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Reproductive Ecology and Hatchling Behavior of Olive Ridley Sea Turtles in Honduras

by

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A Dissertation submitted in partial satisfaction of
the requirements for the degree
Doctor of Philosophy in Biology

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To Fernanda, my little miracle found in Honduras

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ABBREVIATIONS

AHT	After High Tide
ANOVA	Analysis of Variance
CBC	Community Based Conservation
CCL	Curved Carapace Length
CITES	Convention on International Trade in Endangered Species
Cm84	Microsallite # 84 of green turtles (<i>Chelonia mydas</i>)
CMS	Convention of Migratory Species of Wild Animals
DNA	Deoxyribonucleic Acid
E	East
EDTA	Ethylenediaminetetraacetic Acid
GIS	Geographic Information System
GPS	Global Positioning System
IAC	Inter-American Convention for the Protection and Conservation of Sea Turtles
IUCN	International Union for Conservation of Nature
MOT	Mid-Outgoing Tide
N	North
NGO	Non-Governmental Organization
NOAA	National Oceanic and Atmospheric Administration
Or-1	Microsallite # 1 of olive ridley sea turtles (<i>Lepidochelys olivacea</i>)
PCR	Polymerase Chain Reaction

PrDM	Probability of Detecting Multiple Paternity
ProTECTOR	Protective Turtle Ecology Center for Training, Outreach and Research, Inc.
PROTUMAR	The Sea Turtle Conservation Program (Oaxaca, Mexico)
S	South
SDS	Sodium Dodecyl Sulfate
SE	Standard Error
TAMAR-IBAMA	The Brazilian Sea Turtle Conservation Program
TRT	Transitional Range of Temperatures
US	United States
UV	Ultra Violet
W	West

ABSTRACT OF THE DISSERTATION

Reproductive Ecology and Hatchling Behavior of Olive Ridley Sea Turtles in Honduras

by

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Dr. Stephen G. Dunbar, Chairperson

In this dissertation, I investigated the reproductive ecology and hatchling behavior of the olive ridley (*Lepidochelys olivacea*) sea turtle population nesting in Pacific Honduras. I begin by reviewing olive ridley reproduction, human use of this species, and past and present conservation on nesting beaches. I also propose a conservation strategy to improve nesting beach conservation programs in developing countries. In the first of four empirical studies, I used microsatellite markers to assess multiple paternity levels of the Honduran population, and found evidence for multiple paternity in 75% of the nests examined. This rate, higher than expected for a population of solitary nesters, suggests that some females may be coming from Nicaraguan mass nesting beaches. The second study compared thermal profiles, hatching success, and hatchling characteristics of nests left *in situ* on the beach with nests from a local hatchery. I also assessed the effects of retaining hatchlings for 24 hours after emergence. Incubation temperatures were higher, and hatching success was lower, in the hatchery compared to the beach. Mean nest temperature during the second third of incubation was a good predictor of hatching success. Retention for 24 hours reduced hatchling weight, running speed, and active swimming time. In the third study, I compare diurnal and nocturnal hatchling swimming

patterns, finding that hatchlings at night swam near the surface 98% of the time, yet during the day they spent 78% of the time swimming at depth. This diurnal pattern of deep swimming may be used by hatchlings to avoid avian predation. In the fourth study, I investigated the effects of tidal currents on hatchling movements during offshore migration. Hatchlings moved away from the coast during outgoing tides, but were pulled back to the shore during incoming tides. I found that a change in the timing of hatchling releases helped counter backward movements and increased effective distances covered by the hatchlings. This dissertation represents the first comprehensive study on the reproductive ecology of the Honduran olive ridley population. My findings provide useful information for improving ongoing conservation efforts for this species.

CHAPTER ONE

INTRODUCTION

Sea turtles are species-at-risk. The habit of aggregating in predictable areas for feeding or reproducing (Frazier et al. 2007; Plotkin et al. 2012) makes sea turtles especially vulnerable to human over-exploitation. Additionally, some features of sea turtle life history, such as slow growth and late maturity, make it difficult to recover populations that are already depleted.

The life cycle of sea turtles encompasses both land and oceanic stages, often separated by large distances and covering long periods of time. Sea turtles reproduce on land. Reproductive females emerge to lay their eggs on beaches, usually in the same area where they hatched, a behavior called philopatry, or natal homing (Miller 1997). After an incubation period of approximately 60 days, the eggs hatch and the hatchlings crawl to the sea and swim off-shore until they find a main oceanic current where they will drift while developing and growing (Carr 1986; Carr 1987). This journey usually takes two or three years and often involves distances of thousands of kilometers, sometimes across entire ocean basins (Bolten and Balazs 1995). Once they reach the juvenile stage, individuals of some sea turtle species go back to neritic waters and stay in coastal foraging grounds. Individuals of other species may remain in pelagic waters until they reach adult size (Bolten 2003). Sea turtles are long lived, slow growing vertebrates that can take more than 35 years to reach sexual maturity, depending on the species (Hirth 1971). Adult males and females usually aggregate in front of the nesting beaches to mate. Females often lay more than one clutch during the reproductive season, yet in most sea

turtle species they do not reproduce annually, breeding instead every two to nine years (Miller 1997).

Throughout this complex life cycle sea turtles face a variety of threats. Nesting females and developing eggs are vulnerable to land predators, and recently emerged hatchlings become common prey for fish and seabirds (Stancyk 1982). Egg harvesting for human consumption and commerce, both legal and illegal, is widespread in many countries (Campbell 2007; Plotkin et al. 2012). Juvenile and adult sea turtles are still actively captured for meat and other products in some parts of the world (Humber et al. 2014), and they are also unintentionally captured as by-catch in fisheries all over the world (Gilman et al. 2006). Other current threats to sea turtles include the loss of nesting habitats due to human interventions on beaches (McClenachan et al. 2006), marine debris and other types of oceanic pollution (Derraik 2002), and global warming (Hawkes et al. 2009).

There is no doubt that sea turtles need protection. Six of the seven extant species are included in the IUCN's Red List of Threatened Species (IUCN 2014), either as Critically Endangered (hawksbill, *Eretmochelys imbricata*, and Kemp's ridley, *Lepidochelys kempii*), Endangered (green, *Chelonia mydas*, and loggerhead, *Caretta caretta*), or Vulnerable (leatherback, *Dermochelys coriacea*, and olive ridley, *Lepidochelys olivacea*). However, the life patterns of sea turtles, and the wide array of threats they face make sea turtle conservation challenging. First, because conservation resources are limited, researchers must decide where to concentrate conservation efforts, which species and populations are of most concern, and which life stages should receive the most protection. Second, because sea turtles move across national boundaries as well

as through international waters, it is pivotal to coordinate conservation efforts and to approach sea turtle conservation from local, regional, and international levels.

With regard to which life stage or stages should be the primary focus of sea turtle conservation measures, several mathematical and computer models have attempted to represent population dynamics of different sea turtle species, and have provided some insights on the matter. Early models for loggerhead sea turtles (Crouse et al. 1987; Heppell 1998) concluded that management practices focused on the first life stages (eggs and hatchlings on nesting beaches) had low impact on the population and accordingly, protection efforts should concentrate on juveniles or subadults. However, more recent models using data from loggerhead and green turtles suggest that egg survival and hatchling success are also of high importance, and can compensate for losses in other age classes (Mazaris et al. 2005, 2009).

Regarding levels of protection and conservation action, several international agreements, such as the Convention on International Trade in Endangered Species (CITES), and the Convention of Migratory Species of Wild Animals (CMS), include sea turtles under the highest levels of protection (Richardson et al. 2006). Sea turtles are also protected by domestic laws in many countries, yet levels of enforcement and compliance vary widely. In many developing countries where sea turtles occur, governments struggle with financial difficulties, impoverishment, illiteracy, social instability, drug trafficking, corruption, and violent conflicts, all of which take priority over conservation issues. This scenario suggests that, in many areas, the fate of sea turtle populations mostly depends on local communities living in those sites, highlighting the importance of local conservation initiatives.

This dissertation focuses on the conservation of olive ridley sea turtles, *Lepidochelys olivacea*, through field and experimental research, and enhanced nesting beach management. The overriding objective was to investigate the reproductive ecology of the olive ridley population nesting in Pacific Honduras, and to use this information to suggest improvements for the current management protocols of the local conservation project.

In chapter 2, I begin with a review of the reproductive ecology of olive ridleys, the history of human use of the species, and the current conservation practices in nesting beaches. Olive ridleys are the most abundant of the extant sea turtle species, yet their current status, according to the IUCN's Red List, is vulnerable, meaning that they face high risk of extinction in the wild (IUCN 2014). In that chapter, I review the characteristics of the species that have favored human overexploitation, mainly the special mode of reproduction displayed by some populations called mass nesting, or *arribada*. I also review the current conservation status of the species along its range. Olive ridley nesting beaches are located in tropical and subtropical regions of the Pacific, Atlantic, and Indian Oceans. Many of these beaches belong to developing countries with high poverty indexes, where local communities have traditionally used sea turtles and sea turtle eggs as a source of income. Although olive ridleys are legally protected in most countries, the actual levels of compliance tend to be low, and sea turtle captures and egg harvesting are widespread. At the end of chapter 2 I list several reasons why olive ridley conservation research is important, and suggest a conservation strategy for olive ridley nesting beaches, applicable to local conservation projects currently carried out in developing countries. The strategy is based on the concept of Community Based

Conservation, and has the objective of using research and accurate management practices to optimize the results of current operative projects, increasing benefits for both the sea turtle species and local human communities.

Chapters 3 to 6 present examples of the application of this conservation strategy to the olive ridley population that nests in Pacific Honduras. Olive ridleys nest solitarily on several beaches along the south coast of Honduras. These beaches are located in the northeast end of the Gulf of Fonseca, a shallow 80 by 50 km inlet of the Pacific Ocean, with coasts shared by El Salvador, Honduras, and Nicaragua. Olive ridley eggs have been historically used in Honduras for human consumption and commerce. Since the 1940s to the 1970s, almost 100% of eggs deposited on Honduran beaches were harvested (Campbell 2007; Pritchard 2007). However, in 1975 the government established a yearly protected period, *la veda*, to protect the declining population (Minarik 1985). *La veda* encompasses the first 25 days of September. During this period beaches are patrolled in search of nests and eggs are relocated into hatcheries. Currently, there are four operational hatcheries along the Pacific coast of Honduras, at Punta Ratón, Boca del Río Viejo, Cedeño, and El Venado. Punta Ratón, the main research site for this dissertation, comprises the largest hatchery, with more than 200 nests a year. In Punta Ratón, the local community, with the support of the national military, performs beach patrolling, hatchery construction, and hatchery management. During the 25 protected days, people participating in beach patrolling tasks receive compensation in the form of basic food items. When *la veda* finishes, only one or two people remain in charge of the hatchery and receive a small economic compensation for maintaining the eggs until hatched. Hatchery tasks include burying relocated eggs, watching the hatchery during incubation

to prevent human or animal predation, releasing emerged hatchlings, digging up nests after emergence, and recording nest and hatchling data. In the hatchery at Punta Ratón, hatching success tends to be low, likely due to severe construction and management deficiencies. In 2009, the hatchery was built in a lowland area that became flooded, causing all embryos to die, and in 2010 all nests were lost because of excess heat (Dunbar, personal communication). Very often hatchlings are too weak to emerge naturally and the nests are dug up to prevent hatchlings from dying inside the nests. Also, because of their weakness, hatchlings are not released on the beach, but directly into the water. Releases take place during outgoing tides in order for ebb currents to help hatchlings move away from the coast, but only during the night to minimize potential avian predation. This implies that hatchlings are retained for long periods before release, sometimes as long as 24 hours after emergence.

Although the conservation program at Punta Ratón has been in place for 40 years, the only available data during most of this time were the numbers of relocated eggs and released hatchlings per year. In 2007, the Protective Turtle Ecology Center for Training, Outreach and Research, Inc. (ProTECTOR) started a flipper-tagging program with nesting females on the beaches at Punta Ratón and El Venado (Dunbar and Salinas 2008), and deployed several satellite tags on nesting females from the region to monitor their post nesting movements in 2010, 2011 and 2012 (Dunbar et al. 2010; Dunbar and Salinas 2013). However, prior to the research studies reported in this dissertation, no scientific assessment had been performed on the management practices in any of the hatcheries, and no information existed about the fate of the hatchlings released from Honduran beaches.

From 2011 to 2013, I carried out four research projects in Punta Ratón with the aims of increasing scientific knowledge on the reproductive characteristics of the local olive ridley population, and of using this knowledge to suggest practical improvements for the conservation practices currently in place on Honduran beaches.

In chapter 3, I report results of an assessment of the multiple paternity levels of olive ridleys in Honduras, using microsatellite markers. The percentage of multiple paternity in a population is important because it influences effective population size (Sugg and Chesser 1994) and genetic variability (Baer and Schmid-Hempel 1999), and provides information on mating patterns and population structure (Jensen et al. 2006). In this case, a multiple paternity analysis was even more informative because it could provide insights on the origins of individuals within the Honduran olive ridley population. Multiple paternity levels in olive ridley turtles depend on the mode of reproduction in each population, being much higher in *arribada* beaches than in solitary beaches (Jensen et al. 2006). Although female olive ridleys nest in solitary at Punta Ratón and the rest of the Honduran nesting beaches, Pritchard (2007) suggested that these females may actually originate from *arribada* nesting beaches in Nicaragua. If that was the case, the olive ridley nests deposited at Punta Ratón would presumably show high levels of multiple paternity. If not, they would likely show low levels typical of solitary populations. The information obtained on the mating patterns of the population, as well as on the likely origin for the nesting females, have implications for conservation purposes.

In chapter 4, I compare the characteristics of semi-natural nests incubated *in situ* on the beach at Punta Ratón with nests incubated at the local hatchery. Hatcheries are

widely used in sea turtle conservation programs (Mortimer 1999), but may alter incubation temperatures (DeGregorio and Williard 2011) decrease hatching success (Pintus et al. 2009) and affect the physical characteristics and behavior of hatchlings (Türkozan et al. 2001; Koch et al. 2008). I examined incubation temperatures for nests both on the beach and at the hatchery, as well as hatching success, hatchling weight and size, hatchling running speed, and hatchling swimming style. Because previous studies have shown that long retention times may reduce hatchling body condition and locomotion performance (Pilcher and Enderby 2001; van der Merwe et al. 2013), I also assessed the influence of retaining hatchlings for 24 hours on hatchling weight, running speed, and swimming ability. The significance of this study to conservation consisted of assessing the weaknesses of the current conservation program and providing scientifically supported improvements and alternatives.

In chapter 5, I provide results from investigations on levels of in-water predation suffered by hatchlings released from Punta Ratón during the first hours of their offshore migration. Because previous predation studies related shallow waters with high predation rates (Witherington and Salmon 1992; Pilcher et al. 2000), I expected that hatchlings swimming across the shallow waters of the Gulf of Fonseca suffered high incidences of predation by fish. However, actual predation rates were very low. In this chapter, I also provide results of a study that compared the diurnal and nocturnal swimming patterns of the hatchlings. Sea turtle hatchlings are positively buoyant and tend to swim near the surface of the water (Davenport and Clough 1986). However, they are able to swim at depth for variable periods of time, and hatchlings of different sea turtle species have been recorded diving in response to birds or other objects flying overhead (Frick 1976;

Witherington et al. 1995). In this study, I describe a diurnal pattern of deep swimming performed by the Honduran hatchlings, and suggest the possibility of this behavior being an antipredator strategy to avoid detection by birds in the turbid waters of the Gulf of Fonseca. Taking into account that preventing bird predation is one of the goals that determined current protocols for hatchling releases at Punta Ratón, investigating hatchling predator avoidance behaviors and assessing the actual risk of predation was important to evaluate the necessity and adequacy of such protocols.

In chapter 6, I investigate the effects of tidal currents on hatchling offshore migration. Local environmental conditions vary from beach to beach, and therefore, they may affect hatchlings from different areas in different ways. Due to its semi-enclosed shape and its shallow waters, tidal currents in the Gulf of Fonseca are strong and reach speeds five times higher than typical hatchling swimming speeds (Admiralty Hydrographic Office 1951). The small size of sea turtle hatchlings makes them vulnerable to these strong tidal currents. In this study, I assessed the reversal effects of flood tidal currents on hatchling movements, and examined whether changing the current timing of hatchling releases, from mid outgoing tide to the beginning of the outgoing tide, could minimize these effects.

In Chapter 7, I summarize and discuss the conclusions of my research. My results include new findings that will enrich the general knowledge of olive ridley reproductive biology, and practical data useful for improving current management and conservation practices. At the end of chapter 7, I provide suggestions for future directions on continuing and expanding the research of this dissertation.

Goal, Objectives and Hypotheses

The overriding goal of this dissertation was to investigate the reproductive ecology of the olive ridley population nesting in Pacific Honduras, and to use this information to suggest improvements for the current management protocols of the local conservation project.

- The first objective was to assess the levels of multiple paternity of this population.
 - I hypothesized that the levels at Punta Ratón would be low because multiple paternity levels in olive ridley turtles depend on the mode of reproduction, being high in *arribada* beaches and low in solitary beaches.
- The second objective was to compare nest and hatchling characteristics between semi-natural nests incubated *in situ* on the beach, and nests reburied in the local hatchery.
 - Because of the poor hatchery management at Punta Ratón, I hypothesized that:
 - The nests at the hatchery would incubate at higher temperatures and show lower hatching success than the nests at the beach.
 - Hatchlings from the hatchery would be smaller and show lower locomotion performances than hatchlings from the beach.
- The third objective was to investigate the effects of retaining hatchlings for long periods of time after emergence on hatchling characteristics and behavior.
 - I hypothesized that long retention times would reduce hatchling weight, running speed, and swimming ability.

- The fourth objective was to assess the levels of in-water predation suffered by hatchlings from Punta Ratón during the first hours of their off-shore migration.
 - I hypothesized that the rate of predation by fish would be high, because of the shallow depth of the Gulf of Fonseca waters.
- The fifth objective was to compare the diurnal and nocturnal swimming patterns of recently emerged hatchlings, regarding their position in the water column.
 - I hypothesized that hatchlings would spend more time swimming at depth during the day to minimize predation by birds.
- The sixth objective was to determine the effects of tidal currents on the movements of hatchlings during their off-shore migration.
 - I hypothesized that the movements of the hatchlings would be highly affected by tidal currents, moving away from the beach during outgoing tides and back toward the coast during incoming tides.
- The seventh objective was to investigate if a change in the release protocols of the hatchlings would reduce the reversal effect of the flood tidal current.
 - I hypothesized that releasing the hatchlings just after high tide would provide more time for them to move away from the beach during the first outgoing tide and thus, minimize the effects of the following incoming tide.

