Nesting Ecology of Olive Ridley (*Lepidochelys olivacea*) Turtles on Arribada Nesting Beaches

A Thesis

Submitted to the Faculty

of

Drexel University

by

Shaya Honarvar

in partial fulfillment of the

requirements for the degree

of

Doctor of Philosophy

December 2007

ACKNOWLEDGEMENTS

First, I would like to thank my family, especially my mother Shila and my brothers Ashkan and Sina, for all their support and love that they have provided me. Thank you for pushing me to go to the USA for finishing up my MS, even though originally it was only for 9 months and not 7 years.

Thanks to Dieter Schifferli for teaching me how to think like a scientist and Micah Brodsky, for believing in me and supporting me through my graduate years. I am also indebted to Marion and Curtis Brodsky. They have treated me as their daughter and have been my second family. Without them I would not have reached this point.

Of course, I thank my best friends Siona, Gauri, Matt and Laura for putting up with me when I could not talk about anything else except turtles. They probably know as much about turtles as I do at this point. Also thanks to Wasila, Vlademir, Raul and Tsan for their love and support. Thanks to Jack for listening to all my density-dependent and oxygen stories including the G^2 hypothesis…and forcing me to take a walk around the block when I could not think straight anymore. Thanks to Lorna super MAGNUS for helping me get through the last couple of weeks. It must have been hard to live with me!

I thank my colleagues and friends within the department of Bioscience and Biotechnology, especially Annette for all the fun and discussions we had; Eugenia for the good times, good food and providing a roof on top of my head when I needed one. Bibi and Gabi for the fun time during my stay at Playa Grande; and Bryan Wallace for his help dealing with Qubit system.

I am grateful to Roger Blanco Segura, Yorlan Diaz Chavarria, Emily Davis, Luis Gabriel Fonseca Lopez, Grettel Murillo Quiros, Luis Araya Sanchez and may others for helping me in the field. Thanks to Elyzabeth Velez and Rotney for all their help and support during preparation for a new field season.

I will never forget Dr. van den Berghe for all his help and support during my two year stay in Nicaragua. Especially when he was bringing me cheese puffs and tangerines while I was in the field stuck with rice and beans.

I am very grateful to my advisor Dr. James Spotila for the years of expert career guidance. He has been a great mentor, friend, and father-figure. Thanks to Dr. Mike O'Connor for keeping me sane and taking the time to help me improve my statistical analysis skills. I would like to thank the members of my committee, Dr. Susan Kilham, Dr. Jeremy Lee, Dr. Arthur Dunham, Dr. Pamela Plotkin, Dr. Harold Avery for all their support and guidance over the years.

This research was funded by Betz Chair of Environmental Science at Drexel University, Koinonia Foundation, The Leatherback Trust, Lerner-Grey grant for marine research from American Museum of Natural History and Linnaeus Fund and Chelonian Research Foundation and private funds. Approvals were provided by MINAE in Costa Rica, MARENA and Fundacion Cocibolca in Nicaragua and Drexel University IACUC. A special thank you to Thomas Elzey for his support of my project. Finally I would like to thank anyone and everyone else who has helped me on this journey. I am sorry that your name is not mentioned here. That would be due to my inability to type any more words…..

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Nesting Ecology of olive ridley turtles (*Lepidochelys olivacea*)
on Arribada Beaches
Shaya Honarvar
James R. Spotila, Supervisor, Ph.D.

Historically, the olive ridley (*Lepidochelys olivacea*) arribada at Playa Nancite, Costa Rica, was one of the largest arribadas in the eastern Pacific with 70,000 nesting females in a year. Recently that arribada drastically declined. We hypothesized that the decline at Playa Nancite could be due to low hatching success as a result of the high density of nests on the beach, such that recruitment to the population was insufficient to balance losses. To test this hypothesis, we examined density-dependent effects on hatching success and their underlying mechanisms by experimentally manipulating nest densities on the nesting beach. Experimental nest densities affected hatching success with highest density having lowest hatching success. Higher nest density led to lower O_2 levels and higher CO_2 levels in the nest, with greater changes in the latter part of the incubation. Highest temperatures occurred in high nest density areas. Bacterial diversity and richness were higher in the high zone of the beach on Playa Nancite. Bacterial diversity and richness were also studied at another arribada beach, Playa La Flor in Nicaragua. Bacterial diversity and richness were higher in the high zone of the beach on Playa La Flor. Bacterial abundance was not different in different zones of the beach or in different nest densities at both Playa Nancite and Playa La Flor. Bacterial diversity and richness may be important in affecting hatching success of olive ridley eggs. Long term failure in production of hatchlings due to historically high densities probably contributed to the decline of arribadas on Playa Nancite. The effects of egg harvest on olive ridley sea turtle

nesting beaches have been debated for decades. In order to more effectively manage the beach at Playa La Flor, Nicaragua, and potentially other nesting beaches, we developed an experimental protocol to measure the impact of egg harvest on this beach. Management strategies have traditionally involved the removal of eggs that are predicted to have less chance of survival, despite a lack of experimental data supporting this approach. Our findings indicate that even controlled egg harvest has a negative effect on nest hatching success and total hatchling production.

CHAPTER 1: General Introduction

Olive ridley turtles (*Lepidochelys olivacea*) are one of two sea turtle species that have massive synchronous nesting emergences called *arribada* (Spanish term for arrival). During an arribada olive ridley turtles nest at night and the arribada can last from 2 to 10 nights (Cornelius 1982). The cues responsible for this reproductive synchrony may include winds, tidal cycle, lunar phase, rainfall patterns, temperature, photoperiod as well as physiological cues and socially facilitated behavior (Pritchard 1969; Owens et al. 1982; Mendonca and Pritchard 1986; Cornelius and Robinson 1986). Prior to an arribada, groups of turtles are near shore in front of the beach (Cornelius and Robinson 1986; Plotkin et al. 1991). Generally, females lay two clutches of eggs per season, remaining near shore during the internesting period (Plotkin et al. 1994). It has been suggested that olive ridleys are capable of retaining oviductal eggs longer then other sea turtle species (Licht et al. 1982). They will retain the eggs until they receive the appropriate cues to nest (Pritchard 1969; Plotkin et al. 1995). The arribada nesting strategy results in a large number of nesting turtles on the beach at a given time and consequently a large number of hatchlings.

Arribada nesting behavior results in an increase in nest destruction when turtles inadvertently dig up each other's nests (Cornelius et al. 1991). Nest destruction, therefore, is often caused by density-dependent disturbances. However, density-dependent effects on hatching success in olive ridleys that nest in arribadas remain largely untested.

Furthermore, the large number of eggs deposited during successive arribadas may contribute to the buildup of pathogens in the sand, which may debilitate healthy eggs (Cornelius et al. 1991). The physical destruction of eggs and higher pathogen loads may negatively affect hatchling survival. Whether microorganism diversity and abundance is higher in high nest density areas on the beach remains to be tested.

Even though high nest density has a negative effect on hatching success there are still a number of advantages for olive ridley turtles to nest *en masse*. Arribada nesters can find mates more easily and can delay nesting until environmental conditions are suitable for nesting (Bernardo and Plotkin 2007). Olive ridley arribada nesters have high nesting beach fidelity and stay near shore and are inactive during the internesting period. This behavior potentially conserves energy in order to produce larger clutches (Plotkin et al. 1991; Plotkin et al. 1995; Kalb 1999). Producing large clutches may have a positive effect on organismal fitness (McGinley 1989). In addition it has been suggested that predators will consume a certain number of offspring (eggs and hatchlings) regardless of the total clutch size (McGinley 1989). A larger clutch can serve as a predator satiation device and/or social facilitation for hatchling survival (McGinley 1989). High nest density resulting from the arribada nesting strategy could additionally serve as a predator satiation device although this hypothesis needs to be studied more carefully (Bernardo and Plotkin 2007; Eckrich and Owens 1995).

Olive ridley sea turtle is classified as endangered in the IUCN red data book (Groombridge 1994) and is also listed in Appendix I of CITES (Lyster 1985). There are only a few nesting beaches in the world were olive ridleys still nest in arribadas

(Cornelius et al. 1991). The most important nesting beaches are in Costa Rica (Playa Ostional and Playa Nancite), Mexico (La Escobilla), India (Orissa) and Nicaragua (Playa Chacocente and Playa La Flor). Much smaller nesting aggregations occur along the Atlantic coast of South America and western Africa, as well as in the western Pacific and Indian Oceans (Groombridge 1982; Carr and Carr 1991).

Olive ridleys and their eggs are important for socio-economic reasons. For the local communities surrounding an arribada nesting beach, the trade of turtle eggs represents an important economic resource (Cáceres 1992; Campbell 1998). Due to the high level of poverty typical to local people near these nesting beaches, there is an economic demand for harvesting turtles and their eggs (Campbell 1998). It is argued that a regulated legal harvest will satisfy the demand for eggs in the market so that egg prices will decrease. Hence illegal egg harvest will not be valuable and egg poaching will stop. This will in turn keep turtle populations viable. However, in Playa Ostional, Costa Rica a controlled egg harvest is permitted to help the local community and illegal egg poaching still continues (Arauz, *personal communication*). Another argument in support of harvesting olive ridley eggs from the arribada beaches is the fact that due to the high numbers of nesting turtles they end up destroying each other's nests. A large number of nests will be destroyed which could lead to a high pathogen load in the sand and subsequently high levels of infection of healthy eggs (Cornelius and Robinson 1985). It is assumed that by taking eggs early in the arribada that the problem of nest destruction will be reduced. However, there is not sufficient data to support this argument and until now the impact of egg harvest

on hatching success and hatchling production has not been studied (Ballestro et al. 1998).

The general aim of my dissertation is to study both natural and anthropogenic factors that influence population dynamics of olive ridley turtles at Playa Nancite and Playa La Flor. In Chapter 2, I analyze the effects of nest density on hatching success and the underlying mechanisms that may be responsible for these effects. In chapter 3, I describe the impact of egg harvest on nest hatching success and hatchling production. In chapter 4, I analyze the diversity and abundance of microorganism on different parts on the beach and in different nest densities on two important olive ridley nesting beaches. Finally, in chapter 5, I will reassess my findings from chapters 2 through 4 and discuss their implications for a conservation management strategy for olive ridley arribada nesting beaches.

CHAPTER 2: Density-dependent effects on hatching success of the olive ridley turtle, *Lepidochelys olivacea.*

INTRODUCTION

Density-dependent effects regulate populations in the laboratory, but uncertainties continue as to the importance of these effects in nature (Ricklefs and Miller 2000). "By definition, a population is regulated if it persists for many generations with fluctuations bounded above zero with high probability" (Hixon et al. 2002). Factors such as intraspecific competition, predation, parasitism, and pathogens all have increasingly important effects as population density increases, limiting density increase (Gause 1934; Rasmussen 1941; Holling 1959a, Holling 1959b; Caughley 1970; Churcher et al. 2005; Hixon and Jones 2005; Johnson 2006). However, it is difficult to demonstrate these effects experimentally in natural populations.

Effects of density dependence are most obvious for sessile organisms, where space is often a limiting factor (Sousa 1984; Roughgarden et al. 1985; Possingham et al. 1994) resulting in an increase in mortality rate when most of the space is occupied. Motile organisms may have large impacts on other populations in a region by perturbing an area as they move through it by activities such as predation and grazing, as well as non-predatory behaviors (King 1977; Schaal and Leverich 1982; Tilman et al. 1997; Kausrud et al. 2006).

Recent studies have documented density-dependent effects on population dynamics in diverse vertebrates. Carr et al. (2002) used orthogonal manipulations of the presence of predators and territorial competition to determine the source of

density-dependent mortality in coral-reef fish. Density of tadpoles in experimental ponds affected survival and metamorph growth and development (Loman 2004). Altwegg (2003) determined that density-dependent effects played a role in development stages of pool frogs (*Rana lessonae*) and may have played a role in population regulation and dynamics of these frogs.

The importance of effects of nest density on hatchling production in sea turtles has been debated for decades. Bustard and Tognetti (1969) reported that nest density affected hatching success of green turtles (*Chelonia mydas*) in Australia. Cornelius et al. (1991) stated that the high densities of olive ridleys (*Lepidochelys olivacea*) nesting on Playa Nancite and Playa Ostional caused the destruction of many nests and reduced overall hatching success. Girondot et al. (2002), Caut et al. (2006) and Tiwari et al. (2006) used mathematical simulations to predict the effect of nest destruction on hatchling production and to estimate the maximum number of hatchlings (carrying capacity of the nesting beach) that could be produced on a leatherback (*Dermochelys coriacea*) nesting beach in French Guiana and a green turtle nesting beach at Tortuguero, Costa Rica. However, nest destruction is insufficient to explain low hatching success on olive ridley nesting beaches (Cornelius and Robinson 1986) and there are no studies of other mechanisms whereby high densities of nests could affect hatching success in sea turtles.

Olive ridleys nest in massive synchronous nesting emergences: a seasonal, monthly occurrence called an “arribada”. Bernardo and Plotkin (2007) reviewed the arribada phenomena and discussed fitness advantages for its participants. Despite direct effects of mass nesting on adult fitness, olive ridley populations may reach

"local egg-carrying capacity in the sand" such that nest density in sand is very high and hatchling production decreases leading to long-term population declines because of low recruitment of hatchlings (Arauz, *personal communication.*).

To understand population dynamics and to design the most effective conservation and management strategies for an endangered species it is important to understand how density-dependent factors regulate a population and to identify the mechanisms of regulation under natural conditions. Thus, understanding the processes regulating nesting success is essential for improving management decisions for the olive ridley. The population of olive ridleys at Playa Nancite has declined sharply since 1981 (Valverde et al. 1998; Plotkin et al. 1997; Cornelius et al. 1991; Mo and Clusella *unpublished reports*, Valverde*, personal communication*) and this may be due to low egg survival resulting in minimal population recruitment. From 1980 to 1990 hatching success was only 0.8-10% on Playa Nancite (Cornelius et al. 1991). Overcrowding resulted in large numbers of clutches (22.5%) being destroyed by subsequent nesting turtles when they dug up previously laid nests (Cornelius et al. 1991). High densities of nests on the beach may produce a hypoxic environment due to low diffusive conductance of beach sand to respiratory gases and high metabolic activity of developing sea turtle embryos (Ackerman 1977). In addition, because temperature of an egg determines the sex of a developing sea turtle embryo (Morreale et al. 1982), higher temperatures in crowded nests will produce more females.

In this study we have conducted an experiment under natural conditions that successfully isolates the effects of only one variable, nest density. We identified the density at which spatially determined interactions were important

for reproduction of olive ridleys at Playa Nancite, Costa Rica. We determined biophysical factors responsible for low clutch survival, density-dependent effects on hatching success and effects of clutch density on egg temperature. We also determined these variables at Playa La Flor, Nicaragua where nest densities are now as high as densities at Playa Nancite in 1980's. Our objectives were to determine: (1) How experimental nest densities in the range of natural densities affected hatching success, (2) How O_2 / CO_2 levels changed in high versus low nest density areas, (3) If embryo mortality was related to O_2 / CO_2 levels, (4) How nest and sand temperatures changed with nest density, (5) If incubation duration changed with nest density, (6) If nest density affected development stage at which eggs die, and (7) If density affected hatchling mass.

METHODS

We conducted this study from November 2005 – January 2006 at Playa Nancite, Costa Rica, and from January – February 2007 at Playa La Flor, Nicaragua. Playa Nancite is located in Santa Rosa National Park, in Guanacaste Province Costa Rica on the Pacific Ocean. The beach is 1 km long and, in general, 15 to 20 m wide, but unstable (its profile changes with storms). An estuary opens in either the center and/or at the northern end of the beach due to heavy rains and this may wash away a large number of nests. Since the 1970's regulations prevent egg harvest at any time at Playa Nancite. There are no significant anthropogenic impacts on nesting activity.

Playa La Flor is a 1.6 km beach located on the southwest (Pacific) coast of Nicaragua in Rivas Province. Playa La Flor has been a Wildlife Refuge since 1996

(Hope 2002). The beach is protected and number of nesting turtles and egg harvest are monitored by Ministerio del Ambiente y los Recursos Naturales (MARENA) and a nongovernmental organization, Fundación Cocibolca. Turtle egg harvest, permitted and poached, is very high at Playa La Flor (up to 40%).

At Playa Nancite we used the upper part of the beach with the lowest risk of clutches being washed away, for this experiment. We used a randomized, complete block design with 5 replicates of four 1 m x 1 m plots, each with a different density of nests. A control plot had no clutches and three other plots had low (2), moderate (5) and high (9) clutch densities.

We chose experimental nest densities using data from high and low density nesting areas on Playa La Flor, Nicaragua (Honarvar and van den Berghe, *personal observation*) and historical data from Playa Nancite (Cornelius et al. 1991, Mo and Clusella, *personal communications*). We marked each plot with 1.5 m long steel rods driven 1.1 m into the sand, at the corner of each plot. Each plot was in the middle of a 2 m x 2 m area and closed off by wire mesh cage material (2 m x 2 m x 0.5 m). We removed all eggshells and vegetation in each area to a depth of 70 cm by hand. We placed 4 clutches in the buffer zone around each 1 m plot to limit edge effects. During an arribada in November 2005, we collected eggs directly from the cloaca of turtles into a sterile bag to decrease potential contamination by microorganisms and then transported egg-filled bags to each plot. Average clutch size of olive ridleys at Playa La Flor is 95 ±11 (Honarvar and van den Berghe, *personal observation*) and at Playa Nancite is 100 (Cornelius et al. 1991). Each relocated clutch contained 70 eggs to ensure uniformity and 10 other eggs from the same clutch were weighed for initial

egg mass (all extra excavated eggs were reburied in the beach). We relocated eggs to experimental nests within 45 min of collection. We constructed egg chambers with gloved hands, 50-60 cm deep with the bottom of the chamber wider then the top, in the shape of a round bottom flask. We placed a wire mesh cage (40 cm x 40 cm x 10 cm) on top of each nest, burying all sides of the cage in the sand. All plots were protected from other nesting turtles by wooden poles every 50 cm around the whole area. Hourly patrols began on the first day of relocation and continued for a week to prevent predation.

We collected gas samples from one clutch per density treatment per block and from sand at nest depth near a nest for each density treatment in 3 blocks. We placed a 35 mm film canister, perforated with numerous small holes, in the center of a clutch when burying eggs. Tygon tubing (3 mm ID, 60 cm long) allowed for passage of gas from the film canister to the surface. Shut-off valves closed the ends of tubes so that water, air and sand could not enter the clutch. Infrared CO_2 analyzer and a flow through O_2 sensor (Qubit Systems, Ontario, Canada) gave real-time measurements of nest pO_2 and pCO_2. We calibrated sensors using atmospheric air before each use and with a standardized mixture of CO_2 and O_2 in the laboratory at the end of the experiment. A LabPro data logger and laptop computer connected to sensors collected and stored data. A pump drew gas from the nest, through a Drierite desiccant column, the CO_2 sensor, soda lime column and finally, the O_2 sensor. Sample air flow rates were 50 ml/min and the sampling duration was 2 min (Wallace et al. 2004). We used a 30 sec calibration period between each nest sample, in which we measured atmospheric pO_2 and pCO_2 (Wallace et al. 2004). We analyzed data using Logger Pro

software from Qubit Systems. We measured pO_2 and pCO_2 every 5 days during early incubation, every 4 days in the second third of incubation and every 3 days in the last part of incubation until hatchlings emerged. The pO_2 and pCO_2 change more rapidly during the latter part of incubation.

