

The effects of nest management methods on sex ratio and hatching success of leatherback turtles (*Dermochelys coriacea*).

Herrera, Arturo Eduardo^{a, b, c, 1}

^aConservation Biology Centre for Ecology and Conservation
University of Exeter in Cornwall
Tremough Campus
Penryn TR10 9EZ, UK

^bGandoca-Manzanillo National Wildlife Refuge
Marine Turtle Conservation Program of Southern Caribbean,
Talamanca, Costa Rica

^cAssociation ANAI
Apdo. 170-2070
Sabanilla, San José, Costa Rica

Corresponding Author:

Arturo Eduardo Herrera
Conservation Biology Centre for Ecology and Conservation
University of Exeter in Cornwall
Tremough Campus
Penryn TR10 9EZ, UK
E-mail: arturo_e_herrera@hotmail.com / Arturo.Herrera@gmail.com / ah258@ex.ac.uk
Phone: 07896 155182 (UK) / Phone: 859 -533-5446 (United States)

I, Arturo Herrera, do hereby confirm that all original research was carried out by the author and agree with the contents of the manuscript and its submission to the journal. No part of the research has been published in any form elsewhere, and is not being considered for publication elsewhere. Any research in the paper not carried out by the author is fully acknowledged in the manuscript and all appropriate ethics and other approvals were obtained for the research.

¹ Present address: 2020 Santa Anita Drive
Lexington, Kentucky USA
40516-9600

The effects of nest management methods on sex ratio and hatching success of leatherback turtles (*Dermochelys coriacea*) in the southern Caribbean.

Abstract

A study was undertaken on Playa Gandoca (82°37'W, 9°37'N) to determine if different incubation strategies varied temperature during the critical period of sexual phenotype, or influenced temperature sex determination. The study was conducted on Leatherback turtles (*Dermochelys coriacea*) from March 2006 until the end of July 2006. Forty seven nests (n=47) were created and grouped into three categories; hatchery = 15; natural = 12 and relocated = 20. Hatchery mean during middle third of incubation (the period of sexual determination) was recorded as 29.5°C ± 0.4. Relocated mean during the same period was 30.4°C ±0.7 and natural mean was 30.8°C ±0.6. Hatching success also showed that hatchery nests had 71.7% success while relocated had 61.2 % and natural a 50.5% success. Furthermore, a GLM model distinguished that hatchery clutches were cooler compared to other nest types and placing nests in hatcheries can alter sex ratios of hatchlings. Although leatherback turtles are stabilizing in the southern Caribbean region, they are still classified as critically endangered. Given the leatherbacks' wide home range, hatchlings are subject to many predatory threats and vulnerable to human pressure from fishing in the Atlantic pelagic zone. Improved conservation and understanding of this species at Playa Gandoca and worldwide will require a better understanding of the methods behind their nesting ecology not only in this stage of their life cycle but to ensure more viable populations in later stages of their life cycle if leatherbacks are to reach elevated populations.

Keywords: Leatherback; turtle; incubation; temperature-dependent sex determination; reptile; *Dermochelys coriacea* (6 keywords)

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Introduction

It is estimated that there are currently between 26,000 and 43,000 female leatherback (*Dermochelys coriacea*) turtles in the world (Spotila et al., 1996). Leatherback turtles are classified as Critically Endangered (CR - A1abd) on the IUCN Red List 2006 (IUCN, 2006), are listed on Appendix I of CITES and Appendix I of the Convention on Migratory Species (CMS or the Bonn Convention) (Sarti Martinez, 2000). Furthermore, their populations are declining (Spotila et al., 2000; Chan, E. & Liew, H., 1996). In 1995, it was estimated that the number of nesting leatherbacks on twenty-eight beaches and approximated the worldwide population in 1995 was about 34,500 females with a lower limit of about 26,200 and an upper limit of about 42,900 (Spotila et al., 1996). This is less than one third of the 1980 estimate of 115,000 (Ross, 1982) indicating that in less than one generation, the population has declined over 70%.