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CHAPTER TWO

**WHY SHOULD WE CARE FOR THE MOST ABUNDANT AND LEAST
ENDANGERED SEA TURTLE SPECIES?**

**A REVIEW OF OLIVE RIDLEY REPRODUCTIVE ECOLOGY, HUMAN USE,
AND NESTING BEACH CONSERVATION MANAGEMENT**

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Introduction

The olive ridley (*Lepidochelys olivacea*) is one of the smallest (Van Buskirk and Crowder 1994) and the most abundant of the seven extant sea turtle species (Pritchard 1997). Olive ridleys are widely distributed in tropical and subtropical waters of the Pacific and Indian Oceans (Pritchard and Mortimer 1999). They also occur in the Atlantic Ocean, but are absent from the East coast of the United States and the Gulf of Mexico, where *L. olivacea* is replaced by its congeneric species, the Kemp's ridley sea turtle, *L. kempii* (Fretey 1999; Pritchard 2007). Both olive ridleys and Kemp's ridleys exhibit a special mode of reproduction called *arribada* or mass nesting, consisting of the synchronized emergence of hundreds to tens of thousands of females nesting in close proximity on specific beaches during a few days (Bernardo and Plotkin 2007). Only a handful of olive ridley mass nesting beaches exist in the world and are located in India, Mexico, Costa Rica, Nicaragua, Panama, and Suriname (Fig. 1). Besides nesting *en masse*, olive ridley sea turtles also nest solitarily on many tropical beaches worldwide. Solitary nesting is much more common than *arribada* nesting, and several new nesting beaches have been discovered in the last decade (Alava et al. 2007; Kelez et al. 2009; Padmavathy and Anbarashan 2011) (Fig. 1). Olive ridley nesting has been documented along the East Pacific coast, from Sonora and Baja California (Mexico) to Peru. In the West Atlantic they nest in varying densities in Suriname, French Guiana, and Brazil. Extensive nesting has been reported also in the East Atlantic in several countries along the African coast, as well as throughout the Indian Ocean.



Figure 1. Location of Olive Ridley nesting beaches. Black dots indicate countries where olive ridleys nest in solitary. Grey circles indicate countries that have one or more *arribada* beaches. Dots and circles do not indicate the exact position of the rookeries.

In addition to mass nesting behavior, several other characteristics identify olive ridleys and distinguish them from other sea turtle species. Olive ridleys are small, with average weights of 35 kg and rarely exceeding 45 kg (Marcovaldi 1999; Pritchard and Mortimer 1999). The head is triangular in shape, with two prefrontal scales. The common name for the species originates from the olive color typical of the adult carapace, although it can actually range from greenish to dark grey. Typical straight carapace lengths (SCL) for adult female olive ridley sea turtles range from 60.6 cm (Mexico) to 71.5 cm (Sultanate of Oman) (Castellanos Michel et al. 2003). The carapace is nearly round with wide marginal scutes, and shows regional variation. For example, Pacific populations have a steep-sided, flat-topped carapace, possibly associated with their habit of surface basking in relatively cool waters (Pritchard 2007). The presence of 6 – 9 lateral scutes (Wyneken and Witherington 2001), often with asymmetrical configuration (Pritchard and Mortimer 1999), distinguishes olive ridleys from all other hard-shelled sea turtles. The plastron is lighter in color and exhibits a series of conspicuous pores in the inframarginal scutes, also present in the congeneric species, Kemp's ridley. These pores correspond to the openings of Rathke's glands (Pritchard 2007), present in other turtles, yet especially enlarged in the ridleys. While the function of these glands remains unknown, it has been suggested that their secretions may play a role in detection of conspecifics during *arribadas* (Owens et al. 1982). Regarding the distinctive high number of costal scutes, Pritchard (2007) pointed out that this highly variable, multiscutate condition has been reported in hatchlings of other sea turtle species raised under artificial incubation (Mast and Carr 1989). The significance of this observation remains unclear.

Pritchard (2007) described the general morphology of the olive ridley as the most primitive and generalized among the sea turtle species, and proposed that its lack of adaptations to highly specialized ways of life may have represented a formula for survival. The generalist diet and opportunistic feeding habits of the olive ridley (Márquez 1990) underscore this lack of specialization. They may feed on fish, tunicates, mollusks, crustaceans, jellyfish, sea urchins, other invertebrates, or algae (Bjorndal 1997) depending on what is available. In fact, olive ridley diets show important regional differences. In the Indian Ocean olive ridleys were first described as almost exclusively herbivorous (Deraniyagala 1953; Biswas 1982), yet a recent study that analyzed gut contents of a higher number of animals, covering both sexes and different sizes, reported animal prey as the most abundant, with variable algae content depending on age group (Behera et al. 2014). Adult turtles fed mostly on mollusks (47%), followed by algae (12%). Subadults, in contrast, preferred crustaceans (32%), followed by mollusks and fish in similar amounts (23% and 21%, respectively). In the eastern Pacific, olive ridleys are primarily carnivorous (Márquez et al. 1976; Pritchard and Trebbau 1984), feeding both on benthic invertebrates in nearshore areas (Casas-Andreu and Gómez-Aguirre 1980) and on planktonic gelatinous prey in the open ocean (Kopitsky et al. 2004; Polovina et al. 2004). In the Atlantic, a recent study on gut contents of animals stranded in Brazil showed that olive ridleys in the region are benthic carnivorous, whose preferred prey are crustaceans and fish (Colman et al. 2014).

The life cycle of olive ridleys also supports the hypothesis that they are less constrained than other sea turtle species, and highly adaptable to variable environmental factors. Sea turtles exhibit three different types of life history patterns (Bolten 2003).

Flatbacks (*Natator depressus*) are endemic to Australia and display Type 1, which consists in spending all life stages (hatchling, juvenile, and adult) in the neritic zone, close to the coast. All other extant species spend at least some part of their lives in the oceanic zone, usually after hatchlings leave the nesting beaches and drift for several years within oceanic currents. While loggerheads (*Caretta caretta*), hawksbills (*Eretmochelys imbricata*), greens (*Chelonia mydas*), and Kemp's ridleys all return to the neritic zone as juveniles to finish development (Type 2), leatherbacks (*Dermochelys coriacea*) complete development up to the adult stage in the oceanic zone (Type 3). According to Bolten (2003), the olive ridley is the only species that appears to follow either a Type 2 (Australian and West Atlantic populations) or a Type 3 life history pattern (East Pacific populations), perhaps in response to variations in resource availability.

Regional differences also exist in the post-migratory movements of adult olive ridleys. East Pacific populations exhibit nomadic behavior, wandering over vast oceanic areas without targeting specific foraging grounds (Cornelius and Robinson-Clark 1986; Plotkin 1994; Plotkin et al. 1995; Dunbar and Salinas 2013). In a recent satellite telemetry study on post-reproductive migrations, Plotkin (2010) confirmed that olive ridleys from the eastern Pacific do not follow specific migratory corridors and do not show site fidelity to feeding areas. She found that their movement patterns changed in response to an El Niño event and concluded that this high migratory flexibility makes the species less vulnerable to the impacts of climate change. Studies from Australia, however, showed that olive ridleys from that region do use distinct foraging areas and move directly towards them after the nesting season (McMahon et al. 2007; Whiting et al. 2007). Likewise, recovered carcasses of tagged turtles in the western Atlantic suggest that

olive ridleys from that area could be regularly migrating to several specific foraging areas in southern Brazil (Reis et al. 2010). These data reinforce the high habitat plasticity and adaptability of this species.

Reproductive Behavior and Nesting Ecology

Olive ridley sea turtles attain sexual maturity at approximately 13 years of age, relatively early compared with other sea turtle species (Zug et al. 2006). Another peculiarity is that females nest almost annually (Pritchard 1969; Plotkin 1994), with a clutch frequency of 2 – 3 nests per year (Miller 1997). Evidence from tag-return studies in India has shown that the reproductive span for this species is at least 21 years (Pandav and Kar 2000). Olive ridleys are primarily mainland nesters and rarely utilize islands or shores with extensive offshore coral (Pritchard 2007), preferring sandy beaches with high humidity levels near river mouths or estuaries (Casas-Andreu 1978). Regarding nesting behavior, a peculiar feature of the genus *Lepidochelys* is the so-called “dance of the ridley” (Pritchard 2007). After covering the nest, the female starts lifting her body and hammering down on the sand with each side, producing a characteristic sound audible from several meters away (Silas and Rajagopalan 1984). Table 1 provides a summary of the main characteristics of olive ridley reproduction.

Table 1. Main characteristics of olive ridley reproduction

Attribute	Value	Source
Age at sexual maturity	10 years 13 years	(Pritchard 2007) (Zug et al. 2006)
Female size at first reproduction	Usually over 60 cm (range 54-72 cm)	(Hughes and Richard 1974; Ernst et al. 1994)
Track characteristics	Alternate gait About 76 cm wide	(Pritchard 2007) (Spotila 2004)
Substrate preferences	Open sand, some under vegetation	(Spotila 2004)
Nesting duration	Less than 1 hour	(Spotila 2004)
Oviposition duration	Less than 20 minutes	(Vega and Robles 2005; Kumar et al. 2013)
Clutch size	74-126 eggs, mean 105 eggs (Eastern Pacific)	(Ernst et al. 1994; Abreu-Grobois and Plotkin 2008)
Egg weight	40 g 30-38 g	(Spotila 2004) (Ernst et al. 1994)
Egg size	32-45 mm diameter	(Ernst et al. 1994; Pritchard and Mortimer 1999)
Nest size	Flask shaped 30-55 cm deep (commonly 38-43 cm) Egg chamber 17-30 cm wide	(Ernst et al. 1994; Vega and Robles 2005)
Clutch frequency	2-3 (2.2)	(Spotila 2004)
Renesting interval (intra-season)	14 days (Solitary) 17-45 days, usually 28 days (<i>Arribada</i>)	(Spotila 2004) (Bernardo and Plotkin 2007)
Inter-nesting period (inter-season)	They nest almost every year	(Pritchard 2007)
Site fidelity	Low (Solitary), high (<i>Arribada</i>)	(Bernardo and Plotkin 2007)
Incubation period	50-65 days 45-51 days	(Spotila 2004) (Ernst et al. 1994)
Pivotal temperatures	30.5 °C (Nancite, Costa Rica) 29 °C (Gahirmatha, India)	(Spotila 2004; Wibbels 2007)
TRT (transitional range of temperatures)	27-32 °C (Costa Rica) 100% males at <27 °C 100% females at >32 °C	(Spotila 2004)
Hatchling weight	16-19 g	(Ernst et al. 1994)
Hatchling size	CCL 37-50 mm, CCW 31-45 cm	(Ernst et al. 1994)

The most remarkable feature of olive ridley reproduction is the *arribada* or mass nesting behavior undertaken by some populations. The key feature that distinguishes an *arribada* from colonial nesting, typical of green and loggerhead turtles, is the synchronicity of female emergence (Bernardo and Plotkin 2007). Ridleys aggregate in nearshore waters of specific beaches for days or weeks, and assemble closer to the shore a few days before the *arribada* begins (Bernardo and Plotkin 2007; Behera et al. 2010). These turtles have been observed swimming back and forth parallel to the beach, resting on the benthos (Cornelius and Robinson-Clark 1986; Plotkin et al. 1991) or floating with their heads against the waves (Behera et al. 2010), until a high number of them suddenly begin crawling onto the beach, and many others continue doing so for between 3 and 7 days (Bernardo and Plotkin 2007). A number of extrinsic factors have been proposed as proximate cues that trigger initiation of the *arribadas*, such as strong winds or specific moon phases, but none of these cues have proven to be consistent, even for a specific region (Bernardo and Plotkin 2007). Pritchard (1979) and Owens et al. (1982) speculated that chemical communication through Rathke's gland secretions might facilitate *arribada* synchronization. Plotkin found waxy plugs closing the Rathke's pores of olive ridley females captured close to Nancite beach weeks before an *arribada*, but the plugs were absent in females captured once the *arribada* had started (Bernardo and Plotkin 2007). These observations support some type of relationship between Rathke's gland secretions and *arribada* behavior, but more experimental evidence is needed.

There are only a few mass nesting olive ridley beaches in the world, but no specific factors have been discovered that differentiate these beaches from others where solitary nesting occurs (Pritchard 2007). Most well-known *arribada* beaches are located

in the central eastern Pacific: La Escobilla in Mexico, Nancite and Ostional in Costa Rica, Chacocente and La Flor in Nicaragua, and Isla Cañas in Panama (Bernardo and Plotkin 2007). The state of Orissa, in northeast India, contains three mass nesting beaches, Gahirmatha, Rushikulya and Devi River (Behera et al. 2010). Gahirmatha, La Escobilla and Ostional, with estimates of over 100,000 nests per year (Tripathy 2002) are currently the largest mass nesting rookeries in the world. However, the sizes of *arribadas* do not maintain temporal constancy (Pritchard 2007). According to historical records, large *arribadas* occurred in the past in some beaches in Nicaragua and Mexico, which no longer take place (Bernardo and Plotkin 2007). At Eilanti, Suriname, the only mass nesting beach in the western Atlantic, *arribadas* were historically known to be much larger but currently encompass only a few hundred nests per year (Hoeckert et al. 1996; Godfrey and Chevalier 2004). The decrease in size of an *arribada* may be due to human overexploitation in some cases (Limpus 1995), but may be a result of natural causes, as well. For instance, the Costa Rican beach at Nancite has been a nesting site free from human interference since the 1970s (Hope 2002), yet nevertheless has suffered a 90% decline in the number of nests from 1971 to 2007 (Fonseca et al. 2009). *Arribadas* are described as ephemeral reproductive aggregations by several authors (Bernardo and Plotkin 2007; Pritchard 2007), and thus such a reduction may be part of a normal cycle of development and disappearance.

Various selective advantages have been suggested for mass versus solitary nesting that could have driven the evolution of olive ridley *arribada* behavior (Bernardo and Plotkin 2007). The pelagic habits of olive ridleys make intraspecific encounters difficult, so the establishment of reproductive aggregations could help individuals find mates, as

well as increase the rate of multiple mating, which may be advantageous for both sexes (Yasui 1998; Zeh and Zeh 2001). All sea turtle species show high incidences of multiple paternity (Kichler et al. 1999; Moore and Ball 2002; Lee and Hays 2004; Bowen and Karl 2007; Theissinger et al. 2009; Joseph and Shaw 2011; Stewart and Dutton 2011; Duran et al. 2015) although discussions of actual benefits for females remain controversial (Lee and Hays 2004). Bernardo and Plotkin (2007) proposed that the increase in mate encounter rate enabled by *arribada* aggregations supposes a selective advantage for olive ridleys, and might have been involved in the evolution of *arribada* behavior. Recent data showing that multiple paternity is much higher in *arribada* beaches than in solitary beaches (Jensen et al. 2006) supports the idea of a positive correlation between reproductive aggregations and multiple mating, yet implications regarding hatchling fitness have not been ascertained.

Nevertheless, the foremost hypothesis regarding the evolution of *arribada* behavior in ridleys is the predator satiation strategy (Pritchard 1969; Eckrich and Owens 1995). Both eggs and recently emerged hatchlings are exposed to a high risk of predation on the beach. In a mass nesting setting, synchronous nesting and subsequent hatching of hundreds to thousands of nests provide such abundance of resources that terrestrial predators are quickly satiated, and a large proportion of hatchlings survive. An experimental study at Nancite, Costa Rica, seemed to confirm this hypothesis showing that nests from solitary nesters suffered significantly more predation than nests from *arribada* nesters (Eckrich and Owens 1995). However, the validity of these results has been questioned because both experimental nest groups were located in Nancite, an

arribada beach with much higher abundance of predators than typical solitary beaches (Bernardo and Plotkin 2007).

The increased mating opportunities and the predator swamping strategy are not mutually exclusive. In fact, Bernardo and Plotkin (2007) proposed that the *arribada* behavior originally arose as a way to increase mating opportunities, yet was later enhanced because of its additional benefits in reducing predation. Figure 2a depicts a summary of this suggested evolutionary pathway. Because of their pelagic wandering behavior, olive ridleys would otherwise have very few mating encounters and almost no opportunities for multiple mating and multiple paternity and thus, reproductive aggregations would provide the species with these allegedly advantageous reproductive features. Synchronous nesting would then add the advantage of predator swamping.

The fact that only a few populations of olive ridleys perform *arribadas*, however, suggests that the *arribada* strategy is not universally advantageous over solitary nesting. In fact, considering that solitary nesting is widespread, and that solitary nesters could outnumber *arribada* nesters worldwide (Cornelius and Robinson 1985), Bernardo and Plotkin (2007) concluded that two fitness peaks exist for nesting behavior in this species. The coexistence of both reproductive modes is possible because of the advantages and trade-offs inherent in each strategy. Although *arribada* aggregations are supposed to favor multiple mating and decrease nest predation risk, hatching success in *arribada* beaches is usually very low due to high density-dependent mortality (Clusella Trullas and Paladino 2007).

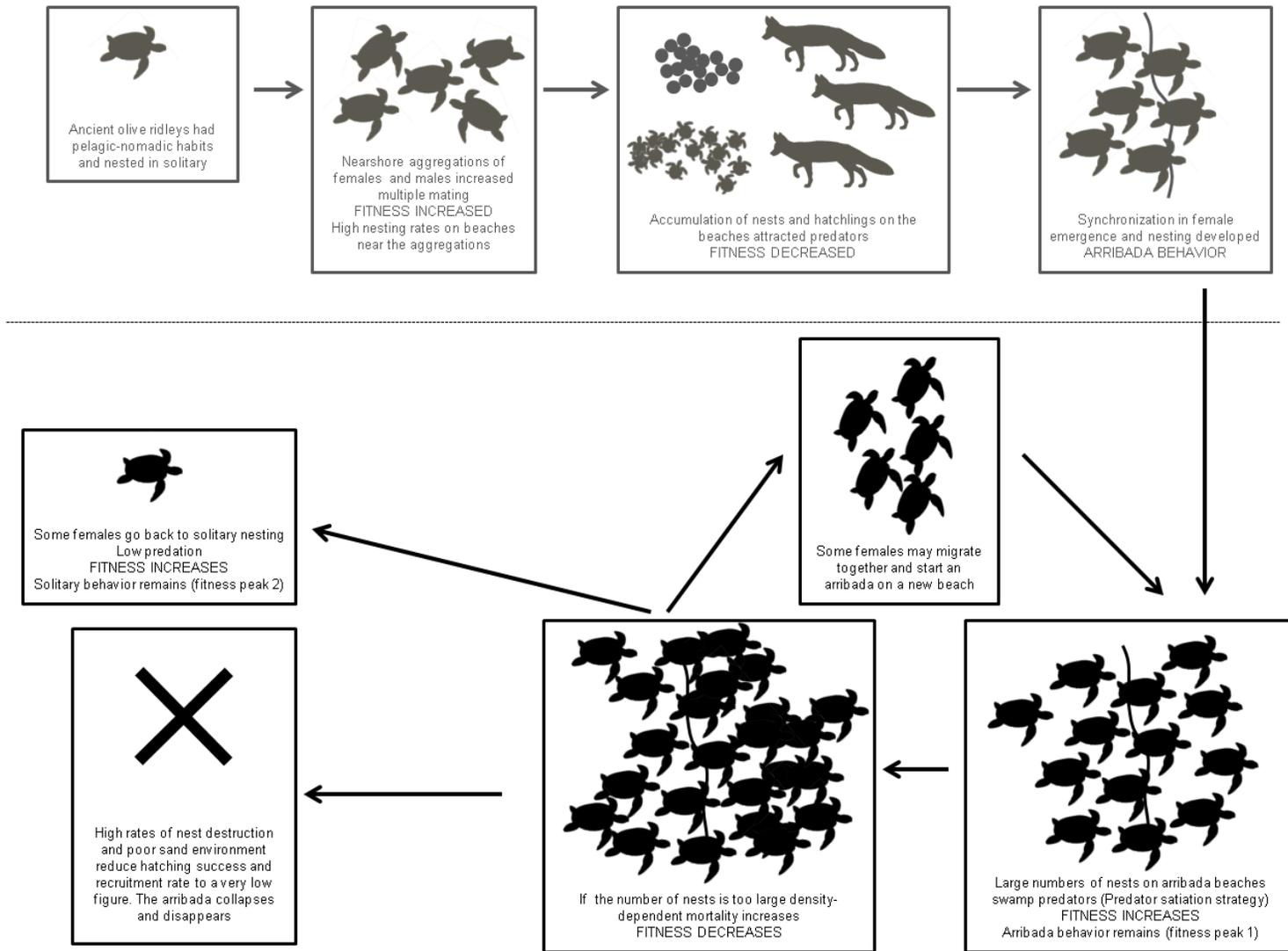


Figure 2. Evolution of the *arribada* behavior (top, grey) according to Bernardo and Plotkin (2007) and cycle of development, collapse and disappearance of an *arribada* beach (bottom, black) according to Pritchard (Pritchard 2007).