We recorded temperature of one clutch per density treatment (including control plot) in 5 blocks and in surrounding sand in 3 blocks with 24-gauge Cu/Cn thermocouples (± 0.05°C) using a hand -held digital thermometer (model HH200A, Omega) at 7:00 am every 3 days.

To ensure that hatching turtles were not kept in cages for an excessive period of time, hourly patrols began on the 44th night of incubation and continued through day 55. Once hatchlings were detected in cages, we counted and released them at the nest. The cage was repositioned over the nest so no stragglers were omitted. Ten hatchlings per nest were randomly chosen and weighed. Five days after the first day of hatching we excavated nests and determined developmental stages of un-hatched embryos following the protocol of Leslie et al. (1996).

At Playa Nancite we recorded number of nesting turtles during the November arribada in a 25 m x 50 m section on the beach with the highest density and the entire beach. During hatching, we counted number of clutches hatched and number of hatchlings per clutch for 10 nests. To compare O_2, CO_2 and temperature from density treatment plots with natural high densities on the beach, we measured these variables at nest depth at 6 randomly chosen locations on a densely used section of the beach near the experimental plots as described above. We also recorded air temperature and amount of rain (via a TRU-Check rain gauge) at 7:00 am every day.

At Playa La Flor we recorded O_2, CO_2 and temperature in a high nest density area (N=10) and in a low nest density area (N=5) at nest depth in sand in January 2007. This was the latter part of incubation for nests from the December 2006 arribada and the early part of incubation for nests from the January 2007 arribada. In addition, we recorded O_2, CO_2 and temperature from three clutches in the high density area of the beach from both December 2006 and January 2007 arribadas. We placed tygon tubing and thermocouples 1 cm from the clutch at 30 cm depth. Number of nesting turtles was counted in a high nest density area 30 m X 100 m and a low nest density area 20 m X 100 m by MARENA during the December 2006 arribada. During hatching, number of clutches hatched in both the low and the high nest density areas of the beach was counted by MARENA.

All statistical analyses were done using SAS 9.1. Separate two-way, mixed model ANOVAs (SAS PROC MIXED, randomized block design) tested whether experimentally modified nest densities affected hatching success, hatchling production, incubation duration and whether temperature, O_2 and CO_2 levels were significantly different in different density plots and blocks. Nest density was a fixed factor, blocks were a random factor and the number of hatchlings/clutch, hatchlings/m^2, incubation duration, temperature, O_2 and CO_2 were response variables. We used a Tukey-Kramer post hoc test to determine significant differences between densities. We used the CONTRAST comparison in SAS to determine significant differences in hatchling production. Hatching success was calculated as number of hatchlings emerged divided by 70 (the original number of eggs). We arcsine transformed hatching success data for analysis. An $\alpha = 0.05$ level was accepted for all

the tests preformed. We analyzed development stage of un-hatched eggs in the different density treatments by separating them into early (stage 0 and 1) and late (stage 2 and 3) stages and running a MANOVA with randomized block design.

We used one-way ANOVA to determine whether, O_2, CO_2 and temperatures in the sand were significantly different at high and low nest densities at Playa La Flor. We also compared high and moderate density experimental plots versus high nest density in the beach at Playa Nancite during latter part of incubation. We used a Tukey post hoc test to determine significant differences between densities. We calculated overall nest hatching success for the nesting beach for the November 2005 arribada at Playa Nancite (data collected in this study) and the December 2006 arribada at Playa La Flor (data collected by MARENA) using number of nests hatched on the nesting beach divided by number of nesting turtles.

Since nest densities on the different sections of the beach were not uniform we did a simulation to calculate the expected range of nest densities from an arribada on Playa Nancite and on Playa La Flor (25 m x 50 m section). We located 1500 and 3000 nests using MATLAB 7.0, which randomly placed nests in each plot. We estimated nest densities using a bivariate normal product kernel technique (Martinez and Martinez 2002) and ran the simulation 1000 times. In addition, we simulated percentage occurrence of nest destruction for 1500 nests randomly distributed on a 25 m x 50 m section of the beach. We used a similar simulation to model nest densities for a 25 m X 50 m section given total number of nesting turtles for the whole nesting season at Playa Nancite (13,000) and Playa La Flor (187,000). In addition, simulation for Playa La Flor also predicted the number of nests/m^2 in 1980s at Playa Nancite.

RESULTS

Density-dependent effects

Experimental nest density had a significant effect on hatching success. Hatching success in high density experimental plots was significantly lower (29.5%) than in moderate (55.9%) and low density (71.6%) plots (two-way ANOVA, $F_{2,73}$ = 13.63, $P < 0.0001$; Fig. 1). Overall hatchling production did not differ in high ($192/m^2$) and moderate ($189/m^2$) density experimental plots, but was significantly higher than in low ($100/m^2$) density experimental plots (two-way ANOVA, $F_{1,8}$ = 12.24, $P = 0.0081$). Only 122 clutches from 1521 nesting turtles at Playa Nancite hatched (8%). Overall nest hatching success of the beach was also 8%.

Density did not have a statistically significant effect on the stage of development at death of embryos or on incubation time (46.2 – 46.9 days). In addition, stage of development did not affect embryonic mortality. There were no statistically significant differences among density treatments in egg mass before incubation (31.2 – 31.8 g) or in hatchling mass (15.5 – 15.6 g).

Gas exchange

Oxygen and CO_2 levels in clutches in all three density treatments remained close to control values during early incubation and changed during the latter part of incubation (Fig 2a and b). The CO_2 concentrations on the 40^{th} and 45^{th} day of incubation were significantly higher in high density plots than in control and low density treatment plots (two-way ANOVA, $F_{3,28} = 91.13$, $P < 0.0001$). On both days 40 and 45, CO_2 concentrations were significantly higher in high density treatment plots versus moderate and low densities. The O_2 concentrations on the 40^{th} and 45^{th}

day of incubation were significantly lower in high density plots than in control plots (two-way ANOVA, $F_{3,28} = 50.11$, $P < 0.0001$). On both days 40 and 45, O_2 concentrations were significantly lower in high density treatment plots versus the other plots.

Temperature

There was 25 mm of rain and air temperature was 25°C ± 2.3 during the incubation period. Temperatures in nests and nearby sand in density treatments were close to values in control plots for the first part of incubation but differed during the latter part of incubation (Fig. 3a). Temperatures for days 37, 40 and 47 of incubation were significantly higher in density plots than in control plots and significantly higher in highest density plots versus moderate and low density plots (two-way ANOVA, $F_{3,45} = 154.61$, $P < 0.0001$).

Gas exchange and temperature under natural conditions on Playa Nancite and Playa La Flor

The O_2 and CO_2 levels in the sand under natural conditions on Playa Nancite had the same pattern as in nests in density treatment plots with values close to control values for early incubation (Fig 2c and d). Mean O_2 levels in the sand during latter part of incubation were significantly different between beach and high and moderate density treatments (one-way ANOVA, $F = 34.55$; $df = 2,45$; $P < 0.0001$). Mean CO_2 levels in the sand during the latter part of incubation were significantly different between beach and high and moderate density treatments (one-way ANOVA, $F_{3,56} = 76.63$, $P < 0.0001$). Sand temperatures during the latter part of the incubation were significantly lower in the beach than in high density plots (one-way ANOVA, $F_{3,56} =$

9.30, $P < 0.0001$; Fig. 3b). There was no statistically significant difference between temperatures in the beach and the temperatures in sand in moderate and low density plots.

Oxygen and CO_2 concentrations in sand at Playa La Flor in high and low nest density areas were significantly different. Mean O_2 level during the latter part of incubation was significantly lower in a high nest density area (18.4%) compared to a low density area (19.1%) on the beach (one-way ANOVA, $F_{1,105} = 27.70$, $P < 0.0001$). Mean CO_2 level in the sand during latter part of incubation was significantly higher in a high nest density area (4.2%) compared to a low density area (2.8%) (one-way ANOVA, $F_{1,127} = 67.53$, $P < 0.0001$). In nests from the December arribada (day 36 of 51 day incubation) O_2 was as low as 15% and CO_2 was as high as 10%. In nests from the January arribada (day 18 of incubation), laid among December nests, O_2 was as low as 16.5% and CO_2 was as high as 8%. Temperature was significantly lower in a low nest density area (32.7°C) compared to a high density area (35.3°C) (one–way ANOVA, $F_{1,114} = 200.56$, $P < 0.0001$). Temperatures of 6 nests incubating in a high density area of the beach from December and January arribadas reached as high as 38°C. Nest hatching success in a high density section was 10% and in a low density section was 16%. Overall nest hatching success was 12% for the December arribada at Playa La Flor.

Simulation

Simulated nest density distributions for a Playa Nancite arribada (1500 nests/25 m X 50 m) and a Playa La Flor arribada (3000 nests/25 m X 50 m) (Fig. 4) indicated that nest densities would not be uniformly distributed and that variable local

nest densities could be explained by random processes. Simulated local nest densities varied from zero to 4-5 nests/m^2 on Playa Nancite (Fig. 4b) and 5-7 nests/m^2 on Playa La Flor during these arribadas (Fig. 4a). The relative frequency of nest densities was highest for approximately 1 nest/m^2 at Playa Nancite and 2-3 nests/m^2 at Playa La Flor (Fig. 5) for the simulated arribadas. Simulation of nest densities for the total number of nesting turtles for the whole nesting season (2005-2006 at Playa Nancite and 2006-2007 at Playa La Flor) indicated highs of 4-5 nests/m^2 for Playa Nancite and 10-16 nests/m^2 for Playa La Flor. Relative frequency of nest densities was highest for approximately 1 nest/m^2 at Playa Nancite and 9 nests/m^2 at Playa La Flor (Fig. 5). Simulation of nest densities for the total number of nesting turtles for the 1980 nesting season at Playa Nancite indicated highs of 10-16 nests/m^2. This simulation predicted 22.7% nest destruction on Playa Nancite and 40.2% on Playa La Flor during one arribada in a given high density section of the beach. It also predicted 87.2% nest destruction for the current nesting season on Playa La Flor and for a nesting season in the 1980's at Playa Nancite.

DISCUSSION

This study is the first experimental demonstration of a density-dependent effect on hatching success in sea turtles. Here space for nests was the limiting factor and an increase in mortality rate occurred as more of the space was occupied. Roughgarden et al. (1985) developed a model that predicted the effect of settlement rate on the demography of sessile marine organism. Space was the limiting factor in recruitment success. Possingham et al. (1994) predicted that if density dependent

predation by starfish on barnacles increased rapidly at some critical prey density, then abundance of the prey would cycle at the same spatial scale as the predation mechanism. Here the mechanism of the density dependent effect was physiological and the overall effect on the population appeared to be a population cycle like that predicted by Possingham et al. (1994) based on predation.

Unlike previous studies on sea turtles, we removed all other density dependent and independent factors that could affect hatching success leaving only one factor, the nest density itself (nests/m^2). Nest density significantly affected hatching success in experimental treatment plots (Fig. 1). High nest density decreased hatching success. There was no significant difference in hatchling production between high and moderate density experimental plots. There was no difference in embryonic stages at which embryos died in the different density plots. To understand how limited space can affect the decline in hatching success in high nest density treatments we measured the gas environment and temperature in which embryos developed.

Gas exchange

Gas exchange has been shown to be important factor affecting population dynamics of pool frogs. Frog embryos stop development or die when the pO_2 is very low due to high number of metabolizing embryos in large gelatinous egg masses (Seymour and Bradford 1995). In estuarine crocodiles and alligators, oxygen has been shown to be a limiting factor and could cause slow growth, development and smaller hatchling size (Booth 2000; Warburton et al. 1995).

In our study experimental nest density affected gas exchange in sea turtle eggs and gas exchange was limited in high nest density plots. This confirmed the

hypothesis of Ackerman (1996) that at high nest density gas exchange of nests will affect other surrounding nests. As nest density increased O_2 concentration decreased and CO_2 concentration increased in the nest and in the surrounding sand (Fig. 2). This was due to increased metabolic activity (increased O_2 consumption) in high density plots. Ackerman (1977) reported that by the end of incubation O_2 can be as low as 12 to 14 kPa (11.8% to 13.8%) while CO_2 levels can be as high as 4 to 6 kPa (3.9% to 5.9%) in green turtle and loggerhead turtle nests. Maloney et al. (1990) measured 2 kPa to 3 kPa (2% to 2.9%) for CO_2 in loggerhead turtle nests in Queensland, Australia. In our high nest density treatments O_2 dropped to 17.2% and the CO_2 rose to 6.2% in nests on Playa Nancite. This was due to the large number of developing clutches in high density plots. The O_2 demand and CO_2 production increase during the second half of the incubation period in a sea turtle nest (Prange and Ackerman 1974; Ackerman 1977; Reynolds 2000; Wallace et al. 2004). In our study, higher production of CO_2 in the latter part of incubation and large number of clutches resulted in higher CO_2 levels in high nest density treatments. Hatching success decreased in our study in high density nests with an elevated CO_2 level suggesting that levels of CO_2 approaching 4-5 % were detrimental to developing sea turtle embryos. Levels of O_2 in our study were higher than those measured by Ackerman (1977) but similar to those measured by Wallace et al. (2004) for leatherback nests in a hatchery at nearby Playa Grande. They attributed higher than expected O_2 levels to tidal pumping. The same mechanism was probably active at Playa Nancite. Both beaches have a 3 m tidal range.

Under natural conditions on the nesting beach O_2 levels in sand were significantly lower and CO_2 concentrations in sand were significantly higher than the gas concentrations found in sand from the high nest density treatments. This was probably due to shorter distances between nests and presence of higher levels of microorganisms in the sand on the nesting beach due to the large number of nests destroyed by nesting turtles (Clusella Trullas and Paladino 2007).

At Playa La Flor the CO_2 and O_2 levels in sand at high nest density were 4.2% and 18.4%, respectively. The CO_2 levels in nests in a high density area on the beach reached 10% at 36 days of incubation. An important difference between Playa La Flor and the experimental plots at Playa Nancite was the large number of nests destroyed during large arribadas. Oxygen uptake and CO_2 release due to the rotting eggs from these nests likely affected gas concentrations on the nesting beach. At Playa La Flor there were 11 arribadas from July 2006 to January 2007. Whether the number of arribadas has a cumulative effect on gas concentration in nests or in the beach (the more arribadas the higher the CO_2 and the lower the O_2 of the sand) requires further study.

Temperature

Temperatures in nests and in sand surrounding the nests were significantly higher in high nest density treatments during the last third of incubation (Fig. 3). However, the absolute difference was small (1°C) and did not affect incubation duration. We found greater changes (2 to 3°C) in the sand temperature in a high density area versus a low density area under natural conditions on Playa La Flor.

Simulation

Simulations predict local nest densities on Playa Nancite as high as 4-5 nests/m^2 for 2005-2006 (Fig. 5), which was comparable to our moderate nest density treatments. However there was a great difference between the hatching success on the beach (8%) and in the moderate nest density treatment plots (55.9%). This was probably due to nest destruction (22.7%), which left large numbers of broken eggs in the sand, as well as egg predation by many species (Cornelius 1991).

In the 1980's local nest density at Playa Nancite could have been as high as 10 to16 nests/m^2/nesting season (Fig. 5). Since then the olive ridley population on Playa Nancite has declined (Valverde et al. 1998). Our data suggest that this decline was probably due to high nest densities in the past that resulted in high numbers of nests destroyed by subsequent nesting turtles (87.2%) and high incubation temperature and CO_2 levels in nests and surrounding sand due to high nest densities and excessive microbial respiration.

The number of nesting turtles at Playa La Flor is increasing (46,000 turtles in 1999-2000 to 187,000 in 2006-2007 nesting season). Currently simulated nest density at Playa La Flor is as high as 5 to 7 nests/m^2/arribada and 10 to 16 nests/m^2/season (Fig. 4a and 5). Nest hatching success for the December 2006 arribada was 12%. It is possible that due to the small population size at Playa La Flor in the past, hatching success was higher resulting in the current high numbers of turtles. This population may crash in the future as did the population on Playa Nancite. There may be population cycles on these beaches similar to the classic rise and decline of the Kaibab deer population in Arizona (Caughley 1970). Long term studies on the

population biology of nesting turtles and hatchling production on these two beaches are necessary to elucidate the interaction of nest density effects on recruitment and the population dynamics of olive ridley turtles.

Density dependence and population dynamics

In general, many factors can cause density-dependent effects at different life stages of an organism leading to regulation of population cycles. This is well known in insects, birds and mammals (Sousa 1984; Miller 2007; Yom-Tov et al. 2007). Many of the best recent examples of density dependent effects at multiple life stages are in amphibians. Altwegg (2003) found that high densities reduced growth of both aquatic larvae and terrestrial juvenile pool frogs (*Rana lessonae*). Loman (2004) reported density-dependent effects on survival of *Rana temporaria* during the tadpole stage in natural ponds. Harper and Semlitsch (2007) determined that density had strong regulative effects on survival, growth and reproductive development of wood frogs (*Rana sylvatica*) and American toads (*Bufo americanus*) in terrestrial enclosures.

In our study we focused on one life stage (egg incubation) and showed that when space was a limiting resource, gas exchange became an important limiting factor leading to higher embryo mortality rates in clutches at high nest densities. Density-dependent effects also occur in the adult stage of sea turtles. Bjorndal et al. (2000) showed that there are density-dependent effects on growth rate in adult green sea turtles. This density-dependent effect is due to their foraging behavior (grazing), in which the turtles concentrate on specific grazing areas.

Therefore, in order to understand population regulation in sea turtles it is necessary to study all life stages of a species. For example, directed and incidental capture of olive ridleys by fisheries has greatly impacted many populations (Frazier et al. 2007). More then 60,000 olive ridleys were captured annually in the 1990s in Pacific Central America (Arauz 1996; Arauz et al. 1998). The combined impact of adult mortality and density-dependent effects on the beach reported here on reduction of recruitment should be considered in assessing the population dynamics of this species. This in turn is critical for developing an effective conservation strategy. Thus, even though olive ridleys are the most abundant sea turtles, population crashes at nesting beaches in Costa Rica and Mexico (Frazier et al. 2007; Cornelius et al. 2007) caution us to obtain a clearer understanding of population regulation at all life stages before allowing exploitation of any life stage.

