

**STUDIES ON THE EFFECT OF TEMPERATURE ON THE  
LEATHERBACK TURTLE (DERMOCHELYS CORIACEA) NEST  
DURING THE EGGS INCUBATIONAL PERIOD.**

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## **ABSTRACT**

Globally, the earth's temperature has been increasing steadily. According to the Intergovernmental Panel for Climate Change (IPCC), the threats of these changes for small islands include the rise in sea levels and the increase in the atmospheric temperature. Scientists believe that this increase in temperature has caused the population of the leatherback turtle, *Dermochelys coriacea*, to become statistically mainly female. The reduced number of males can have a significant effect on the population of the leatherback turtle. In this research project, it is proposed that monitoring the leatherback population and nesting area, modification of the nest incubation temperatures and genetic testing to determine the genetic control of the population. These ideas are proposed in order to discover and prove that global climate change is having an effect on the leatherback turtle clutch sex determination.

## **1.0 INTRODUCTION**

Having first appeared on Earth during the Cretaceous period about 70 million years ago, the leatherback sea turtle, *Dermochelys coriacea*, can be aptly described as a living fossil, having changed little in structure and morphology for almost 30 million years.

Individuals of this sea turtle can reach 6.5 feet (2 meters) in length and weigh up to 2,000 pounds (900 kilograms), and can live for up to 70 years. The main migratory pathway of the leatherback turtle extends throughout the Pacific, Atlantic, and Indian Oceans as well as the Mediterranean Sea.

At present, according to the IUCN Red List, 6 out of the 7 species of sea turtles are declared either endangered or critically endangered. This is inclusive of the leatherback sea turtle which was declared as critically endangered as of the year 2000 due to estimates in population sizes inferred from nesting abundance studies made world-wide (Hilton-Taylor 2000).

In the Caribbean, it is estimated that 150-200 leatherbacks nest annually and since then that number has increased (Bacon 1970).

Leatherbacks nest in high energy beaches which are clear of any obstructions or debris which would hinder their approach to shore. Most of these beaches are found more or less on the east coast of Trinidad. Nesting areas on the east coast are Matura, Fishing pond and Manzanilla. Nesting areas are also found on the north coast in places such as Grande Riviere, Blanchisseuse and recently at Las Cuevas. Las Cuevas is not a high energy beach as compared to some of the beaches in Blanchisseuse and it is not known why leatherbacks have chosen to nest at this location. Grande Riviere, Matura and Fishing pond have the highest number of nesting turtles. There have also been reports of an increase in nesting turtles at Manzanilla.

Each female lays about 7 clutches of eggs every ten days before returning to the north to feed. Each clutch of eggs contains about 75 to 120 eggs. About 2% of these eggs hatch and about 1% of the hatchlings survive to maturity.

There are two different types of eggs yolk, fertilised eggs and yolkless which are unfertilised eggs. The unfertilised eggs are deposited last and these help to form a layer to help maintain a constant environment for the fertilised eggs. Oxygen and temperature helps the nest to collapse when eggs have hatched.

The eggs in a nest would all be of one sex but the positioning of the nest would determine the sex of the nest. Nests which are laid further to the back beach would have warmer conditions. These nests would produce female hatchlings. Nests which are laid closer to shore would have slightly cooler conditions and would therefore produce male hatchlings. It is predicted that if there is an increase in ambient temperatures, there is the possibility of increased female hatchlings.

The leatherback turtles are tourist attractions and can be seen as a source of income for some Caribbean countries and other small islands. Climate change can affect these countries via sea level rise. Sea level rise can be caused by increased temperature, both directly and indirectly. Increase in temperature can directly affect the ocean via heating. Heating causes expansion and therefore the expansion of the ocean would cause the sea level to rise. Indirectly, increase in temperature affects polar caps and glaciers which would cause melting and also cause the sea level to rise.

Sea level rise would ultimately affect these islands as there would be a loss in beaches. A loss in beaches would result in the loss of nesting areas for the leatherback turtles. A loss in the leatherback turtle population due to climate change will also affect tourism and jobs. While climate change would affect animals and other organisms in the environment, there will be a chain reaction which would ultimately affect the human population either directly or indirectly.

The sea turtle plays a major role in the local economy, drawing hundreds of tourists each year to beaches such as Grande Riviere, Matura, Manzanilla, Turtle Beach, Las Cuevas and Fishing Pond. These beaches are just a few of the nesting grounds of this species

within the country. Locally, the turtle has been traditionally hunted for its meat and eggs, and although it is now illegal to hunt them, poaching is still a widespread practice. The conservation of this species has now become a world-wide effort and Trinidad and Tobago, being one of the primary nesting sites in the world for this species of sea turtle, has recently been undertaking measures to protect and conserve the females that visit our shores. Turtle patrols and PIT tagging has spread from Matura and Grande Riviere to other beaches such as Fishing Pond and Turtle Beach, Tobago.