The causes of leatherback decline are numerous (Chacon et al, 1996; Troeng et al, 2002). Natural threats can include those caused by predators which prey on eggs and/or hatchlings (Maros et al, 2005; Troeng, 2000) and some insects lay their eggs in the turtle clutch for their larvae to feed (Maros et al, 2005). The commercial use of leatherback products and incidental fishing cause juvenile and adult mortality, resulting in major threats to this sea turtle species (Chevalier & Girondot, 2000). The dynamics of the world's population structure shows the extent of this threat (Baillie and Groombridge, 1996; Spotila et al., 2000). Conservation programs should protect and manage current populations while fostering future offspring especially for species threatened with extinction that may rely on conservation strategies during stressful periods.

Many conservation activities regarding marine turtles focus on present nesting populations and raising future success by preserving current turtle eggs. International conservation bodies and conventions, such as IUCN and the Convention on Biological Diversity, protect current

populations of adult and juvenile turtles dispersed through many national bodies of water (Sarti Martinez, 2000). Conservation activities focus on improving hatchling recruitment by safeguarding egg clutches to protected areas, such as hatcheries (IUCN, 2006). Hatchlings foster the success of its species by reaching sexual maturity and reproducing.

In many reptiles, as well as amphibians, gender is driven by the incubation temperature of eggs (Mrosovsky and Yntema, 1980; Bull, 1985; Pieau et al, 1995). This phenomenon has been dubbed temperature dependent sex determination (TSD). TSD is when the temperature exposed to the egg clutch during a thermo-sensitive stage of development determines sexual phenotype. All turtle species exhibit TSD (Mrosovsky and Yntema, 1980; Pieau et al., 1995; Godley et al, 2001) with the sensitive period occurring in the middle third of incubation (Mrosovsky, 1994). Pivotal temperatures are around 29°C depending on the species (Davenport, 1997). The pivotal temperature is defined as the constant temperature during incubation which gives 50% individuals of each sexual phenotype (Mrosovsky and Pieau, 1991; Mrosovsky and Yntema, 1980; Spotila et al, 1987). For turtles, below this pivotal temperature, mostly males are produced and above this temperature, mostly females (Rees and Margaritoulis, 2005; Spotila et al, 1987; Mrosovsky, 1994). The pivotal temperature for leatherbacks is 29.5°C (Rees and Margaritoulis, 2005; Spotila et al, 1987; Mrosovsky, 1994).

Given the influential nature of temperature affecting sexual phenotype, this must be taken into consideration when implementing management strategies. There are a number of conservation practices that involve thermal changes, such as the transplant of eggs (Mrosovsky, 1994). For example, incubating eggs in Styrofoam boxes was a popular practice to protect eggs and increase the rates of hatchlings. Unfortunately, Styrofoam boxes were cooler than the nesting beach sand and skewed the sex ratio below the pivotal temperature to males (Dutton et al, 1985). In Malaysia, the thirty percent infertility of leatherback eggs was attributed to a lack of males in the

population (Chan and Liew, 1996). This insufficiency has been linked to previously protecting eggs in the warmer, female producing season and less protection of potential males clutches in cooler seasons (Mrosovsky, 1994). Eggs incubated under artificial conditions such as hatcheries or incubators influence sex ratios (Dutton et al., 1985; Whitmore and Dutton, 1985). A hatchery is an artificially constructed and protected area on the beach (Chacon pers comm., 2006). It is usually delimited by a physical defense and monitored. The use of hatcheries has been criticized since it might influence the sex ratio of the hatchlings depending on location (Marcovaldi and Laurent, 1996). Handling methods to transport turtle eggs can change the initial temperature of the eggs, disrupting incubation time or harming the clutch (Chan and Liew, 1996). These factors may lead to long phases of imbalanced female-to-male percentages that Fisher stated to be standard if the cost of producing either sex is equal (Fisher, 1930). Conservation techniques may not take into account that translocation, although used for protection of at-risk nests, may alter sex ratios by biasing temperatures during the middle third of incubation as was found by Whitmore and Dutton (1985). Furthermore, hatcheries might influence the natural sex ratio (Marcovaldi and Laurent, 1996) leading to agitated results in sex ratios (Saltz and Rubenstein, 1995).