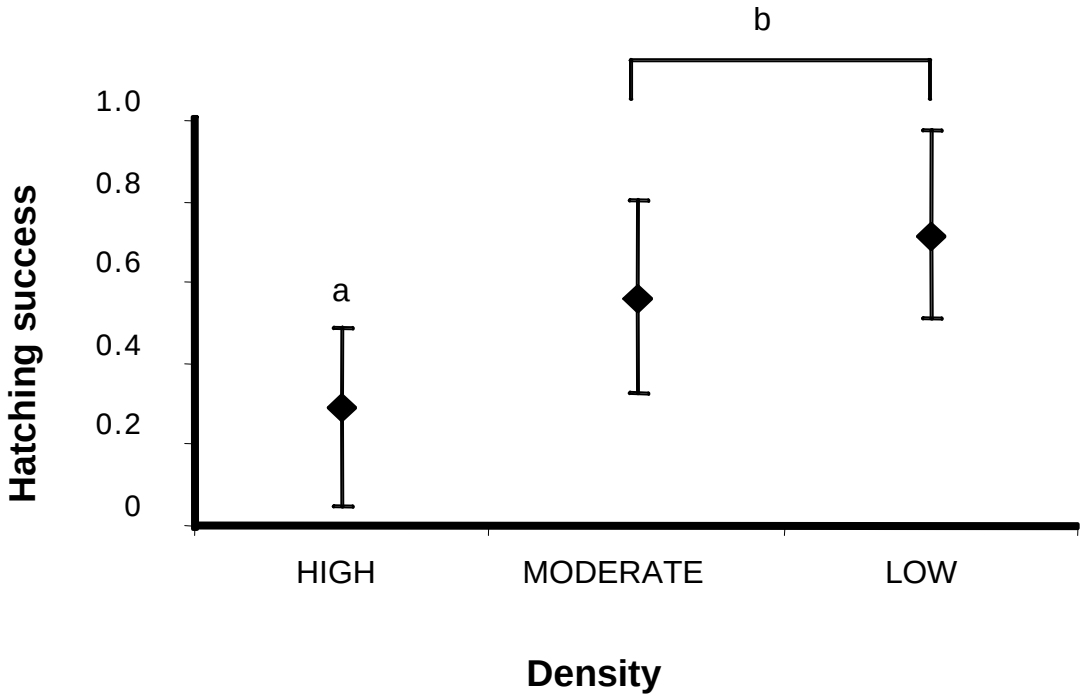

Figure 1. Hatching success (mean ± CI) measured in different experimental density treatment plots at Playa Nancite, Costa Rica. Hatching success in high density treatment plots was significantly lower compared to moderate and low density plots indicated by different lowercase letters.

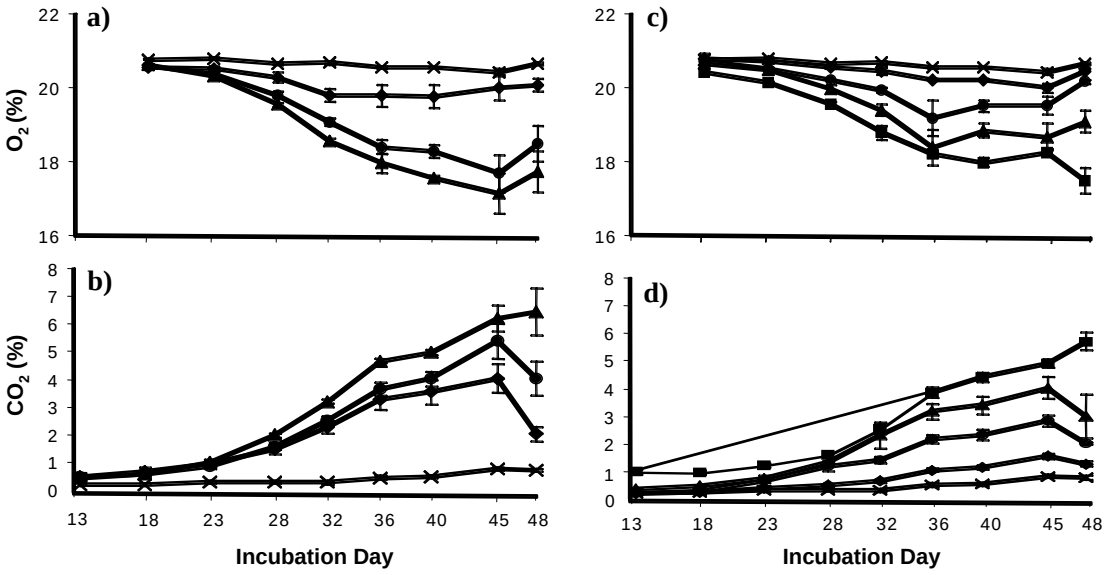

Figure 2. Oxygen and CO_2 in nests and in sand (mean ± SE) during incubation at Playa Nancite. Oxygen in nests (a) in sand (c). Carbon dioxide levels in nests (b) and in sand (d) in different density treatment plots (*Squares = beach, triangles = high density, circles = moderate density, diamond = low density*) shown in the graph along with control plots (*X = control*) which contained no nests.

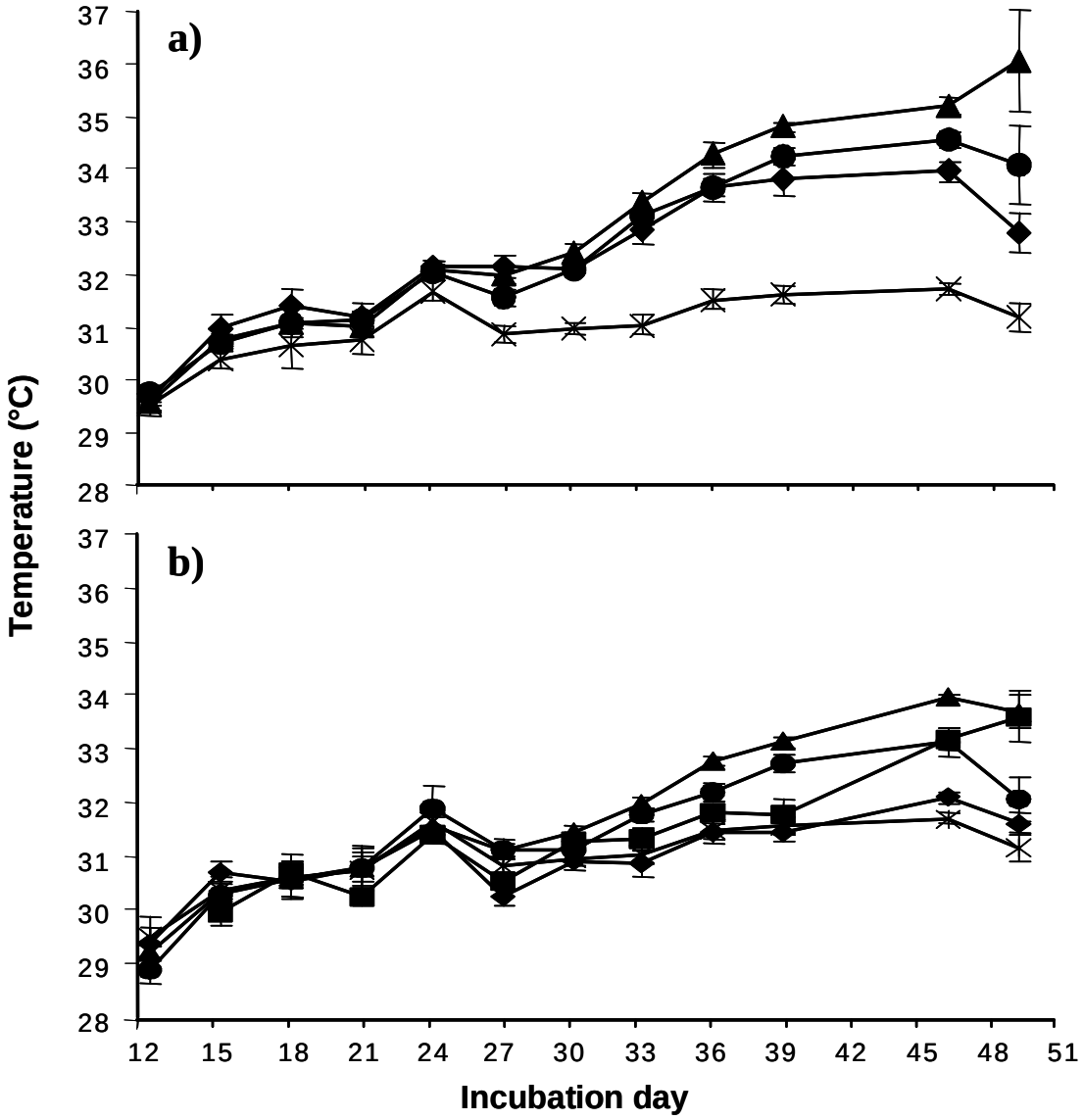

Figure 3. Temperatures in nests and in sand (mean ± SE) during incubation at Playa Nancite. Temperature in nests (a) and in sand (b) in different density treatment plots (*Squares = beach, triangles = high density, circles = moderate density, diamond = low density*) are shown in the graph along with control plots (*X = control*) which contained no nests.

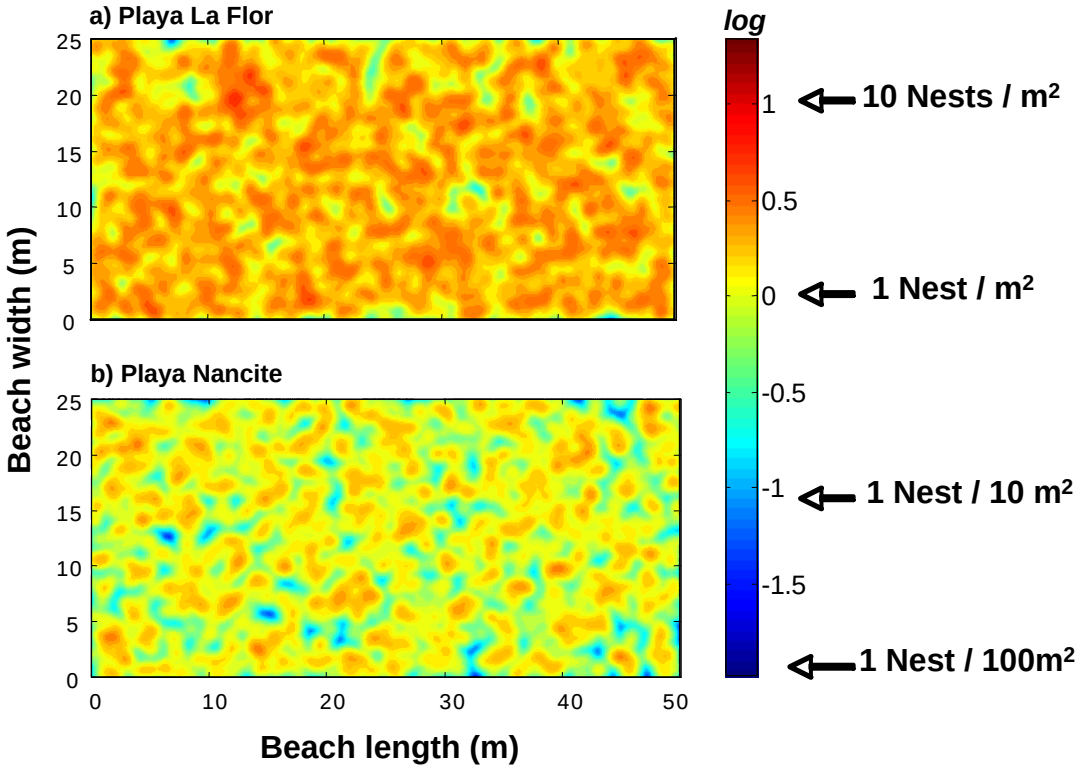

Figure 4. Simulated nest densities from one arribada on the nesting beach at 2005 levels of nesting. Playa La Flor (a) and Playa Nancite (b). Color scale of densities is given as a log scale.

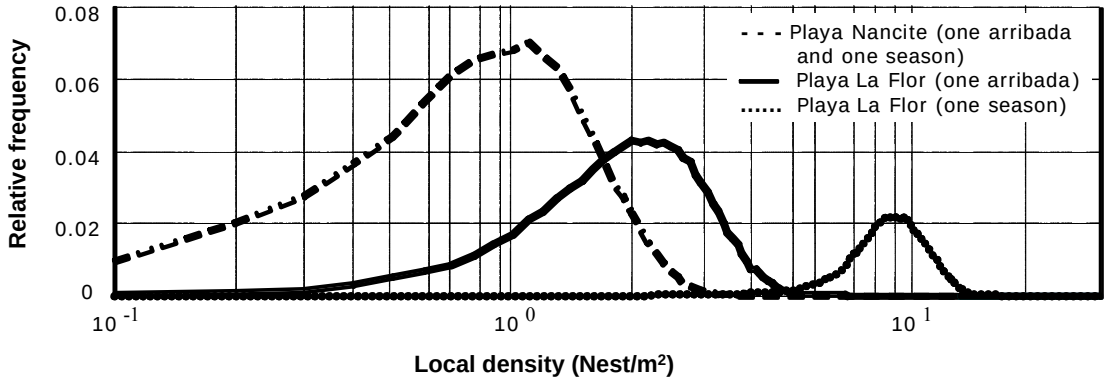

Figure 5. Simulated frequency of number of nests/m^2 at Playa Nancite and Playa La Flor. Relative frequency of number of nests/m^2 for one arribada and for the entire season at Playa Nancite (*dashed line*), Relative frequency of number of nests/m^2 for one arribada at Playa La Flor (*solid line*) and for the entire nesting season at Playa La Flor (*dotted line*).

CHAPTER 3: Impact of egg harvest on the olive ridley, *Lepidochelys olivacea,* population at Playa La Flor, Nicaragua.

INTRODUCTION

Utilization of natural resources, including wildlife, may be of significant economic, political, social, and cultural importance to a society (Campbell 1998; Hope 2002). The benefit derived from wildlife use can play a role in the response of community members to conservation efforts and may significantly influence the outcome of scientific investigations and conservation projects. Consideration of the views of, and effects on, local communities should be addressed during the design and implementation of conservation projects. Major threats to individual species survival and biodiversity include habitat destruction, habitat fragmentation and degradation, introduction of invasive species and over-harvesting of wildlife resources (Bucher 1992; Holdaway and Jacomb 2000; Eng-Heng Chan 2006). While long-term studies are important for studying the biology and population status of any organism under consideration, the effects of wildlife harvest on a population should be examined before implementing harvest management programs.

The olive ridley sea turtle, *Lepidochelys olivacea,* is classified as endangered in the IUCN red data book (Groombridge 1994) and is listed in Appendix I of CITES (Lyster 1985). To date, scientific research on the olive ridley turtles has focused largely on biology, despite decades of debate about the viability of egg harvest on nesting beaches (Cornelius & Robinson 1985; Ballestero et al. 1998; Mrosovsky 1997; Cornelius et al. 2007; Campbell 2007; Witherington and Frazer 2003). Although a few studies have examined the economic benefits and socio-economic

influences of harvesting olive ridley eggs (Campbell 1998; Hope 2002), there have been no definitive studies examining the effects of egg harvest on the population dynamics of the olive ridley turtle. The impact of harvesting on olive ridley population was studied at Playa Ostional but due to weakness of the counting methodology no conclusions were able to be drawn (Ballestro et al. 1998).

Olive ridley turtles are the most abundant sea turtles in the world. They exhibit a unique nesting strategy whereby large numbers of turtles emerge on nesting beaches in a synchronous phenomenon, called an "Arribada," (a Spanish term for arrival). The Arribada nesting behavior by these turtles results in a density-dependent destruction of previously laid clutches (Cornelius and Robinson 1985). Current management strategies include egg harvest and are based on the rationale that clutches laid early in an arribada are likely to be destroyed by turtles nesting later in the same arribada, or in subsequent arribadas (Campbell 1998). Clutch destruction, in conjunction with increasing microbial load throughout the nesting season (due largely to deposition of the nutrient rich egg content from disturbed nests being deposited in the sand) may decrease hatching success and hatchling production on the nesting beach (Cornelius et al. 1991, Campbell 1998). To date, there have been no conclusive studies showing the effect of nest removal on hatching success and hatchling production.

Worldwide, few beaches remain where olive ridley turtles still nest in arribadas (Spotila 2004). The most important nesting beaches in the Americas are located in Costa Rica, Mexico and Nicaragua, and they benefit from varying degrees of protection or management. In Costa Rica, Playa Ostional Wildlife Refuge was

established in 1985. Since then a controlled egg harvest has been enforced, in which eggs are only collected during the first 36 hours of each arribada (Campbell 1998). Playa Nancite is located in Santa Rosa National Park, Costa Rica, and is fully protected by geographic isolation in addition to legislation. There is no egg harvest permitted at Playa Nancite and significant poaching is absent (Cornelius et al. 1991). In Mexico, a nationwide ban on egg harvest has been in place since 1927. Despite this ban, high levels of egg poaching, and land based slaughter of nesting adults, continued through the 1990s, leaving La Escobilla, in Oaxaca, as Mexico's only surviving arribada beach (Trinidad & Wilson 2000, Peñaflores et al. 2001). In Nicaragua, Playa Chococente is managed with a controlled egg harvest between July and January of each year, while unlimited egg harvest is permitted for the rest of the year (Stewart 2001, Hope 2002). Playa La Flor, Nicaragua, was officially declared a wildlife refuge in 1996 (Hope 2002). A program of controlled egg harvest is in place throughout the arribada nesting season at Playa La Flor, which extends from July to February each year. Peak nesting activity and the largest arribadas typically occur between August and October. Since 1992, MARENA (Ministerio del Ambiente y Recursos Naturales), the Nicaraguan military, and local community leaders have worked together to protect nests from July to February of each year. From 1998 until present, Fundación Cocibolca (a non governmental organization) has been responsible for management of the nesting beach, beach protection and for monitoring the egg harvest, on a contractual basis.

The trade of turtle eggs represents an important economic resource for the population surrounding Playa La Flor (Cáceres 1992). A semi-controlled egg harvest

of 4% has occurred since 1993 (Arauz 1996, Hope 2002). Historically, in an average year, each family from the surrounding communities would receive from 7 to 8 dozen turtle eggs per season, which might be used for consumption or for sale (Arauz 1996). During the nesting seasons from 2003 to 2005 the controlled egg harvest was increased to an estimated 10% of the eggs deposited on the beach. From this 10% harvest, approximately half was given to the surrounding communities, where each family allotment was increased to an average 10 dozen eggs per season. The remaining eggs were used as a payment to individual community members who physically participated in the egg harvest (Fundación Cocibolca reports in 2003- 2004 and 2004-2005 nesting season).

In order to more effectively manage the nesting beach at Playa La Flor, and potentially other beaches where the same nesting strategy is employed, it is important to develop an accurate, quantitative estimate of the impact of egg harvest on this population of olive ridley turtles. Answers to the following questions are crucial to the development of a more effective management strategy: 1) What is the status of olive ridley nesting population at Playa La Flor, Nicaragua? 2) How does removal of a newly laid clutch on destroyed nest affect overall hatching success and hatchling production under natural conditions?