The leatherback turtle is a reptile whose nesting sex ratios depends on external influences such as the temperature of the sand and the temperature within the nests, with a lower temperature resulting in the increased proliferation of males and a higher temperature resulting in the increased probability of female hatchlings being produced. According to Janzen 1994, global warming may affect reptile species that exhibit temperature-dependent sex-determination, and this may be inclusive of the leatherback sea turtle. With the rise of Earth's temperatures due to global warming, the increased temperatures of the atmosphere may have a direct effect on the temperature of beach sand and leatherback nests.

Hatchling sex is determined by the temperature at which a clutch is incubated (Hendrickson 1958; Standora and Spotila 1985; Binckley et al. 1998). According to Hillel 1998, there are two main mechanisms for gaseous transport within the soil, diffusion and convection. The gradient of the partial pressure of the gases within the soil provide the propulsion for diffusion to occur whereas convection occurs when the gradient of total gas pressure creates the bulk flow of gas particles from a zone of higher pressure to a zone of lower pressure (Hillel 1998).

## ***2.0 AIM & OBJECTIVES***

The aim of this research is to analyze the following question. “How is the projected change in global temperature going to possibly affect the sex determination of the leatherback turtle and ultimately its genetic stability?” The proposal is further subdivided into three major areas to fully explore this concept.

Temperature is projected to increase global however it is still to be determine if this change in temperature has a significant effect on the leatherback nesting population

Temperature affects the sex determination of the leatherback and therefore with this predicted increase in the ratio of male to female become statically more female

What possible effects does it have on the population genetics stability arising from the predicted production of more females?

### **3.0 BACKGROUND INFORMATION**

#### **3.1 Remote Sensing**

The use of remote sensing can determine a beach profile, surface topography, moisture and depth and, vegetation biomass. Monitoring flooding and surface topography are some of the data that can be obtained through remote sensing. It is crucial to obtain this data since studies have suggested that there are other factors that affect the population the hatchlings. By using this advanced technology along with data collected on the surface, it will give scientists and researchers a complete image of the nesting areas of the leatherback turtle.

Remote sensing is a tool that gives detailed information about the status of the land. It can also be used in conjunction with information of the surface to give a complete picture of the status of the environment. This information is important to the study as it gives a detail picture of the type of environment the leatherback turtle is nesting.

Another feature used to track the leatherback turtle is satellite tracking, which can give more insight into their migratory pattern and other habits of the species. This is important to be able to determine of the entire population numbers and where other metapopulation are forming.

#### **3.2 Other factors affecting the nest**

There are many factors that affect the nest of a leatherback turtle (*Dermochelys coriacea*). Biotic factors such as egg predation and bacteria just to name a few can affect the nest and ultimately the validity of the research. Some abiotic factors affecting the nesting sites of the leatherback turtle are beach erosion, sand particle size and level of

phosphorus oxide. Then to be able monitor to limit externalities that could effects to this study should also be monitored.

### **3.3 Sex determination by temperature**

About 50% of the leatherback turtle eggs survive the incubation period. (Wallace et al 2003, see Bell et al. 2003). There are two ways in which the sex of a leatherback turtle can be determined at the hatchling stage. The first is through histology of the embryo and the other through temperature.

Gonad differentiation is sensitive to the temperature in the middle of incubation period. The critical temperature for leatherback turtles is estimated to be 29.25 to 29.50 °C. At this critical temperature the nest should be producing at a ratio of 1:1 male to female hatchlings. The higher the temperature the eggs incubate at the faster they will hatch. The length of time for leatherback turtle incubate in the nest before hatching is between 6-12 weeks. Within the nest a microclimate is created

### **3.4 Phylogeography**

The use of genetics is a tool to understanding the population structure of the leatherback turtle. This can be used to assess current rookery sizes and the degree of genetic structure between them. However there are some scientists that believe that the number and mass of the metabolizing leatherback turtle would affect the maximum temperature of the nest. This paper focuses on abiotic factors specifically the temperature affecting the sex determination.

### **3.5 Climate Predictions Current**

According to the Intergovernmental Panel on Climate Change IPCC warming of the climate is now evident as global temperatures are increasing and sea level is rising due to meltdown of ice. See figure one.

