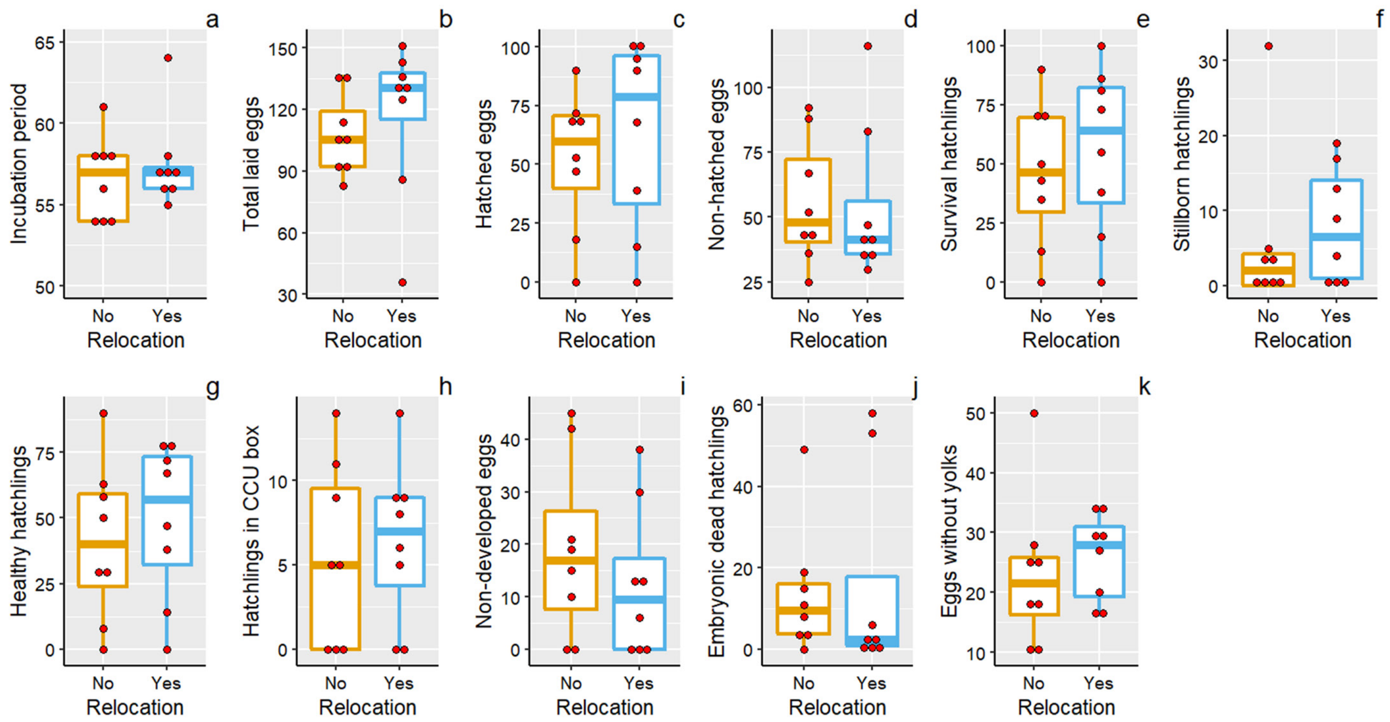

Figure 2 Data are shown on nonrelocated nests (No) and relocated nests (Yes) for the following parameters: incubation period (a), total laid eggs (b), number of hatched eggs (c), number of non-hatched eggs (d), number of survival hatchlings (e), number of stillborn hatchlings (f), number of healthy hatchlings (g), number of hatchlings in critical care unit (CCU) box (h), number of non-developed eggs (i), number of embryonic dead hatchlings (j), and number of eggs without yolks (k).

Table 1 Mean ± standard deviation of incubation period (days), total laid eggs, and hatching parameters between relocated and nonrelocated groups.