METHODS

Study site

Playa La Flor is situated on the southern Pacific coast of Nicaragua, bounded by Punta Brasilito to the north and Punta La Flor to the south, 15 km north of the

Costa Rican border. Playa La Flor is in the Province of Rivas, approximately 18 km southeast of the town of San Juan del Sur. 1.6 km long, this nesting beach lies within the 8 km^2 boundary of the refugio de Vida Silverster La Flor. In general, the beach ranges from 15 to 20 m wide, and is unstable (its profile changes throughout the season).

Clutch removal experiments

Clutch removal experiments were carried out during four consecutive arribadas and their respective hatching periods, from August 2004 to January 2005. Due to extremely high poaching levels, the data from the August, 2004 arribada was omitted from this analysis. We chose a 300 m section of beach on which six pairs of a 6 m X 6 m experimental and a control plots were established, in random locations. Each control and experimental plot was treated as a set and they were considered to be a block during data analysis. This yielded 6 blocks total, with 2 randomized treatment plots per block.

We recorded the number of nests deposited in each plot and applied a dab of white paint to a scute on the carapace of each nesting turtle. Paint was only applied after the process of covering the nest had begun, to ensure that each count corresponded to one completed nest. If a nesting turtle excavated eggs from a previously laid clutch (eggs can be observed lying on the surface surrounding the new nest chamber) this nesting site was deemed to contain two clutches and was recorded as a "double clutch" and the number of nests destroyed in this fashion was recorded. Newly laid clutches were removed from these "double-clutch" experimental plots immediately after nesting, while double-clutch eggs were left in control plots. The

removed eggs were given to park rangers for distribution among community members. We also recorded the number of nests poached from each plot. Poached nests were defined as clutches that were observed being removed by poachers (we filled the holes with sand immediately after this discovery) or any empty nest cavity within either experimental or control plots that were discovered during the day.

On Playa La Flor, the incubation of olive ridley eggs takes between 44 and 55days. We initiated hourly patrols on the 43rd night, which continued until 55 days after clutch deposition. We positioned wire mesh cages over each nest before hatching started, in order to ensure that we counted every emerged hatchling and attributed it to the proper clutch. We recorded the total number of clutches hatched per plot (nest hatching success) and the number of emerged hatchlings per clutch.

Data analysis

All statistical analyses utilized SAS 9.1. Three separate linear regressions were used to evaluate the nesting trends of the olive ridley population from 1998 to 2006 (the number of turtles nesting), the number of arribadas occurring per nesting season and the duration of each arribada (number of days for which an arribada lasted) from 1998 to 2004.

Mixed model ANOVA's (SAS PROC MIXED, with randomized block design) were used to compare the number of clutches (both before and after poaching) in control plots to experimental plots. Nest density was treated as a dependent variable and the statistical blocks were treated as a random factor. Both treatment of the plots (whether double clutches were removed or not) and the time of an arribada (September, October or November) were considered fixed factors.

We compared the number of double clutches in different treatment plots, using mixed model ANOVAs (SAS PROC MIXED, randomized block design). The number of double clutches in a plot was assigned as the dependent variable and the number of clutches was a covariate. Blocks were treated as a random factor, while treatment and the month of the arribada were considered to be fixed factors.

A generalized linear model (SAS PROC GENMOD, binomial distribution) was used to estimate the degree of egg poaching in each block (spatially) and over the four months that arribadas occurred (temporally). The response variable was set as the number of clutches poached divided by the total number of clutches per plot, Block and the time of the arribada were established as classification variables.

Nest hatching success was evaluated using SAS PROC GENMOD, binomial distribution. The response variable was defined as the number of hatched clutches divided by the total number of clutches per plot (double clutches were included but poached clutches were omitted), while treatment, block and time of arribada were all considered classification variables.

We analyzed hatchling production in different treatment plots using SAS PROC GENMOD, poisson distribution. The response variable was defined as hatchling production and the treatment, block and month that an arribada occurred were considered classification variables.

RESULTS

Utilizing historical records, it was established that there was an increasing trend in the number of clutches laid per season, from 1998 to 2006 (Fig. 6). The number of arribadas per nesting season and the duration of those arribadas showed no statistically significant change over the same time period (Table 1).

In the clutch removal experiment, the nest density and the number of double clutches (both before and after poaching) did not differ significantly between the control plots and the experimental plots (Table 2). No significant difference was detected between the number of clutches laid in the experimental plots without the double clutches removed versus the control plots. A significant difference was detected in the number of nests laid (both before and after poaching) during the three arribadas (PROC MIXED, before poaching: $F_{2,25} = 4.75$, $P = 0.02$; after poaching: $F_{2,25} = 3.35$, $P = 0.05$). After poaching, there were 736 nests laid during the September arribada whereas only 461 were deposited during the October arribada.

The proportion of clutches poached did not differ significantly in control versus experimental plots (Table 2). However, there was a significant difference in the spatial (PROC GENMOD, $\chi^2_{5,48} = 28.06$, $P < 0.0001$) and temporal (PROC GENMOD, $\chi^2_{3,48} = 172.82$, $P < 0.0001$) distribution of poaching (Table 2). Poaching levels were the highest in blocks 1 (12.0%) and block 6 (17.5%) that were at the distant ends of the experimental area (Table 2). In the August 2004 arribada, 45% of all the clutches laid were poached, whereas only 2.0% of the clutches were poached during the October 2005 arribada (Table 2).

Nest hatching success was significantly different between the control plots and the experimental plots (PROC GENMOD, $\chi^2_{1,36}$= 18.66, P < 0.0001). Nest hatching success in the control plots was 10.7% versus 3.1% in the experimental plots, where double clutches were removed (Table 2). Nest hatching success varied significantly based on both spatial (PROC GENMOD, $\chi^2_{5,36}$= 166.14, P < 0.0001) and temporal (PROC GENMOD, $\chi^2_{2,36}$= 22.33, P < 0.0001) parameters (Table 2). The highest nest hatching success occurred in block 6 (32.0%) and the lowest in block 1 (2.4%) (Table 2). Hatching success was higher in both the September (8.3%) and October (6.7%) arribadas than in the November (3.5%) arribada (Table 2).

Mean hatchling production was significantly higher in the control plots than in experimental plots (PROC GENMOD, $\chi^2_{1,36}$= 31.96, P < 0.0001). Hatchling production in the control plots was 4124 hatchlings, versus1998 in the experimental plots (Table 2). The mean hatchling production varied significantly based on both spatial (PROC GENMOD, $\chi^2_{5,36}$= 153.00, P < 0.0001) and temporal (PROC GENMOD, $\chi^2_{2,36}$= 95.92, P < 0.0001) parameters (Table 2). Hatchling production was highest in block 6 (2137 hatchlings) and lowest in block 3 (81 hatchlings) (Table 2). The September 2004 arribada had the highest hatchling production (3724 hatchlings) of the nesting season (Table 2).

DISCUSSION

Historical records indicate an increase in the number of turtles that arrived at Playa La Flor over the 9 years from 1995 to 2004 (Fig. 6). While the trend may be accurate, there is likely an overestimation of population size. These numbers

represent an estimate of the number of turtles that crawled up the beach but did not differentiate between turtles that did nest and those that did not. Furthermore, there was no correction made to account for the fact that the same turtle might nest multiple times during the same nesting season. More accurate counting methodologies would improve the accuracy of the raw data and the estimates of the nesting population (Gates et al. 1996; Valverde et al. 1998). Further analysis of the historical data showed that there are no statistically significant differences in the number and duration of arribadas from year to year (Table 1).

The clutch removal experiment was carried out under natural conditions with natural nest densities. The amount of poaching and the number of double clutches occurring in the treatment plots were not significantly different. The data shows a decrease in nest hatching success and hatchling production linked to the removal of double nests. In general the more turtles that nest on a beach, the more clutches are destroyed by turtles (Cornelius and Robinson 1985; Cornelius et al. 1991). It has been suggested that egg harvest could minimize the number of clutches destroyed by other turtles on the nesting beach and that hatching success and hatchling production would therefore increase (Cornelius and Robinson 1985). The data collected in this study did not support that suggestion.

In this study, we removed the clutches that had the lowest chance of survival, the double clutches. The hatching success of double clutches has previously been reported to be lower (36.7 %) than in single clutches (58.5 %) at Playa La Flor (Von Mutius 2000). In theory, this nest removal should result in more space availability for subsequently nesting turtles. Our results show that control plots, where no

manipulation or disturbance occurred, produced more hatchlings and had a higher nest hatching success than experimentally treated plots. A total of 111 double clutches were laid in the experimental plots. Assuming that the nesting turtle destroys the clutch that was previously laid and that we remove her clutch, a total of 222 clutches are removed from the experimental plots. Even if all 222 nests were considered to be equally viable (an overestimation), with a 10.7% nest hatching success (equal to that in the control plots) only 24 clutches would have hatched. The average hatchling production per nest in the control plots was 35 hatchlings. With an average of 35 hatchlings per nest, 24 clutches would produce 840 hatchlings. There is a great difference between the 840 hatchlings potentially produced from the removed nests and the 2126 hatchling disparity observed between the experimental plots and control plots.

The data clearly indicates that there are other mechanisms affecting nest hatching success and hatchling production in the experimental plots. We hypothesize that this is due to disturbance of the nest environment, resulting from human intervention during egg harvest. In most biological systems natural disturbance can have significant effects on an individual's physiology, behavior and/or ecology, and may affect the fitness of certain phenotypes (Karr and Freemark 1985). In general there are two types of disturbances, physical (i.e. fires, floods, very high tides or waves) and biological (i.e. predation, grazing, etc.) (Sousa 1984). In our study, discrete disturbances caused by turtles can damage clutches and kill eggs, but it may create an opportunity for a new clutch to become established. The disturbance caused by humans during the egg harvest appears to have a more severe detrimental effect on

nest hatching success and on hatchling production. The harvest of double clutches appears to disturb the nest environment in ways that other turtles do not, and lowers hatching success and hatchling production by mechanisms that are not yet apparent.

In our study, spatial effects were identified which had significant impact on nest hatching success and on hatchling production (Table 2). Blocks 5 and 6 had the greatest nest hatchling success (19.1% and 32% respectively) and the highest hatchling production (2081 and 2137 respectively). These two blocks were located furthest away from the vegetation and closer to the high tide line than the other four blocks. It has been shown in green turtles and in leatherback turtles that nests laid below the high tide line or very high on the beach, too near to vegetation, have a decreased chance of survival (Whitmore and Dutton 1985; Bjorndal and Bolten 1992; Kamel and Mrosovsky 2004).

We observed significant temporal effects on nest hatching success and hatchling production (Table 2). The September arribada had the highest nest hatching success (8.3%) which resulted in the highest hatchling production (3724 hatchlings). October and November months are the height of the rainy season in Nicaragua. It has been hypothesized that the microbial load increases as the nesting season progresses, due to an increase in nutrient rich egg mater on the beach (Cornelius and Robinson 1985). The rains may also exacerbate the situation by creating a moist environment which favors microbial growth. In addition, a large number of olive ridley nests are lost due to extremely heavy rains and to the unusually high tides experienced during the rainy season at Playa La Flor (*personal observation*).

The exact mechanisms by which human disturbance, spatial effects and temporal effects exert force on nest hatching success and hatchling production are not yet known. Further investigation will be needed to identify the mechanisms negatively affecting the nest environment and represent important subjects for future research efforts.

Our study indicates that, in contrast to dogma, human disturbance of the nesting beaches (for any reason) is more detrimental to nest hatching success and hatchling production than no interference at all. It was, therefore, premature to conclude that because density-dependent effects exist on olive ridley nesting beaches that hatching success would not be negatively affected by egg harvest or by relocation of nests to hatcheries. It is important to consider the biological implications of disturbance, spatial effects and temporal effects on nest hatching success and hatchling production when designing conservation and management strategies.

The Nicaraguan people living near the ocean have utilized turtle eggs for both consumption and as a source of income for many generations. There is considerable pressure from surrounding communities to continue the egg harvest, as a cultural right and as a source of income. When this pressure has not been satisfied, uncontrolled poaching has been the consequence. Despite protection, Playa La Flor suffers from a high level of poaching, illegal trade, corruption and occasional mass invasion by poachers that may result in an off take of 45% or more (Personal Observation 2003 and 2004 nesting seasons).

It is important to have hard science as a reference to help guide policy, conservation efforts and management programs, so that these natural resources can be utilized in a

sustainable fashion and will be available for generations to come. It is inappropriate to continue harvesting wildlife resources without a thorough study and understanding of the effects of harvest on the population dynamics of the organism under consideration. Conservation efforts based on untested dogma, can end up having detrimental effects on the very resource that we are trying to preserve. Regardless of the intention, the effects of these efforts, such as inadvertent over-harvesting or other unidentified negative effectors, can have potentially catastrophic effects on the resource in question, potentially leading to population collapse or even extinction.

Table 1 Number and duration of arribadas at Playa La Flor, 1998-2006 (* 2005 data is not available, 2006 data was not included in the data analysis).

Year	Number of arribadas	Duration of arribadas (mean ± SE)
1998	9	3.7 ± 0.3
1999	8	5.9 ± 0.6
2000	7	7.1 ± 0.7
2001	8	6.1 ± 1.2
2002	9	7.6 ± 1.5
2003	9	4.7 ± 0.4
2004	7	5.3 ± 1.2
2005*	?	?
2006*	11	8.0 ± 1.0

Table 2 Direct counts of number of nests, destroyed nests and hatchling production. Percent poaching and hatching success data are the estimated least squares means from the SAS output.
***** Data from the August arribada is not included in any analysis due to high poaching rate.

	Blocks						Months				Treatment	
	1	2	3	4	5	6	Aug*	Sept	Oct	Nov	Cont	Exp
Nests	311	312	408	368	293	188	133	736	461	683	931	949
Destroyed Nests	46	37	77	43	29	17	10	106	76	67	138	111
Poaching (%)	12.0 ±2.1	9.2 ±2.0	8.6 ±1.7	7.3 ±1.4	9.7 ±1.8	17.5 ±3.0	45.4 ±4.4	6.2 ±1.0	2.0 ±0.7	14.4 ±1.4	10.4 ±1.3	10.3 ±1.3
Hatching success (%)	2.4 ±1.0	2.8 ±1.4	0.65 ±0.4	9.5 ±1.8	19.1 ±2.7	32.0 ±4.0	N/A	8.3 ±1.4	6.7 ±1.5	3.5 ±0.7	10.7 ±1.5	3.1 ±0.8
Hatchling production	110	350	81	1363	2081	2137	N/A	3724	855	1543	4124	1998

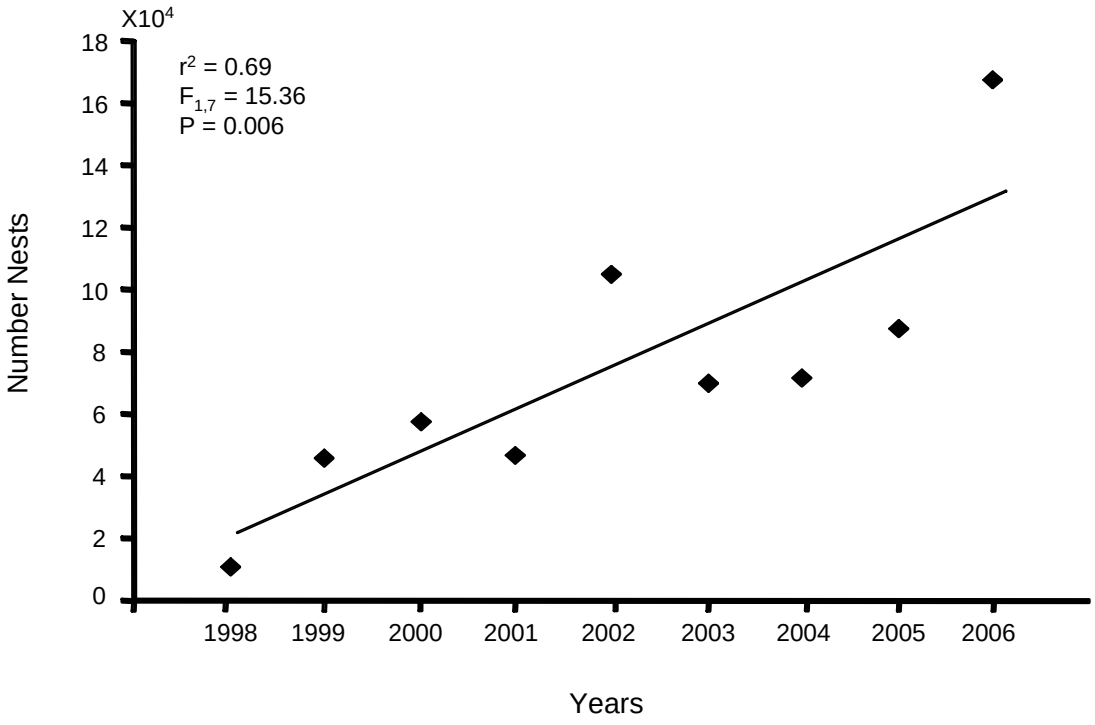

Figure 6. Olive ridley population trend from 1998 to 2006 at Playa La Flor.

CHAPTER 4: Microbial community structure in sand on two olive ridley arribada nesting beaches, Playa La Flor, Nicaragua and Playa Nancite, Costa Rica.

INTRODUCTION

Microorganisms have the potential to be important selective forces in the evolution of oviparous organisms through their interaction on development and on mortality, as indicated by studies in birds (Pinowski et al. 1991; Nuttall 1997; Mills et al. 1999). For instance, presence of fungi and gram- negative bacteria on chicken eggshells can destroy the water resistance properties of the shell leading to digestion of the protective shell cuticle and facilitating microbial infection (Cook et al. 2005; Board et al. 1979). Several species of bacteria and fungi have been isolated from failed eggs and dead embryos in different bird species (Stewart et al. 2000; Mills et al. 1999; Lombardo et al. 1996; Kozlowski et al. 1991). Analyses of egg failure in freshwater turtles have identified *Salmonella spp.* as a pathogen (Ewert 1979). Using culture-dependent methodologies the presence of microorganisms on the egg exterior and/or in embryonic tissue has been described in several species of sea turtles including the loggerhead turtle (Ragotzkie 1959; Wyneken et al. 1988; Peters et al. 1994; Awong-Taylor et al. 2007), the green turtle (Bustard and Greenham 1968; Solomon and Baird 1980; Whitmore and Dutton 1985), the leatherback turtle (Whitmore and Dutton 1985; Solomon and Tippett 1987; Eckert and Eckert 1990) and the olive ridley turtle (Mo et al. 1990 and 1992; Acuña-Mesén 1992; Acuña et al. 1999). Despite the potential importance of microorganism diversity and its effects on hatching success, microorganism diversity and abundance has not been explored on natural sea turtle beaches.