The IPCC states that with medium confidence about 20% to 30% of species are likely to be at risk to extinction. The leatherback turtle can be considered one of those species. The sex of the leatherback turtle is determined by the temperature at incubation and more specifically at the third stage of incubation. With projections from leading scientists that surface temperatures will increase it can only be predicted that sex determination will be affected.

In figure one taken from the fourth assessment report of the summary for policy makers it is seen that from 1970-2004 the earth's surface temperature has significantly increased. Any small change in the surface temperature will ultimately affect the temperature rookery of the leatherback turtle which will test leading skewed sex determination in the population.

Data collected by the IPCC states that seasonal ocean and surface temperature has increased by 0.6 to 1.0°C since 1910. There is also much data that shows the maximum and minimum number of warm days has increased.

Changes in physical and biological systems and surface temperature 1970-2004

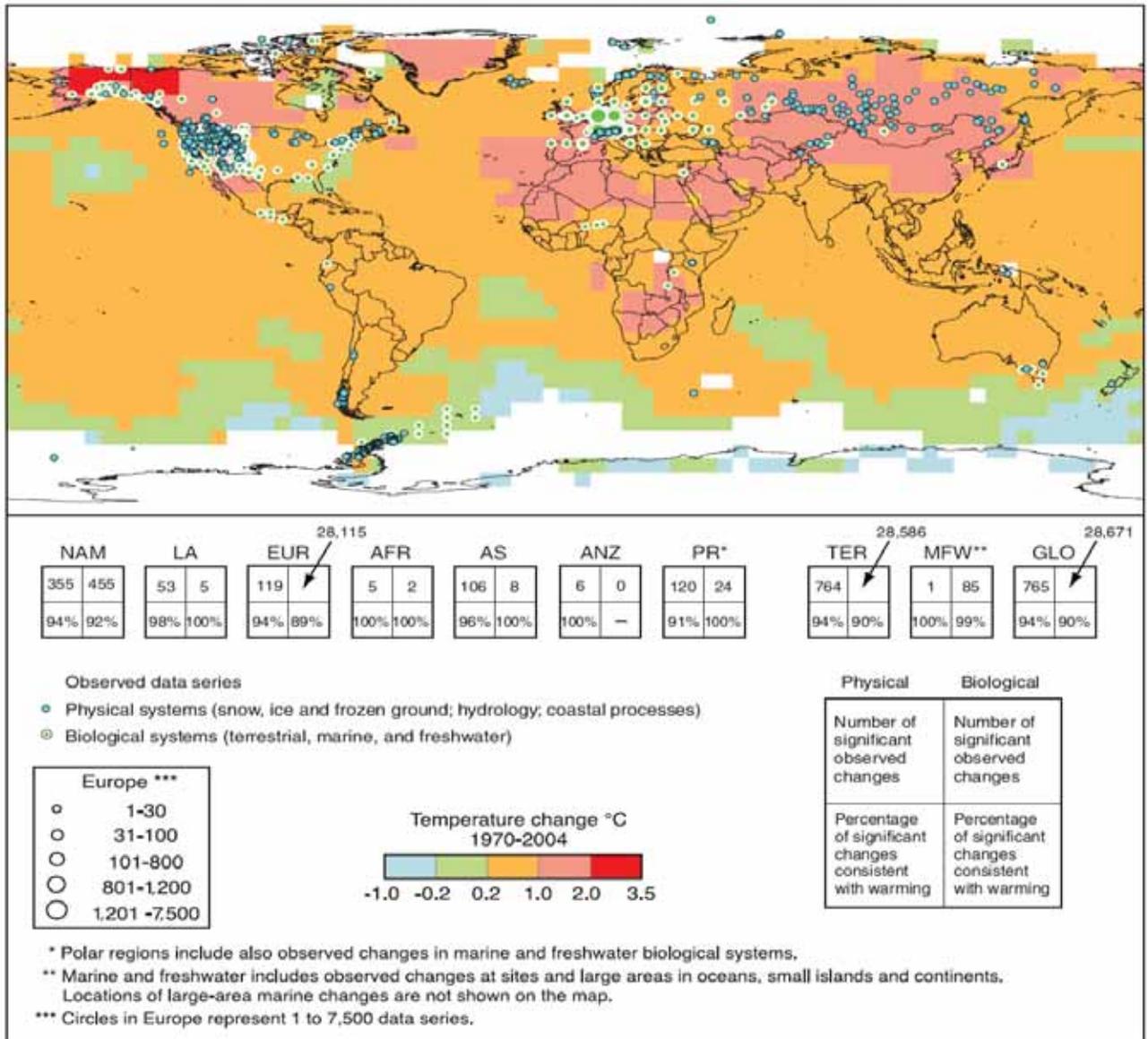


Figure SPM.2. Locations of significant changes in data series of physical systems (snow, ice and frozen ground; hydrology; and coastal processes) and biological systems (terrestrial, marine and freshwater biological systems), are shown together with surface air temperature changes over the period 1970-2004. A subset of about 29,000 data series was selected from about 80,000 data series from 577 studies. These met the following criteria: (1) ending in 1990 or later; (2) spanning a period of at least 20 years; and (3) showing a significant change in either direction, as assessed in individual studies. These data series are from about 75 studies (of which about 70 are new since the TAR) and contain about 29,000 data series, of which about 28,000 are from European studies. White areas do not contain sufficient observational climate data to estimate a temperature trend. The 2 x 2 boxes show the total number of data series with significant changes (top row) and the percentage of those consistent with warming (bottom row) for (i) continental regions: North America (NAM), Latin America (LA), Europe (EUR), Africa (AFR), Asia (AS), Australia and New Zealand (ANZ), and Polar Regions (PR) and (ii) global-scale: Terrestrial (TER), Marine and Freshwater (MFW), and Global (GLO). The numbers of studies from the seven regional boxes (NAM, EUR, AFR, AS, ANZ, PR) do not add up to the global (GLO) totals because numbers from regions except Polar do not include the numbers related to Marine and Freshwater (MFW) systems. Locations of large-area marine changes are not shown on the map. (Figure 1.2)

Figure 1 source [http://www.ipcc.ch/pdf/assessment-report/ar4/syr/ar4\\_syr\\_spm.pdf](http://www.ipcc.ch/pdf/assessment-report/ar4/syr/ar4_syr_spm.pdf)