Low hatching success compounds the problem of population decline and thus understanding causes of low hatching success would be an important step towards preventing extinction in this species (Ralph et al, 2005). Do current conservation practices change hatching success? Would transplanting eggs cause such a change in hatching mortality that it compounds the issue of hatching success? Are these practices detrimental or beneficial? Understanding such issues is fundamental not only for leatherbacks, but other critically endangered marine turtle species that use similar conservation tools.

Translocation of nests either to a hatchery or another area of the beach is important where nest predation or human traffic on the beach is evident (Chacon pers comm., 2006). However, handling methods to transport turtle eggs may alter the temperature of the clutch, disrupting incubation time or hatching success. Therefore, in this study, I aim to discover whether translocation of nests may create a disparity in temperatures, creating an unbalanced natural sex ratio. Furthermore, I plan to ascertain the role that translocating eggs can have on hatching success, determining if there is a considerable distinction between natural and translocated nests and hatching success. The results from this study are imperative to the successful implementation of conservation strategies for not only leatherback turtles, but all marine turtles, as altering sex ratio can have exponential effects throughout their life history cycle.

Materials and Methods

Study Site - Gandoca Beach, 8.5 kilometers long, is situated on the southeastern Caribbean coast of Costa Rica and an important nesting site for leatherback turtles in the southern Caribbean Central America (Chacón, 1999). Playa Gandoca, along with Tortuguero, Pacuare and Chiriquí beach, comprise the world's fourth largest rookery (Troeng et al, 2004). Playa Gandoca ($82^{\circ}37'W$, $9^{\circ}37'N$) is found in the Gandoca/Manzanillo National Wildlife Refuge on the southern Caribbean border of Costa Rica and Panama and under management of ANAI Sea Turtle Conservation Project. As shown in Figure 1, the refuge encompasses 4436 hectares of marine and 5000 hectares of terrestrial area (Chacón, 1999). On a successful nesting season, over 600 nests can be laid at Gandoca beach. It is a dynamic and high energy black sand beach with no slope to a steep slope in some areas. The berm width and configuration varies widely and changes from season to season and within the nesting season itself. This is a result of long shore currents, riptide, storm waves and high spring tides. In general, this beach has a poorly developed berm during most of the year and is littered by debris washed in by the nearby Sixaola River.

Determining Nest Type - Three types of turtle nests were categorized in the research. The first type was natural, or in-situ, nests. These are nest sites chosen by nesting females not moved from the originally selected location. A nest was left natural when the distance from the nest to the sea was enough so that there was no danger of tidal inundation, traffic, predation or erosion. The other two categories of nests were relocated and hatchery nests. These are clutches moved from the originally chosen spot due to one or a combination of the previously stated factors. For example, a clutch below the high tide water mark would be relocated to a safer area on the beach or the hatchery. Hatchery nests are simply nests that are relocated to the hatchery. Relocated nests are nests that are not relocated to the hatchery but from the originally selected site to a safer area of the nesting beach.

The selected egg clutch to be relocated was moved to its final destination less than 2 hours after initial nesting. Eggs were relocated by catching them in a plastic bag placed below the cloaca. The artificial nest chamber, whether hatchery or relocated, was hand dug as closely to the original nest specifications and measurements.

Temperature Collection – Temperature collection started on March 30th 2006 and continued until July 25th 2006. Temperature samples were taken three times a day. One was taken from 11:00 until 13:00 (mid-day sample). Another was taken between 16:00 to 18:00 (afternoon sample). The third set was taken from 23:00 to 01:00 (midnight sample). The reason for the large time variance in collecting temperature data was to ensure multiple measurements and diel patterns throughout a 24 hour period. Cu/Cn Type J and K thermocouples were placed in the center of the nest to achieve uniform standard. After 10 to 15 eggs were placed in the nest, regardless of nest type, the thermocouple was placed in the center of the clutch. Also, nests with the thermocouples placed in them were marked with tape as relocated, hatchery or natural and

could be visually identified as an experimental nest. Nest temperature was read by a HH501AJK Handheld Digital Thermometer© manufactured by Omega Engineering, Inc.