Olive ridley turtles nest in high densities, which is referred to as an "arribada" (Spanish term for arrival). There are only a few nesting beaches in the world where olive ridleys still nest in arribadas (Cornelius et al. 1991). The most important nesting beaches are in Costa Rica (Playa Ostional and Playa Nancite), Mexico (La Escobilla), India (Orissa) and Nicaragua (Playa Chacocente and Playa La Flor) (Spotila 2004).

The numbers of nesting olive ridleys seem to be increasing at Playa La Flor from 46,000 in 1999, 71,000 in 2004 to 167,000 in 2006 (data from chapter 3). Olive ridleys deposited 340,000 clutches on Playa Nancite in 1981, 200,000 in 1982, 52,000 in 1983 and 185,000 in 1984 on the 1 km nesting beach (Cornelius et al. 1991). Recently the number of nesting turtles has drastically declined to 13,000 clutches in 2005 and 17,000 in 2006 per nesting season (Chapter 1; Unpublished data Valverde *Pers Comm*). One hypothesis for the olive ridley population decline at Playa Nancite is low egg survival resulting in minimal population recruitment. The arribada nesting behavior results in destruction of large number of clutches by subsequent nesting turtles that inadvertently dig out previously laid clutches (Cornelius et al. 1985). It has been hypothesized that due to the large numbers of nests destroyed during arribadas, the organic content (broken eggs) in the sand would increase resulting in high microbial build up in nests (Cornelius et al. 1985; Cornelius et al. 1991). Microorganisms can debilitate healthy eggs through resource consumption and micro-environmental changes that may result in decreased hatching success (Cornelius et al. 1985; Cornelius et al. 1991). Cornelius and Robinson (1985) speculated that egg harvest may increase hatching success by reducing the number of decomposing eggs in the beach. Since then, this speculation has been the basis of many management

programs for olive ridley arribada nesting beaches in the world (Cornelius et al. 1991; Campbell 1998; Hope 2002). To date, it has not been established whether microbial diversity and abundance differ on different parts of the nesting beach and or in different nest densities where the turtle clutches are incubated for an extensive amount of time. Thus, in order to make accurate management decisions for these important arribada nesting beaches, microbial studies may prove to be crucial.

Since less then 5% of microorganisms are cultivatable, in order to study changes in microbial community structure and diversity, polymerase chain reaction (PCR)-amplified rRNA gene based molecular techniques are necessary to avoid limitation of culture-based studies (Head et al. 1998; Anderson and Cairney 2004; Hackl et al. 2004). Terminal restriction fragment length polymorphism (TRF, or T-RFLP) is a DNA-based analysis that allows rapid comparison of complex bacterial communities. The method is based on differences in the positions of restriction sites in specific DNA sequences from different microbes; detection and determination of the lengths of digested fragments allow for an estimate of the number of different microbial species found in a sample. This methodology has been used to study changes in microbial structure, diversity and abundance in agricultural soil, grassland forest soils and biological soil crusts (Hackl et al. 2004). The TRF methodology has also been used to study the impact of recreation derived activities on changes in microbial structure, diversity and abundance (Nogales et al. 2007). A possible limitation of TRF analysis is that, each peak in a profile could represent a number of TRF of the same size originating from different 16S rRNA genes leading to an underestimate of microbial diversity in a sample. Regardless, TRF is still a useful

method to assess the similarity of soil bacterial communities allowing spatial heterogeneities and temporal changes to be detected in highly diverse bacterial communities without the need to know the identity of every peak in every profile (Lukow et al. 2000). In addition, this problem can be prevented by using the appropriate number and types of restriction endonucleases, resulting in TRF profiles that more accurately reflect the natural diversity of microbial population (Engebretson and Moyer 2003; Osborne et al. 2006).

In this study, we used 16S rRNA gene-based TRF community analysis to answer the following questions: 1) Are there differences in diversity and abundance of the bacterial communities in the sand on different parts of two nesting beaches, Playa La Flor and Playa Nancite? 2) Are there differences in diversity and abundance of bacterial communities in sand in different nest densities on these two arribada nesting beaches?

METHODS

Site description and sampling

We collected sand at the end of the olive ridley nesting season at Playa Nancite, Costa Rica in January 2007 and Playa La Flor, Nicaragua in February 2007. Playa La Flor is a 1.6 km long beach situated on the southern Pacific coast of Nicaragua. Playa Nancite is 1.1 km long beach located on the northwest coast of Costa Rica on the Pacific Ocean in Guanacaste Province. Both nesting beaches are 15 to 20 m wide, and unstable (their profile changes with storms).

We collected sand from both nesting beaches at different nest densities and on different beach zones above the tide right after hatching of the November arribada (Table 3). Different zones on the beach were defined as follows. The width of the beach was divided into three zones: high on the beach was the area closest to the vegetation (0-5 m), middle was 5-10 m from vegetation and low on the beach was the area closest to the high tide line, 10-15m from vegetation. Nest density was defined as follows: High nest density (~ 2000 nests/100 m^2), moderate nest density (~1000 nests/100 m^2), low nest density (~500 nests/ 100 m^2) from the latest arribada, and a control where there were no nests present. We collected 3 samples randomly (~10m apart) from each nest density in each zone of the beach at nest depth (30 – 35 cm depth) by digging down a nest cavity using sterile gloved hands. For each sample, three 50 ml sub-samples of sand from each nest cavity were collected in sterile tubes (Table 3). We transported sand samples to the laboratory and stored them at -20ºC within 24 hours of collection until analysis.

Microbial community DNA extraction

We extracted DNA from sand samples using a modified PowerMax™ soil DNA isolation kit (MoBio, Solana Beach, CA, USA) protocol. We took 2 g of sand of each sub-sample from each nest cavity, mixed in a 50 ml sterile tube and extracted DNA. To ensure high yields of genomic DNA, we added the power bead solution from the kit to the sand samples followed by three freeze (-20ºC) and thaw (60ºC) cycles. Then we used the PowerMax™ soil DNA isolation kit protocol for further extraction. We modified the last step of DNA extraction to ensure clean DNA for further analysis. Only 1.5 ml of the C6 elution buffer was used followed by

centrifugation and collection of DNA in a sterile tube. We repeated this step three times and each time collected DNA in a separate sterile tube. Using a spectrophotometer (Spectronic, Genesys 2.0) we quantified the DNA in each tube and only used DNA in tubes that had a 260/280 ratio <1.9 indicating relatively high purity. We mixed the replicates from the same nest cavity and re-quantified the DNA.

PCR with 16S rRNA gene primers

The extracted DNA was used as template for PCR (three replicate reactions for each sample) using the 16S rRNA primers: 8F primer labeled with 6-carboxyfluorescein (6-FAM 5' AGAGTTTGATCCTGGCTCAG 3') and 926R (5' CCGTCAATTCCTTTRAGTTT3') (Muyzer et al. 1995; Hackl et al. 2004). The PCR mixture (50µl) contained 60 to 120 ng of extracted DNA, 1X reaction buffer, dNTP (200µM), $MgCl_2$ (0.5 mM), primers (0.2 µM) and 0.5 units of *Taq* DNA polymerase (Boehringer). The *Taq* polymerase used in this study had the highest resistance to humic acid (Tebbe et al. 1993) among commercially available *Taq* polymerases. Three independent PCRs were performed for each sample as follows: initial denaturing step of 5 min at 95ºC, 30 cycles of denaturing, annealing and extension (30 s at 95ºC, 1 min at 53ºC and 2 min at 72ºC respectively) followed by a final extension of 10 min at 72ºC. The three PCR products from the same sample were pooled to reduce PCR bias and the products were purified with a MinElute PCR purification kit (Qiagen, Inc., Chatsworth, Calif.) with final elution volume of 20 µl.

TRF profiles

Approximately 300 ng of fluorescently labeled PCR product was digested with 10 units of AluI restriction enzyme (Invitrogen) (Hackel et al. 2004) and purified

with the PowerMax kits as previously described as before. Aliquots of 5 µl were mixed with 10 µl master mix containing 1 ml loading buffer (deionized formamide; Fluka) and 50 µl DNA fragment length standard (ROX 500; PE Applied Biosystems Inc., Foster City, Calif.). The fluorescently labeled TRFs were then detected using ABI 3100 automated DNA sequencer (PE Applied Biosystems, Inc.).

Statistical analysis of TRF profiles

All TRF profiles were analyzed and normalized with GeneMapper version 4.0. We included TRFs of 50 to 500 bp in length and with peak heights of ≥ 50 fluorescence units (Hackl et al. 2004). The TRF analysis produced two types of output, an electropherogram and a table with numerical data. Number and height of peaks in each electropherogram produced by the TRF analysis represented the number and abundance of phylotypes (bacteria only defined by their 16S rRNA sequence) (Dunbar et al. 2000). The analysis also quantified the size (bp) of each peak, the height of each peak and the area under each peak. The size of each peak was calculated to reference to the internal standard and the peak height was calculated by relative amount of fluorescence detected in each sample.

We used SAS 9.1 and MATLAB 7.0 to carry out the following analysis. We used Principal Component Analysis (PCA) to study the major variation patterns in phylotypes in the TRF data both on Playa Nancite and Playa La Flor. Only fragments that occurred twice or more in all samples were included in the analysis and peak height was used as a parameter. We also computed PCA using only the 30 most commonly occurring fragments. We used the scores of the first ten components from the PCA in a multivariate analysis of variance (MANOVA) to compare means of the

PCA scores from different zones on the beach and different nest densities on the two nesting beaches.

To measure similarities between samples from different zones of the beach and different nest densities the PCA scores (fragment heights were log transformed and included all phylotypes) were subjected to cluster analysis using the Euclidean distance measure. We constructed two dendograms using complete linkage clustering (farthest neighbor clustering) and average clustering using the unweighted pair group method with arithmetic mean (UPGMA). In addition, we converted peak heights from TRF profiles of all samples to binary data (presence and absence of a peak). We calculated Jaccard coefficient matrix for all samples and used this matrix in a cluster analysis. We constructed two dendograms using farthest neighbor clustering and UPGMA.

We calculated the Shannon-Weiner diversity index as follows: $H = -\sum(p_i)(\log_2 p_i)$, where p = proportion of an individual peak height relative to the sum of all peak heights. We calculated Simpson's diversity index using the following formula: $D = 1 - [-\sum(p_i)^2]$. Evenness for Shannon-Weiner index was calculated as follows: $E = H/H_{max}$ where $H_{max} = \log_2 (S)$ and S = total number of distinct TRF sizes in a profile (phylotype richness). Evenness for Simpson's index was calculated as follows: $E = D/D_{max}$ where $D_{max} = 1-(1/S)$. Only fragments that occurred two or three times per three replicate samples were considered in these analyses. We calculated both diversity indices because Shannon-Weiner diversity index is most sensitive to abundance of rare species and Simpson's diversity index was more sensitive to changes of more abundant species. Two–way ANOVA detected significant

differences in diversity among different parts of the beach (zones) and different nest densities at Playa La Flor and Playa Nancite. Both zones and nest density were classification variables and Shannon-Weiner or Simpson's diversity index were the dependent variables. We used the Student-Newman-Keuls test (SNK) as a post-hoc analysis.

Both species richness and abundance were calculated for all the samples individually. Richness was defined as total number of different fragments per sample and abundance was the sum of the total heights of different fragments per sample (only fragments that occurred 2 or 3 times per three replicated samples were included in the analysis). We carried out three MANOVA's for Playa La Flor to find out whether there were differences in means of different samples, samples taken from different zones on the beach and in different nest densities. Both abundance and richness were treated as dependent variables and different samples, zones on the beach or nest density were classification variables. We carried out two-way MANOVA for Playa Nancite where both richness and abundance were the dependent variables and zones on the beach and nest density were both classification variables. We used two-way ANOVA's together with SNK to discover where the differences that were detected in MANOVA's lay. Both zones on the beach and nest density were the classification variables and richness or abundance were the dependent variables for both Playa Nancite and Playa La Flor. To be able to run a two-way ANOVA using the Playa La Flor data we only considered two different zones on the beach (high and low).

RESULTS

The PCA using all fragment heights present did not reveal any patterns. However, the PCA of the 30 most commonly occurring fragments at Playa Nancite showed different distribution patterns at different nest densities in different zones on the beach (Fig 7). At Playa Nancite, TRF profiles from the control and moderate nest density, high on the nesting beach (MH) were separated from the other TRF profiles along the first principal component (Fig 7). The TRF profiles from moderate densities in the middle part of the beach (MM) were separated from TRF profiles from high nest density, middle on the beach (HM) and high nest density, low part of the beach (HL) along the second principal component (Fig 7). At Playa La Flor the TRF profile from low nest density, low on the beach (LL) had a different pattern both on the first and second principal component axes compared to the other samples (Fig 8). The MANOVA's on the scores of the first 10 components for both Playa Nancite and Playa La Flor indicated that there were significant differences in means between TRF profiles at different nest densities and zones of the beach (Table 4). Dendograms of the cluster analysis using the PCA scores indicated that samples from the same nest density within a zone on the beach clustered together (Fig 9a and b). The TRF profiles from high nest density within the high zone of Playa Nancite (HH) were most different from all other profiles. In addition, the TRF profiles from high nest density within the high zone of Playa La Flor (LFHH) also differed from all other profiles. The farthest neighbor analysis (Fig. 9b) indicated that TRF profile from high density area in the low and middle zone of the beach (HL and HM) on Playa Nancite differed from the remaining profiles.

Dendograms of the cluster analysis using Jaccard coefficient matrix indicated that samples from the same nest density within a zone on the beach clustered together (Fig 10a and b). The TRF profiles from moderate nest density within middle and high zone of Playa Nancite (MM and MH) were most different from all other profiles. The farthest neighbor analysis (Fig. 10b) indicated that TRF profile from high nest density within the high, middle and low zone of the beach (HH, HM and HL) on Playa Nancite differed from the remaining profiles.

We calculated phylotype diversity for each sample by Shannon-Weiner and Simpson's index of diversity, both for Playa Nancite (Table 5) and Playa La Flor (Table 6). Two-way ANOVA and post-hoc SNK tests showed that the fragment diversity calculated using Shannon-Weiner diversity index was significantly different between moderate (3.00) and high (3.63) nest density at Playa Nancite (Table 7). There were no significant differences found when diversity was calculated using Simpson's diversity index at Playa Nancite (Table 7). There was no significant difference detected between zones of the beach at Playa Nancite using both Shannon-Weiner and Simpson's diversity indexes. The interaction of zone of beach and nest density had a significant effect on the Shannon-Weiner index at Playa Nancite ($P = 0.0352$) (Table 7). Similarly, Two-way ANOVA and SNK confirmed that the phylotype diversity calculated using the Shannon-Weiner diversity index was significantly different between samples from higher zone on the beach (4.05) compared with samples taken from the low zone on the beach (2.72) at Playa La Flor (Table 7). Diversity was not significantly different between the two nest densities using Shannon-Weiner diversity index at Playa La Flor. Using Simpson's diversity

index, there were significant differences in samples from high (0.71) and low (0.88) nest density and high (0.89) and low (0.69) zones on the beach at Playa La Flor (Table 7). The interaction of beach zone and nest density was also significant at Playa La Flor (Table 7).

At Playa Nancite two-way MANOVA confirmed that there were significant differences in abundance and richness of the TRF fragments in different nest densities (Table 8). Two-way ANOVA and SNK confirmed that there were significant differences in TRF fragment richness in high (23 phylotypes) versus moderate (13 phylotypes) nest densities at Playa Nancite (Table 9; Fig 11). There were no significant differences found in phylotype richness at different zones of the beach at Playa Nancite (Table 9). There were no significant differences found in phylotype abundance at Playa Nancite (Table 9; Fig 11). At Playa La Flor MANOVA confirmed that there were significant differences in abundance and richness of the phylotype in different samples and at different zones on the beach (Table 8). Two-way ANOVA and SNK confirmed that there were significant differences in phylotype richness in the high zone on the beach (28 fragments) versus the low zone on the beach (18 fragments) (Table 9; Fig 12). There were no significant differences found in phylotype richness between different nest densities on the beach (Table 9). There were no significant differences found in phylotype abundance at Playa La Flor (Table 9; Fig 12). The interaction of beach zone and nest density was significant at Playa La Flor (Table 9).

DISCUSSION

Understanding microbial community structure of soil is important in conservation management and environmental monitoring. In the present study we adapted a TRF methodology to rapidly assess microbial community structure, diversity and abundance on sea turtle nesting beaches.

There were differences in TRF profiles from high nest density and moderate nest density areas of Playa Nancite (Fig 7; 9 and 10). Phylotype richness and diversity of bacteria changed at different nest densities at Playa Nancite and in different zones of the beach at Playa La Flor. Phylotype abundance did not change in different zones of the beach or in different densities at both Playa Nancite and Playa La Flor.

Phylotype diversity according to the Shannon-Weiner index and richness were both higher in high nest density at Playa Nancite. The high diversity and richness may have been the result of the presence of broken eggs that provided organic matter for bacterial growth. In addition, the large number of nesting turtles may have introduced more bacterial species to the sand at high nest densities. Cloacal bacterial species can be introduced from nesting turtles into the nest via eggs (Wyneken et al. 1988).

Phylotype diversity according to Simpson's index was different at different nest densities at Playa La Flor (0.71 in high and 0.88 in low density). Simpson's diversity index is more sensitive to the more abundant species. This suggests that the more abundant bacteria are less diverse at high nest density areas at Playa La Flor.

Phylotype diversity according to Shannon-Weiner index and richness was significantly higher in the high zone on the beach compared to the low zone of the beach at Playa La Flor. The zone closest to the high tide gets washed out completely

during high tides. The zone closer to vegetation has less chance of being washed and the accumulation of broken eggs in this area over time may contribute to more bacterial diversity and richness. Bacterial diversity and richness in different zones became important in very high nest densities especially at Playa La Flor (11 arribadas, 167,000 clutches total).

Cornelius and Robinson (1985) suggested that higher nest densities would produce higher abundance of microorganisms on the nesting beach due to the higher number of incubating and broken eggs. A large number of olive ridley nests are destroyed during arribadas and the broken eggs in the sand are good media for bacterial growth. Microbial load will increase and this may cause decreased hatching success and hatchling production (Cornelius et al. 1991). However, in our study bacterial abundance was not statistically different in different zones of the beach and in different nest densities at Playa Nancite and Playa La Flor suggesting that bacterial abundance may not be an important factor causing reduced hatching success.

High bacterial richness of cultures from un-hatched, non-viable eggs is associated with reduced hatching success (Wyneken et al. 1988). Hatching success was also lower in high nest densities versus low nest densities in experimental plots on Playa Nancite (Chapter 2). In our study bacterial diversity and richness was higher in the high density area at Playa Nancite suggesting that bacterial diversity and richness may be important in affecting hatching success of olive ridley eggs on this nesting beach. More studies are needed to identify different bacterial species on the nesting beach.