## **4.0 SEX DETERMINATION AND TEMPERATURE**

### **4.1 Proposed Experimentation**

#### **Justification**

The Leatherback Turtle, *Dermochelys coriacea*, follows the life history, characteristics and strategies adopted by fellow sea turtles. These include its high mortality, great longevity life history strategy and its exhibition of temperature-dependent sex determination (TSD) with pivotal temperatures close to 29°C (Davenport 1998). The turtle's dependence on temperature can lead to its detriment with elevated global concerns on the effects of Climate Change.

Scientists from the IPCC states that the Caribbean is expected to have a 1 degree rise in surface temperature which may affect the Leatherback turtle TSD. This effect has yet to be statically proven in Trinidad and Tobago. The hypothesis therefore suggests that the ratios will be statistically more female is based on numerous researches done by various scientists. What it also shows is that decrease male population numbers can have major detrimental effects on the leatherback turtle surviving due to its lack of ability to adapt to environmental changes.

As a Small Island Developing State (SIDS), we should be naturally concerned with these effects because of our Geography and Topography. Since Trinidad and Tobago are among the few tropical countries in the Western Atlantic Ocean where this endangered creature goes to lay its eggs (Sankar-Øyan 2000), investments should be made in the conservation and management of the leatherbacks in light of the increasingly evident and futuristic effect of climate change on our island.

We therefore suggest the following experiments:

- *The Effects of Climate Change on Nesting Sites (Sand Temperature)*
- *Hatchery Design and Production of Male Hatchling*

### **4.2 The Effects of Climate Change on Nesting Site**

Objective:

To determine how the increase in atmospheric and land temperature affect the temperature of the sand/ nesting site for Leatherback Turtles due to Climate Changes caused by Global Warming.

Material:

Thermometers, gloves

Methodology:

Major nesting beaches on the coast of Trinidad and Tobago are to be chosen ahead of experiment commencement along with determination of the mean rainfall and temperature during the nesting season for both day and night. Identify sites for temperature recording. Temperature of the sand at each site shall be taken for both day and night. The thermometer is to be gently placed into the sand at a predetermined depth and left for a predetermined time for each temperature reading.

*This procedure is to be repeated with each Leatherback Turtle Nesting Season to obtain a database of information that will allow for well informed conclusions to be made about the effects of temperature rise on the coastal sand temperature.*

Suggested Table for data collection:

Year	Beach	Site	Temp. of day	Temp. of Night	Temp. of sand at Day	Temp. of sand at Night
		A	°C	°C	°C	°C
		B	°C	°C	°C	°C
		C	°C	°C	°C	°C

**4.3 Hatchery Design and Production of Male Hatchling**

Objective:

The objective of this study is to determine under which artificial shading condition the male hatchling population will predominate.