The large number of turtles on the beach causes high levels of nest destruction when they incidentally dig up nests laid by previous turtles (Cornelius et al. 1991), and the accumulation of organic matter increases microbial content in the sand, which consequently affects the normal development of the embryos (Cornelius et al. 1991; Valverde et al. 1998). A number of studies (Clusella Trullas and Paladino 2007; Honarvar 2007; Honarvar et al. 2008; Honarvar et al. 2011; Bézy et al. 2014) document a strong correlation between high-density nesting and high CO₂ levels, low O₂ levels, high bacterial and fungal content, increased incubation temperature, and low hatching success. *Arribadas* may be advantageous in their first stages because they increase hatching survival by overwhelming predators (Pritchard 2007), but after some time the quality of the sand environment becomes poor, leading to high embryo mortality, low hatchling production, low recruitment, and population decline. This scenario may explain the decrease in nesting females observed in Nancite during the last decades (Fonseca et al. 2009), as well as the discrepancy between historical and current locations of *arribada* beaches (Pritchard 2007). Table 2 provides data on historical records and current trends on olive ridley *arribada* beaches.

Table 2. Estimates of historical and current abundances, as well as trends for olive ridley populations on *arribada* beaches. Information from Hoeckert (1996), Evans and Vargas (1998), Pandav et al. (Pandav et al. 1998), Pandav (2000), Hope (2002), Shanker (2003), Pritchard (2007), Bernardo and Plotkin (2007), Cornelius et al. (2007), Honarvar et al. (2008), Ocana (2012), Plotkin et al. (2012) and Valverde (2012).

Country	Beach	Year of discovery	Historical data	Current data
Mexico	Mismaloya [†] / Tlacoyunque [†]		> 20,000 females (1970s) each Collapsed from fishing pressure	Depleted < 5,000 nests/year
Mexico	Chacahua		20,000-50,000 (1974) Collapsed from fishing pressure	Depleted, irregular trend 2,000-15,000 nests/year
Mexico	Morro Ayuta		No historical data	Stable or increasing 10,000-100,000 nests/year
Mexico	Ixtapilla*	1994	No significant nesting before 1994	> 150,000 nests/year (2008-2010)
Mexico	La Escobilla	Before 1950	180,000-400,000 females (1970s) Declined from fishing pressure 200,000 nests in 1991	Increasing until 2006 Now stable > 1,000,000 nests/year
Nicaragua	Boquita [†] / Masachapa [†] / Pochomil [†]		Former <i>arribada</i> rookeries; collapsed from egg harvesting pressure	Extinct as <i>arribada</i> rookeries
Nicaragua	Chacocente/ La La Flor		Legal harvesting projects since 1983 (Chacocente) and 1993 (La Flor) No estimates on effects	> 325,000 turtles/year (combined data for both rookeries 1993-1999) Increasing
Costa Rica	Nancite	1971	One of the largest <i>arribada</i> rookeries in the Eastern Pacific > 140,000 turtles/year (1970s) Collapsed from natural causes	Depleted but stable 2,000-12,000 turtles/ <i>arribada</i>

Costa Rica	Ostional	1971 1940s (local reports)	200,000-1,100,000 turtles/ <i>arribada</i> (1988-1997) Legal harvesting project since 1987	Decline from historical levels Currently increasing or stable High variability: 3,500-475,000 turtles/ <i>arribada</i> (2000-2006)
Panama	Isla Cañas		15,000-60,000 nests (1990s) Legal harvesting project	Decreasing 5,000-12,000 turtles/ <i>arribada</i>
Suriname	Galibi Nature Reserve [†] (Eilanti)	1960s	<i>Arribada</i> rookery > 3,000 in 1968 Collapsed from egg harvesting, fishing related mortality and natural erosion	Depleted A few hundred nests
India	Several beaches [†]	1708	Report by Hamilton “Prodigious number of sea tortoises resort to lay their eggs” between Cunnaca (Maipura) and Balasore (Budha Balanga)	Disappeared Precursor of Gahirmatha <i>arribada</i> ?
India	Gahirmatha	1974	Largest rookery in the world in the 1970s	1,000-100,000 turtles/ <i>arribada</i> Decreasing
India	Devi River	1981	Considerable reduction Not monitored until the 1990s	> 25,000 turtles in 1997
India	Rushikulya	1994	High fluctuation in numbers	10,000-200,000 turtles/ <i>arribada</i>

[†] Former *arribada* rookeries that are currently heavily depleted (< 5,000 nests) or where *arribadas* do not occur anymore

*New *arribada* rookery developed in the last two decades

Several behavioral differences exist between solitary and mass nesting females. Solitary nesters show low nest site fidelity (Kalb 1999), and may switch between different beaches separated by dozens to hundreds of kilometers within a single nesting season (Schulz 1971; Tripathy and Pandav 2008). Conversely, *arribada* nesters generally show high site fidelity and remain close to the nesting beach during the entire season (Pandav et al. 2000). *Arribada* nesters show larger relative clutch sizes (Plotkin and Bernardo 2003; Kumar et al. 2013) and higher multiple paternity levels (Jensen et al. 2006). Moreover, the internesting period is longer in *arribada* nesters (28 days) than in solitary nesters (14 days) (Pritchard 1969; Kalb 1999).

Some of these differences have been proposed to be adaptive. The larger clutch size in *arribada* nesters might be an additional adaptation for predator satiation, or a consequence of differential energy requirements between the two strategies. Given that solitary nesters travel during the internesting period while *arribada* nesters remain relatively inactive, the latter likely have more energy available to allocate for reproduction. As previously discussed, the high multiple paternity rates may be also a selective advantage of *arribada* behavior. Likewise, longer internesting periods would be advantageous at *arribada* beaches for several reasons. First, they may help nesting synchronization, by “waiting” for as many females as possible to complete egg development and be ready to nest at once (Hamann et al. 2002). Olive ridleys have, in fact, the capacity to modify the length of the internesting period, as demonstrated by a group of nesting females from Nancite which in 1991 delayed oviposition for 63 days in response to a period of heavy rainfall, and emerged synchronously after the rain ceased (Plotkin et al. 1997). Second, longer internesting periods may reduce the destructive

effects that new *arribadas* cause on previously deposited nests. Because the incubation period for olive ridleys takes an average of 55 days, nests from one *arribada* are in risk of being dug up only during the following one, whereas if the internesting period was 14 days each new *arribada* would affect nests from several previous nesting events. Third, internesting periods of 28 days may well reduce thermal interference between nests from successive *arribadas*. In high-density nesting sites the temperature increase that each nest experiences due to metabolic heating affects neighboring nests (Maulany et al. 2012; Duran and Dunbar *In prep*), and may cause low hatching success and decrease hatchling fitness if temperatures become excessively high. Metabolic heating is substantial mostly during the second half of the incubation (Broderick et al. 2001; DeGregorio and Williard 2011; Damazo 2014) and thus, the periods of high metabolic heating of two *arribadas* separated by 28 days should be less likely to overlap.

The differences between solitary and mass nesting olive ridleys have been considered large enough to classify them as distinct Management Units, even when they share the same general nesting area (Wallace et al. 2010). However, recent data suggests that the distinction between *arribada* and solitary nesters is not as sharp as it may first appear. Some females are known to use a mixed strategy, switching between mass nesting and solitary nesting, even during a single nesting season (Kalb 1999; Bernardo and Plotkin 2007). Solitary females from Australia (Hamel et al. 2008) and Brazil (Matos et al. 2012) showed internesting periods longer than expected, and the latter also showed high nesting site fidelity typical of *arribada* nesters. Plot et al. (2012) found reproductive synchrony and long internesting intervals (28 days) in a small non-*arribada* population in French Guiana. On the other hand, low site fidelity has been reported for *arribada*

females recorded nesting alternately between different rookeries (Cornelius and Robinson 1985; Pandav 2000; Tripathy and Pandav 2008). Given the temporary nature of *arribada* beaches, some of these intermediate behaviors may reflect residuals from previous *arribadas* or the initial steps in the formation of new *arribada* beaches. In any case, the behavioral and physiological flexibility of olive ridleys allows them to capitalize on the ephemeral advantages of mass nesting and to survive after its eventual collapse. The ability to move between beaches, to alter the length of the interesting period, and to switch between *arribada* and solitary behavioral modes are crucial adaptations that may assist in colonizing new areas when this happens (Tripathy and Pandav 2008; Duran et al. 2015). Figure 2b represents the reproductive polymorphism of olive ridleys suggested by Pritchard (2007), with the life cycle of *arribada* beaches and its connection with solitary nesting, including the two fitness peaks described by Bernardo and Plotkin (2007).

The *arribada* behavior has been the focus of many research projects on olive ridleys for several decades, but important questions remain unanswered. Why *arribadas* are only performed by *Lepidochelys* sea turtles is still a mystery, given that other species also show reproductive aggregations but have never evolved synchronous nesting. Experimental evidence is lacking on the actual benefits of *arribada* vs solitary nesting. *Arribada* nests are supposed to suffer less relative predation and yield fitter hatchlings than solitary nests, but these hypotheses have yet to be investigated. The specific role of Rathke's glands in nesting synchronization has not been accurately assessed, as well as other physiological mechanisms, such as the specific mechanisms olive ridleys use to delay ovoposition at will. Recent studies on nest environment and hatching success in *arribada* beaches provide information that may be used to generate useful mathematical

models of the life cycle of *arribada* beaches in ecological time, and genetic and satellite tracking studies may enlighten the relationship between neighboring solitary and *arribada* populations.

Human Interaction with Olive Ridleys: History of Use and Abuse

Sea turtles have been utilized by humans since prehistoric times as a source of food and other goods (Frazier 2003). The first records of olive ridley exploitation in the Eastern Pacific date back to 5,500 years before present, and it has been suggested that this ancient use affected numbers, densities, and geographic distributions with consequences that still persist (Frazier 2003; Spotila 2004). The reproductive behavior of olive ridleys makes this species highly vulnerable to human overexploitation (Cornelius and Robinson 1985; Plotkin et al. 2012). The dense aggregations of males and females close to the shore, as well as the gatherings of females by the thousands to lay eggs in small beach areas, are very conspicuous. Even solitary nesters are easy targets because they usually nest on sandy beaches and do not avoid human contact. From archeological artifacts and chronicles of European explorers we know that pre-Colombian cultures captured olive ridleys and widely used a number of sea turtle products, such as meat, shells, skins, and eggs (Plotkin et al. 2012).

In modern times, the history of olive ridley exploitation shows a parallel pattern in different parts of its range, beginning with sustainable subsistence levels that progressively turned into unsustainable large-scale, commercial and industrial harvesting, followed by population collapses and subsequent government regulations and protective

measures, which resulted in different outcomes depending on the particular circumstances of each region.

Before the second half of the twentieth century, most of the traditional harvest, both of adults and eggs, was kept at sustainable subsistence levels (Plotkin et al. 2012). However, in the 1940s and 1950s local subsistence use was progressively replaced by much larger commercial and industrial exploitations. The industrial fishery that flourished in Mexico in the 1960s replacing crocodile skins with turtle skins in the fabrication of luxury goods, killed at least 1.3 million turtles, most of them olive ridleys, in less than two decades (Plotkin et al. 2012). In Ecuador, almost 500,000 olive ridleys were captured and slaughtered for meat and skin from 1970 to 1981 (Plotkin et al. 2012). The total estimate for the Eastern Pacific fisheries was 2.5 million animals in three decades, not counting the black market (Plotkin et al. 2012). In eastern India, a large-scale commercial fishery captured more than 50,000 olive ridley sea turtles each year from the 1970s to the mid-1980s (Spotila 2004; Cornelius et al. 2007). These turtles were consumed locally or transported by road and train to the main cities in the interior of the country. In addition to local and national markets, India was one of the major suppliers of turtle products for Europe, Australia and other western countries (Tripathy and Choudbury 2007). In Suriname, indigenous people from coastal areas used sea turtle products beginning in the late 1600s. However, in the twentieth century, pressure on nesting populations increased steadily due to high national and international demand for meat and eggs (Campbell 2007; Cornelius et al. 2007). More than 1,500 females were taken yearly from 1933 to 1940, which encompassed between 15% and 50% of the total Suriname nesting population (Godfrey and Chevalier 2004).

From the 1930s to the 1970s, olive ridley egg harvesting reached its highest intensity on most beaches (both solitary and *arribada*) in the species' range. Harvesting rates over 90% were reported for Eilanti beach in Suriname, for many solitary beaches in the eastern Pacific, and for several Indian nesting sites (Campbell 2007; Cornelius et al. 2007; Plotkin et al. 2012).

As a result of the uncontrolled harvest of adults and eggs, most olive ridley populations experienced severe declines. All historical *arribada* rookeries in Mexico became severely depleted (Plotkin et al. 2012), and several solitary beaches lost their turtles (Spotila 2004). Two *arribada* beaches in Nicaragua disappeared due to egg overharvesting (Nietschmann 1975), and widespread declines have been reported all along the Pacific coast of Central America (Cornelius et al. 2007). In the western Atlantic, the Eilanti *arribada* beach in Suriname collapsed (Hoeckert et al. 1996), and the nesting population of Guyana was extirpated (Cornelius et al. 2007). Declines of solitary nesting olive ridleys were registered throughout the Indian Ocean in India, Bangladesh, Myanmar, Malaysia, Pakistan, and Sri Lanka (Cornelius et al. 2007). The *arribada* population nesting at Gahirmatha, India, also decreased, and *arribadas* failed to appear for three consecutive years (Shanker et al. 2003).

Growing awareness by local governments regarding the detrimental consequences of human overharvesting of the olive ridley nesting populations fostered the implementation of different types of protective measures. Mexico banned sea turtle egg harvesting beginning as early as 1927 (Campbell 2007). When adult olive ridley catches declined in the late 1960s, the government halted the fishery for two years and put the industry under the control of a private firm to encourage a more sustainable use of the

resource (Campbell 2007). Protection of nesting beaches also began during this period. Although these measures likely postponed the final collapse (Cliffon et al. 1995), population numbers continued decreasing, eventually leading the Mexican government to permanently close the fishery in 1990 and decree a total ban on harvesting of all species and subspecies of sea turtles (Peñaflores et al. 2000).

To protect their nesting beaches, several East Pacific countries established protected coastal areas. Nicaragua created the Chacocente-Rio Escalante Wildlife Refuge in 1983 (Stewart 2001), and Playa La Flor was declared a wildlife refuge in 1996 (Hope 2002). Isla Cañas, in Panama, was made a part of the Panama National Wildlife system (Plotkin et al. 2012). One measure common to some countries, such as Nicaragua and Honduras, was the establishment of a yearly non-harvesting period or *veda* (the Spanish word for “closure”), coincident with the peak of the nesting season (Minarik 1985; Campbell 2007).

In Costa Rica, the harvest of sea turtles and their eggs has been prohibited since 1966 under the Wildlife Conservation Law 4551 (Campbell 2007), and similar measures have been implemented worldwide. Suriname protected some of their nesting beaches beginning in 1954, when the Game Ordinance and the Nature Preservation Ordinance came into force. In 1969 Eilanti beach was declared a natural reserve and a complete ban on egg harvesting began to be enforced in 1970 (Campbell 2007). The Indian Wildlife (Protection) Act of 1972 stopped the national trade of olive ridley products in India since 1977, when all species of sea turtles were included in the Schedule I of this law (Tripathy and Choudbury 2007). In addition, three Indian National Parks and Sanctuaries, Bhitarkanika Wildlife Sanctuary, Bhitarkanika National Park, and the Gahirmatha Marine

Wildlife Sanctuary, included sea turtle conservation goals. In the state of Orissa specific conservation measures were implemented, such as effective beach protection at Gahirmatha and the declaration of offshore coastal waters as a ‘No Fishing Zone’ (Patnaik and Kar 2000).

In addition to developing domestic laws geared toward the protection of olive ridleys and other sea turtle species in their territories, most countries became signatories of international agreements, such as the Convention on International Trade in Endangered Species (CITES) and the Convention of Migratory Species of Wild Animals (CMS), both of which grant sea turtles the highest levels of protection (Richardson et al. 2006).

This wide array of protective measures instituted over the last four decades has resulted in a number of different outcomes. For the species as a whole, the population appears to be increasing. In fact, the status of the olive ridley sea turtle on the IUCN Red List was reviewed in 2007, and changed from Endangered to Vulnerable because of these increasing numbers (Abreu-Grobois and Plotkin 2008). The estimate for the current world population of olive ridleys is about 2 million nesting females and more than 4 million individuals including males and juveniles (Spotila 2004). For an estimate of the Eastern Pacific population, Eguchi et al. (2007) used aerial surveys and calculated approximately 1.4 million animals. Despite these seemingly large figures, current abundance is well below historical levels, which estimate 500 million turtles in pre-Colombian times, and about 10 million for Mexico alone prior to 1950 (Spotila 2004).

When considering individual populations, some are clearly increasing. There are even reports of a new *arribada* rookery developing on a Mexican beach with no historical record of hosting olive ridley nesting (Plotkin et al. 2012). One encouraging example of

population increase due to successful protective measures is the rookery at La Escobilla, Mexico. The number of nests at this nesting site rebounded from 55,000 nests in 1988 to more than a million in 2000, and the annual frequency of *arribadas* has doubled since the fishery was closed (Plotkin et al. 2012). The number of nests in Brazil has shown a significant upward trend during the last two decades (Godfrey and Chevalier 2004), allegedly due to the conservation efforts of the NGO Projeto TAMAR-IBAMA (the Brazilian Sea Turtle Conservation Program) (Da Silva et al. 2007). Increasing trends have also been reported for the two Nicaraguan *arribada* beaches, Chacocente and La Flor, (Plotkin et al. 2012) and for the French Guiana population (Kelle et al. 2009). Three causes have been suggested for the increase in the latter case: long-term conservation efforts, movement of females from the neighboring Suriname population, and past underestimates due to poor beach monitoring (Kelle et al. 2009).

Despite all these optimistic data, olive ridleys are not increasing in all areas of their range. In some sites where protective measures have been in place for decades, populations have not recovered or are still decreasing. Except La Escobilla, all other former Mexican *arribada* beaches remain depleted (Márquez et al. 1998; Plotkin et al. 2012), and the populations at many solitary beaches in the country persist at low levels (Abreu-Grobois and Plotkin 2008). Likely reasons for this situation are the current unsustainable levels of active turtle hunting and egg poaching inflicted upon most olive ridley populations despite the ban and the supposed protection of nesting beaches (Castellanos Michel et al. 2003; Garcia et al. 2003; Koch et al. 2006). The situation in coastal West Africa is even more severe. The lack of basic data on historical nesting makes it difficult to assess the actual impact of human harvest of olive ridleys in this

area, but egg harvest and slaughter of nesting females are widespread, and the decline of most populations is evident (Cornelius et al. 2007). In India and throughout the Indian Ocean, most olive ridley populations are also declining. Although egg poaching (Bhupathy and Karunakaran 2003; Tripathy and Choudbury 2007) and some adult exploitation for meat consumption (Mohanraj 2011) still exist, the main cause for the ongoing decline in Indian waters is the high rate of by-catch in mechanized coastal trawler fisheries (Shanker et al. 2005).