At Playa La Flor, we measured hatching success, O_2, CO_2 and temperature levels in high and low nest densities from the same sampling site at the same time as we took sand samples for TRF analysis (see chapter 2). Hatching success was lower (10%) in the high nest density part of the beach compared to the low density part of the beach (16%). Lower hatching success correlated with lower O_2 level (18.4%) and higher CO_2 levels (4.2%) in sand. Lower hatching success was also correlated with higher temperatures (35.3°C). We did not observe any differences in abundance of bacteria at Playa La Flor in different nest densities which suggest that bacteria abundance does not effect O_2, CO_2, temperature and hatching success on the nesting beach. It is unlikely that differences in richness and diversity of bacteria would affect the levels of O_2 and CO_2 in the sand on olive ridley nesting beaches.

It is crucial to identify the bacterial species on the nesting beach in order to determine if pathogenic species are present that could cause reduced hatching success. It is possible that decomposition of organic material by fungi could contribute to lower O_2 and higher CO_2 in the sand on olive ridley nesting beaches. A number of studies have looked at the presence of fungi on turtle and alligators eggs (Schumacher et al. 1990; Mo et al. 1990; Acuña-Mesén 1992; Phillott et al. 2001). *Fusarium oxysporum*, *F. solani* and *Pseudallescheria boydii* are common soil fungi (Rippon 1982; Burgess 1981). *Fusarium solani* occurs in failed olive ridley eggs (Acuña-Mesén 1992) and *F. oxysporum* occurs on egg membranes of American alligators (Schumacher et al. 1990). These three fungal species also occurred in green and loggerhead sea turtle nests (Phillott et al. 2001). Another fungus, *Monosporium apiospermum,* also occurs in olive ridley nests in Costa Rica (Acuña-Mesén 1992).

Fungal community structure, diversity, richness and abundance at these two nesting beaches remain to be studied.

In the present study we have looked at the impacts of high number of turtles nesting on the beach on changes in microbial community structure, diversity and abundance. The TRF methodology was a rapid way to assess the diversity, richness and abundance of bacteria on turtle nesting beach. Despite differences in richness and diversity, the role of bacteria in hatching success is still not clear. Cloning studies together with TRF studies will be useful to identify the different bacteria species present on the beach and potential pathogens to turtle eggs. Studies of fungal diversity and abundance and their effects on olive ridley eggs are needed.

Table 3. Sampling sites, beach zones and nest densities.
The numbers represent the number of samples/subsamples.

Site	Beach Zone	Nest density			
		High	moderate	Low	Control
Playa Nancite	High	3/3	3/3	N/A	N/A
	Middle	3/3	3/3	N/A	3/3
	Low	3/3	3/3	NA	N/A
Playa La Flor	High	3/3	N/A	3/3	N/A
	Middle	3/3	N/A	N/A	N/A
	Low	3/3	N/A	3/3	N/A

Table 4. Multivariate analysis of variance (MANOVA) of scores of the first 10 components from PCA in relation to samples, zones of the beach and nest density for both Playa Nancite and Playa La Flor (a = 0.05). Wilks' Lamda, Hotelling-Lawley Trace and Roy's Greatest Root tests showed significance (data not shown here).

Effect	Playa Nancite	value	F	Hypothesis df	Error df	Sig.
Samples	Pillai's Trace	3.934	2.58	50	35	0.002
Zone	Pillai's Trace	2.155	2.55	30	30	0.0063
Nest density	Pillai's Trace	1.554	3.49	20	20	0.0037
Effect	**Playa La Flor**					
Samples	Pillai's Trace	3.760	6.26	40	16	0.0001
Zone	Pillai's Trace	1.863	5.43	20	8	0.0096
Nest density	Pillai's Trace	0.953	8.02	10	4	0.0298

Table 5. Abundance, richness, Shannon-Weiner diversity index (H), Evenness, Simpson's diversity index (D) are all given for different nest densities and zones at Playa Nancite, Costa Rica.

Nest Density	Sampling zone	Abundance (Total TRF Height)	Richness (S)	Shannon-Weiner (H)	Shannon-Weiner (H_{max})	Evenness (H/H_{max})	Simpson (D)	Simpson (D_{max})	Evenness (D/D_{max})
No Nest	Control 1	1038	7	2.55	2.81	0.91	0.81	0.86	0.94
No Nest	Control 2	9678	26	3.79	4.70	0.81	0.88	0.96	0.92
No Nest	Control 3	11444	25	3.74	4.64	0.81	0.88	0.96	0.92
High	High 1	22896	33	4.69	5.04	0.93	0.95	0.97	0.98
High	High 2	13246	27	4.04	4.75	0.85	0.91	0.96	0.95
High	High 3	13244	16	3.65	4.00	0.91	0.90	0.94	0.96
High	Middle 1	15198	27	4.23	4.75	0.89	0.93	0.96	0.96
High	Middle 2	6006	19	3.11	4.25	0.73	0.79	0.95	0.83
High	Middle 3	19230	31	4.46	4.95	0.90	0.94	0.97	0.97
High	Low 1	4819	15	2.59	3.91	0.66	0.69	0.93	0.74
High	Low 2	8478	19	3.24	4.25	0.76	0.84	0.95	0.89
High	Low 3	5085	18	2.65	4.17	0.64	0.71	0.94	0.75
Moderate	High 1	11204	10	2.91	3.32	0.87	0.85	0.90	0.94
Moderate	High 2	5585	6	2.21	2.59	0.85	0.72	0.83	0.87
Moderate	High 3	4786	9	2.18	3.17	0.69	0.67	0.89	0.75
Moderate	Middle 1	7245	9	2.75	3.17	0.87	0.81	0.89	0.91
Moderate	Middle 2	18411	16	3.80	4.00	0.95	0.92	0.94	0.98
Moderate	Middle 3	13395	16	3.63	4.00	0.91	0.90	0.94	0.96
Moderate	Low 1	13405	21	3.72	4.39	0.85	0.90	0.95	0.94
Moderate	Low 2	11157	23	3.66	4.52	0.81	0.89	0.96	0.93
Moderate	Low 3	4713	11	2.21	3.46	0.64	0.66	0.91	0.73

Table 6. Abundance, richness, Shannon-Weiner diversity index (H), Evenness, Simpson's diversity index (D) are all given for different nest densities and Zones at Playa La Flor, Nicaragua.

Nest Density	Sampling Zone	Abundance (Total TRF Height)	Richness (S)	Shannon-Weiner (H)	Shannon-Weiner (H_{max})	Evenness (H/H_{max})	Simpson (D)	Simpson (D_{max})	Evenness (D/D_{max})
High	High 1	10581	30	4.50	4.91	0.92	0.94	0.97	0.97
High	High 2	7935	39	4.28	5.29	0.81	0.88	0.97	0.90
High	High 3	20557	32	4.62	5.00	0.92	0.95	0.97	0.98
High	Low 1	3260	7	1.22	2.81	0.43	0.37	0.86	0.43
High	Low 2	5262	10	2.33	3.32	0.70	0.71	0.90	0.78
High	Low 3	3526	6	1.44	2.59	0.56	0.45	0.83	0.54
High	Middle 1	30238	35	4.78	5.13	0.93	0.96	0.97	0.98
High	Middle 2	24108	33	4.76	5.04	0.94	0.96	0.97	0.98
High	Middle 3	8338	12	3.06	3.59	0.85	0.83	0.92	0.91
Low	High 1	14608	29	4.15	4.86	0.86	0.92	0.97	0.95
Low	High 2	3547	26	4.39	4.70	0.93	0.94	0.96	0.98
Low	High 3	6619	9	2.38	3.17	0.75	0.74	0.89	0.84
Low	Low 1	11135	25	3.39	4.64	0.73	0.85	0.96	0.89
Low	Low 2	17736	31	4.05	4.95	0.82	0.91	0.97	0.94
Low	Low 3	11063	30	3.88	4.91	0.79	0.89	0.97	0.92

Table 7. Two-way analysis of variance for phylotype diversity using Shannon-Weiner and Simpson's diversity index at Playa Nancite and Playa La Flor.

Site	Dependent variable	Source	SS	df	MS	F	P-value
Playa Nancite	Shannon-Weiner's diversity index	Zone	1.296	2	0.647	1.81	0.2061
		Nest density	1.753	1	1.753	4.89	0.0472
		Zone*Nest density	3.213	2	1.607	4.48	0.0352
Playa Nancite	Simpson's diversity index	Zone	0.0291	2	0.0145	1.93	0.1882
		Nest density	0.00694	1	0.00694	0.92	0.3567
		Zone *Nest density	0.0472	2	0.0236	3.12	0.0808
Playa La Flor	Shannon-Weiner's diversity index	Zone	5.336	1	5.336	12.61	0.0075
		Nest density	1.239	1	1.239	2.93	0.1254
		Zone *Nest density	6.454	1	6.454	15.25	0.0045
Playa La Flor	Simpson's diversity index	Zone	0.117	1	0.117	10.39	0.0122
		Nest density	0.081	1	0.081	7.08	0.0288
		Zone *Nest density	0.140	1	0.140	12.40	0.0078

Table 8. Two-way multivariate analysis of phylotype abundance and richness in relation to zone on the beach and nest density for Playa Nancite and one-way multivariate analysis of phylotype abundance and richness in relation to samples, zone on the beach and nest density for Playa La Flor.

Playa Nancite	Class		value	F	Hypothesis df	Error df	Sig.
	Zone	Pillai's Trace	0.576	2.43	4	24	0.0758
	Density	Pillai's Trace	0.696	12.59	2	11	0.0014
	Zone*Density	Pillai's Trace	0.644	2.85	4	24	0.0457
Playa La Flor							
	samples	Pillai's Trace	1.106	3.09	8	20	0.0192
	Zone	Pillai's Trace	0.612	2.65	4	24	0.0581
	Nest density	Pillai's Trace	0.0721	0.47	2	12	0.6381

Table 9. Two-way analysis of variance for phylotype richness and abundance at Playa Nancite and Playa La Flor.

Site	Dependent variable	Source	SS	df	MS	F	P-value
Playa Nancite	Richness	Zone	24.777	2	12.388	0.42	0.668
		Nest density	392.000	1	392.000	13.21	0.0034
		Zone *Nest density	259.000	2	129.500	4.37	0.0376
Playa Nancite	Abundance	Zone	90486476.4	2	45243238.2	1.87	0.1961
		Nest density	18607033.4	1	18607033.4	0.77	0.3975
		Zone *Nest density	130396609.8	2	65198304.9	2.70	0.1078
Playa La Flor	Richness	Zone	261.333	1	261.333	6.82	0.0311
		Nest density	56.333	1	56.333	1.47	0.2600
		Zone *Nest density	833.333	1	833.333	21.74	0.0016
Playa La Flor	Abundance	Zone	11731518.8	1	11731518.8	0.51	0.4972
		Nest density	15383880.8	1	15383880.8	0.66	0.4390
		Zone *Nest density	148297852.1	1	148297852.1	6.39	0.0353

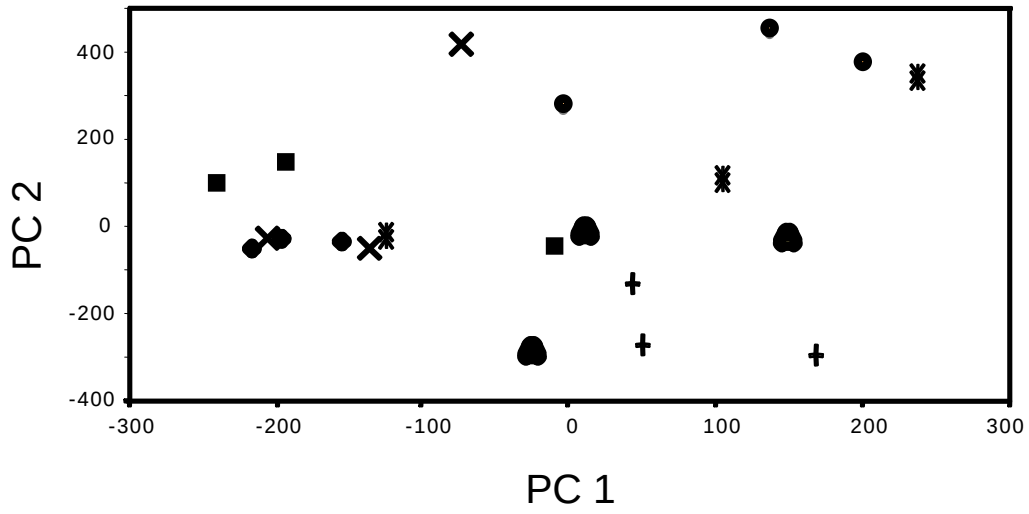

Figure 7. Score plot from PCA of TRF profiles from Playa Nancite. High nest density, High on the beach (*square*=HH); High nest density, Middle on the beach (*triangle*=HM); High nest density, Low on the beach (*cross*=HL); Moderate nest density, High on the beach (*X*=MH); Moderate nest density, Middle on the beach (*circle*=MM); Moderate nest density, Low on the beach (*star*=ML) and Control (*diamond* =CC).

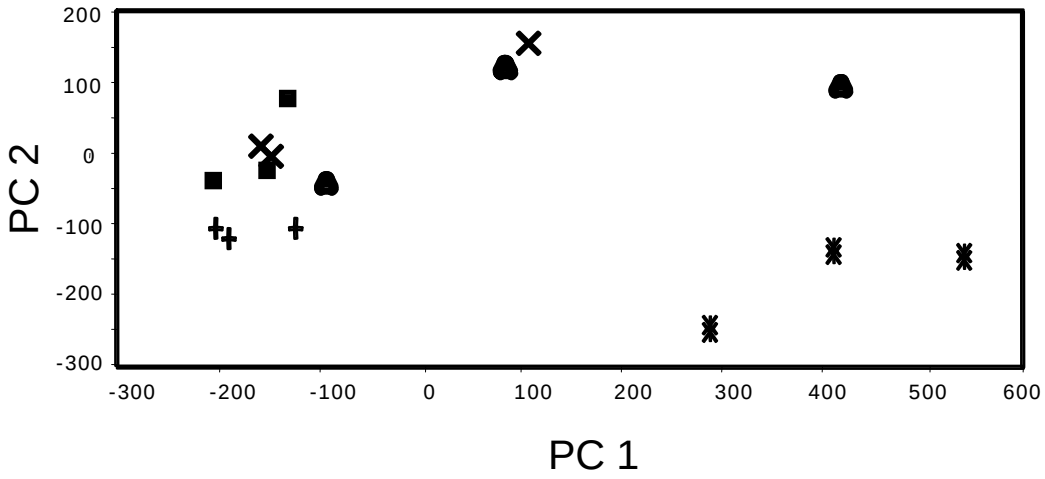

Figure 8. Score plot from PCA of TRF profiles from Playa La Flor. High nest density, High on the beach (square=LFHH); High nest density, Middle on the beach (triangle=LFHM) ; High nest density, Low on the beach (cross=LFHL); Low nest density, High on the beach (X=LFLH); Low nest density, Low on the beach (star=LFLL).

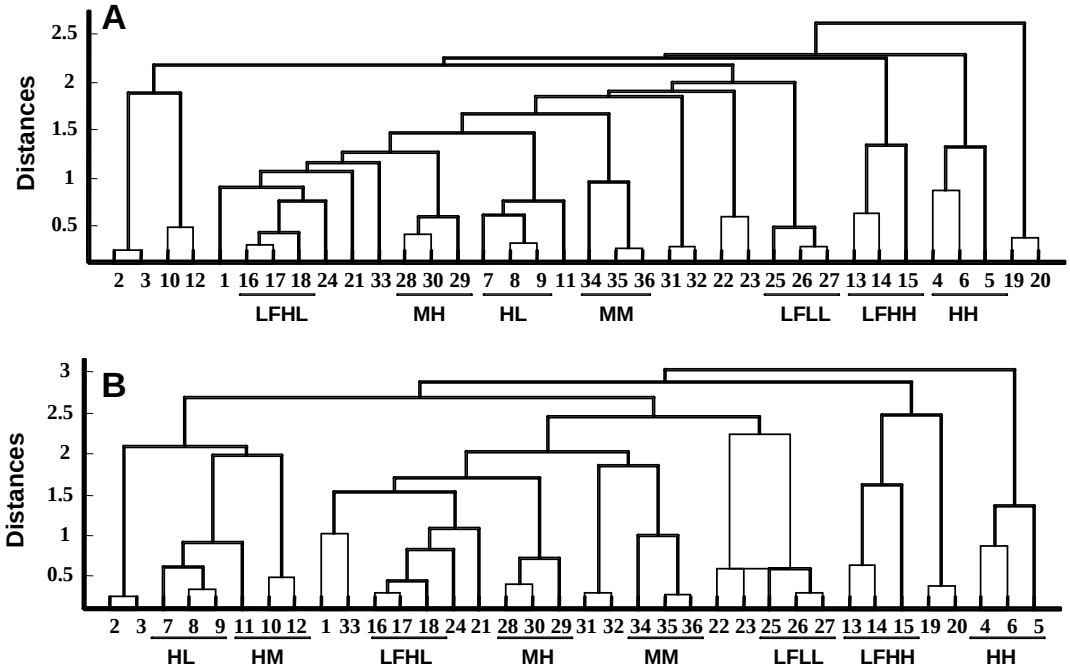

Figure 9. Dendogram of TRF profile from all sand samples using data generated from PCA including all phylotypes. The dendograms were generated using euclidean distance values, using A) UPGMA and B) farthest neighbor methods. Playa nancite: High nest density, High on the beach (HH); High nest density, Middle on the beach (HM) ; High nest density, Low on the beach (HL); Moderate nest density, High on the beach (MH) and Moderate nest density, Middle on the beach (MM). Playa La Flor: High nest density, High on the beach (LFHH); High nest density, Low on the beach (LFHL); Low nest density,Low on the beach (LFLL).