A control was set randomly one meter from experimental nests, parallel from the tide line. The control consisted of temperature logger (Tinytag Plus Explorer © or a HOBO® Pendant™ Data logger) to record sand temperature throughout the life of the nest. The control was set at a depth of 70 centimetres, the average depth of a leatherback clutch (Chacon and Machado, 2005). The nest and control were monitored on a continuous basis for the full incubation period or until the end of the experimental research date, July 25, 2006. Temperature fluctuations were recorded between distinct nest types and the control for each type as well.

Temperature data was analyzed in SAS software using MANOVA. Hatchery, relocated and natural temperature data were compared against each other. The data was compared to each category and within the control. The controls were analyzed with its appropriate pair as well as within the controls using JMP software. Hatching success was obtained by General Linear Model (GLM) with binomial distribution and done on SAS software.

Hatching Success - Nests were marked for exhumation when hatchling tracks were discovered and exhumed 3 - 5 days after the last hatchling track was recorded. Nests that did not show tracks but known to be present were checked 3 days after the normal 60 day incubation period. Nests were excavated manually using latex gloves. Hatching success of nests was predicted by methods laid out by Dutton & Whitmore (1985). The hatching success from a nest was calculated using the following formula:

$$\text{Hatching success (\%)} = (\text{Hatched} / \text{Total Number of eggs}) \times 100$$

Sex Ratio Determination:

Unfortunately, the only true way to determine sex is by invasive methods of examining hatchling gonads (Mrosovsky and Yntema, 1980) through a microscope. Given the population status of leatherbacks, it is counter-productive to forfeit a sizeable number of hatchlings to determine sex. Instead, a less-invasive method was used by calculating the mean temperature during the middle third of incubation and using the formula used previously by Chevalier et al. (1999). This gives an estimation of sex ratio.

$$sr(t) = \frac{1}{1 + e^{\left(\frac{1}{5}(P-t)\right)}}$$

This equation used is fully described in Chevalier et al. (1999) and based on the best fit curve of the sex ratio. This estimation is not a positive determination of the exact sex ratio of the hatchlings in this study. Since it is based on the best fit curve, the actual sex ratio will be slightly different than this estimated ratio.

Results

Overall forty-seven ($n = 47$) nests were collected for this study. Twelve nests were left in-situ (natural), 20 nests were relocated to safer areas (relocated) and 15 nests were taken to the hatchery (hatchery). As shown in Figure 2, clutches were collected throughout the nesting season at Playa Gandoca. This was done to ensure that natural temperature fluctuations throughout the nesting season did not mask the true results. Three natural controls, two relocated controls and three hatchery controls were created. The collection of the sand temperature, displayed in Figure 3 are shown as averages throughout the course of the entire nesting season

The results determined that the natural nest mean temperature of all twelve nests over the course of all twelve incubation periods was 30.6° Celsius. Hatchery mean temperature of all fifteen nests throughout the incubation period was 29.0° Celsius. Relocated mean nests temperature throughout incubation of all twenty nests was 30.1° Celsius.

Control for Hatchery, Relocated and Natural

The relocated control mean was 29.9° C, natural control mean was recorded as 29.8° C and the relocated control mean was 29.9° Celsius. The controls within themselves were analyzed to measure differences within the sites. The control for hatchery was not significantly different from relocated control (DF =44, F = 0.027, p = 0.98) but differed from natural nests (DF = 44, F = 2.119, p = 0.0398). Natural and relocated controls were also found to be significantly from each other (DF = 44, F = 2.222, p = 0.032).