Hatchery Condition and Design will therefore be varied to mimic or induce various sand ambient nesting temperatures which would be inversely proportional to the level of shade.

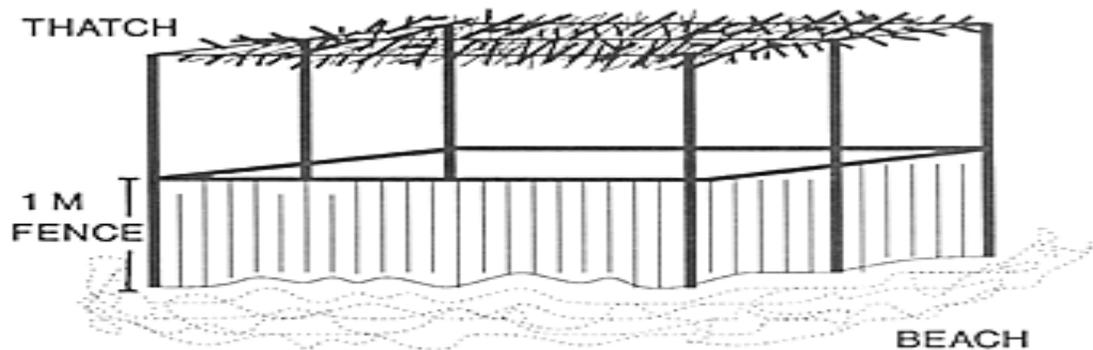
Therefore, we hypothesized that the Level of shade  $\propto 1/\text{nest temperature}$ .

#### Material:

Thatched roof - mainly coconut branches or other shielding material easily sourced on the site.

Galvanize, Plastic sheeting, Metal poles, Nails

#### Design:



Source: Marine Turtle Newsletter, Higginson, Vasquez 1989

#### Methodology:

Major nesting beaches on the coast of Trinidad and Tobago are to be chosen ahead of experiment commencement along with determination of the mean rainfall and temperature during the nesting season for both day and night. Various leatherback turtles are to be tracked on land to determine their site for nesting which should be marked for later identification.

The temperature of the sand is to be measured before construction of the hatchery and recorded. The hatchery shall be constructed over the nesting site ONLY AFTER THE FEMALE TURTLE MOVES. The eventual temperature shall be monitored over the period of incubation. Some of the nesting sites chosen by the female turtle shall not be

altered by the hatchery construction. The temperature of these nests shall also be measured and recorded simultaneously with the nests that are shaded. Hatchlings and late stage embryos that failed to hatch are to be collected freshly dead to determine their sex histological by observing their gonads.

*This procedure is to be repeated with each Leatherback Turtle Nesting Season to obtain a database of information that will allow for well informed conclusions to be made on the attempts to mitigate the effects of climate change on the male population of this endangered species.*

Suggested Table for data Collection:

Beach	Hatchery #	Year	Design Description	Sample size (N)	# of Male	# of Female	% Male
	1						
	2						
	3						
	Uncovered/ Exposed		Nest description	Sample size (N)	# of Male	# of Female	% Male
	1						
	2						
	3						

Constant: The Basal dimension of the hatchery (to be determined)

Manipulated Variable: Height of fence, type of material used for roof, height of pole.

## **5.0 DISCUSSION AND EXPECTATION**

This experiment was designed in an attempt to mitigate the increased atmospheric and surface temperatures due to raises in Greenhouse Gases (GHGs) emission which contribute to Global Warming. From the results we can determine which hatchery design contributed to a greater percentage of male hatchlings when compared to females within the same nest. It is hoped that an elevation in the male population will increase the frequency of female to male interaction and mating.

The purpose of the Shade/ Hatchery Design is to:

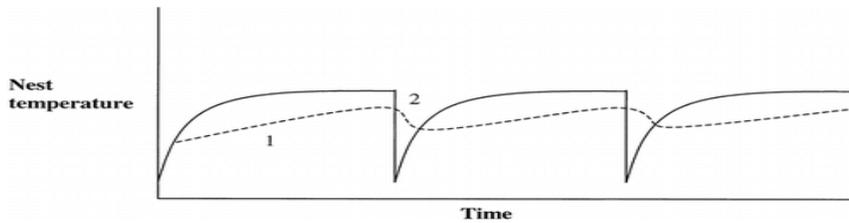
- To trap cool sea breeze within the enclosed nesting site to decrease the sand temperature which will influence the sex outcome of the hatchlings?
- To shield and protect the eggs and hatchlings from predators
- To protect the eggs from direct exposure to UV radiation (sunlight) and heavier rain fall during the seasonal rainy period.