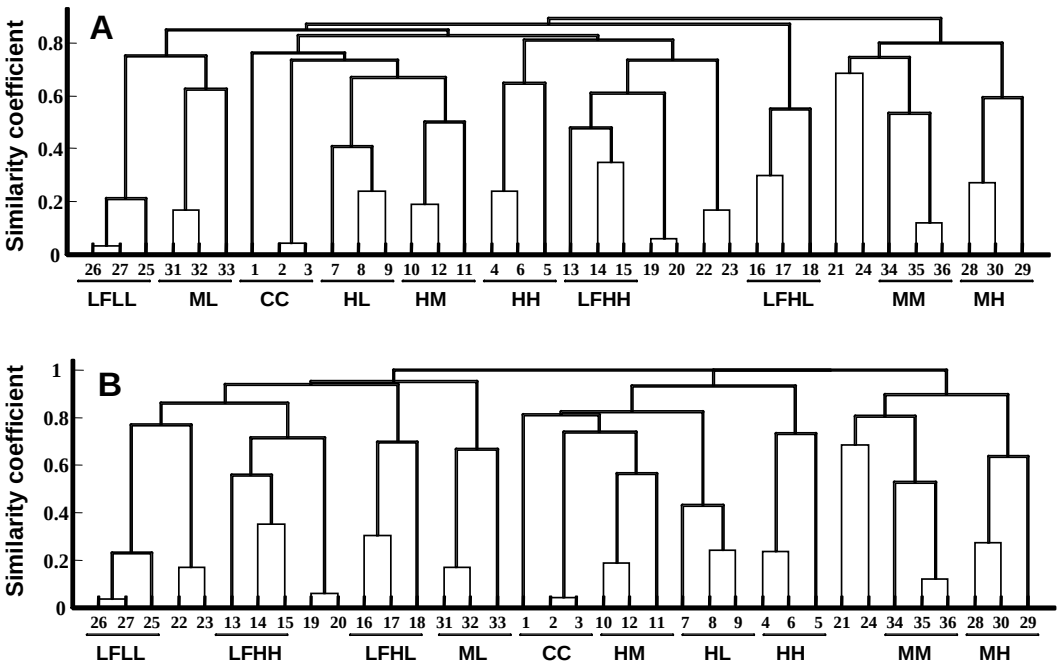

Figure 10. Dendogram of TRF profile from all sand samples generated using Jaccard distance values, using A) UPGMA and B) farthest neighbor methods. Playa nancite: High nest density, High on the beach (HH); High nest density, Middle on the beach (HM) ; High nest density, Low on the beach (HL); Moderate nest density, High on the beach (MH); Moderate nest density, Middle on the beach (MM);Moderate nest density, Low on the beach (ML) and Control (CC). Playa La Flor: High nest density, High on the beach (LFHH); High nest density, Low on the beach (LFHL); Low nest density, Low on the beach (LFLL).

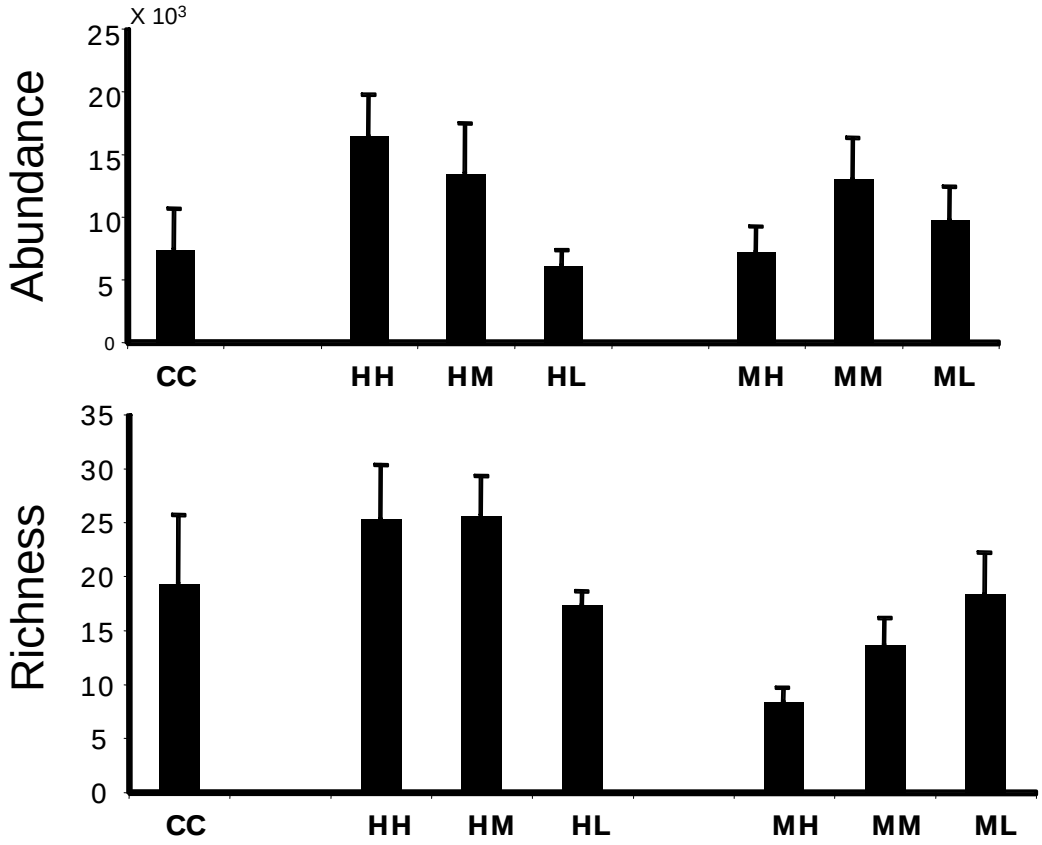

Figure 11. Phylotype abundance and richness at Playa Nancite. High nest density, High zone of the beach (HH); High nest density, Middle zone of the beach (HM) ; High nest density, Low zone of the beach (HL); Moderate nest density, High zone of the beach (MH); Moderate nest density, Middle zone of the beach (MM); Moderate nest density, Low zone of the beach (ML) and Control (CC). Error bars represent Standard error.

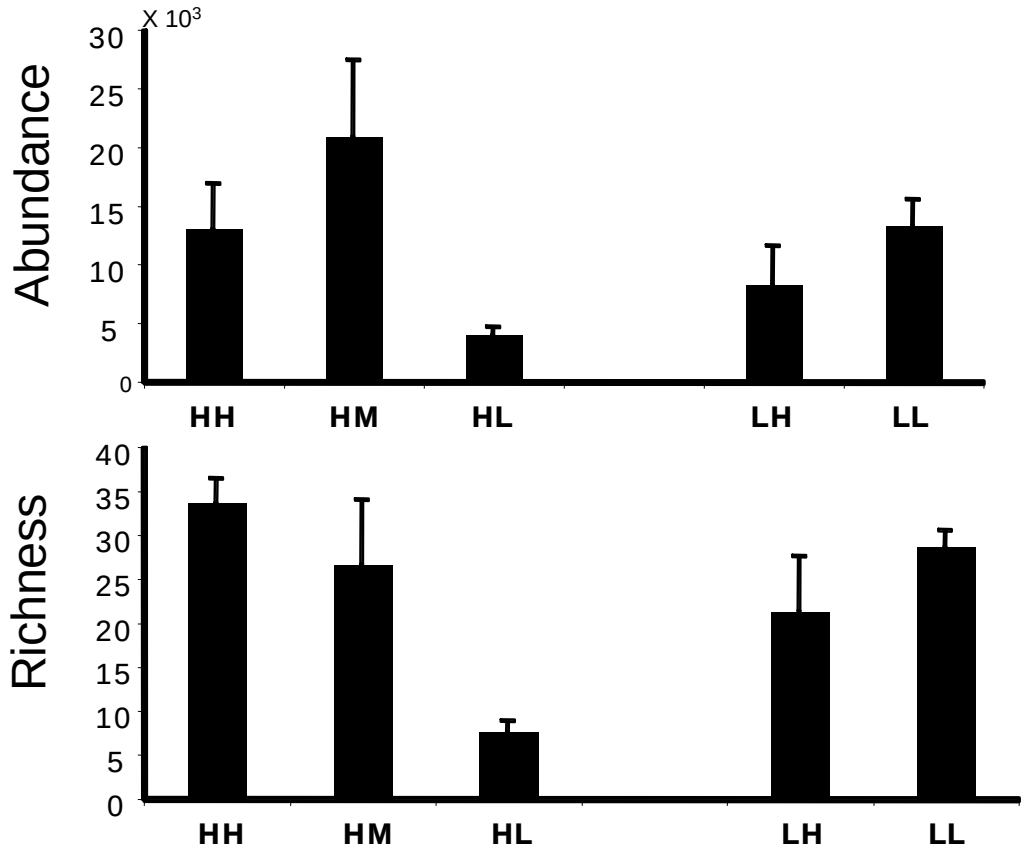

Figure 12. Phylotype abundance and richness at Playa La Flor. High nest density, High zone of the beach (HH); High nest density, Middle zone of the beach (HM) ; High nest density, Low zone of the beach (HL); Low nest density, High zone of the beach (LH); Low nest density, Low zone of the beach (LL). Error bars represent Standard error.

CHAPTER 5: General Conclusion, implications for conservation and management

I have demonstrated that there is a density-dependent effect on hatching success in olive ridley sea turtles. High nest density lowered hatching success. High nest density also caused a decrease in O_2, an increase in CO_2 and an increase in temperature. The higher nest densities in the past probably had an even greater effect on gas concentrations and may have contributed to the low hatching success reported in the past. Bacterial abundance did not differ in different nest densities or zones of the nesting beach but bacterial diversity and richness were both higher in high nest densities at Playa Nancite and higher in high zones of the nesting beach at Playa La Flor, suggesting that bacterial diversity and richness may be important in affecting hatching success of olive ridley turtles on arribada beaches.

Since high nest density has a negative effect on hatching success, the obvious question is: Should we thin out the density on the nesting beaches for better hatching success? If so, from which parts of the beach and how many clutches should we remove? What should we do with these eggs? Should eggs be harvested and used by local people as is done at Playa Ostional, Costa Rica? Should we build hatcheries and protect the eggs and add more hatchlings to the population? Or, should we leave nature to deal with it? These questions all remain to be answered for each specific beach and for each olive ridley population under consideration. It would be premature and overly simple to conclude that because there are density-dependent effects on hatching success we should harvest eggs or relocate nests to hatcheries. Before implementing harvest management programs the effects of wildlife harvest on a

population should be examined. As evident from this thesis, even harvesting eggs that are predicted to have a lower chance of survival had a negative impact on nest hatching success and hatchling production. To accurately estimate the impact of egg harvest on olive ridley populations further studies are necessary. The effects of egg harvest on both hatching success and hatchling production need to be studied in different zones of the beach and at different nest densities at varying times during the nesting seasons.

Further, building hatcheries large enough to accommodate the number of olive ridley clutches that would need to be relocated is very hard, time consuming and expensive. Additionally, we could be altering the gene pool and/or relaxing the selection pressure against nesting in areas that lessen their chance of survival.

Clearly more research is needed before we can be confident that egg harvest would not have unforeseen effects on the olive ridley population. Until the above mentioned issues have been investigated the minimal benefits of egg harvest are greatly out weighed by the harm that it could cause.

LIST OF REFERENCES

1. Ackerman, R.A. 1977. The respiratory gas exchange of sea turtle nests (*Chelonia, Caretta*). Respiratory Physiology 31: 19-38.

2. Ackerman, R.A. 1996. The nest environment and the embryonic development of sea turtles. Pages 83-106. In P.L. Lutz and J.A. Musick, eds. The Biology of Sea Turtles. CRC Press, Boca Press, FL.

3. Acuna, M.T., Diaz, G., Bolanos, H., Barquero, C., Sanchez, O., Sanchez, L.M., Mora, G., Chaves, A. and Campos, E. 1999. Sources of Vibrio mimicus contamination of turtle eggs. Applied and Environmental Microbiology 65: 336-338.

4. Acuña-Mesen, R.A. 1992. Monosporium apiospermum Saccardo (Fungi, Deuteromycetes), associated with sea turtle eggs *Lepidochleys olivacea* (Escholtz 1829) in Costa Rica. Brenesia 38: 159-162.

5. Altwegg, R. 2003. Multistage density-dependence in an amphibian. Oecologia 136: 46-50.

6. Anderson, I.C. and Cairney, J.W.G. 2004. Diversity and ecology of soil fungal communities: increased understanding through the application of molecular techniques. Environmental Microbiology 6: 769-779.

7. Arauz, R.M. 1996. A description of the Central American shrimp fisheries with estimates of incidental capture and mortality of sea turtles. Pages 5-9. In Keinath, J.A., D.E. Barnard, J.A. Musick, and B.A. Bell (compilers). Proceedings of the Fifteenth Annual Symposium on Sea Turtle Biology and Conservation. NOAA Technical Memorandum NMFS-SEFSC-387. 355 pages.

8. Arauz, R., Vargas, R., Naranjo, I. and Gamboa, C. 1998. Analysis of the incidental capture and mortality of sea turtles in the shrimp fleet of Pacific Costa Rica. Pages 1-5. In S.P. Epperly and J. Braun (compilers). Proceedings of the Seventeenth Annual Symposium on Sea Turtle Biology and Conservation. NOAA Technical Memorandum NMFS-SEFSC-415. 294 pages.

9. Awong-Taylor, J., Craven, K.S., Griffiths, L., Bass, C. and Muscarella, M. 2007. Comparison of biochemical and molecular methods for the identification of bacterial isolates associated with failed loggerhead sea turtle eggs. Journal of Applied Microbiology. Early online articles.

10. Ballestero, J., Arauz, R.M. and Rojas, R. 1998. Management, conservation, and sustained use of olive ridley sea turtle eggs

(*Lepidochelys olivacea*) in the Ostional Wildlife Refuge, Costa Rica: an 11 year review. Pages 4-5. In F.A. Abreu-Grobois, R. Briseno-Duenas, R. Marquez and L. Sarti, (compilers). Proceedings of the Eighteenth International Sea Turtle Symposium. NOAA Technical Memorandum NMFS-SEFSC-436. 293 pages.

11. Bernardo, J. and Plotkin, P.T. 2007. An evolutionary perspective on the arribada phenomenon and reproductive behavioral polymorphism of olive ridley sea turtles (*Lepidochelys olivacea*). Pages 59-87. In P.T. Plotkin, ed. Biology and Conservation of Ridley Sea Turtles. Johns Hopkins University Press, Baltimore, MD.

12. Bjorndal, K.A. and Bolten A.B. 1992. Spatial distribution of green turtle (*Chelonia mydas*) nests at Tortuguero, Costa Rica. Copeia 1992: 45-53.

13. Bjorndal, K.A., Bolten, A.B. and Chaloupka, M.Y. 2000. Green turtle somatic growth model: evidence for density dependence. Ecological Applications 10: 269-282.

14. Board, R.G., Loseby, S. and Miles, V.R. 1979. A note on microbial growth on eggshells. British Poultry Science 20: 413-420.

15. Booth, D.T . 2000. The effect of hypoxia on oxygen consumption of embryonic estuarine crocodiles (*Crocodylus porosus*). Journal of Herpetology 34: 478-481.

16. Bucher, E.H. 1992. The causes of extinction of the passenger pigeon. Current Ornithology 9: 1-36.

17. Burgess, L.W. 1981. General ecology of the Fusaria. Pages 225-235. In P.E. Nelson, T.A. Toussoun and R.J. Cook, eds. Fusarium: Diseases, Biology, and Taxonomy. Pennsylvania State University Press, University Park, PA.

18. Bustard, H.R. and Greenham, P.M. 1968. Physical and chemical factors affecting hatching in the green sea turtle, *Chelonia mydas* (L.) Ecology 49: 269-276.

19. Bustard, H.R. and Tognetti, K.P. 1969. Green sea turtles: a discrete simulation of density-dependent population regulation. Science 163: 939-941.

20. Cáceres, G. 1992. Manejo y conservación de tortugas marinas durante los últimos 10 anos. Ed. IRENA. Managua. 22p.

21. Campbell, L.M. 1998. Use them or lose them? Conservation and the consumptive use of marine turtle eggs at Ostional, Costa Rica. Environmental Conservation 25: 305-319.

22. Carr, M.H., Anderson, T.W. and Hixon, M.A. 2002. Biodiversity, population regulation, and the stability of coral-reef fish communities. Proceedings of Natural Academy of Sciences 99: 11241-11245.

23. Carr, T. and Carr, N. 1991. Surveys of turtles of Angola. Biological Conservation. 58: 19-29.

24. Caughley, G. 1970. Eruption of ungulate populations, with emphasis on Himalayan thar in New Zealand. Ecology 51: 53–72.

25. Caut, S., Hulin, V. and Girondot, M. 2006. Impact of density-dependent nest destruction on emergence success of Guianan leatherback turtles (*Dermochelys coriacea*). Animal Conservation 9: 189-197.

26. Churcher, T.S., Ferguson, N.M. and Basanez, M.G. 2005. Density dependence and overdispersion in the transmission of helminth parasites. Parasitology 131: 121-132.

27. Clussella Trullas, S. and Paladino, F.V. 2007. Micro-environment of olive ridley turtle nests deposited during an aggregated nesting event. Journal of Zoology 272: 367-376.

28. Cook, M.I., Beissinger, S.R., Toranzos, G.A., Rodriguez, R.A. and Arendt, W.J. 2005. Microbial infection affects egg viability and incubation behavior in a tropical passerine. Behavioral Ecology 16: 30-36.

29. Cornelius, S.E. and Robinson, D.C. 1985. Counting turtles in Costa Rica. WWF Monthly Report for August (Project 3085). Cambridge, UK: World Conservation Monitoring Center.

30. Cornelius, S.E., Arauz, R., Fretey, J., Godfrey, M.H., Márquez, M.R. and Shanker, K. 2007. Effect of land-based harvest of Lepidochelys. Pages 231-251. Biology and Conservation of Ridley Sea Turtles. Johns Hopkins University Press, Baltimore, MD.

31. Cornelius, S.E. and Robinson, D.C. 1986. Post-nesting movement of olive ridley turtles tagged in Costa Rica. Vida Silvestre Neotropica 1: 12-23.

32. Cornelius, S.E., Ulloa, M.A., Castro, J.C., Mata del Valle, M. and Robinson, D.C. 1991. Management of olive ridley sea turtles (*Lepidochelys olivacea*) nesting at Playas Nancite and Ostional, Costa Rica. Pages 111-135. In J.G. Robinson and K.H. Redford, eds. Neotropical Widlife Use and Conservation. The University of Chicago Press, Chicago and London.

33. Dunbar, J., Ticknor, L.O. and Kuske, C.R. 2000. Assessment of microbial diversity in four southwestern United State soils by 16S rRNA gene terminal restriction fragment analysis. Applied and Environmental Microbiology 66: 2943-2950.

34. Eckert, K.L. and Eckert, S.A. 1990. Embryo mortality and hatching success in In Situ and translocated leatherback sea turtle (*Dermochelys coriacea*) eggs. Biological Conservation 53: 37-46.

35. Eckrich, C.E. and D.W. Owens. 1995. Solitary versus arribada nesting in the olive ridley sea turtles (*Lepidochelys olivacea*): a test of the predator-satiation hypothesis. Herpetologica. 51(3): 349-354.

36. Eng-Heng, C. 2006. Marine turtles in Malaysia: On the verge of extinction? Aquatic Ecosystem Health & Management 9: 175-184.

37. Engebretson, J.J., and Moyer, C.L. 2003. Fidelity of select restriction endonucleases in determining microbial diversity by terminal-restriction fragment polymorphism. Applied Environmental Microbiology 69: 4823-4829.

38. Ewert, M.A. 1979. The embryo and its egg: development and natural history. Pages 333-413. In M. Harless and H. Morlock, eds. Turtles: Perspectives and Research. John Wiley and Sons, New York, NY.

39. Frazier, J., Arauz, R., Chevalier, J., Formia, A., Fretey, J., Godfrey, M.H., Marquez, M.R., Pandav, B. and Shanker K. 2007. Human-turtle interactions at sea. Pages 253-295. In P.T. Plotkin, ed. Biology and Conservation of Ridley Sea Turtles. Johns Hopkins University Press, Baltimore, MD.