Natural Nests – Of the potential fourteen nests that were included in the study, 2 natural nests had their thermocouples removed (N3 and N8) and consequently not included. The natural mean temperature during middle third of incubation of all twelve nests was 30.8 C. ANOVA analysis with repeated measures revealed that natural temperatures were significantly higher from the hatchery nests (DF = 44, F= 2.36, p= 0.02) but not from relocated category (p = 0.51). During the critical period of middle third of incubation, again, no difference between the mean temperature and its control was found (DF, 11, F= 0.482, p = 0.639). As seen in Figure 3 a. and Figure 3.b, natural nests were warmer than other nest types.

According to the established pivotal temperature of 29.5° Celsius, an estimated 74.1% of the natural nests used in this experiment were projected to produce females while a predicted 25.9% produced males.

Hatchery Nests – Incubation period hatchery nests were significantly cooler from the natural nest category (DF = 44, F= 2.36, p= 0.02) but not from relocated category (p = 0.056) as shown in Figure 4.a and 4.b. Overall hatchery mean temperature did not depart significantly from its control either (DF =14, F = 1.43, P = 0.175) seen in Figure 4.c. However, in a matched ANOVA

analysis during the middle third compared with hatchery controls, there was a significantly cooler temperature between hatchery clutches and the controls (DF = 14, F = 4.65, p= 0.00). Mean temperature for clutches during middle third of incubation was 29.5° C while hatchery control was calculated at 30.5° C during the same interval, as demonstrated in Figure 4.d. This indicates that nests were undergoing a drop in temperature considerable enough to alter the sex ratio estimates.

Fifteen hatchery nests were included in this experiment. The hatchery mean temperature of all fifteen nests through the middle third of incubation was 29.5, but varied around the pivotal temperature (33.2° C to 28.6° C). 45.6% from the hatchery category of this experiment were projected to create females while 54.4% of the hatchery nests were estimated to be males.

Relocated Nests - From the potential 24 nests in the study, one was poached (R1), two had their thermocouples taken out (R11 and R12) and one was lost (R20) due to erosion. Overall mean temperatures of all relocated nests during the middle part of incubation was 30.4° Celsius with the lowest recorded temperature during the critical period at 27.7° Celsius (R4). The highest recorded temperatures during the critical period came later in the season, with the high mean temperature recorded at 32.9° Celsius. MANOVA analysis clearly illustrated that relocated nests were not statistically or significantly different from hatchery (DF=44, F= 3.19, p = 0.056) or natural nests (p = 0.51). In Figures 4.a and 4.b, relocated nests not were shown to be significant to the other nest types. Again, hatchery nests did not differ from its control during its incubation period (DF =19, F =1.18, p = 0.25), nor the crucial middle period (DF = 19, F = 1.04, p = 0.31) as demonstrated in Figures 4.c. and Figure 4.d.

Twenty relocated nests were created for this experiment. According to the equation stated previously, the estimation of males was 30.4% from the relocated nests and approximately 69.6% females.

Sex Ratio - Overall, an estimated 37 % of hatchlings were surveyed to be males and 63 % observed to be females. Hatchery nests comprised 17.1% males and 14.8 % of overall females. Natural nests consisted of 6.6 % male and 18.9 % female totals of the 47 observed nests while relocated nests comprised 29.3% females overall and 13.2 % males of all the nests. Natural nests comprised 18.75% of the total nests observed, while Relocated and Hatchery consisted of 43.75% and 37.5% of the total, respectively.

Hatchling Success Results - Of the 47 nests that were obtained, six natural nests, fourteen relocated and twelve hatchery nests were used for hatchery success results. This gave a possible 32 nests to use in the GLM analyses. Twenty-eight (n=28) were used since four nests had incomplete data. Sand compaction was directly due to the relocation of a nest or the placement of natural nests in a formerly favorable area and included in hatching success, despite low percentages attained from those nests. Three natural nests and two relocated nests that were washed away that were not included in the final results. The reason is because both natural nests and relocated nests did not reach the middle third of incubation. Furthermore, one relocated nest was unable to be found or possibly poached and not included in the final estimates.