Beach Conservation Strategies and Management Techniques

During the last 50 years, olive ridley nesting populations have been subjected to a large variety of management policies, from indiscriminate use of adults and eggs to strict protection. This section provides a review of past and present conservation strategies and management techniques applied to olive ridleys in both *arribada* and solitary beaches. The relative success of each approach is briefly assessed, as well as the advantages and shortcomings related to their implementation. Note that these strategies and techniques are not all mutually exclusive, and thus more than one have been, or might be, applied simultaneously to a specific population.

Adult Exploitation

The harvesting of adults from nesting beaches and offshore reproductive aggregations has been justified as a valid management strategy within the sustainable use approach to conservation (Pritchard 2007). Theoretically, it is possible to take a number of individuals from a stable or increasing natural population without causing ecological damage if the harvest is low enough to ensure long-term sustainability (Campbell 2002).

However, taking into account that sea turtles are long-lived, slow maturing animals that undertake long migrations and suffer several threats throughout their life cycle, it is difficult to assess the exact number of animals that may be safely extracted (Mace and Reynolds 2001; Campbell 2002). To our knowledge, no modeling has been applied to olive ridley populations to evaluate the possible consequences of adult exploitation on population dynamics, yet such models on other sea turtle species have shown that increasing adult mortality, even in relatively low amounts, could severely threaten the future of some populations (Crouse et al. 1987; Heppell 1998; Chaloupka 2002). Historically, exploitations based on adult captures, such as the industrial fishery developed in Mexico in the 1960s and 1970s, had severe consequences for olive ridley populations, causing heavy declines and depletion of *arribada* beaches (Alava et al. 2007). The genetic bottlenecks caused by this depletion modified the allelic frequencies and altered the genetic pools of several olive ridley populations in the region (Rodríguez-Zárate et al. 2013). Even if it were possible to calculate a sustainable harvest rate and have the means to enforce it, a general consensus against the exploitation of adult turtles exists among sea turtle experts (Campbell 2002), and most domestic regulations (Patnaik and Kar 2000; Campbell 2007) and international agreements regarding sea turtle conservation (Richardson et al. 2006) specifically forbid this practice. Currently, the harvest of adult turtles is excluded from most olive ridley conservation programs, with a yearly estimate of less than 300 animals total which are legally hunted in countries that still allow direct take of sea turtles (Humber et al. 2014). Unfortunately, the use of sea turtle meat is deeply rooted in some traditional cultures, and the illegal take of olive

ridleys for human consumption continues at high rates in several parts of their range (Koch et al. 2006; Cornelius et al. 2007; Mohanraj 2011).

Controlled Egg Harvesting in Arribada Beaches

To extract a portion of the eggs from *arribada* beaches and use them for human consumption has been justified from a conservation standpoint due to the high embryonic mortality rates typical for these beaches (Cornelius et al. 1991). The best known example of implementation of this strategy is the controlled egg harvesting program in Ostional, Costa Rica (Campbell 1998). Local residents are allowed to harvest eggs during the first 36 hours of each *arribada*, as the majority of those eggs would be destroyed by the digging of successive nesting turtles. The harvesting process and the commercialization of eggs are undertaken by a community association, which also organizes activities to enhance sea turtle conservation, such as beach cleanups, hatchling liberations, and beach patrolling to discourage illegal egg harvesting. The economic profits of the project cover government taxes, operating expenses, wages for 200 associates, and funding for community projects. The program remains controversial among the sea turtle community because it encourages consumptive use (Campbell 2007) and because some doubts exist about its long-term sustainability (Valverde et al. 2012). However, the sea turtle population at Ostional appears to be stable or increasing (Chaves et al. 2005), and hatching success is higher in harvested areas than in non-disturbed areas (Mehta et al. 2000).

Other egg harvesting strategies have been tried in Nicaraguan *arribada* beaches (Campbell 2007) with lower levels of success than the Ostional program. In a comparative study, Hope (2002) concluded that the high level of community participation

and the structured system of commercialization in Ostional encouraged conservation, whereas the lack of organization and infrastructure in Nicaragua promoted overexploitation.

Traditional Egg Harvesting and Protected Periods (Vedas)

International agreements, such as the Convention on the Conservation of Migratory Species of Wild Animals (CMS) and the Inter-American Convention for the Protection and Conservation of Sea Turtles (IAC), pursue complete protection and total banning of the use of sea turtles, eggs, and any other turtle products (Hykle 1999; Richardson et al. 2006). However, the text of such agreements provides exceptions to satisfy the economic needs of traditional subsistence users (Richardson et al. 2006). The signatory countries have the responsibility to incorporate the requirements of the agreements into their domestic legislation, but terms such as “subsistence needs” or “traditional communities” are not clearly defined and allow for a wide array of interpretations. In Malaysia, for instance, a percentage of eggs is transferred to hatcheries, while the rest are legally marketed by licensees (Shanker and Pilcher 2003).

To satisfy the needs of coastal communities, several Central American countries allow variable levels of egg harvesting. A common conservation measure is to ban egg harvesting during specific periods of the year called *vedas* (Cornelius et al. 2007). The length of these periods, the level of enforcement, and the specific ways the protection is implemented vary among countries. The protected period in Nicaragua extends from July 1st to January 31st. The nests remain *in situ*, and the levels of illegal harvesting are high (Hope 2002). In Honduras, the *veda* period only encompasses the first 25 days of September, coincident with the peak of the nesting season. The main nesting beaches are

protected by the military, and local communities participate in collecting the eggs and relocating them to hatcheries (Dunbar and Salinas 2008; Dunbar et al. 2010).

The effectiveness of these types of conservation measures has been questioned due to the low percentage of eggs effectively protected in most cases (12% in Honduras, 10% in Guatemala, less than 1% in El Salvador) (Spotila 2004).

Strict Protection

Only a few olive ridley nesting beaches are completely protected from human disturbance. One of these is Playa Nancite, a remote 1-km beach included in the Guanacaste Conservation Area (Santa Rosa National Park) in Costa Rica. Despite total protection from harvesting since the 1970s, the number of nests at Playa Nancite has shown a steady decline for more than 20 years (Valverde et al. 1998; Fonseca et al. 2009). The alleged reason for this decline is a very low hatching success caused by a poor nesting environment (Fonseca et al. 2009), although fishery related mortality may also have contributed (Spotila 2004; Pritchard 2007).

Hatcheries

The option that provides the least amount of disturbance to natural nesting processes is always recommended when managing nesting beaches, and thus to keep nests *in situ* is preferable to relocating them (Mortimer 1999). However, transferring the eggs to a hatchery is a common practice when nests on the beach face high risks of being destroyed by natural or anthropogenic causes (Mortimer 1999). The use of hatcheries on olive ridley solitary nesting beaches has been reported throughout their range (Silas and Rajagopalan 1984; Garcia et al. 2003; Spotila 2004; Tisdell and Wilson 2005; Cornelius

et al. 2007; Dunbar et al. 2010; Maulany et al. 2012), but its contribution to population maintenance or recovery varies widely (Cornelius et al. 2007). Hatchery management is often deficient and tends to cause a decrease in hatching success respective to normal values for natural nests (Eckert and Eckert 1990; Garcia et al. 2003; Pintus et al. 2009; Duran and Dunbar *In prep*). In addition, relocation of eggs to hatcheries is known to alter incubation temperatures (DeGregorio and Williard 2011; Sieg et al. 2011; Duran and Dunbar *In prep*), potentially affecting both the sex ratio of the hatchlings as well as the length of the incubation period. Conversely, if both the translocation process and the hatchery environment are carefully monitored, it is possible to closely reproduce natural conditions, minimizing negative effects and achieving high hatching success rates (Garcia et al. 2003).

Headstarting

Headstart programs for sea turtles consist of keeping hatchlings in captivity for variable periods of time, ranging from a few days to several months, and releasing them afterwards (Pritchard et al. 1983). The rationale for these programs is to spare the neonates from the large array of threats that jeopardize them during the hatchling stage and thus increase their chance of survival (Bowen et al. 1994). Headstarting as a practice is limited to those species lacking or providing minimal parental care (Escobar et al. 2010). The most famous sea turtle headstart program took place from 1978 to 1992 on Padre Island, Texas, as part of an ambitious project to save the highly decimated Kemp's ridley sea turtle population from extinction (Bowen et al. 1994; Shaver and Wibbels 2007). The value, success level, and consequences of this program remain controversial

(Allen 1990; Woody 1990; Woody 1991; Allen 1992; Shaver 1996; Shaver and Wibbels 2007; Shaver and Rubio 2008). Headstarting is not a widespread practice in olive ridley management, but it has been reported from hatcheries in Sri Lanka (Tisdell and Wilson 2005), Peru (Kelez et al. 2009), southern Thailand (Chantrapornsyl 1992) and Honduras (Dunbar 2011). These types of programs are usually initiated by well-intended persons, yet with little knowledge of sea turtle biology. Studies on headstarted green and hawksbill turtles suggest that headstarting may alter the natural behaviors and movements of the animals (Okuyama et al. 2006; Okuyama et al. 2010). These data, along with the lack of evidence on actual success (Perran Ross 1999), suggest headstarting is not recommended.

Community Based Conservation

Community Based Conservation (CBC) refers to conservation initiatives designed to simultaneously promote nature preservation and human development by actively involving local communities during all stages of the project (Kellert et al. 2000; Campbell and Vainio-Mattila 2003). Although the concept of CBC appears widely in current sea turtle literature, it has not always been properly used. CBC often refers to projects with differing levels of participation by local people, but true CBC requires that local communities be involved in the entire decision-making process and aims to eventually turn management rights of the traditional natural resources over to the communities. Campbell (2002) found that most sea turtle experts were reticent to assign rights for resources to the local people and opposed allowing them control over resources and their management. This attitude is reflected in many sea turtle conservation projects that only incorporate local people for specific tasks whereas the design, implementation,

and management of the project rely on NGOs or governmental agencies (De Vasconcellos Pegas 2009). One example of a true CBC project with high participation of the community and successful outcomes both for sea turtle conservation and for human development is the previously described egg harvesting program at Ostional, Costa Rica, (Campbell 1998). Another conservation initiative focused on olive ridleys that meets the expectations of CBC is The Sea Turtle Conservation Program (PROTUMAR) in Oaxaca, Mexico (Barragan 2012). This program is operated by the Network for Oaxacan Coastal Wetlands, a regional organization created and managed by community groups interested in nature conservation. PROTUMAR involves approximately 50 people from 5 communities located on nesting beaches, which run turtle camps and carry out beach patrolling, egg relocation to hatcheries, hatchery management, and hatchling releases. From 2005 to 2010 this program protected almost 4,000 olive ridley nests and released more than 190,000 hatchlings, whereas before the program started almost 100% of the nests were poached (Vannini et al. 2011). PROTUMAR also organizes ecotourism activities, but the financial benefits do not suffice to cover the living costs of the families involved in the project (Vannini et al. 2011; Barragan 2012).

Non-Consumptive Use (Ecotourism)

Several sea turtle conservation projects aim to obtain economic profit for local communities by using the resource in a non-consumptive way, i.e. one which does not involve the extraction of the animals or their eggs from the ecosystem. The preferred option is turning these coastal communities into ecotourism destinations. Some examples of olive ridley conservation projects based on ecotourism and other non-consumptive

uses are The Sea Turtle Conservation Program (PROTUMAR) in Oaxaca, Mexico (Barragan 2012), discussed in the section *Community Based Conservation*, and the TAMAR-IBAMA project (the Brazilian Sea Turtle Conservation Program) in Brazil (De Vasconcellos Pegas 2009). The TAMAR-IBAMA project is a cooperative effort between the Brazilian Government and a non-profit organization, which promotes ecotourism and other alternative sources of income for the coastal communities formerly dependent on sea turtle products. In 2007 TAMAR was providing jobs for more than 1,000 people, 85% of them coastal community residents.

It has been suggested that ecotourism yields higher economic benefits than direct resource exploitation (Campbell 2002; Tisdell and Wilson 2005), but that it may not be a viable alternative for specific sites (Denman 2001). Additionally, the development of ecotourism projects has some environmental drawbacks that must be taken into account (Campbell 2007; Meletis and Campbell 2007), as they may require compromises in management practices that are potentially harmful for the protected species. For example, tourism-based olive ridley hatcheries in Sri Lanka hold hatchlings in small artificial sea water ponds for several days in order to show them to the tourists (Tisdell and Wilson 2005). This is highly detrimental because hatchlings exhaust the energy reserves needed for offshore migration (Mortimer 1999).

Which of these strategies should prevail in olive ridley management policies is a current topic of debate among the sea turtle community. Especially controversial is the issue of sustainable consumptive use, since most sea turtle experts do not view consumptive use favorably (Campbell 2002; Spotila 2004). Although egg harvesting is better tolerated than the taking of adults, the majority of sea turtle biologists would prefer

to eliminate even successfully controlled egg harvesting programs and instead promote non-consumptive uses, such as ecotourism (Campbell 2002). Uncertainty regarding the actual impact of harvesting programs (Valverde 1999; Valverde et al. 2012) is a common argument used against them, but according to Campbell (2007) this attitude could be based more on philosophical preconceptions than on actual conservation-related facts. Nevertheless, some scholars within the sea turtle community are open to consumptive use as a viable conservation strategy for specific cases (Campbell 1998; Campbell et al. 2007; Godfrey et al. 2007; Mrosovsky 2010). Due to its worldwide abundance and its *arribada* behavior, the olive ridley sea turtle is probably the best candidate for such use, although the actual viability of the strategy would require a careful assessment on a case-by-case basis (Godfrey et al. 2007). Mrosovsky (2010) suggested that an enhanced management of *arribada* beaches designed to increase hatching success would provide an excess of eggs useful for both serving the needs of the local communities and strengthening the sea turtle population.

Another issue of concern regarding current conservation strategies is the extremely high rate of illegal harvesting, both of eggs and adults, that takes place in most of the olive ridley range despite extensive nesting beach protection efforts and domestic and international regulations (Seminoff et al. 2012). This illegal harvesting is likely hampering the recovery of several nesting beaches (Koch et al. 2006). Several factors, such as the high economic revenues yielded by the sale of sea turtle products, family and community tradition, lack of viable economic alternatives, low risk of punishment, and government corruption, have been identified as key motivators for people to engage in such illegal behavior (Mancini and Koch 2009; Mancini et al. 2011; Tanner 2013). An

additional factor reported by Senko et al. (2011) was the people's lack of belief that the turtles are actually endangered.

Conclusions

The olive ridley sea turtle is the most abundant and least endangered of all sea turtle species, with a current estimated population of more than 2,000,000 nesting females around the world. The generalist habits of olive ridleys, along with their high behavioral and physiological flexibility, have allowed this species to adapt to a variety of different habitats, to respond to changing environmental conditions, and to remain resilient after severe population declines. However, neither its abundance nor its high adaptability makes the olive ridley invulnerable to anthropogenic threats. In fact, in recent history its special mass reproductive behavior facilitated such overexploitation that several nesting populations have disappeared, and many others remain depleted today.

Because of its high abundance relative to other sea turtle species, and its conservation status recently downgraded to Vulnerable on the IUCN Red List, the olive ridley sea turtle is of low priority on the conservation agenda of many countries and conservation agencies. This translates into scarce funding both for research and conservation, and low political priority regarding effective enforcement of conservation laws and habitat protection measures (Plotkin 2007). As a result, current levels of illegal egg harvesting and adult hunting are extremely high throughout the range of this species, and neither the means for enforcement nor the political will to address the problem exists in most cases.

This situation does not bode well for olive ridleys. According to Bandura's Social Learning Theory (Bandura and McClelland 1977), when everyone breaks the law, illegal

behavior becomes socially acceptable. Moreover, local communities feel the legitimacy of engaging in illegal behavior when they perceive conservation regulations as unjust, externally imposed, or arbitrary (Mancini et al. 2011). Many olive ridley rookeries are located in areas of extreme poverty (Hope 2002; Mrosovsky 2010), where local people have historically relied on the use of sea turtle products as part of their traditional diet or as an important source of income (Hope 2002; Campbell 2007; Cornelius et al. 2007). Therefore, restriction laws usually generate strong rejection and lack of compliance (Mancini et al. 2011).

In light of the shortage of resources and the fact that intervention by government agencies is unlikely, any significant enhancement of olive ridley nesting beach conservation will depend on the involvement of those living in close proximity to the rookeries. The common patterns of severe human poverty and high levels of illegal harvesting present in many olive ridley nesting regions suggest that the only way to be successful in conservation is by providing some type of benefit to humans, as well. Although the high diversity of countries where olive ridleys nest makes it difficult to design a unique conservation protocol that could be used along their range, a general strategy based on CBC would be widely applicable. In order to be successful, the main goal of such a strategy should be simple: to improve the current situation for each nesting beach regarding both sea turtles and local people.

The ideal for most sea turtle conservationists is to eventually turn poachers into protectors and locations of egg exploitation into ecotourism destinations. However, this is not always feasible, and when possible, requires high financial investment and a long-term project. Likely, a more realistic strategy would be to focus efforts on optimizing

resources currently available, both financial and human, to obtain even slightly better conservation results in each nesting beach.

A detailed elaboration of a general strategy to improve olive ridley conservation in nesting beaches is provided in Figure 3. This strategy has been designed as a 5-stage process: assessment, analysis, planning, implementation, and evaluation. CBC defining features have been incorporated in every step of the process.

During the analysis stage, it is important that the specific objectives are not overly ambitious. Some examples of feasible objectives are to increase the number of local people involved in conservation, to reduce the levels of illegal harvesting, and to increase hatching success in hatcheries or beach protected areas.

Given the generalized consumption and commerce of olive ridley eggs still present in many countries, it is recommended that the planning and implementation stages contemplate some levels of consumptive use, if necessary. This approach, likely challenging for most sea turtle conservationists, has several advantages. Allowing sustainable levels of consumptive use shows respect for the traditions and culture of the local communities, which become a common source of conflict when ignored (Campbell 2007). Moreover, communities that view sea turtle eggs as long-term profitable resources under their responsibility will have an incentive to protect turtles and beaches, and to help enforce conservation regulations (Mancini et al. 2011).