40. Gates, C.E., Valverde, R.A., Mo, C.L., Chaves, A.C., Ballesteros, J. and Peskin, J. 1996. Estimating arribada size using modified instantaneous count procedure. Journal of Agricultural, Biological, and Environmental Statistics 1: 275-287.

41. Gause, G.F. 1934. The Struggle for Existence. Williams and Wilkins, Baltimore, MD.

42. Girondot, M., Tucker, A.D., Rivalan, P., Godfrey, M.H. and Chevalier, J. 2002. Density–dependent nest destruction and population fluctuations of Guianan leatherback turtles. Animal Conservation 5: 75-84.

43. Groombridge, B. (Compiler). 1994. 1994 IUCN Red List of Threatened Animals. Gland, Switzerland: IUCN.

44. Hackl, E., Zechmeister-Boltenstern, S., Bodrossy, L. and Sessitsch, A. 2004. Comparison of diversities and compositions of bacterial populations inhabiting natural forest soils. Applied and Environmental Microbiology 70: 5057-5065.

45. Harper, E.B. and Semlitsch, R.D. 2007. Density dependence in the terrestrial life history stage of two anurans. Oecologia 153: 879-889.

46. Head, I.M., Saunders, J.R. and Pick, R.W. 1998. Microbial evolution, diversity and ecology: a decade of ribosomal RNA analysis of uncultured microorganisms. Microbiology Ecology 35: 1-21.

47. Hixon, M.A., Pacala, S.W. and Sandin, S.A. 2002. Population regulation: Historical context and contemporary challenges of open vs. closed systems. Ecology 83: 1490-1508.

48. Hixon, M.A. and Jones, G.P. 2005. Competition, predation, and density-dependent mortality in demersamarine fishes. Ecology 86: 2847-2859.

49. Holdaway, R.N. and Jacomb, C. 2000. Rapid extinction of the Moas (Aves: *Dinornithiformes*): model, test, and implications. Science 287: 2250-2254.

50. Holling, C.S. 1959a. Some characteristics of simple types of predation and parasitism. The Canadian Entomologist 91: 385-398.

51. Holling, C.S. 1959b. The components of predation as revealed by a study of small mammal predation of the European pine sawfly. The Canadian Entomologist 91: 293-320.

52. Hope, R.A. 2002. Wildlife harvesting, conservation and poverty: the economics of olive ridley egg exploitation. Environmental Conservation 29: 375-384.

53. Johnson, D.W. 2006. Predation, habitat complexity, and variation in density-dependent mortality of temperate reef fishes. Ecology 87: 1179-1188.

54. Kalb, H.J. 1999. Behavior and physiology of solitary and arribada nesting olive ridley sea turtles (*Lepidochelys olivacacea*) during the internesting period. Unpublished Ph.D. dissertation. Texas A&M University, College Station, TX.

55. Kamel, S.J. and Mrosovsky, N. 2004. Nest site selection in leatherbacks, *Dermochelys coriacea*: individual patterns and their consequences. Animal Behaviour 68: 357-366.

56. Karr, J.R. and Freemark, K.E. 1985. Disturbance and vertebrates: an integrative perspective. Pages 153-168. In S.T.A. Pickett and P.S. White, eds. The Ecology of Natural Disturbance and Patch Dynamics. Academic Press, New York, NY.

57. Kausrud, K., Mysterud, A., Rekdal, Y., Holland, O. and Austrheim, G. 2006. Density-dependent foraging behaviour of sheep on alpine pastures: effects of scale. Journal of Zoology 270: 63-71.

58. King, T.J. 1977. The plant ecology of ant-hills in calcareous grasslands: I. Patterns of species in relation to ant-hills in southern England. The Journal of Ecology 65: 235-56.

59. Kozlowski, S., Malyszko E., Pinowski, J. and Kruszewicz, A. 1991. The influence of pathogenic fungi on the mortality of sparrow (*Passer spp.)* eggs and nestling. Pages 167-169. In J. Pinowski, B.P. Kavanagh and W. Gorski, eds. Nestling Mortality of Granivorous Birds Due to Microorganisms and Toxic Substances. PWN-Polish Scientific Publishers, Warsaw Poland. Preliminary report.

60. Leslie, A.J., Penick, D.N., Spotila, J.R. and Paladino, F.V. 1996. Leatherback turtle, *Dermochelys coriacea*, nesting and nest success at Tortuguero, Costa Rica, in 1990-1991. Chelonian Conservation and Biology 2: 159-168.

61. Licht, P.D., Owens, W., Cliffton, K. and Penaflores, C. 1982. Changes in LH and progesterone associated with the nesting cycle and ovulation in the olive ridley sea turtle, *Lepidochelys olivacea*. General and Comparative Endocrinology 48: 247-253.

62. Loman, J. 2004. Density regulation in tadpoles of *Rana temporaria*: a full pond field experiment. Ecology 85: 1611-1618.

63. Lombardo, M.P., Thorpe, P.A., Cichewicz, R., Henshaw, M., Millard, C., Steen, C. and Zeller, T.K. 1996. Communities of cloacal bacteria in tree swallow families. The Condor 98: 167-172.

64. Lukow, T., Dunfield, P.F. and Liesack, W. 2000. Use of T-RFLP technique to assess spatial and temporal changes in the bacterial community structure within an agricultural soil planted with transgenic and non-transgenic potato plants. FEMS Microbiology Ecology 32: 241-247.

65. Lyster, S. 1985. International wildlife law: an analysis of international treaties concerned with the conservation of wildlife. The Research Center for International Law, University of Cambridge. Grotius Publ. Ltd., Cambridge.

66. Maloney, J.E., Darian-Smith, C., Takahashi, Y. and Limpus C.J. 1990. The environment for development of embryonic loggerhead turtle (*Caretta caretta*) in Queensland. Copeia 1990: 378-387.

67. Martinez, W.L. and Martinez, A.R. 2002. Computational Statistics Handbook with MATLAB. Chapman & Hall/CRC, Boca Raton, FL.

68. McGinley, M.A. 1989. The influence of a positive correlation between clutch size and offspring fitness on the optimal offspring size. Evolutionary Ecology 3: 150-156.

69. Mendonca, M.T., and Pritchard, P.C.H. 1986. Offshore movements of post nesting Kemp's ridley sea turtles (*Lepidochelys kempi*). Herpetologica. 12: 373-381.

70. Miller, T.E.X. 2007. Demographic models reveal the shape of density dependence for a specialist insect herbivore on variable host plants. The Journal of Animal Ecology 76: 722-729.

71. Mills, T.K., Lombardo, M.P. and Thorpe, P.A. 1999. Microbial colonization of the cloacae of nestling tree swallows. Journal of Avian Biology 29: 314-321.

72. Mo, C.L., Salas, I. and Caballaro, M. 1990. Are fungi and bacteria responsible for olive ridley's egg loss? Pages 249-252. In T.H. Richardson, J.I. Richardson, and M. Donnelly (compilers). Proceedings of the Tenth Annual Workshop on Sea Turtle Biology and Conservation. NOAA Technical Memorandum NMFS-SEFC-278. 286 pages.

73. Mo, C.L., Caballero, M. and Salas, I. 1992. Microorganism infection of olive ridley eggs. Pages 81-84. In J.I. Richardson and T.H. Richardson (compilers). Proceedings of the Twelfth Annual Workshop on Sea Turtle Biology and Conservation. NOAA Technical Memorandum NMFS-SEFSC-361. 274 pages.

74. Morreale, S.J., Ruiz, G.J., Spotila, J.R. and Standora, E.A. 1982. Temperature-dependent sex determination: current practices threaten conservation of sea turtles. Science 216: 1245-1247.

75. Mrosovsky, N. 1997. A general strategy for conservation through use of sea turtles. Journal of Sustainable Use 1: 42-46.

76. Muyzer, G., Teske, A. and Wirsen, C.O. 1995. Phylogenetic relationships of Thiomicrospri species and their identification in deep-sea hydrothermal vent samples by denaturing gradient gel electrophoresis of 16S rDNA fragments. Archives of Microbiology 164: 165-172.

77. Nogales, B., Aguilo-Ferretjans, M.M., Martin-Cardona, D., Lalucat, J. and Bosch, R. 2007. Bacterial diversity, composition and dynamics in and around recreational coastal areas. Environmental Microbiology 9: 1913-1929.

78. Nuttall, P.A. 1997. Viruses, bacteria, and fungi of birds. Pages 271-302. In D.H. Clayton and J. Moore, eds. Host-Parasite Evolution: General Principles and Avian Models. Oxford University Press, New York, NY.

79. Osborne, C.A., Rees, G.N., Bernstein, Y. and Janssen, P.H. 2006. New threshold and confidence estimates for terminal restriction fragment length polymorphism analysis of complex bacterial communities. Applied and Environmental Microbiology 72: 1270-1278.

80. Owens, D.W., Grassman, M.A. and Hendrickson, J.R. 1982. The imprinting hypothesis and sea turtle reproduction. Herpetologica. 38: 124-135.

81. Penaflores, C., Vasconcelos, J., Albavera, E. and Jiménez, M.C. 2001. Especies sujetas a proteccion especial. Tortoga golfina. Pages 1001-1021. In M.A. Cisneros, L.F. Belendez, E. Zarate, M.T. Gaspar, L.C. Lopez, C. Saucedo, and J. Tovar, eds. Sustentabilidad y Pesca Responsible en Mexico. Evaluacion y Manejo. 1999-2000. Publicado en CD. México: Instituto Nacional de la Pesca/SEMARNAT.

82. Peters, A., Verhoe Ven, K.J.F. and Strijbosch, H. 1994. Hatching and emergence in the Turkish Mediterranean loggerhead turtle, *Caretta caretta*: natural causes for egg and hatchling failure. Ecology. 60: 946-955.

83. Phillott, A.D. and Parmenter, C.J. 2001. The distribution of failed eggs and the appearance of fungi in artificial nests of green (*Chelonia mydas*) and loggerhead (*Caretta caretta*) sea turtle nests and the subsequent appearance of fungi. Australian Journal of Zoology. 49: 713-718.

84. Pinowski, J., Kavanagh, B.P. and Gorski, W. 1991. Nestling Mortality of Granivorous Birds Due to Microorganisms and Toxic Substances. PWN-Polish Scientific Publishers, Warsaw, Poland.

85. Plotkin, P.T., Rostal, D.R., Byles, R.A. and Owens, D.W. 1997. Reproductive and developmental synchrony in female *Lepidochelys olivacea*. Herpetologica 31: 17-22.

86. Plotkin, P. T. 1994. Reproductive behavior of the olive ridley turtle (*Lepidochelys olivacea*) in the eastern Pacific Ocean. PhD. dissertation. Texas A & M University, College Station, TX.

87. Plotkin, P.T., Polak, M. and Owens, D.W. 1991. Observation on olive ridley sea turtle behavior prior to an arribada at Playa Nancite, Costa Rica. Mar. Turtle Newsl. 53: 4.

88. Plotkin, P.T., Byles, R.A., Rostal, D.C. and Owens, D.W. 1995. Independent versus socially facilitated oceanic migrations of the olive ridley, *Lepidochelys olivacea*. Mar. Biol. 122: 137-143.

89. Possingham, H.P., Lindenmayer, D.B., Norton, T.W. and Davies, I. 1994. Metapopulation viability analysis of the greater glider *Petauroides volans* in a wood production area. Biol Conserv 70: 343-353.

90. Prange, H.D. and Ackerman, R.A. 1974. Oxygen consumption and mechanisms of gas exchange of green turtle (*Chelonia mydas*) eggs and hatchlings. Copeia 1974: 758-763.

91. Pritchard, P.C.H. 1969. Sea turtles of the Guianas. Bull. Florida State Museum. Biol. Ser. 13:85.

92. Ragotzkie, R.A. 1959. Mortality of loggerhead turtle eggs from excessive rainfall. Ecology 40: 303-305.

93. Rasmussen, D.I. 1941. Biotic communities of Kaibab Plateau, Arizona. Ecological Monographs 3: 229-275.

94. Reynolds, D.P. 2000. Emergence success and nest environment of natural and hatchery nests of the leatherback turtle (*Dermochelys coriacea*) at Playa Grande, Costa Rica, 1998–1999. M.S. thesis. Drexel University, Philadelphia, PA.

95. Ricklefs, R.E. and Miller, G. 2000. Ecology. 4th edition. W. H. Freeman and Company, New York, NY.

96. Rippon, J. W. 1982. Medical Mycology: The Pathogenic Fungi and the Pathogenic Actinomycetes (2nd ed.). pp 797. W. B. Saunders and Co., Philadelphia, PA.

97. Roughgarden, J., Iwasa, Y. and Baxter, C. 1985. Demographic theory for an open marine population with space-limited recruitment. Ecology 66: 54-57.

98. Schaal, B.A. and Leverich, W.J. 1982. Survivorship patterns in an annual plant community. Oecologia 54: 149-151.

99. Schumacher, J. and Cardeilhac, P.T. 1990. Mycotic infections of egg membranes in American alligator (*Alligator mississippiensis*). In Francis-Floyd, ed. Proceedings of the 21st Annual IAAAM Conference, Vancouver, B.C. 138-140.

100. Seymour, R.S. and Bradford, D.F. 1995. Respiration of amphibian eggs. Physiological Zoology 68: 1-25.

101. Solomon, S.E. and Baird, T. 1980. The effect of fungal penetration on the eggshell of the green turtle. Pages 434-435. In P. Bredero and W. de Priester (compilers). Proceedings of the Seventh European World Congress on Electron Microscopy, The Hague, the Netherlands.

102. Solomon, S.E. and Tippett, R. 1987. The intra-clutch localization of fungal hyphae in the eggshells of the leatherback turtle (*Dermochelys coriacea*). Animal Technology 38: 73-79.

103. Sousa, W.P. 1984. The role of disturbance in natural communities. Annual Review of Ecology and Systematics 15: 353–391.

104. Spotila, J.R. 2004. Sea Turtles: A complete guide to their biology, behavior, and conservation. pp. 228. Johns Hopkins University Press. Baltimore, MD.

105. Stewart, R. and Birch Rambo, T. 2000. Cloacal microbes in house sparrows. The Condor 102: 679–684.

106. Stewart, A.Y. 2001. Poached modernity: parks, people, and politics in Nicaragua, 1975-2000. Ph.D. dissertation. Rutgers University, New Brunswick, NJ.

107. Tebbe, C.C. and Vahjen, W. 1993. Interference of humic acids and DNA extracted directly from soil in detection and transformation of recombinant DNA from bacteria and a yeast. Applied Environmental Microbiology 59: 2657-2665.

108. Tilman, D., Lehman, C.L. and Kareiva, P. 1997. Population dynamics in spatial habitats. Pages 3-20. In D. Tilman and P. Kareiva, eds. Spatial Ecology. Princeton University Press, Monograph Series, Princeton, NJ.

109. Tiwari, M., Bjorndal, K.A., Bolten, A.B. and Bolker, B.M. 2006. Evaluation of density-dependent processes and green turtle *Chelonia mydas* hatchling production at Tortuguero, Costa Rica. Marine Ecology Progress Series 326: 283-293.

110. Trinidad, H. and Wilson, J. 2000. The bio-economics of sea turtle conservation and use in Mexico: history of exploitation and conservation policies for the olive ridley (*Lepidochelys olivacea*). In Proceedings of the International Institute of Fisheries Economics and Trade Conference. Corvallis, OR.

111. Valverde, R.A., Cornelius S.E. and Mo, C.L. 1998. Decline of the olive ridley sea turtle (*Lepidochelys olivacea*) nesting assemblage at Nancite beach, Costa Rica. Chelonian Conservation and Biology 3: 58-63.

112. Von Mutius, A. 2000. Nesting and hatching success of olive ridley sea turtles in La Flor, Nicaragua. M.S. thesis. Ludwig-Maximilians-Universität, Munich, Germany.

113. Wallace, B.P., Sotherland, P.R., Spotila, J.R., Reina, R.D., Franks, B.R. and Paladino, F.V. 2004. Biotic and abiotic factors affect the nest environment of embryonic leatherback turtles. Physiological Biochemical Zoology 77: 423–432.

114. Warburton, S.J., Hastings, D. and Wang, T. 1995. Responses to chronic hypoxia in embryonic alligators. The Journal Experimental Zoology 273: 44-50.

115. Witherington, B.F. and Frazer, N B. 2003. Social and economic aspect of sea turtle conservation. Pages 355-384. In P.L. Lutz, J.A. Musick and J. Wyneken, eds. The Biology of Sea Turtles. Volume II. CRC Press, Boca Raton, FL.

116. Whitmore, C.P. and Dutton, P.H. 1985. Infertility, embryonic mortality and nest-site selection in leatherback and green sea turtle in Suriname. Biological Conservation 34: 251-272.

117. Wyneken, J., Bruke, T.J., Salmon, M. and Pederson, D.K. 1998. Egg failure in natural and relocated sea turtle nests. Journal of Herpetology 22: 88.

118. Yom-Tov, Y., Yom-Tov, S., MacDonald, D. and Yom-Tov, E. 2007. Population cycles and changes in body size of the lynx in Alaska. Oecologia 152: 239-244.

VITA

Shaya Honarvar
Major Advisor: James R. Spotila, Ph.D.

Education

Master of Science , 2004	University of Pennsylvania, USA
Ingenieur (B.S./M.S.), 2000	Hogeschool van Utrecht, The Netherlands

Professional experience

2005-2007 Teaching assistant, Drexel University, Philadelphia, Pennsylvania.
2000-2004 Teaching assistant, University of Pennsylvania, Philadelphia, Pennsylvania.
2001 Research assistant, neurobiology laboratory, University of Pennsylvania, Philadelphia, Pennsylvania.
2000&2002 Research assistant, School of Veterinary Medicine, University of Pennsylvania. Philadelphia.

Publications

Lattal, K. M., S. **Honarvar**, and T. Abel (2004) Effects of post-session injections of anisomycin on the extinction of a spatial preference and on the acquisition of a spatial reversal preference. Behavioural Brain Research 153(2), 327-339.

Honarvar, S., B.-K. Choi, and D. M. Schifferli (2003) Phase variation of the 987-like CS 18 fimbriae of human entertoxigenic *Escherichia coli* is regulated by site-specific recombinases. Molecular Microbiology 48(1), 157-171.

Honarvar, S., O'Connor, M. P., Spotila, J. R. Density-dependent effect on hatching success of the olive ridley turtle, *Lepidochelys olivacea.* Oecologia (In Press).

Grants

2005 Koinonia Foundation. $1,000
2004 Linnaeus Fund and Chelonian Research Foundation. $1,000
2003 Lerner-Grey grant for marine research from American Museum of Natural History. $2,000

Awards

2007 27th Annual Symposium on Sea Turtle Biology and Conservation. Archie Carr Best Student Presentation Award.
2007 Drexel University's College of Arts and Sciences Research Day. Dean's Graduate Research Award for Natural Sciences: poster presentation.
2006 Drexel University Eighth Annual Research Day. Honorable mention: poster presentation.