On initial review the hatching success for natural nests was 50.5%, 61.2% for relocated and 71.1% success for hatchery nests. Hatching success results revealed that nest type is significant and clearly illustrated in Figure 4.e. Hatchery nests had much higher success than either relocated or natural nests. (DF=1, $X^2 = 41.46$, $Pr > X^2 = 0.000$). In Figure 4.e, natural and relocated nests were not shown to be significantly different. This illustrates that nests placed in the hatchery were more likely to have higher hatching success than natural relocated nests.

Discussion

For leatherback sea turtles, the pivotal temperature for sex determination is 29.5°C (Mrosovsky and Yntema, 1980). Nest temperature varied within the season and individually but even high temperatures recorded during incubation were within the normal range of leatherback clutches (Ackerman, 1997). Controls placed within the hatchery displayed a significantly lower temperature than the beach controls, possibly giving a disparate starting point than relocated or natural nest types. Relocated nests did not offer substantial evidence that this translocation method altered sex ratio from natural nests or hatchery nests. Whether during the entire incubation period or the crucial middle period of incubation, relocated nests stayed in line with its control as demonstrated in Figure 4c and 4.d. Natural nests also did not depart considerably from its control during the entire incubation period or the critical period of temperature-dependent sex determination.

The results from this study demonstrate a clear difference of cooler hatchery temperatures during critical periods in comparison to natural nests during the same period throughout the season. As seen in Figure 4.a and 4.b, although neither was shown to be significantly different from relocated nests, there is evidence to differentiate between hatchery and natural nest types. Though hatchery nests did not vary significantly during the entire incubation, there was evidence that it varied during the important determining period of sexual phenotype. Throughout the three categories, hatchery nests demonstrated enough evidence that being placed in a hatchery would alter the sexual phenotype to males. These results validate that clutches placed in a hatchery can be significantly different during the critical period, distorting natural ratios as shown in Figure 4.e. However, estimates from the sex ratio calculated 37% males and 63 % females from this experiment (n = 47), favoring females. The reason is 31.9% nests in the study were hatchery.

Manipulation of the sex ratio is not just a theoretical argument because of its current use in marine turtle conservation programs. There are management and conservation implications of temperature-dependent sexual differentiation: almost any management procedure involving sea turtle eggs has the potential to alter the sex ratio of the hatchlings produced (Godfrey et al, 1997). If natural conditions bias one sex, then it would be understandable for conservation programs using relocating methods to follow suit (Vogt, 1994). Using this case as an example, the slant toward females could increase overall long-term population in the Caribbean, a population thought to be stabilizing or in slight decline (Troeng et al, 2004). The natural ratio is followed and the Caribbean population could potentially recover. However, males are needed to ensure survival for this species. Large sex ratio disproportions could manifest and individuals may never contribute to future success of the overall population since the other sex is possibly absent. Hatcheries can be beneficial in boosting the male population and general population in the southern Caribbean. An important caveat must be included when making statements of biasing sex ratios; knowledge of the adult population is vital of how to move in the future. A change of 1 or 2° Celsius can make a substantial difference to the sex ratio of hatchlings and future populations. Current conservation methods can benefit greatly by assessing adult ratios because of its effect on future nesting seasons. Another factor to point out is the question of manipulating the sex ratio itself. The conundrum is there is little to say what the effect of manipulation of sex ratios will have in boosting or crippling the populations of endangered species, like leatherbacks. The idea of sex ratio manipulation should not be dismissed or encouraged, but local population estimates should be included before these practices are put into place (Mrosovsky and Godfrey, 1995).

Hatching Success

The relocation of nests to protected hatcheries is a common conservation tool widely used throughout marine turtle projects (Grand and Beissinger, 1997). As shown, type of relocation

strategy is significant. Hatchery nests had higher success than either relocated or natural nests (71.1%). The relocation of leatherback clutches to the hatchery could increase future viable populations by sheer biomass. Previous meta-analyses demonstrate that translocation due to poaching and predation has a higher survival probability on many beaches (Grand and Beissinger, 1997). Although relocation to a hatchery will not ensure the survival of this species, it can prolong extinction and may reverse population decline (Spotila et al., 1998). Commercial use of leatherback products and incidental fishing resulting in juvenile and adult mortality cause major threats to this species. The dynamics of the world population structure shows the extent of the threat. The incidental take and mortality of sea turtles as a result of trawling activities have been well documented in the Gulf of Mexico and around the Atlantic (Godley et al, 1996; Eckert, 1997; James et al, 2005). Relatively low hatching success of the leatherback turtle (Bell et al, 2004) and its critically endangered status indicates future experiments should investigate causes of low hatching success in order to increase hatchling production in this species (Ralph et al, 2005).