### **Assumption:**

- Sand Type and texture is the same throughout the coast surface
- The depth of each nest is similar enough to be a negligible variable.

### **Limitation:**

- Decrease in atmospheric temperature during the rainy season will alter the temperature of the sand. The sand enclosed by the hatchery will decrease in temperature which might create a male bias environment thus influencing our final results and conclusion.
- The method does not take into consideration the effect of the heat generated into the metabolizing egg.
- The sex ratio of the leatherback turtle is said to be female biased over the majority of time (1), interspersed with brief male biases when new nest sites are colonized (2).



Source: Freedberg,S and Wade M,J, 2001

## 6.0 GENETIC TESTING

Genetic testing is done to determine the degree of rookery and overall genetic structure of the leatherback turtle found in Trinidad. This study is done by isolating the mtDNA which is an inherited maternal mitochondrial deoxyribosenuclei acid (DNA).

A method to carry out this experiment is as follows:

1. Skin samples can be collected from the mature female leatherback turtle while it is laying it eggs on the beaches.
2. Samples are also collected and preserved from the dead turtle eggs after the emergence of the hatchlings.
3. Samples were then preserved in 20% dimethyl sulphate solution saturated with laboratory grade salt.
4. Isolate the DNA using standard phenol chloroform DNA extraction techniques or by using fast prep DNA isolation kit.

A complete explanation of these steps and more can be found in the appendix.

**Table 3.** mtDNA haplotype frequencies at major rookeries in the western and Indo-Pacific.<sup>a</sup>

Rookery	Haplotype					
	A	D	E	F	H	I
PNG-Kamiali	1	1	1			15
Papua-Jamursba-Medi	1	1	1			28
Papua-War Mon						9
Solomon Islands <sup>b</sup>	2	1	1	1	3	40
Malaysia-Terengganu <sup>b</sup>	3	2	3		1	

<sup>a</sup> mtDNA = mitochondrial deoxyribonucleic acid.

<sup>b</sup> Data for Terengganu and Solomon Islands from Dutton et al. (1999).

Figure 2 source (Dutton et al 1999)

The information collected above shows that there was a lack of genetic differentiation between the leatherback turtles nesting in the Papua, Solomon and Malaysia. This common marker could indicate that there is a possible meta population with limited gene flow in the population.

This experiment shows that the population is fragmented and can indicate that future projections of population begin to have a long term decline. The results from this experiment show that there is a significant size of nest aggregation and this is a meta population.

This experiment can be replicated and done in Trinidad and Tobago to prove that there is limited gene flow in the population.

## **7.0 CONCLUSION**

The conservation of leatherback turtle in Trinidad and Tobago is a vital part of the tourism industry. The new threats they face are due to the increase in the global surface temperature. This increase in surface temperature can possibly affect the sex ratio of the Leatherback turtle. The tests proposed by this paper are vital to determine if the results predicted is actually a feasible outcome through viable testing.

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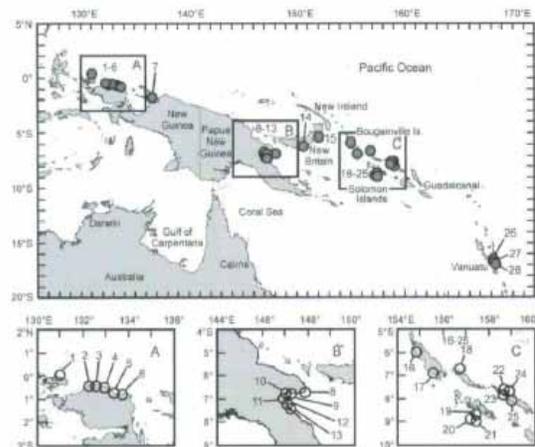
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## **10.0 Appendix**



**Figure 1.** Locations of significant (>20 nests/season) nesting sites for leatherbacks identified in the western Pacific. Names of the nesting sites are given by the corresponding numbers of the locations listed in Table 1.

and in demonstrating the existence of distinguishable stocks for management purposes (Moritz 1994; Dutton et al. 1999). A global survey of leatherbacks identified an eastern Pacific genetic stock, consisting of rookeries in Mexico and Costa Rica, that was distinct from the single rookery sampled in the western Pacific in the Solomon Islands and the Indo-Pacific rookery at Terengganu, Malaysia (Dutton et al. 1999). Because the other regional nesting sites were not sampled in this previous study, the extent of stock structuring among the western Pacific leatherback rookeries remains unknown. An understanding of genetic stock structure is necessary to accurately define management units for conservation (Moritz 1994).