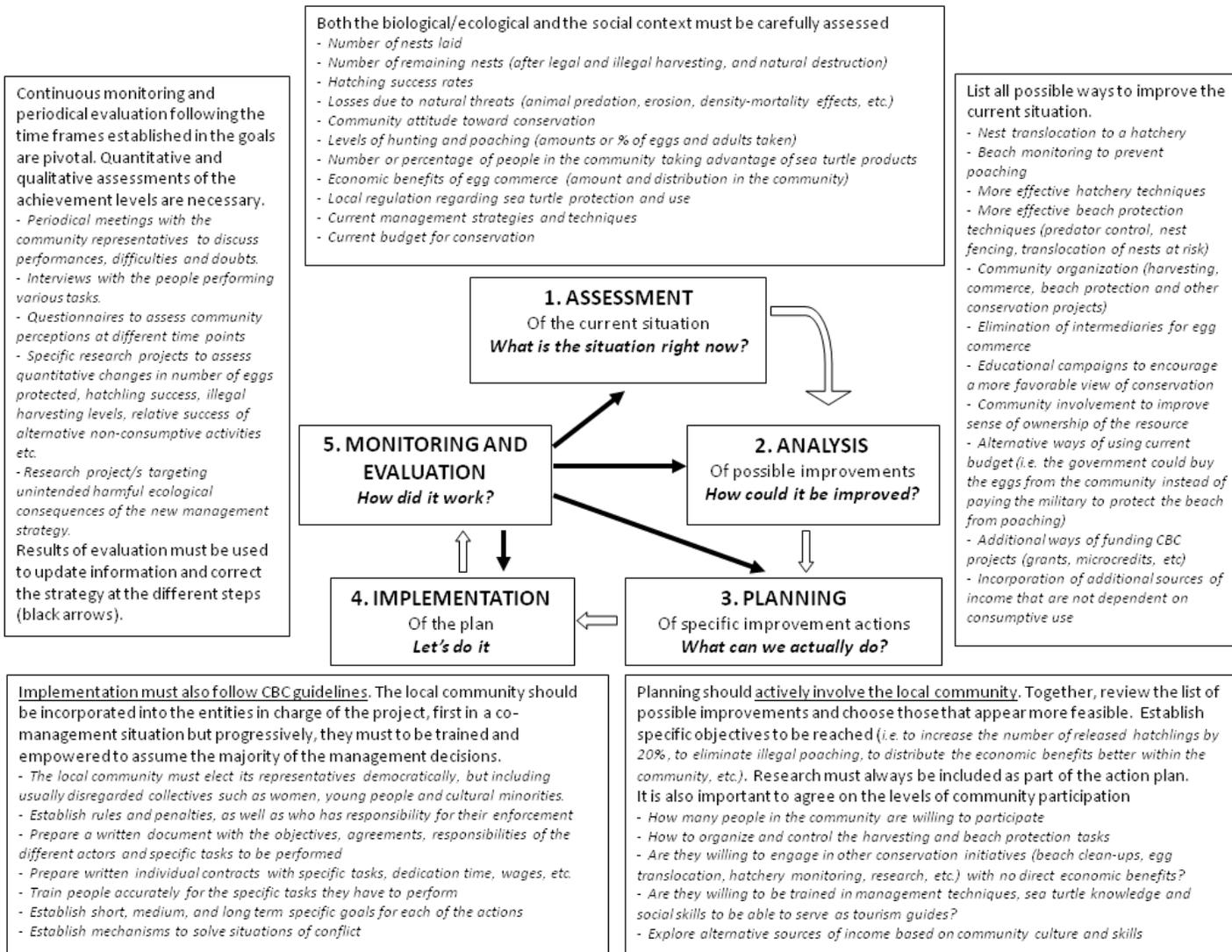


Figure 3. Management strategy for Olive Ridley rookeries, based on sustainable consumptive use and Community Based Conservation (CBC). The central part shows the general steps. The outer part elaborates on the steps and presents examples (*italics*) to apply in specific situations.

To prevent past failures, such as consumptive use programs resulting in overexploitation of the population, continuous monitoring of the project and periodic evaluation of biological and social outcomes are crucial (Valverde 1999). The careful application of enhanced beach management techniques is expected to yield increased hatching success and an excess of usable eggs (Mrosovsky 2010), but even if the number of released hatchlings remained stable, to engage local communities of former poachers in a coordinated conservation work within a legal framework would be a successful achievement in itself.

Those who see non-consumptive use as the ideal can apply this strategy as a temporary middle step that may aid in turning local community attitudes more favorably toward sea turtle conservation. Other actions, such as educational campaigns and the promotion of alternative non-consumptive conservation activities should be also carried out (Valverde 1999). If the development of a non-consumptive project based on ecotourism is the final goal, some tourism activities may be implemented simultaneously with the egg harvesting program. Although consumptive use and ecotourism are often presented as incompatible, several examples exist both outside and inside the sea turtle field supporting their effective combination (Meletis and Campbell 2007). Additional, more creative initiatives can also be sought in order to foster the transition towards non-consumptive uses. For example, Senko (2009) investigated what the effects on sea turtle meat consumption would be if physicians informed their patients of the health problems related with such consumption, and Nichols and Palmer (2006) requested that Pope Benedict XVI state that sea turtle meat is not fish in order to reduce its generalized consumption in some countries during the Christian season of Lent. The positive

influence that religious teachings can have on sea turtle conservation has been previously demonstrated. In the mid-1980s sea turtles were reclassified from halal (permitted food) to haram (prohibited food) in the Islamic teachings. After that, turtle harvest and consumption decreased significantly in some countries (Macrae and Whiting 2014).

This paper has reviewed the characteristics of olive ridleys that make them unique among the sea turtles, as well as how these characteristics have modeled the relationship between humans and olive ridleys through ancient and modern history. Diverse management techniques have been discussed and a general strategy suggested for improving conservation in nesting beaches. The question, however, remains: Are these efforts worthwhile? Why should we care for the most common and least endangered sea turtle species? There are several reasons why studying and conserving olive ridleys is important:

First, the olive ridley is one of the least studied species among sea turtles (Bjorndal 1999). Little is known about the oceanic part of its life cycle, and a number of questions about *arribada* behavior still remain unanswered (Bernardo and Plotkin 2007; Plotkin 2007; Plotkin 2007). Future studies on this species might yield valuable information on sea turtle evolution, physiology, and adaptability to environmental variation and climate change.

Second, due to its morphological and behavioral similarities with its congeneric species, the Kemp's ridley, research studies may be able to use olive ridleys as a proxy for this more endangered species, providing useful information for its protection and conservation.

Third, despite its abundance and high resilience, past human overexploitation has driven several olive ridley populations to the brink of extinction. Continuous research aimed at informing and improving conservation measures may prevent this from happening again.

Fourth, the presence and abundance of olive ridleys in tropical nesting beaches coincident with areas of high human malnourishment and poverty render this species and its eggs a valuable food resource for many human populations (Mrosovsky 2010), making it crucial to assess whether a sustainable long-term use of this resource is possible, and what type of use or uses would be acceptable.

Finally, the wide nesting range of the olive ridley and its high abundance on nesting beaches makes this species especially accessible to human contact. Olive ridleys may thus be used as a flagship species to promote the conservation of all sea turtle species and their habitats, as well as marine ecosystems in general (Frazier 2005).

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CHAPTER THREE

**HIGH FREQUENCY OF MULTIPLE PATERNITY IN A SOLITARY
POPULATION OF OLIVE RIDLEY SEA TURTLES IN HONDURAS**

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Abstract

Females of all seven living species of sea turtles are known to be polyandrous and show multiple paternity. The frequency of multiple paternity varies among species, and among populations of the same species. In the olive ridley sea turtle (*Lepidochelys olivacea*), multiple paternity levels correlate with the abundance of individuals in the mating system, being much higher in *arribada* (mass nesting) rookeries than in solitary nesting sites. We used two highly polymorphic microsatellite markers (Cm84 and Or1) to assess the level of multiple paternity in an olive ridley solitary population nesting in the Gulf of Fonseca, Honduras. We found evidence of multiple paternity in 6 out of 8 clutches (75 %), with a minimum number of two fathers in four clutches, and a minimum of three in the remaining two clutches. This high level of multiple paternity in a small solitary population suggests that some of the females nesting in Honduras may be coming from proximal Nicaraguan *arribada* nesting beaches. Historical evidences and recent satellite telemetry data support this hypothesis. In addition, we show that multiple paternity studies can be effectively performed in the absence of maternal samples, and that pooled DNA samples can be used with results comparable to individual hatchling sampling in multiple paternity analyses.

Keywords: microsatellites; *Lepidochelys olivacea*; maternal samples; pooled samples; nest-site fidelity; inter-beach movement

Introduction

To make effective management decisions and improve current conservation projects on nesting beaches, it is important to accurately estimate population size, population structure, and reproductive behavior. In populations where polyandry occurs, multiple paternity influences the effective population size (Sugg and Chesser 1994) and the genetic variability within a population (Baer and Schmid-Hempel 1999). Multiple paternity studies yield valuable information regarding mating patterns, and help in understanding population structure (Jensen et al. 2006). Recent studies have shown evidence of multiple paternity in all sea turtle species: green (*Chelonia mydas*) (FitzSimmons 1998; Lee and Hays 2004), loggerhead (*Caretta caretta*) (Moore and Ball 2002; Zbinden et al. 2007), leatherback (*Dermochelys coriacea*) (Crim et al. 2002; Stewart and Dutton 2011), hawksbill (*Eretmochelys imbricata*) (Joseph and Shaw 2011), flatback (*Natator depressus*) (Theissinger et al. 2009), olive ridley (*Lepidochelys olivacea*) (Hoeckert et al. 1996; Jensen et al. 2006) and Kemp's ridley (*Lepidochelys kempi*) (Kichler et al. 1999), with high inter- and intraspecific variability (Uller and Olsson 2008). In the case of olive ridleys, Jensen et al. (2006) showed that multiple paternity strongly depends on reproductive patterns, with *arribada* nesters showing much higher rates than solitary nesters. They suggested that the frequency of multiple paternity depends primarily on the abundance of individuals in the mating system, and calculated the relationship between population size and multiple paternity levels for the genus *Lepidochelys*.

Because of their abundance, high polymorphism content, codominance, easy detection, and transferability among studies, microsatellites are ideal molecular markers for paternity studies (Aggarwal et al. 2004). For assessing multiple paternity in sea turtle

clutches, it is not unusual to analyze both the mother and the offspring. Once the maternal alleles for each microsatellite marker have been identified in the hatchlings, the remaining alleles constitute the paternal contribution. Multiple paternity can be inferred in egg clutches laid by a single mother from the presence of more than two paternal alleles, with three alleles meaning at least two fathers, and five alleles meaning at least three fathers. The analysis of individual offspring genotypes allows estimating the paternal contribution of each father, and in some cases, identifying actual multiple paternity with less than five total alleles, when the observed distribution of alleles is not compatible with only one father (Jensen et al. 2006).

Although determination of multiple paternity in single clutches based on knowledge of the maternal and individual offspring genotypes is the ideal and most informative procedure for multiple paternity studies, this protocol may sometimes be impractical or impossible. In some cases, samples from mothers may be unavailable, such as in conservation projects where beaches are monitored and nests collected during morning patrols (Zbinden et al. 2007; de Santos Loureiro 2008; Godgenger et al. 2009). In other cases, the high number of samples required in typical multiple paternity studies (Hoeckert et al. 1996; Hoekert et al. 2002; Jensen et al. 2006; Stewart and Dutton 2011), become cost prohibitive. Taking into account that it is the total number of different alleles in each clutch that establishes both presence and frequency of multiple paternity in a population, maternal samples are not strictly necessary for detecting multiple paternity. In the absence of female samples, the presence of five or more alleles in a single nest evidences at least two contributing males, and thus, multiple paternity. Pearse et al. (2002), Theissinger et al. (2009), and Valenzuela (2000) have previously applied this

criterion in turtle multiple paternity studies that lacked maternal samples for a number of clutches. The specific genotypes of individual offspring are not essential information either. Given that multiple paternity can be correctly assessed knowing the total number of alleles in a clutch, it may be advantageous in terms of time and budget to obtain this information via pooled samples from hatchlings in a clutch, rather than by individually analyzing each hatchling sample. The use of pooled samples for microsatellite analysis has been successful in human forensic and epidemiological studies with high numbers of individuals (Pacek et al. 1993; Sham et al. 2002). However, due to the frequent presence of PCR artifacts, the electrophoretic patterns of pooled samples tend to be complex and difficult to interpret (Schnack et al. 2004). Preliminary DNA pooling is recommended for paternity studies, to reduce the number of tests required to identify potential parents for an individual progeny (Curnow and Morris 1998), but is rarely used in multiple paternity studies. Gosselin et al. (2005) pooled eggs from individual pleopods in a multiple paternity study on the American lobster, *Homarus americanus*, following Urbani et al. (1998), who previously used this method with the snow crab, *Chionocetes opilio*. To our knowledge, the use of pooled DNA samples has not been reported to date in multiple paternity studies on sea turtles or any other vertebrates.

Olive ridley sea turtle eggs have been economically exploited in the South coast of Honduras since the 1940s (Campbell 2007). Significant population declines led to the implementation of conservation measures by the Honduran government in 1975, which established an ongoing yearly protected period when the collection of eggs is forbidden and the eggs are relocated to hatcheries (Minarik 1985). However, studies on the olive ridley population nesting in Honduras are scarce. The environmental NGO *Protective*

Turtle Ecology Center for Training, Outreach, and Research, Inc. (ProTECTOR) has been monitoring the nesting beaches since 2007 (Dunbar and Salinas 2008; Dunbar et al. 2010), yet until now, no genetic studies have been performed.

Olive ridley nesting beaches in Honduras are located within the Gulf of Fonseca, a shallow-water inlet of the Pacific Ocean, 80 km long and 50 km wide, sheltered by islands at its entrance (Lemay et al. 2007) (Fig. 4). Punta Ratón (13.26570N, 87.51228W) is the main nesting beach in the country, with an estimated number of 400 – 500 nests per season (Dunbar, personal communication). Three other nesting beaches are known along the east coast of the Gulf: El Venado (13.11581N, 087.42725W), which receives approximately 200 – 250 nests per season; along with smaller sites at Boca del Río Viejo and Cedeño, with approximately 80 – 140 nests each per season (Dunbar, personal communication). According to historical reports, 100 % of eggs from Punta Ratón were consumed for more than three decades (1940s-1970s) (Campbell 2007) before the establishment of protection measures. The fact that the turtle population nevertheless persisted caused Pritchard (2007) to speculate that females nesting at Punta Ratón may, in reality, come from *arribada* populations at the Chacocente and La Flor beaches in Nicaragua.

The main goals of our study were: 1) to assess the levels of multiple paternity in the olive ridley sea turtle population nesting in the South coast of Honduras, and 2) to use levels of multiple paternity to estimate population size and origin of the nesting females present in the Gulf of Fonseca. Besides specific information about the Honduran olive ridley population, this study also allowed us to investigate new methods to determine sea turtle multiple paternity, with a potentially wider application.

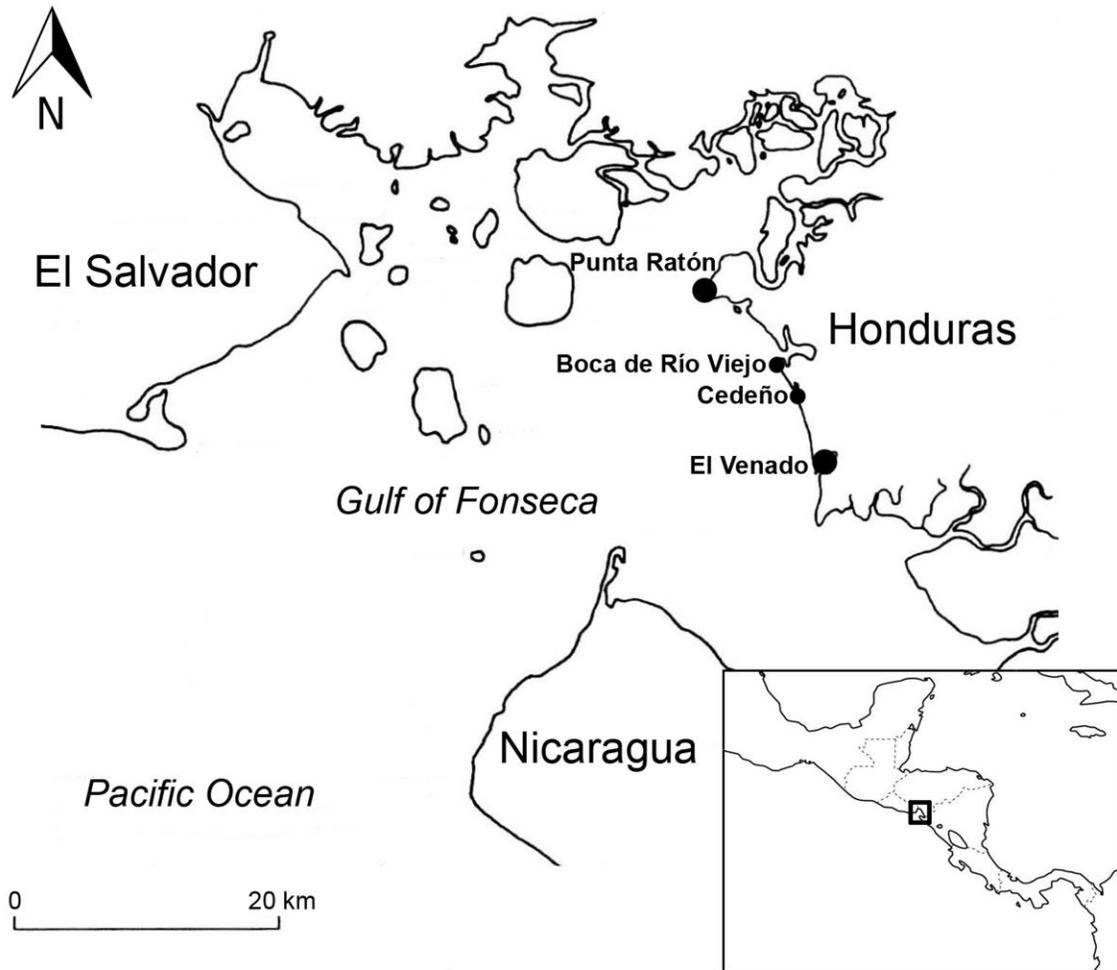


Figure 4. The Gulf of Fonseca. Circles indicate the four main nesting beaches for *L. olivacea* on the South coast of Honduras. Large circles indicate the two main *L. olivacea* nesting beaches at Punta Ratón and El Venado, the field sites for this study.

Two additional goals were 1) to confirm that multiple paternity studies can be effectively performed without female samples, and 2) to evaluate the potential of using one-per-clutch pooled samples to detect multiple paternity, rather than individually analyzing multiple hatchling samples.

Methods

Field Sampling

During the 2011 – 2013 nesting seasons (August to November), we collected blood and tissue samples from 26 nesting females at Punta Raton and El Venado (Fig. 4). Blood samples (1 – 2 ml) from the dorsal cervical sinus were collected from 15 females at Punta Raton and 5 females at El Venado. In 6 cases we collected tissue samples from females nesting at Punta Ratón by cutting a small piece of skin (<25 mm²) from the soft tissue of the posterior edge of the left rear flipper. We marked all sampled females with flipper tags on the left front flipper. Hatchlings from three nests of the 2012 season and six nests of the 2013 season were also sampled. Immediately after emergence, we randomly selected 20 hatchlings from each nest and took blood samples (<0.1 ml) from the dorsal cervical sinus. We monitored sampled hatchlings for one hour after the procedure to ensure normal behavior, and released them as soon as possible after observance. Blood and tissue samples were stored at room temperature in cell lysis buffer [10 mM Ethylenediaminetetraacetic acid (EDTA), 2% sodium dodecyl sulfate (SDS), 10mM Tris-base - 8.0pH]. Samples from 2011 and 2012 were kept at room temperature until June of 2013, when they were imported to the US and stored at 4 °C in the laboratory. Samples from the 2013 season were imported to the US and stored at 4 °C in

the laboratory in November, 2013. All samples were analyzed between July and December, 2013.