Parameters	Nest relocation	
	Yes (N = 8)	No (N = 8)
Incubation period (days)	57.50 ± 2.78	56.63 ± 2.56
Total laid eggs	117.25 ± 38.07	107.88 ± 19.65
Number of hatched eggs	63.50 ± 40.42	52.13 ± 29.95
Number of survival hatchlings	56.50 ± 35	46.50 ± 30.45
Number of healthy hatchlings	49.13 ± 29.83	41.00 ± 29.98
Number of hatchlings in critical care unit (CCU) box	6.38 ± 4.75	5.50 ± 6.38
Number of stillborn hatchlings	8.00 ± 7.62	5.63 ± 10.84
Number of non-hatched eggs	53.75 ± 30.03	55.75 ± 24.37
Number of non-developed eggs	12.50 ± 14.48	19.00 ± 17.02
Number of embryonic dead hatchlings	15.38 ± 24.88	13.63 ± 15.64
Number of eggs without yolks	25.88 ± 7.28	23.13 ± 12.68

Table 2 Poisson distribution, a generalized linear model to compare differences in the rate values of hatching parameters between relocated and nonrelocated groups (reference).

Parameters	Estimate	Standard error	P value
Incubation period (days)	-0.068	0.066	0.304
Number of hatched eggs	0.114	0.066	0.084
Number of survival hatchlings	0.111	0.070	0.111
Number of healthy hatchlings	0.097	0.075	0.193
Number of hatchlings in critical care unit (CCU) box	0.064	0.206	0.755
Number of stillborn hatchlings	0.269	0.195	0.167
Number of non-hatched eggs	-0.120	0.068	0.076
Number of non-developed eggs	-0.502	0.129	<0.001*
Number of embryonic dead hatchlings	0.038	0.132	0.776
Number of eggs without yolks	0.029	0.101	0.774

* Significant using Poisson distribution.

DISCUSSION

Our study found that nest relocation of leatherback turtles, in overall, had no negative effects on hatching parameters. Although no difference of hatching rate between relocated and nonrelocated nests was found in this study, the relocated nests exhibited an advantage in the decrease rate of non-developed eggs. Therefore, moving eggs of leatherback turtles to more favorable locations are encouraged. This outcome is similar to that of previous studies which also recommended relocating the nests of sea turtles (Furler, 2005; Reboul et al., 2021; Wineken et al., 1988). Furler (2005) determined the hatching success of leatherback sea turtles of natural and relocated nests on Gandoca Beach, Costa Rica. Relocated nests showed a similar hatching success as natural nests, indicating that the handling of the eggs, the reconstruction of the nests, and the new nest site selection did not affect their hatching success negatively. Wineken et al. (1988) investigated hatching success between natural and relocated nests of the loggerhead sea turtles located on Jekyll Island, Georgia. Eggs in undisturbed natural nests were found to have lower hatching success than relocated eggs. Relocation was not detrimental to hatching success. The authors suggest that as long as the eggs are not traumatized, are moved early (prior to the establishment of egg membranes) and placed in safe areas, relocating nests is an effective conservation method. More recently, Reboul et al. (2021) determined the effectiveness of natural and artificial shade on reducing green turtle incubation temperature at Chagar Hutang beach, Redang Island, Malaysia. The result showed that incubating eggs in clutches within tree shade does not compromise clutch hatching success and is a viable strategy to reduce clutch temperature and increase the proportion of male hatchlings. The authors recommend relocating nests at high risk of inundation or predation to natural tree shade.