The objective of this paper is to update information on leatherback nesting in the western Pacific by 1) identifying all known leatherback nesting beaches and 2) providing minimum estimates of current rookery sizes. For each of these sites, we assess current threats and indicate where gaps in information exist. Finally, we use mtDNA analysis to examine the degree of genetic population structuring among the key rookeries in the western Pacific. This paper lays the groundwork for future research and describes the geographic extent of the western Pacific leatherback stock.

## METHODS

*Population Status and Threats.* — In May 2004, a working group (WG) convened that included representatives from Papua (Indonesia), PNG, Solomon Islands, and Vanuatu (Kinan 2005). This WG consisted of researchers, managers, and tribal community leaders with extensive local knowledge. The WG reviewed collective knowledge and mapped out nesting sites that were either documented or believed to have more than 20 nests per season. The WG drew on several sources of information from internal reports, gray literature in local languages, field notes, and

personal observations to compile a matrix of information on population size and threats (Kinan 2005). Where possible, the WG attempted to estimate the number of nests laid per season at each site. Given the inherent error in these estimates, in addition to the annual variability in numbers of nests laid characteristic of marine turtles, a range was given for numbers of nests laid each year based on observations since 1999. The intent was to provide a minimum estimate for the number of nests laid annually by leatherbacks in the western Pacific as a basis for comparison to the previous estimate reported in Spotila et al. (1996). To make the estimates comparable, we divided the number of nests by 5 to estimate the number of females per year as described in Spotila et al. (1996). This approach makes the assumption that the average number of clutches laid by leatherbacks in the western Pacific is five. However, the number of nests laid per females is unknown for these populations. It is important to emphasize that we are only using this simplistic approach to provide a conservative population estimate for comparative purposes.

*Genetic Population Structure.* — Skin samples were collected from nesting females or salvaged from dead hatchlings from Jamursba-Medi (Wembrack, Warnamed, and Batu Rumah; Fig. 1), and Wemmon in Papua; from Kamiali in PNG; and from Solomon Islands (Sasakolo; Dutton et al. 1999; Fig. 1). Hatchling samples came from nests after emergence, taking care not to sample more than 1 nest per female. Skin samples were preserved in a 20% dimethyl sulphoxide solution saturated with laboratory grade salt, as described in Dutton et al. (1999). DNA was isolated by using either standard phenol/chloroform extraction techniques (Sambrook et al. 1989) or by using the Fast Prep DNA isolation kit (Bio101®). Amplification of mtDNA was performed by polymerase chain reaction (PCR) by using the primers HDCM2 and LTCM2, designed to target 496 bp at the 5' end of the control region of the mitochondrial genome (see Dutton et al. 1999). Template DNAs were amplified in 50  $\mu$ L PCR reactions on a Perkin Elmer 480 thermocycler by using the following profile: initial denaturation at 94°C for 2 minutes, followed by 36 cycles of 1) DNA denaturing at 94°C for 50 seconds, 2) primer annealing at 52°C for 2 minutes, and 3) primer extension at 72°C for 1 minute 30 seconds, concluding with a final primer extension for 5 minutes at 72°C. The size of the amplified products were determined by using electrophoresis in a 2% agarose gel stained with ethidium bromide. PCR products were then purified by using the Qiaquick PCR Purification Kit (Qiagen, Valencia, CA) and stored at 4°C. Direct cycle sequencing reactions of the light strand were performed on 2  $\mu$ L purified PCR product combined with 2  $\mu$ L ABI Prism dRhodamine Terminator Cycle Sequencing Kit, 3  $\mu$ L primer LTCM2, and 5  $\mu$ L purified water. The labeled extension products were then purified via ethanol precipitation and analyzed with an Applied Biosystems model 377 or 310 genetic analyzer. The sequences were

edited by using Gene Codes Sequencher 4.1. Haplotype frequencies were compared between the sampled nesting sites at PNG and Papua, and with published data from Solomon Islands and Malaysia (Dutton et al. 1999) by using a  $\chi^2$  test to determine genetic homogeneity. Monte Carlo procedures were used to correct the probabilities obtained by the  $\chi^2$  test for multiple tests and small samples (Roff and Bentzen 1989).