Microsatellite Analysis

Total genomic DNA was extracted from tissue samples following Prager and Stoneking (1999). For DNA extraction from blood samples, a modification of the same protocol was used (Table 3). DNA concentration was checked with a NanoDrop 2000c UV-Vis Spectrophotometer (Thermo Scientific, MA) and adjusted to 50 - 100 ng/μl. Pooled samples for each nest were prepared by mixing 1 μl of each of the hatchling samples together and adjusting the final concentration to 50 – 100 ng/μl. The microsatellite primers Cm84 and Or-1 (Table 4) were selected for the paternity analysis because they had shown high variability and effective amplification in previous studies with Eastern Pacific olive ridley populations (Jensen et al. 2006). Microsatellites were amplified with fluorescent-labeled primers in 50 μl PCR reactions containing 50 – 100 ng of nuclear DNA, 10 pmol forward 6FAM 5-end labeled primer, 10 pmol reverse unlabelled primer (Integrated DNA Technologies, IA), and 25 μl of Maxima Hot Start PCR Master Mix (Thermo Scientific, MA).

Thermal cycling was initiated with a 4 min denaturing step at 95 °C for both Cm84 and Or-1, followed by 35 cycles of 30 sec denaturation at 95 °C (Cm84 and Or-1), 30 sec annealing at 56 °C for Cm84 (55 °C for Or-1), and a 30 sec (1 min for Or-1) extension at 72 °C for both Cm84 and Or-1, and a final extension of 5 and 10 min at 72 °C for Cm84 and Or-1, respectively. PCR products were run on a 5 % polyacrylamide gel (Bio Rad, CA) at 60 W for 2 h to confirm DNA presence.

Table 3. Extraction protocol for reptile blood (modified from Prager and Stoneking, 1999)

1. Add 200 μ l of cell lysis buffer [10 mM EDTA, 2% SDS, 10mM Tris-base - 8.0pH] to 100 μ l of blood sample (already diluted with cell lysis buffer), along with 9 μ l of 20 mg/ml Proteinase K. Following thorough mixing, incubate the sample in a 55 °C water bath for 15-20 minutes.
2. Following incubation, remove the sample from the water bath and cool to room temperature. Then add 4 μ l of 10 mg/ml RNase A, mix, and place in a 37 °C water bath for 1 h.
3. Cool the sample to room temperature and add 100 μ l of 7.5 M ammonium acetate. Vortex mix the sample for 10 s and place on ice for 10-15 min.
4. Remove the sample from the ice and centrifuge in a microcentrifuge at top speed (ca. 13-14k rpm) for 5 min. Draw off as much of the supernatant as possible and transfer to a new microcentrifuge tube. Centrifuge the supernatant again at top speed for 5 min.
5. Transfer the supernatant from the second spin to a new 2 ml tube containing 900 μ l of isopropanol and invert gently about 20 times to mix and precipitate the DNA. Refrigerate at -20°C for 2 hours to overnight.
6. Centrifuge the sample at top speed for 5 min. to precipitate the DNA into a pellet at the bottom of the tube. After centrifugation, pour off the isopropanol and wash the pellet with 500 μ l of 70% ethanol. Centrifuge the sample again at top speed for 5 min. and air dry until all traces of ethanol have evaporated.
7. Suspend the DNA pellet in 30 μ l of ultrapurified water.

Table 4. Microsatellite markers. Primer sequence, annealing temperature, allele length, number of alleles (NA), number of individuals analyzed (n), overall expected (H_E), and observed (H_O) heterozygosity for the two markers used in the study.

Locus	Primer sequence (5'-3')	Annealing temperature (°C)	Allele length (bp)	NA (n)	H_E	H_O
Cm84	TGTTTTGACATTAGTCCAGGATTG ATTGTTATAGCCTATTGTTCAGGA	58	321- 348	14 (158)	0.89	0.83
Or-1	CCCCTTGTGTTCTGAAATCCTATGA CAGGCATAGGGAAAAATCAGAGGTA	55	148- 191	13 (158)	0.85	0.92

Following PCR using template DNA from individual hatchlings, a separate pooled sample of the PCR products for each nest was prepared by mixing 1 µl of each PCR product from the nest. In a second PCR reaction, the pooled DNA mentioned in the previous paragraph was used as the template. The PCR products were sent to an external laboratory (Genewiz, NJ), where fragment analysis was performed on an ABI3730 DNA Analyzer (Life Technologies, CA). PCR products from 17 - 20 hatchlings were analyzed from each of 9 nests totaling 158 individuals, plus two pooled samples for each nest, one pooled before PCR and one pooled after PCR. Maternal samples were only available for the six 2013 season nests, yet only three of them yielded useful PCR products. In these cases, products from adult females were run along with the products from their offspring. In addition, 26 samples from non-related adult females and 6 hatchlings from different nests were analyzed to assess population diversity. Results from the DNA Analyzer were visualized using Geneious 6.1.7 created by Biomatters.

Data Analysis

Population Analysis

PCR products from 32 individuals were analyzed to estimate the allele frequencies for Cm84 and Or1 in the population, yet only 27 genotypes were obtained, corresponding to 15 nesting females from Punta Raton, six nesting females from El Venado and six hatchlings randomly selected from nests with no maternal samples (one hatchling per nest). We assumed that all the sampled animals were unrelated. Data were checked for departure from Hardy-Weinberg equilibrium, genotypic linkage disequilibrium, and the presence of null alleles using GENEPOP 4.2.2 (Rousset 2008).

Paternity Analysis

For those nests with known maternal genotypes, paternal alleles were inferred from offspring genotypes once maternal alleles were accounted for, and confirmed using GERUD 2.0 (Jones 2005). For nests with no maternal samples, offspring genotypes were directly analyzed with GERUD 2.0 to determine all possible maternal genotypes and the corresponding paternal genotypes for each case. We also used GERUD 2.0 to calculate exclusion probabilities. To calculate the probability of detecting multiple paternity with unknown parents for the number of offspring sampled in the study we used the PrDM software (Neff and Pitcher 2002) and GERUDsim 2.0 (Jones 2005). GERUDsim 2.0 uses a simulation approach to determine the ability of GERUD 2.0 to correctly determine the number and genotype of sires for specific progeny.

We confirmed paternity results obtained with GERUD 2.0 using COLONY (Wang 2004). Because we sought to assess the minimum number of sires required to explain offspring genotypes (MIN estimates), we used the MIN method from Sefc and Koblmüller (2009). COLONY calculations include the possibility of two error classes: null alleles (Class I), and typing errors and mutations (Class II). We used error rates of 0.05 for both classes (Wang 2004).

GERUD 2.0 and COLONY estimates for multiple paternity were obtained from the analysis of individual samples. Results from pooled samples were visually analyzed using Geneious 6.1.7, and the sizes and total number of alleles present in each clutch were compared with those obtained from the individual samples of the same clutch. For samples that contained high levels of unresolved peaks, we used the program Poolfitter v1.1 (Schnack et al. 2004) to remove stutter noise and identify the true allelic peaks (Fig.

5). When interpreting Poolfitter outcomes, we removed peaks lower than 0.1 of the total frequency and consider the remaining peaks to be true alleles.

Results

Population Variability

Both loci were highly polymorphic, with 16 and 13 alleles found at microsatellites Cm84 and Or1, respectively. Although Cm84 is a dinucleotide repeat, several alleles for this locus differed by only 1 bp, a fact previously observed in other studies (Hoekert et al. 2002). Expected heterozygosities were 0.89 for Cm84 and 0.85 for Or1. Observed heterozygosities were slightly lower for Cm84 (0.83) and slightly higher for Or1 (0.92). No loci exhibited significant departure from Hardy–Weinberg equilibrium ($P > 0.05$), and no linkage disequilibrium was detected between loci ($P > 0.05$). The test for null alleles showed possible null alleles at very low frequency for one locus (Cm84; 0.0471), likely caused by scoring errors (Jensen et al. 2006).

Multiple Paternity

Individual Samples

Multiple paternity was inferred when the total number of alleles per locus was 5 or more, and confirmed by GERUD 2.0 outcomes. One case with less than 5 alleles at one locus was also identified as resulting from multiple paternity when the distribution of alleles across loci could not be attributed to only one father. We considered alleles present in just one locus and only one offspring likely resulting from mutation events or scoring errors (Jensen et al. 2006).

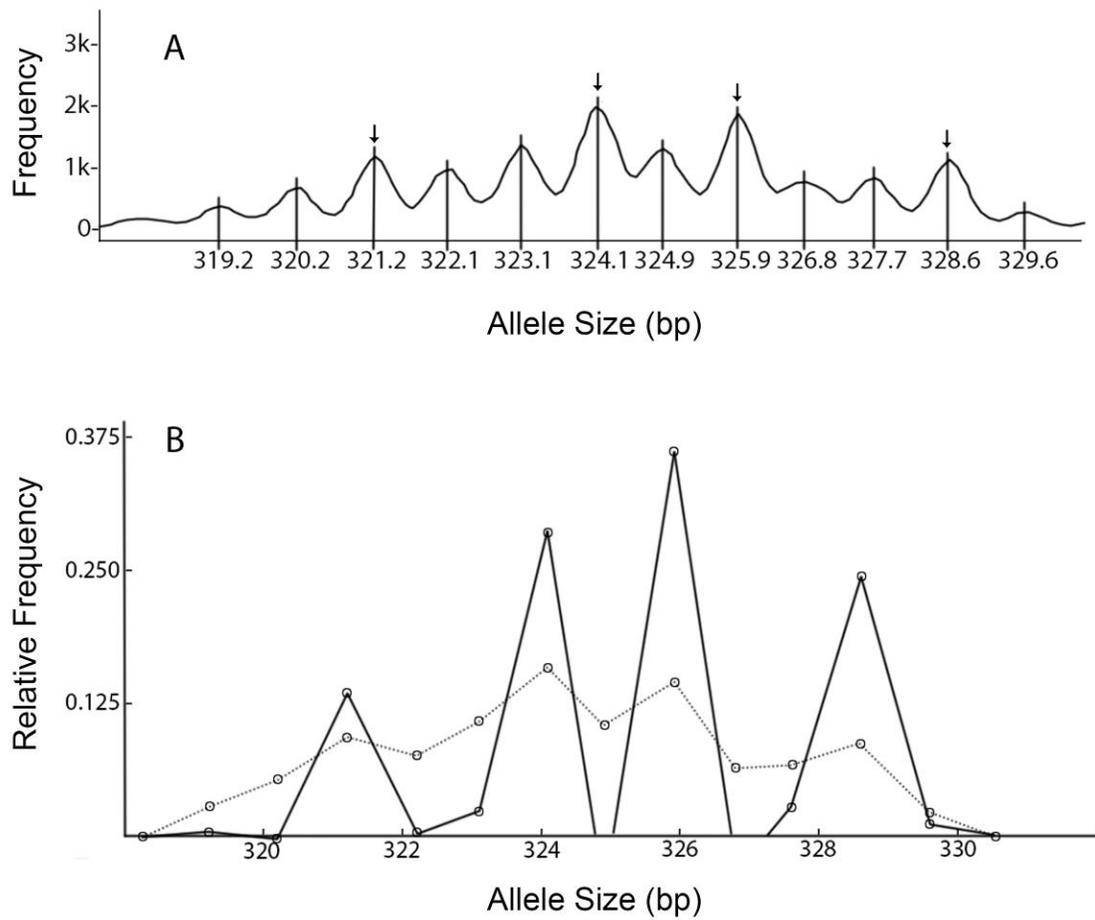


Figure 5. Analysis of Cm84 pooled samples. (A) Electrophoretic pattern of nest H1 from Geneious 6.1.7, showing a high number of stutter peaks almost indistinguishable from the true allelic peaks (shown with arrows). (B) Stutter correction with Poolfitter v1.1. Dotted line shows the original pattern and solid line shows the corrected pattern. Individual peaks are represented as circles. X axis shows allele sizes, Y axis shows relative frequencies.

After removing the corresponding hatchlings from the analysis, the sample size of offspring analyzed per clutch ranged from 12 to 19, with a mean of 16 ± 1 SE. The highest number of alleles in a single clutch was seven and the highest minimum number of fathers identified by GERUD 2.0 was three (Table 5). Only three maternal samples yielded DNA and were used in the analysis. For each of the three nests with known maternal genotypes (B1, B3 and H26), results from GERUD 2.0 on multiple paternity and minimum number of fathers were the same whether or not maternal samples were included in the analysis. Among the nine clutches studied, one (B2) yielded results incompatible with only one mother, probably due to contamination. From the eight remaining clutches, two (25 %) showed no evidence of multiple paternity, while four (50 %) had a minimum of two fathers, and two (25 %) had a minimum of three fathers (Table 5). The two clutches sired by a single father (B3 and N38) showed low variability. Among the offspring in clutch B3, three different alleles were found at Cm84 and only two at Or1 (Table 6). The female was homozygous at Cm84 (326/326) and both parents shared the same genotype at Or1 (148/168). In clutch N38, we found only three different alleles at each locus. Not having a maternal sample for this clutch, the software GERUD 2.0 was unable to yield a single solution for maternal and paternal genotypes. However, in all four possible combinations, parents shared one allele at Cm84 (325) and either also shared one allele at Or1, or one of the parents was homozygous (Table 6).

Table 5. Multiple paternity results. The table shows the number of hatchlings originally analyzed in each nest and the final number after removing the hatchlings with just one ‘extra’ paternal allele at one locus. The number of different alleles at the microsatellite loci Cm84 and Or1 is given, both the total found and the final number after removing probable mutations and scoring errors. The minimum number of fathers inferred by the program GERUD 2.0 was calculated using the final values. Because COLONY incorporates error rates in its calculations, the minimum number of fathers inferred by COLONY was calculated using the whole data.

Nest	No. of hatchlings analyzed (final No.)	Cm84 alleles (final No.)	Or1 alleles (final No.)	Minimum Number of Fathers GERUD 2.0	Minimum Number of Fathers COLONY
B1	19 (18)	7(6)	7	3	4
B2	17	7	7	No results ^a	4
B3	19	3	2	1	1
H1	18(17)	6(5)	4	2	2
H2	17	4	7	3	3
H26	18(12)	9(5)	6(5)	2	2
N37	16(14)	4(2)	5(4)	2	2
N38	18	3	3	1	1
N40	16(13)	7(5)	6(5)	2	3

^aNest B2 results were not compatible with just one mother, probably due to contamination. This nest was removed from the study.

Table 6. Genotypes of nests sired by only one male (B3 and N38)

(A) Nest B3 genotypes. Maternal and offspring genotypes were obtained from samples, the paternal genotype was inferred by GERUD 2.0

Maternal genotype		Number of offspring	Offspring genotypes		Genotype frequency	Inferred paternal genotype	
Cm84	Or1		Cm84	Or1		Cm84	Or1
326/326	148/168	19	326/337	168/168	2	322/337	148/168
			326/322	148/168	4		
			326/337	148/168	4		
			326/337	148/148	3		
			326/322	168/168	3		
			326/322	148/148	3		

(B) Nest N38 genotypes. Offspring genotypes were obtained from samples. Parental genotypes were inferred by GERUD 2.0. The software was unable to identify one single pair of mother-father genotypes, but found four different possible combinations.

Number of offspring	Offspring genotypes		Genotype frequency	Inferred genotypes			
	Cm84	Or1		Parent 1		Parent 2	
				Cm84	Or1	Cm84	Or1
18	325/325	152/168	4	325/341	148/168	325/344	152/152
	341/344	152/168	3				
	325/344	148/152	1	325/341	148/152	325/344	152/168
	325/344	152/168	2				
	325/341	152/168	2	325/341	148/152	325/344	148/168
	325/341	148/152	3				
	341/344	148/152	3	325/344	152/168	325/341	148/168

With the loci and sample sizes used, the combined exclusion probability was 0.92 for the clutches with one known parent, and 0.81 for those with neither parent known. The probability of detecting multiple paternity (PrDM) assuming equal paternal contributions was high when sampling 10 offspring (PrDM = 0.96) and only slightly higher when sampling 15 (PrDM = 0.98). A skewed paternal contribution of 1 : 4 would still give a PrDM > 0.9 when sampling 15 offspring. However, a very skewed case of 1 : 9 would reduce the PrDM to 0.72.

Simulation analyses with GERUDsim 2.0 for an offspring of 15 indicated that multiple paternity would be detected in 97.1 % of clutches for equal contributions (8 : 7) and in 91.4 % in case of a very skewed paternal contribution (13 : 2).

Paternity reconstructions using COLONY confirmed GERUD 2.0 outcomes. Because COLONY reconstructions already take into account both mutations and typing errors, as well as null alleles, we used the entire data set, without removing the extra alleles that appeared in just one locus in a single hatchling. COLONY found multiple paternity in the same 6 nests as the GERUD 2.0 analysis, although the minimum estimated number of fathers was higher in two nests (Table 5). The number of hatchlings analyzed ranged from 16 to 19. Two nests (25%) were sired by only one male and 6 nests (75%) showed multiple paternity, with a minimum of two fathers in three nests, a minimum of three fathers in two nests, and a minimum of four fathers in one nest. Because COLONY analysis includes the possibility of errors in the data, this program could make a parental reconstruction of nest B2, which GERUD 2.0 considered incompatible with only one mother. According to COLONY, this clutch was sired by at least 4 fathers. COLONY tends to overestimate the number of parents when the number

of loci analyzed is low (Jones et al. 2007; Sefc and Koblmüller 2009), while GERUD 2.0 is considered more accurate when paternity analyses can be run individually with less than 6 sires (Jones 2005). For this reason, we chose to use COLONY results only for confirmation of our GERUD 2.0 outcomes, and excluded nest B2 from the analysis.

Pooled Samples

Both Or1 and Cm84 pooled samples showed stutter peaks, although Or1 could be resolved by visual comparison with a few individual samples. Cm84 were corrected using Poolfitter v1.1.

Alleles detected in the Or1 pooled samples were the same in both pooled samples for each nest, before and after PCR (Fig. 6), and coincided with the alleles identified through individual analyses (Table 7). For some alleles with very low frequencies, such as those present in only one hatchling, the peaks were small and could easily be confused with noise (Figs. 6B and 6B'). After removing these small peaks, the total number of alleles detected in the combined samples was lower than the number obtained from the analysis of the individual samples in 3 out of 8 clutches (37.5 %). In two clutches (B1 and H26) the sample pooled before PCR was more informative than the one pooled after PCR, revealing one additional allele (Fig. 6B, Table 7). When a total of 5 or more alleles were used as an indicator of multiple paternity, and results from pooled and individual samples were compared, results from both approaches coincided in 6 clutches (75 %) (Table 7). The remaining two clutches, H26 and N40, showed evidence of multiple paternity when the samples were individually analyzed, yet only 4 different alleles appeared in the pooled samples (Table 7).