At egg laying, some fertilized eggs of sea turtles could have already died. Moreover, fertile eggs at laying have no external signals of embryonic development (Abella et al., 2017). Incubation temperature influences several factors in sea turtle egg incubation. Most temperature-dependent sex determination turtles produce males at lower incubation temperatures and females at higher temperatures; the effects of global increased thermal fluctuations could accelerate feminization of species (Phillott and Godfrey, 2020; Valenzuela et al., 2019). Besides hatchling sex, incubation temperature affects egg development including development time, size, mass, and amount of yolk content converted to hatchling tissue (Booth et al., 2004; Foley, 1998; Reece et al., 2002). In undeveloped eggs of leatherback turtles, eggshell calcium content decreased by 25.7%. Sand temperature had a significant influence upon the successful development of *D. coriacea* embryos. Low sand temperatures were associated with increased incubation period and slower growth rate (Bilinski et al., 2001). Perhaps, nest relocation may protect eggs from temperature fluctuation during incubation period, which reduced the rate of non-developed eggs in this study. Unfortunately, we cannot prove this assumption because we did not have a complete record of the incubation temperature.

In order to increase hatching rate, not only non-developed eggs should be reduced, but also embryonic dead. Embryonic mortality in sea turtle eggs can occur due to unsuitable or extreme temperature, moisture, subsand flooding, excessive rainfall, inundation, collapse of egg chambers during piping and hatching emergence, respiratory gas availability in the nest, environment, or inappropriate movement while handling during sensitive periods (Miller et al., 2017; Phillott and Godfrey, 2020). The majority of embryonic mortality occurred at Stage 6 (intra-oviducal arrested development to oviposition) or earlier, before the embryo attached to the eggshell membrane and resulted in the formation of a white spot (Phillott and Godfrey, 2020). In the nest relocation process, reburial should occur within 1-6 hour to minimize movement-induced injury to embryos, and the negative effects of changes in temperature and moisture content of the eggs (Eckert et al., 1999).

It is widely questioned by different research groups about the impact that relocation can have on the sex ratio of offspring for sea turtles. In leatherback turtles, Sieg et al. (2011) determined how primary sex ratios, estimated from incubation temperatures, were affected by egg clutch translocation to a beach hatchery. Hatchery translocation decreased metabolic heating and female bias. Thus, it is a strong indication that hatchery translocation should be used cautiously.

Limitations of this study include the small sample size and missing records. The hatching rate was higher in relocated nests compared to nonrelocated nests but not significant ($P = 0.084$). However, the p value of less than 0.1 suggests a certain trend toward significance and this finding may hold biological significance. Increasing sample size of nests may allow to find the significant differences between two groups. We did not have access to an effective technique for sexing newborn turtles. Also, we did not have a complete record of the temperature, humidity, lighting, depth, and proportion of sand in the nests. We encourage future studies to record sex ratio of offspring and environmental factors, which are beneficial for evaluation of nest success.

CONCLUSION

The leatherback turtle nest relocation program has shown positive results in terms of decreasing the rate of non-developed eggs. These results provide scientific evidence to support the moving of nests of the leatherback turtle to safer and more optimal locations whenever it is deemed necessary.

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AUTHOR CONTRIBUTIONS

P. Kaewmong conceived and designed the experiments, performed the experiments, analyzed the data, authored and reviewed drafts of the paper, approved the final draft.
V. Punyapornwithaya analyzed the data, prepared figures and tables, authored and reviewed drafts of the paper, approved the final draft.
C. Wongfu analyzed the data, prepared figures and tables, authored and reviewed drafts of the paper, approved the final draft.
W. Prasitwiset analyzed the data, prepared figures and tables, authored and reviewed drafts of the paper, approved the final draft.
P. Jongjit conceived and designed the experiments, performed the experiments, analyzed the data, authored and reviewed drafts of the paper, approved the final draft.
A. Boonkasemsanti conceived and designed the experiments, performed the experiments, analyzed the data, authored and reviewed drafts of the paper, approved the final draft.
K. Kittiwattanawong conceived and designed the experiments, performed the experiments, analyzed the data, authored and reviewed drafts of the paper, approved the final draft.
P. Bansiddhi analyzed the data, prepared figures and tables, authored or reviewed drafts of the paper, approved the final draft.

CONFLICT OF INTEREST

The authors declare that they hold no conflicting interests with regards to the publishing of this manuscript.

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