While a well managed hatchery can have high rates of hatching success, the justification of having the tools that will skew the ratio from the natural percentages must be addressed. An adult population assessment of the south Caribbean region must be included to properly move forward. The leatherback population in the Caribbean not only depends on hatching success, but conservation throughout the life history cycle. To prevent leatherback sea turtles from extinction will require a comprehensive knowledge about their life history as well as their nesting ecology.

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Figure Legends:

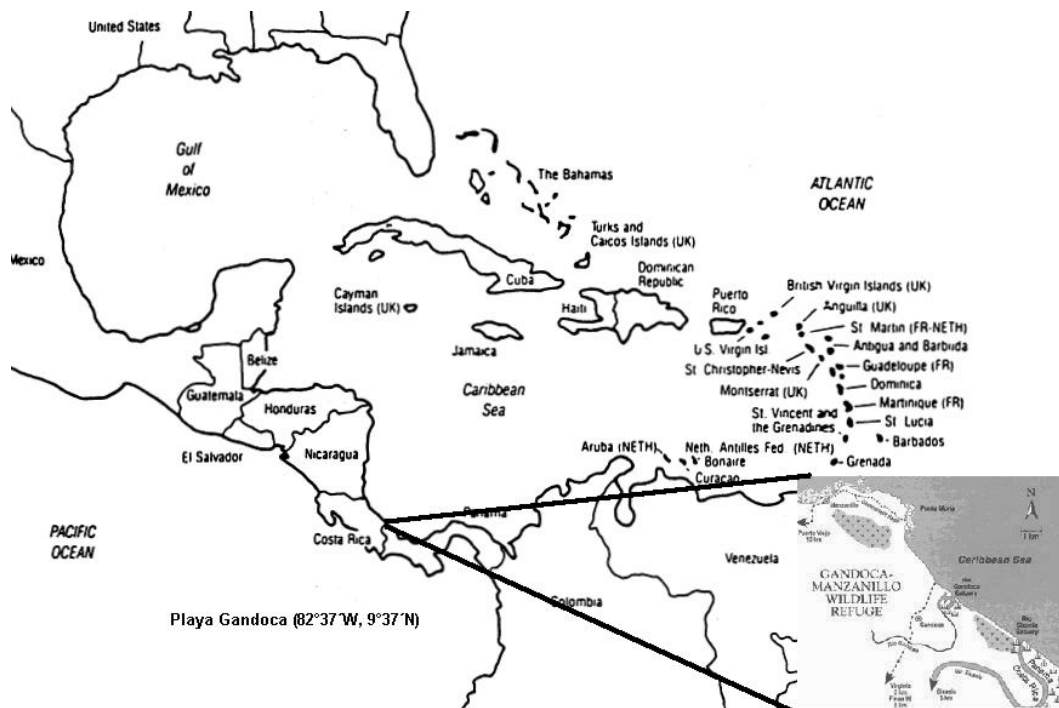
Figure 1: Gandoca-Manzanillo Wildlife Refuge area showing the location of the study site in Playa Gandoca, Costa Rica ($82^{\circ}37'W$, $9^{\circ}37'N$). Playa Gandoca, along with Tortuguero, Pacuare and Chiriquí beach, comprise the world's fourth largest rookery. An average of 500 nests can be laid during one nesting season.

Figure 2: Nest category collected per week over the course of the nesting season. Relocated clutches are shown in black, natural nests are indicated by labeled in white and relocated are displayed in grey. They are stacked and shown how many of each nest type were collected weekly. The weeks are displayed on the X-axis while number of nests collected is displayed on the Y-axis.