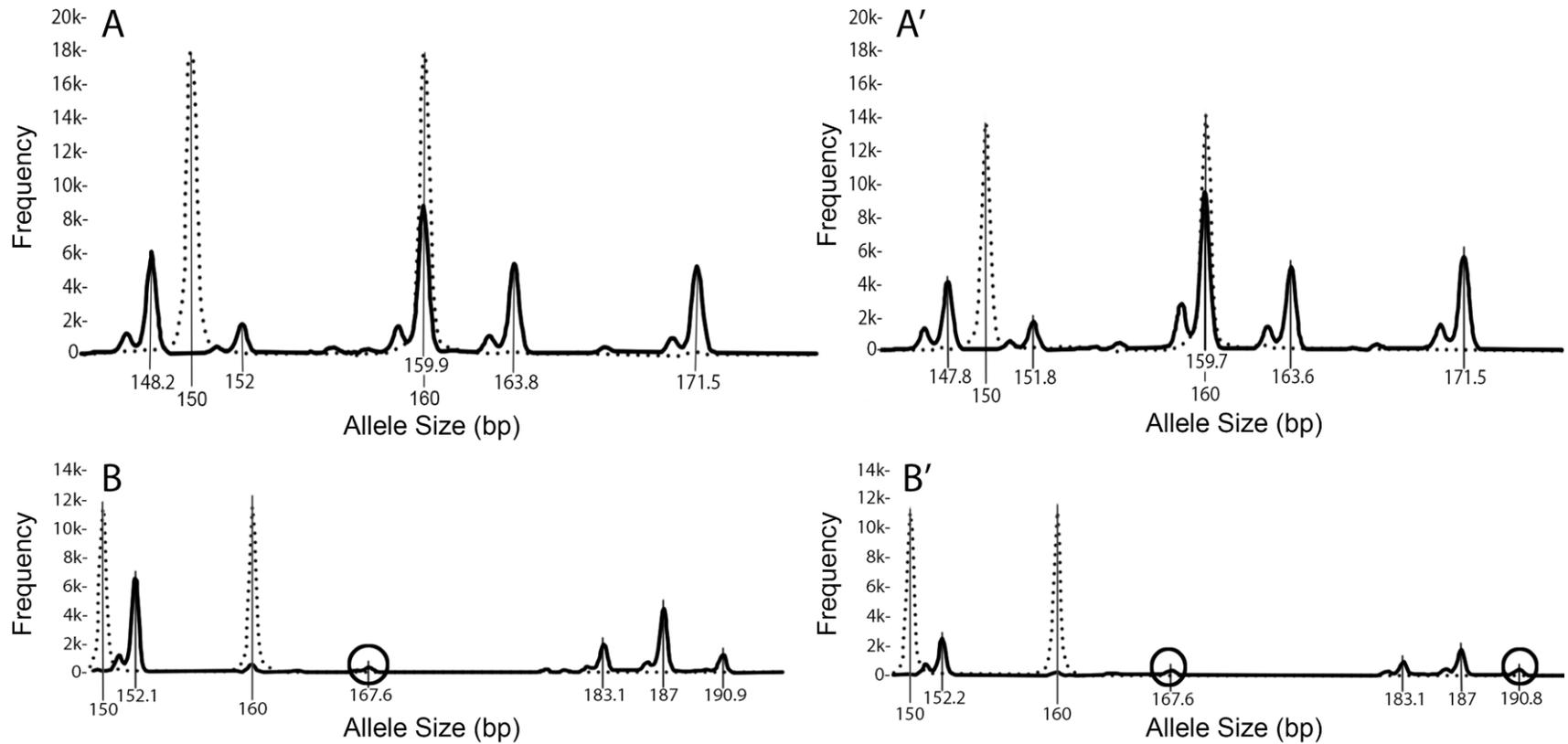


Figure 6. Examples of alleles identified in Or1 pooled samples, visualized with Geneious 6.1.7. We show the graph for nests N37 (A, top) and H26 (B, bottom), both for samples pooled before PCR (A, B; left), and pooled PCR products (A', B'; right). Solid line peaks indicate different alleles present in the nest, dotted line peaks represent reference scale sizes. Numbers indicate allele sizes in bp. Y axis shows frequencies in relative fluorescence units. Small peaks that may be indistinguishable from noise are circled.

Table 7. Pooled samples results. Number of Or1 and Cm84 parental alleles from individual and pooled samples, along with accuracy of results from pooled samples to correctly detect multiple paternity.

Nest	Or1				Cm84			Correct multiple paternity detection (Or1 & Cm 84)
	No. of alleles from individual samples (Final No. after removing possible mutations)	Total No. of alleles from samples pooled before PCR	Total No. of alleles from samples pooled after PCR	Correct multiple paternity detection	No. of alleles from individual samples (Final No. after removing possible mutations)	Total No. of alleles from samples pooled before PCR	Correct multiple paternity detection	
B1	7	6	5	Yes	7(6)	8	Yes	Yes
B3	2	2	2	Yes	3	3	Yes	Yes
H1	4	4	4	No ^a	6(5)	4	No	No
H2	7	7	7	Yes	4	3	No ^b	Yes
H26	6(5)	4	3	No	9(5)	4	No	No
N37	5(4)	5	5	Yes	4(2)	3	No ^b	Yes
N38	3	3	-	Yes	3	3	Yes	Yes
N40	6(5)	4	4	No	7(5)	5	Yes	Yes

^aThe analysis of the pooled sample for this nest assessed the correct number of alleles (4), but the distribution pattern of the alleles in the individual samples indicated the presence of two fathers. ^bInformation from Cm84 for nests H2 and N37 did not allow to infer multiple paternity because the total number of alleles was less than 5 both in the individual sample and in the pooled sample analysis. However, information from Or1 individual samples revealed multiple paternity in both nests.

Clutch H1 showed only 4 alleles both in the individual and pooled analyses, not allowing multiple paternity to be inferred. However, the distribution of alleles in the individual samples was not compatible with only one father. Two hatchlings from this clutch were homozygous for allele 152, meaning that the mother and the father shared this allele, increasing the minimum number of paternal alleles to three. This fact, along with information from locus Cm84, strongly suggested multiple paternity for this clutch.

Because Or1 samples pooled before PCR gave better results than pooled PCR products, we only used the Cm84 samples pooled before PCR. After stutter correction with Poolfitter, the number of alleles detected in the Cm84 pooled samples was lower than the higher number found through individual analysis in five cases (62.5%), equal in two (25%), and higher in one (12.5%) (Table 7). In spite of these differences, pooled samples gave us the same information as individual samples regarding multiple paternity in 6 out of 8 clutches (75%). Under both approaches, nests B1 and N40 contained 5 or more different alleles, indicating more than one sire, whereas nests B3, N38, H2 and N37 showed 4 or fewer alleles, not allowing the inference of multiple paternity. In the remaining two nests (25%), H1 and H26, the pooled sample did not indicate evidence of multiple paternity, while the analysis of individual samples did.

Adding the outcomes of the analysis of both Or1 and Cm84 pooled samples, we obtained evidence of multiple paternity for 4 out of 8 nests (B1, H2, N37 and N40), whereas 4 nests (B3, H1, H26 and N38) appeared to be sired by only one male each. These results coincided with the results obtained from the analyses of individual samples in six cases (75%). Nests H1 and H26 showed evidence of multiple sires when samples were

analyzed individually, although multiple paternity was not detected in any of the pooled samples from these nests.

Discussion

This study documents the presence of high levels of multiple paternity in the olive ridley population nesting within the Gulf of Fonseca, Honduras. Although sample size (8 clutches), as well as the number of hatchlings analyzed per clutch (12 – 19) were relatively low, multiple paternity was unambiguously found in 75 % of the nests, with a minimum of two contributing males in 66 % of them, and a minimum of three contributing males in the remaining 33 %. In the interpretation of results, we used a conservative approach, meaning that both the level of multiple paternity in the Honduran olive ridley population and the minimum number of fathers per clutch may actually be higher than detected.

Even if slightly underestimated, multiple paternity levels detected here fell within the range demonstrated in previous studies. Hoekert et al. (2002) found 20 % multiple paternity in the olive ridley population nesting in Surinam and, in a study on the Pacific coast of Costa Rica, Jensen et al. (2006) detected highly different levels of multiple paternity between a solitary population nesting at Playa Hermosa (30%) and the *arribada* population nesting at Ostional (92%). These two geographically close populations showed no evident genetic differentiation, suggesting that differences in multiple paternity levels among olive ridley populations may well be caused by extrinsic factors rather than being a genetically determined phenomenon (Jensen et al. 2006). The authors concluded that the abundance of individuals in the mating system was the dominating factor related to multiple paternity.

Although several benefits have been proposed to explain the evolution and selection of polyandry and multiple paternity (Zeh and Zeh 2001; Uller and Olsson 2008), no direct benefits have been demonstrated to date for sea turtle females. In fact, studies on green turtles (Lee and Hays 2004; Wright et al. 2013) highlighted a potential cost to polyandry, and suggested that females mate several times in response to male coercion to avoid the potentially higher costs of aggressive male harassment. The advantages of multiple mating for males are obvious and thus it is likely that polyandry and multiple paternity in sea turtles are actually driven by the benefits to males mating as often as possible with as many females as possible (Lee and Hays 2004). Due to the ease of finding females for successive mating encounters, large aggregations with high densities of mature individuals off *arribada* rookeries provide optimal mating opportunities for males, giving rise to the potential for high levels of multiple paternity. However, in a solitary population with a relatively small offshore aggregation, expected multiple paternity levels are likely to be much lower. A trend in increasing multiple paternity with increasing female breeding population size has been reported by Ireland et al. (2003) using data from different sea turtle species. Jensen et al. (2006) detected the same trend when plotting multiple paternity data from ten rookeries against breeding population size. When taking into account only data for the genus *Lepidochelys*, they found a significant fit to an exponential regression (Fig. 7).

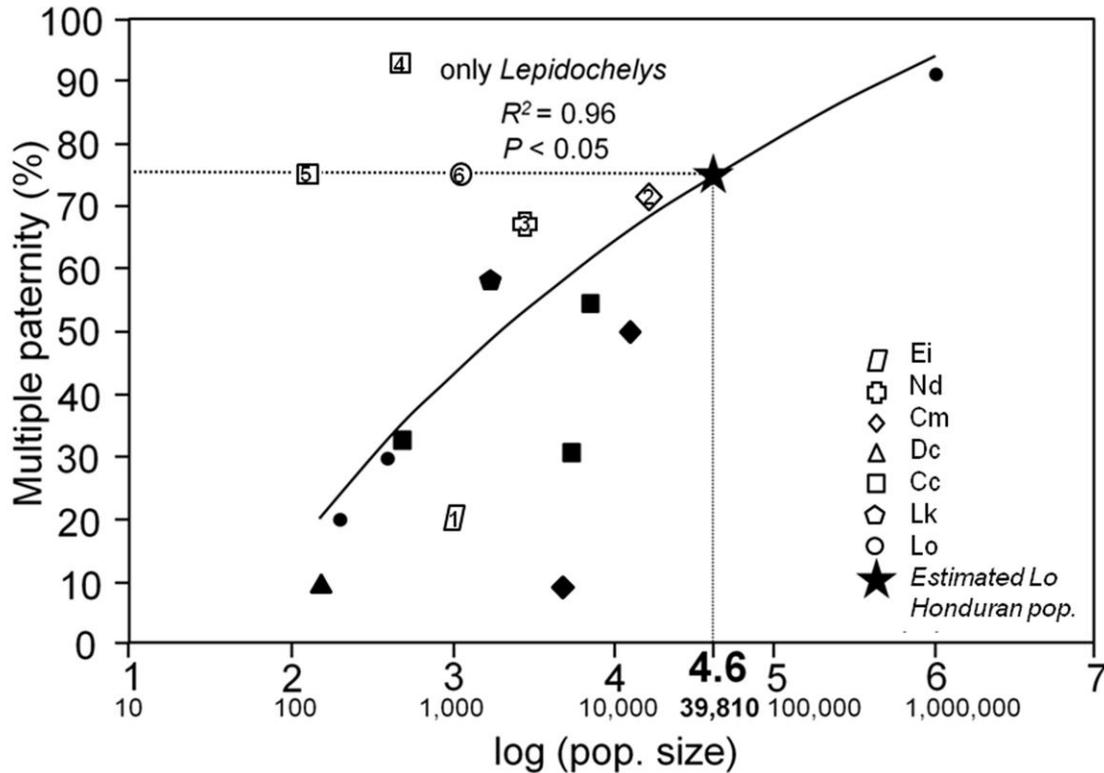


Figure 7. Estimated size of the Honduran *L. olivacea* population (indicated by a star) derived using the exponential regression graph from Jensen et al. (2006). Solid symbols indicate data from the original graph by Jensen et al. (2006). Open symbols indicate data from recent studies: (1) Joseph and Shaw (2011), (2) Joseph (2006), (3) Theissinger (2009), (4) Zbinden (2007), (5) Lasala et al. (2013) and (6) this study. When the study did not report data on adult female population size, an estimate was calculated as the total number of clutches divided by the mean clutch frequency, adjusted by the estimated remigration interval. Ei: *Eretmochelys imbricata*, Nd: *Natator depressus*, Cm: *Chelonia midas*, Dc: *Dermochelys coriacea*, Cc: *Caretta caretta*, Lk: *Lepidochelys kempi*, Lo: *Lepidochelys olivacea*.

Recent multiple paternity studies confirmed the general trend of higher levels of multiple paternity associated with larger populations (Fig. 7). Joseph and Shaw (2011) found 20% multiple paternity in a small population of hawksbill sea turtles from Sabah Turtle Islands, Malaysia, whereas a several fold times larger population of green turtles from the same area showed multiple paternity levels of 71% (Joseph 2006). However, some discordant data have also appeared (Fig. 7). A study on flatbacks from Queensland, Australia, by Theissinger et al. (2009), showed multiple paternity in 69% of the clutches in a population with an estimated size of only 2,650 adult females. Zbinden et al. (2007) reported the highest multiple paternity rate found in a sea turtle population (93%) in the Mediterranean loggerhead rookery of Zakynthos, Greece, with an estimated breeding population size of less than 500 females. Additionally, Lasala (2013) studied a small loggerhead nesting beach on Wassaw Island, Georgia, and found multiple paternity in 75% of the clutches although the population size did not exceeded 200 females. One suggested explanation for finding extremely high rates of multiple paternity in small populations is that the most influencing factor is not the actual number of animals present, but their density within the mating area (Zbinden et al. 2007). In the Zakynthos rookery, turtles aggregate in a narrow area of the Bay of Laganas, 9 km long by 1 km wide, where densities may reach 54 individuals/km² (Schofield et al. 2009). In Georgia, the ocean floor off Wassaw Island drops sharply in a feature known as the Georgia Bight, which may limit the size of the area for loggerheads to congregate, also resulting in a dense concentration of turtles (Lasala et al. 2013).

Our current study on Honduran olive ridleys has also yielded anomalously high rates of multiple paternity. When we used Jensen's regression graph (Jensen et al. 2006) to

calculate the breeding population size corresponding to the multiple paternity level found in the current study (75 %), the result approached 40,000 individuals (Fig. 7). Although information on this population is scarce, data from beach monitoring compiled by ProTECTOR during the last seven years strongly suggest that the actual population size is approximately two orders of magnitude lower than this figure. Female breeding population size can be estimated as the total number of clutches laid in the population, divided by the mean clutch frequency, adjusted by the estimated remigration interval (Ireland et al. 2003). One thousand appears to be a generous estimate of the number of nests deposited annually along all nesting beaches in the South coast of Honduras. Solitary olive ridleys lay two to three clutches per year (Miller 1997), with a mean interesting interval of 14 days, and tend to nest annually (Pritchard 1969; Plotkin 1994). From these data, we estimate a breeding population size of 333 to 500 females. Even if the number of nests is doubled to 2,000 (supposing that many nests may remain undetected on non-monitored beaches), and the less common remigration interval of 2 years is used, the estimated breeding population size would be 1,333 to 2,000 females, still far short of the calculated 40,000 using Jensen's regression.

Although the density of animals in the breeding area has not been calculated, high density is not likely to be the best explanation for increased multiple paternity rates in this case. The Gulf of Fonseca is an important area for artisanal fishing in Honduras (Dunbar et al. 2012), and more than 300 small boats fish daily in the Honduran waters of the gulf, covering most of its area (Box and Bonilla 2009). If the sea turtle density was as high as those reported in Costa Rica mass nesting beaches, or in the Zakynthos rookery in Greece, fishers should be reporting sea turtle encounters very frequently during the nesting season.

Yet, reported sea turtle sightings are only occasional. In this case, behavioral information specific to olive ridleys may help explain the observed discrepancy between the expected and observed multiple paternity levels. Cheloniids (hard-shelled sea turtles) are known for their high fidelity, both to feeding areas (Broderick et al. 2007) and to nesting beaches (Plotkin 2003). Olive ridleys, however, lack the highly specialized life history stages characteristic of the other cheloniid species (Pritchard 2007), showing high behavioral plasticity and adaptability to variable environmental factors. Recent studies of the Eastern Pacific population found that *L. olivacea* do not follow specific migratory corridors, do not show site fidelity to feeding areas, and change their movement patterns in response to climatic events, such as El Niño Southern Oscillation (ENSO) (Plotkin 2010). Nest site fidelity tends to be low for *L. olivacea* solitary nesters (Kalb 1999), which may switch between beaches separated by dozens to hundreds of kilometers within a single nesting season (Schulz 1971; Tripathy and Pandav 2008). *Arribada* nesters generally show high site fidelity and remain close to the nesting beach during the entire season (Pandav et al. 2000), yet reports of *arribada* females nesting alternately between different rookeries are not uncommon (Cornelius and Robinson 1985; Pandav 2000; Tripathy and Pandav 2008). Some females are also known to use a mixed strategy, switching between mass and solitary nesting, even during a single season (Kalb 1999; Bernardo and Plotkin 2007). Low nest site fidelity along with the possibility of females moving back and forth from mass nesting to solitary beaches may explain the disparity of results we obtained when estimating the size of the Honduran olive ridley population.

If the Gulf of Fonseca population is not discrete, but contains a number of females coming from proximal *arribada* beaches, multiple paternity levels above normal values for

a small solitary population may be expected. Pritchard (2007), who worked on the beach at Punta Ratón during the 1960s and recorded 100 % egg collection and consumption by community members, also proposed this hypothesis. Essentially, complete egg collection occurred from at least the 1940s and until 1975, when the Honduran Government established the first *veda* protected period. Although the population should have been extirpated from the region, nesting females continued emerging to nest in Punta Ratón during the ensuing decades. Pritchard (2007a) suggested that instead of being returning hatchlings from Honduran nesting beaches, these females were probably coming from Nicaraguan mass nesting beaches. The closest mass nesting beaches to Honduras are Chacocente and La Flor, located approximately 220 km and 290 km south, respectively, from the mouth of the Gulf of Fonseca, and both are within the distance range of observed inter-beach movements for olive ridleys (Tripathy and Pandav 2008). Further support for the presence of external nesting females in the Gulf of Fonseca comes from recent satellite telemetry that showed a nesting female satellite tagged at El Venado, Honduras, leaving the Gulf and moving south along the coast of Nicaragua to subsequently reach the shore, presumably in an attempt to nest (Dunbar and Salinas 2013). Beach exchange is a complex strategy that olive ridleys may use to colonize new areas or new beaches (Tripathy and Pandav 2008), or to distribute the reproductive resources between *arribada* and solitary beaches to increase offspring survival. In any case, results of this study, along with recent satellite telemetry and historical data on the Honduran olive ridley population suggest that at least a number of the females nesting within the Gulf of Fonseca may be coming from close proximity *arribada* beaches, with Chacocente or La Flor in Nicaragua being likely candidates. Further research will be needed to confirm this hypothesis. Although genetic

analysis of mitochondrial DNA has been previously used to identify the origin of sea turtles found in feeding grounds (Sears et al. 1995; Bowen et al. 1996) and to establish phylogeography and population structure of a sea turtle species in specific regions (Encalada et al. 1996), we do not recommend this approach due to the low levels of genetic differentiation that Eastern Pacific olive ridleys show between populations (Briseño-Dueñas 1998; López-Chávez 2000). Satellite telemetry may likely be a better option for confirming that females are moving from mass nesting beaches in Nicaragua to the Honduran waters within the Gulf of Fonseca.