## RESULTS AND DISCUSSION

*Nesting Distribution and Abundance.* — Several previously undescribed nesting sites were identified. In most cases, there was local knowledge of leatherback nesting; however, no census work was carried out, and the number of nests could not be estimated (Table 1, Fig. 1). We estimate an approximate total of 5000–9100 leatherback nests are laid each year among all the beaches identified in the western Pacific (Table 1). The rookery at Papua, Indonesia, remains the largest and best studied (Hitipeuw et al. 2007), with 3 beaches at Jamursba-Medi containing the bulk of the nesting. However, there are several sites along the northwest coast of Papua that had historic reports of high-density nesting, where the status is now unclear. An aerial survey conducted in July 2005 only detected relatively low densities of nests along the stretch of coastline northwestward from Jamursba-Medi to Sorong (S.R. Benson, unpubl. data). In addition, Wermom appears to have much greater nesting activity than previously thought (Hitipeuw et al. 2007). Little is known about the vast, isolated coastline stretching southeastwards from these beaches to the border of PNG (Fig. 1), thereby underscoring the need for aerial surveys to identify unknown leatherback nesting sites along the coastline from the Bird's Head (Vogelkop) Peninsula of Papua to the PNG border. In PNG, there is scattered nesting along the north coast and around the Island of New Britain and Bougainville (Table 1; Fig. 1). This has been confirmed by recent aerial surveys (Benson et al. 2007) that identified Buang-Buasi and Kamiali as 2 areas of higher-density nesting. We recommend establishing these 2 areas as index sites for long-term monitoring to determine nesting abundance trends in PNG. The other sites listed for PNG in Table 1 can essentially be considered beaches that are used by the same nesting population that nests in the Huon Gulf. The estimates of total nests laid annually at all the sites in the Huon Gulf range from 500 to 1150 (Table 1). This range reflects the annual variability in nests and is based on preliminary data from 3 years of aerial surveys (S.R. Benson and V. Rei, unpubl. data). There was sporadic monitoring of nesting within a 4-km stretch of beach demarcated at the Kamiali Wildlife Management Area (KWMA), and 40–89 nesters were tagged annually between 1999 and 2005 (Kisakao 2004; Kinan 2005; Benson et al. 2007; N. Pilcher, unpubl. data). Nest counts from this effort underestimate the total nesting activity for PNG and the Huon Gulf, and we consider the numbers

provided in Table 1 from the aerial surveys as the most reliable estimates currently available. More recently, beach monitoring was initiated at the sites in Labu-Tale, Buang-Buassi, and Paiawa (Table 1; N. Pilcher, *pers. comm.*), and it is hoped that this expanded monitoring will provide more accurate estimates of annual abundance in the future.

Satellite telemetry suggests that nesters tagged at Kamiali tend to lay subsequent clutches in the same season at other sites, as well as within and adjacent to the KWMA. There is also an indication of movement by nesters between Kamiali and Bougainville (Benson et al. 2007). However, Bougainville is closer to the Solomon Islands than the Huon Gulf, and it is possible that some leatherbacks nesting in the Solomon Islands tend to also lay clutches on Bougainville. This would account for the variability in nest counts reported for Bougainville (Table 1), however, the extent of within-season movement between nesting sites is unknown. The Solomon Islands are more important than previously thought, with scattered nesting reported from several sites in the western province of Isabel (Table 1; Fig. 1). Surveys have been incomplete, and it is likely that nesting activity described in Table 1 underestimates the true population size. Vaughan (1981) reported leatherback nesting at 61 beaches in Isabel and the western province but many with just a few scattered nests. In 1996, 40 leatherbacks were sampled over a 7-day period for the genetic study at Sasakolo (Dutton et al. 1999; D. Broderick, *pers. comm.*). Given the scattered nesting around the islands in the region, it is possible that the size of this nesting population is on the order of hundreds, rather than tens of females. Extensive aerial surveys in conjunction with complete monitoring and tagging at multiple sites should be a priority for a more accurate assessment of leatherback population abundance in the region.

By using the numbers given in Table 1, we estimated a total of 1113 females nesting annually (FNA) following methods reported in Spotila et al. (1996). This number might be larger, because there are still areas where undocumented nesting occurs throughout the Island of New Guinea and beyond, such as in Thailand and Vietnam. This minimum estimate is larger than that of Spotila et al. (1996) who estimated 700 females for the western Pacific rookeries. Use of these new estimates would have produced a regional population estimate of 2782 breeding females in the western Pacific by applying the same simplified methods Spotila et al. (1996) used (multiplying FNA by 2.5) to derive their 1996 estimates for the regional populations. This is likely a conservative estimate and depends on the assumption that the average number of nests laid per female is 5 (Spotila et al. 1996). If leatherbacks lay fewer nests on average then the estimated number of females derived from the nest counts will be greater and vice versa, so that estimates would range from 2100–5700 females based on estimates ranging from ca. 840–3200 FNA (Table 2). This illustrates the problem of drawing conclusions on population status from estimates