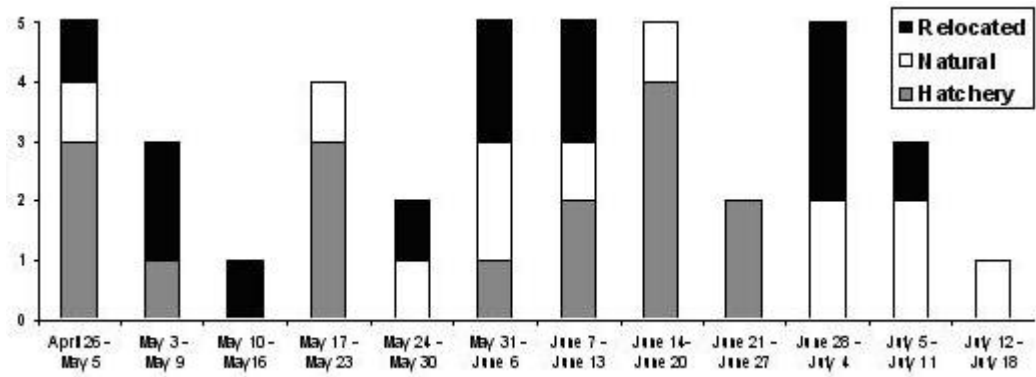
Figure 3: Overall mean nest temperature by nest category.

Figure 4: (a) Showing overall temperature mean by nest category. The large bar displays the range of all nests and shows that overall nest types varied while (b) shows the critical middle incubation temperature mean per nest type. The large bar also displayed the range of each individual nest per nest category and further displays the difference in temperature during critical sexual phenotype determination between nest types. (c) Overall mean temperature of all nest types with control mean per nest type. Control is displayed in gray while mean per nest type is shown in black. (d) The critical middle incubation mean with of all nests with control at the same critical period per nest type. The control is displayed in gray while mean per nest type is shown in black. (e) Shows Hatching success by nest type. The large bars display the range of each category while each dot represents an individual nest. Hatchling success is shown in percent and finally (f.) is the estimated female proportion of hatchlings in Playa Gandoca based on this study.

Figures:

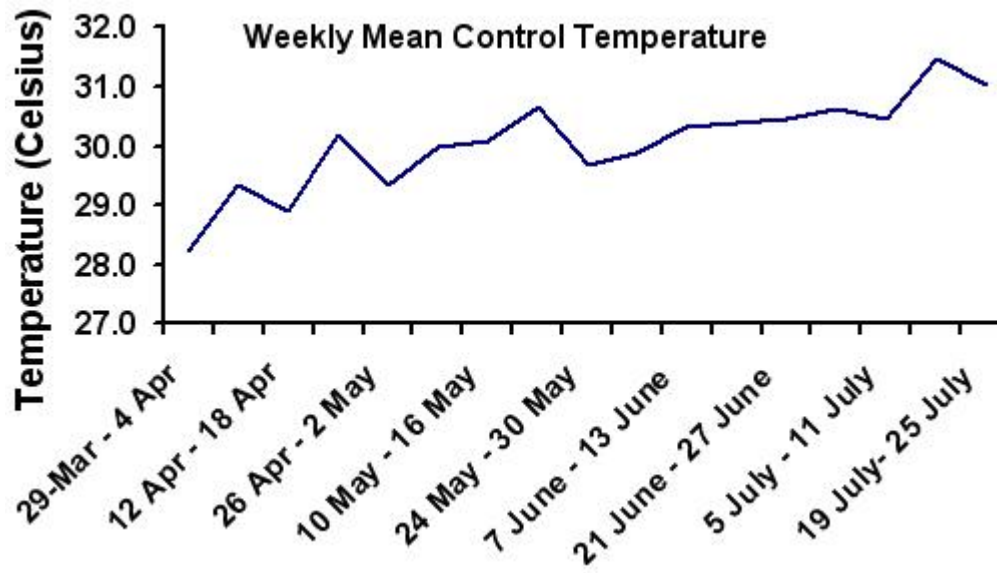


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