Regarding the methodological goals of this study, we have shown that it is possible to perform successful multiple paternity studies in the absence of female samples. While the availability of maternal samples reduces uncertainty regarding the exact genotype of males and allows calculation of paternal contributions, in cases where minimal access to females is possible, such studies can still provide accurate estimates of multiple paternity levels in the population, as well as of the number of contributing males per clutch. Likewise, the use of pooled samples has the potential to be a valuable tool for sea turtle multiple paternity research, at least for obtaining fast and relatively inexpensive preliminary results. Although the analysis of pooled samples was less informative than the analysis of individual samples, the same alleles found in individual samples appeared in pooled samples and the presence or absence of multiple paternity was correctly revealed in 75% of clutches when pooled samples for both markers were used. Underestimation in pooled samples may occur in the case of alleles with very low frequencies, or when one or both parents is homozygous. However, both issues might be addressed with the use of several microsatellite markers. In cases where the presence of stutter PCR artifacts makes

difficult to identify true allelic peaks, we suggest using specific software for stutter correction, such as Poolfitter (Schnack et al. 2004). Even if stutter correction is not needed, we strongly recommend analyzing at least some individual samples, and visually identifying the shape of the peaks for each particular locus. This will help distinguish true peaks from artifacts in an electropherogram from a pooled sample. Because pooling samples after DNA extraction yields slightly better results than pooling the PCR products, we also recommend pooling DNA samples of equal concentration before using the pooled sample as the template in PCR.

Multiple paternity studies are pivotal in sea turtle research. They provide information about reproductive behavior that it is difficult to observe directly. Multiple paternity levels impact effective population size and diversity, and thus need to be considered for management and conservation purposes. In the current study, high multiple paternity levels found in Honduran olive ridleys imply that the effective population size and diversity are likely higher than expected for this population. The results of this study also inform our understanding of possible origins of the nesting females outside the Gulf of Fonseca. Further studies are needed to confirm this interpretation. To know the origin, migration patterns, and reproductive behavior of Honduran nesting olive ridley turtles will help in designing more effective conservation plans for the population, as well as informing international cooperative management strategies for this species.

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CHAPTER FOUR

DO COMMUNITY-MANAGED SEA TURTLE HATCHERIES WORK? COMPARING NEST AND HATCHLING CHARACTERISTICS BETWEEN HATCHERY NESTS AND SEMI-NATURAL BEACH NESTS

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Abstract

Context

Relocation of sea turtle eggs to hatcheries is a common conservation strategy. To study the effects of hatchery conditions on nest and hatchling characteristics is essential to assess the appropriateness of this management technique and improve its success.

Aims

The present study aimed to compare thermal profiles, hatching success, and hatchling characteristics between semi-natural nests (dug by nesting females transported by humans to a specific beach site) and nests relocated to a hatchery. We also assessed the effects on hatchling body condition and locomotion performance of retaining hatchlings for long periods of time after emergence.

Methods

Incubation temperatures and hatchling success were compared between four nests at the beach and four at the hatchery. Hatchling body mass, curved carapace length, running speed, and swimming style were measured at emergence and 24 hours later, and compared between sites and between measurements. Linear mixed models were used to analyse the data.

Key results

Mean metabolic heating (nest temperature minus sand temperature) was significantly higher in the hatchery than at the beach (1.9 vs 0.6 oC). Hatching success in the hatchery was significantly lower than at the beach (24.1 vs 83.2 %), and the mean

temperature during the second third of the incubation was found to be a good predictor for hatching success. Retention time significantly reduced hatchling body mass and locomotion performance, for both beach and hatchery hatchlings.

Conclusions

The high temperatures reached at the hatchery during the second incubation third likely caused the observed decreases in hatching success, hatchling size, and locomotion performance. Hatchlings retained for long periods suffered substantial water loss, and wasted energy resources needed for their offshore migration.

Implications

Accurate nest temperature control and early release of emerged hatchlings are pivotal features for successful sea turtle hatchery management.

Additional keywords

Lepidochelys olivacea, egg relocation, conservation management, metabolic heat, incubation temperatures, hatching success

Introduction

Sea turtle nests that remain *in situ* are often threatened by both natural and anthropogenic causes. At some nesting sites, nests may be destroyed by beach erosion processes, or inundated during high tides (Eckert and Eckert 1990; Garcia *et al.* 2003; Lee Lum 2005; Tanner 2013; Ellepola *et al.* 2014). Predators, such as fire ants (*Solenopsis invicta*), monitor lizards (*Varanus spp.*), coyotes (*Canis latrans*), mongooses (*Herpestes javanicus*), dingoes (*Canis lupus dingo*), dogs (*Canis familiaris*), and pigs (*Sus scrofa*) are known to feed on sea turtle eggs (Stancyk 1982; Cornelius *et al.* 1991; Allen *et al.* 2001; Whiting *et al.* 2007; Engeman *et al.* 2010; Leighton *et al.* 2010; Bevan *et al.* 2014; Ellepola *et al.* 2014). In some instances, predators may result in reducing the hatching success of some populations to zero (Maulany *et al.* 2012b). Egg poaching for human consumption constitutes a severe problem on many nesting beaches (Garcia *et al.* 2003; Shanker and Pilcher 2003; Chacón-Chaverri and Eckert 2007), and has been considered the direct cause of sea turtle population declines in Mexico and Central America (Cornelius *et al.* 2007; Plotkin *et al.* 2012). In the specific case of olive ridleys (*Lepidochelys olivacea*) nesting on *arribada* (mass nesting) beaches, females often destroy previously deposited eggs (Cornelius *et al.* 1991). Moreover, the extreme temperatures present at some beaches may result in arrested hatchling development during part of the nesting season (Valverde *et al.* 2010), while the fungal and bacterial microenvironment surrounding the eggs may considerably reduce hatching success (Clusella Trullas and Paladino 2007; Bézy *et al.* 2014).

Although some controversy exists regarding their use (Lee Lum 2005; Mrosovsky 2006; Mrosovsky 2008; Pike 2008), sea turtle hatcheries comprise a wide-spread strategy to avoid threats to *in situ* nests (Mortimer 1999). In many sea turtle conservation programs,

sea turtle eggs deposited on beaches are relocated to hatcheries and cared for until hatchlings emerge and are released back to the sea. Hatchery use has both advantages and disadvantages. On one hand, the high level of control of the nest environment may increase hatching success and survival under specific circumstances that cause low success in natural nests (Clusella Trullas and Paladino 2007; Maulany *et al.* 2012a). Factors such as temperature and humidity can be kept near optimal values by using shading structures and artificially moisturizing nests if necessary, while natural predators and human poachers are prevented from accessing nests. Hatcheries facilitate research and are useful resources for environmental education and awareness (Pike 2008). At some sites, hatcheries have become tourist attractions and important income sources (Tisdell and Wilson 2005). On the other hand, hatcheries are unnatural settings and relocating eggs from the beach to a hatchery may affect hatchling development and physiology. Hatcheries tend to reduce hatching success (Eckert and Eckert 1990; Pintus *et al.* 2009; Sieg *et al.* 2011). Moreover, hatcheries are known to alter incubation thermal regimes (DeGregorio and Williard 2011; Sieg *et al.* 2011), which in turn affects incubation duration (Matsuzawa *et al.* 2002; Sönmez *et al.* 2011), sex ratios (Morreale *et al.* 1982; Tiwol and Cabanban 2000; Sieg *et al.* 2011), and hatchling size and performance (Booth *et al.* 2004; Burgess *et al.* 2006; Booth and Evans 2011; Sönmez *et al.* 2011; Read *et al.* 2013). Saving hatchlings from eggs that would have been lost in natural settings may also act against natural selection and could alter the genetic composition of sea turtle populations, resulting in females prone to nesting in non-suitable habitats (Mrosovsky 2006).

Studies elsewhere have investigated the effects of hatcheries by comparing nest and hatchling characteristics between nests kept *in situ* and hatchery nests (Furler 2005;

Clusella Trullas and Paladino 2007; Koch *et al.* 2008; Sieg *et al.* 2011; Sönmez *et al.* 2011; Maulany *et al.* 2012b). Results obtained in these studies vary widely, from hatcheries being considered detrimental to hatching success (Frazier 1991; Almeida and Mendes 2007), to hatcheries considered successful and recommended for increased hatching success (Furler 2005; Clusella Trullas and Paladino 2007; Maulany *et al.* 2012a). This disparity of results may be explained by the large differences existing among sea turtle hatcheries around the world. Fidelity in following recommended parameters for hatchery construction and management increases success and minimizes negative impacts (Garcia *et al.* 2003), yet conditions in many sea turtle hatcheries are far from ideal due to logistic, financial, or political reasons. Many hatcheries that are part of government conservation programs are managed by local communities with or without guidance of specialized nonprofit organizations. The training of community hatchery managers, their awareness of conservation issues, and their motivation to participate are pivotal factors affecting the final outcomes.

A sea turtle conservation program based on egg relocations to hatcheries has been in place in Honduras since 1975. Prior to this, almost 100% of olive ridley nests deposited along beaches of Pacific Honduras were harvested and used for human consumption (Campbell 2007; Pritchard 2007). This conservation program consists of a yearly period of nest protection, *la veda*, that encompasses the first 25 days of the month of September. Outside of this period, commerce of sea turtle eggs is legal and accounts for an important source of income for many coastal families. In Punta Ratón, the main nesting beach for olive ridleys in the country, local community members strongly compete for nests. To speed the nesting process and get the eggs earlier, they dig artificial body pits in the upper

part of the beach and actively transport sea turtle females from the surf to that area. During the *veda* period, nesting beaches are patrolled at night and nests encountered are relocated to hatcheries. Beach patrolling, hatchery construction and hatchery management are performed by the local community and supported by the national military. In the hatchery at Punta Ratón hatching and emergence success tend to be low. Nests are buried only 30 – 40 cm from each other, which may allow metabolic heating from one nest to affect neighboring, nests. Hatchlings often are too weak to emerge naturally and nests need to be excavated to prevent hatchlings from dying inside the nests. Hatchling releases take place during outgoing tides, but only during the night to minimize avian predation. Accordingly, hatchlings are often retained in the hatchery for long periods, ranging from 12 – 24 h. Both excessively high incubation temperatures and long retention times after emergence are known to have negative impacts on hatchling condition and locomotor performance (Pilcher and Enderby 2001; Booth and Evans 2011; Maulany *et al.* 2012a; Read *et al.* 2013; van der Merwe *et al.* 2013; Wood *et al.* 2014), but the exact effects of the current management practices at Honduran olive ridley hatcheries have not been scientifically investigated to date.

For this study, we took advantage of the local tradition of actively moving females from the surf to ovoposit in specific places on the beach to obtain semi-natural nests which were kept *in situ*. We hypothesized that incubation temperatures at the beach would be lower than temperatures at the hatchery under current conditions, and that hatching success, hatchling body condition, and locomotion performance (running speed and swimming ability) would be higher for the semi-natural nests than for the transferred nests. We also

hypothesized that retaining hatchlings for 24 hours would reduce running speed and swimming ability.

Methods

Study Site

We conducted this research from September to November of 2013 at Punta Ratón (13.26570N, 87.51228W), the main nesting beach for olive ridley sea turtles along the Pacific coast of Honduras, in the Gulf of Fonseca (Fig 8). The beach at Punta Ratón is approximately 8 km long, although most nesting events occur on the southern end, on a sand bar of less than 2 km length.

Nests used for this study were located in two different experimental sites. The semi-natural nests were deposited in an elevated area of the beach at the beginning of the sand bar, at the spring high tide line. We called them semi-natural because although they were dug by female turtles, the nesting location was chosen by us. The relocated nests were situated at the local hatchery, 200 m inland from the beach front.

Site Preparation and Nest Selection

The beach site had an approximate area of 40 m². We cleaned the site of garbage, sticks and logs, and surrounded it with spiny tree branches to keep livestock out. Four female turtles that emerged within 200 m of the area were transported to the beach site by fishers from the local community. These nesting turtles were allowed to freely wander within the area and complete the nesting process without major interference. We approached each female once during oviposition to deposit a thermo datalogger in the middle of the clutch.

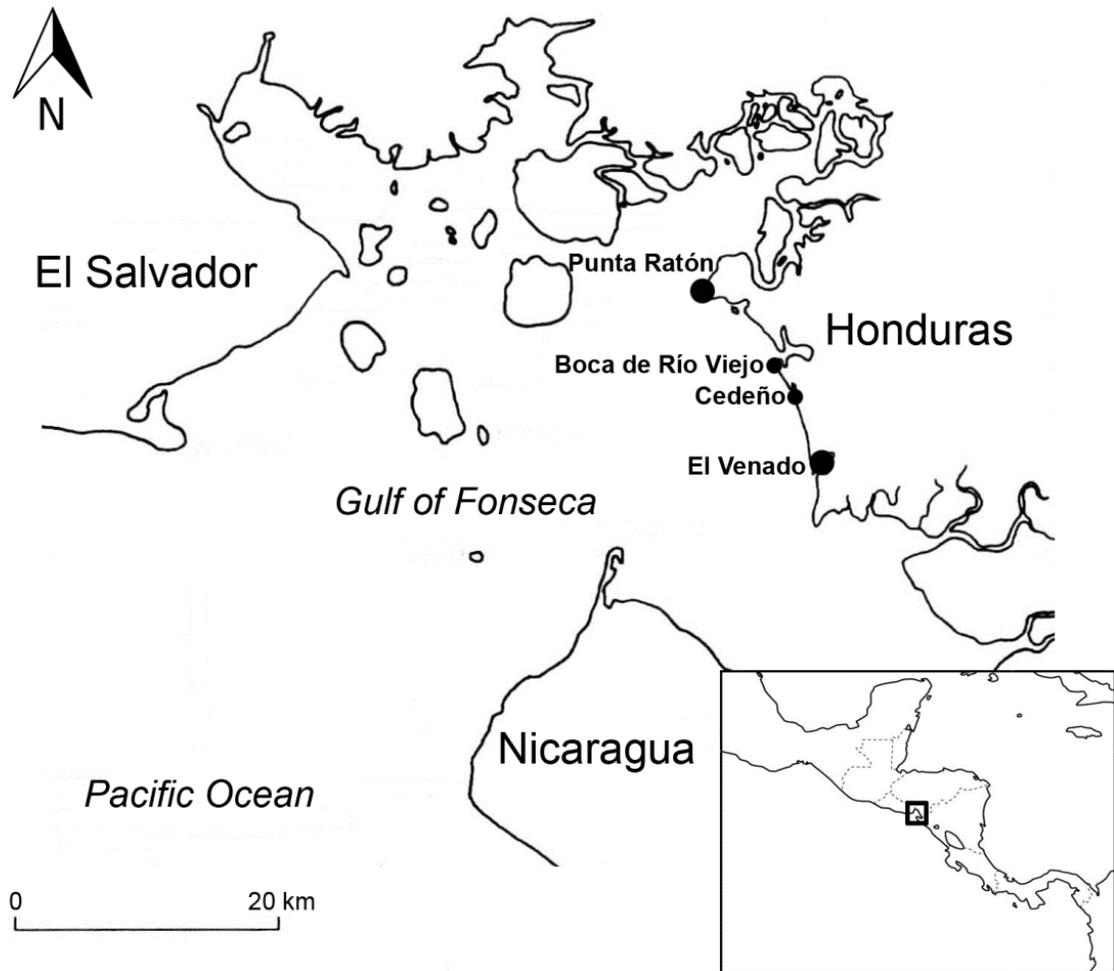


Figure 8. The Gulf of Fonseca. Location of the hatcheries along the Pacific coast of Honduras.

After females finished covering the nests, we measured and flipper-tagged them. The four semi-natural nests were left *in situ*. Distances between each pair of semi-natural nests exceeded 2 m. We triangulated the position of each nest at the beach site and removed any physical marks to reduce the potential for poaching. At day 42 of incubation, three days before the expected hatching date, we placed a protective wire enclosure around the neck of each nest chamber and checked them four times per day, looking for any sign of emergence.

The hatchery had approximate dimensions of 10×6 m and was prepared by local community members. Because the hatchery was not on the beach itself, they dug up and removed the soil, and then covered it with approximately 40 – 50 cm of beach sand. The hatchery area was surrounded by a wire mesh to protect nests from predators and poachers. Nests deposited along the beach during the *veda* period were relocated to the hatchery and reburied at an approximate depth of 40 cm (the typical depth for olive ridley natural nests). Time between initial deposition, relocation, and burying was less than 1 hour for all hatchery nests used in the study. Nests at the hatchery were buried in lines, with 10 nests per line. Distances between lines, as well as distances between nests within each line were 30 – 40 cm. All nests in the hatchery were protected by individual wire enclosures.

Thermal Regimes

We measured incubation temperatures using DS1922L iButton dataloggers (Maxim Dallas Semiconductor, San Jose, CA) located approximately in the middle of each clutch. Dataloggers were programmed to record temperature once per hour during the whole incubation period. To protect the thermo dataloggers from humidity, we placed them inside rubber balloons. At the beach site, we deposited the dataloggers in the nests while the

females were laying the eggs. After each nest was deposited at the beach site, we dug a 30-cm deep pseudo-nest 1 m from the real nest, and deployed a datalogger to use as a control. On the same or subsequent night that each of the beach nests was laid, we randomly selected one nest relocated to the hatchery to include it in the study. We deployed a datalogger in the middle of the clutch while the nest was being buried by hand. We also dug a corresponding control pseudo-nest at the edge of the hatchery and deployed a datalogger in it. We selected nests from flipper tagged females, to ensure all nests in the experiment were laid by different mothers.

Dataloggers were recovered when nests were excavated at the end of the incubation period. As soon as dataloggers were recovered, the stored data were downloaded to a computer. We then calibrated the dataloggers by introducing them, along with a mercury thermometer, into a thermal chamber at a constant temperature of 32 °C. Data from the dataloggers were adjusted accordingly. We used Microsoft Excel (Microsoft Corporation, Redmond, WA) to plot temperature profiles of nests and pseudo-nests at both sites, and to calculate mean daily temperatures, daily temperature ranges, and the total and consecutive number of days that each nest experienced mean temperatures over 35 °C. We then used IBM SPSS Statistics (Version 22; IBM Corporation 1989, 2011) to calculate mean temperatures for each third of the incubation period, and mean temperatures for the entire incubation. We calculated metabolic heating by subtracting daily mean pseudo-nest temperatures from daily mean nest temperatures. Metabolic heating was calculated for the overall incubation period and for each of the incubation thirds.

Hatching Success

Each nest was excavated 46 days after being laid. We counted live hatchlings, dead hatchlings and unhatched eggs. The sum of these three numbers was considered the total number of eggs for the clutch. We calculated hatching success as the total number of hatched neonates (hatchlings both live and dead) divided by the total number of eggs. Hatching success was calculated for four experimental nests at the beach site (B1, B2, B3 and B4) and eight nests at the hatchery, for a total of twelve nests. The eight nests from the hatchery included four nests containing thermal dataloggers (H26, H67, H93 and H94), two additional nests used for the hatchling body condition and performance experiments (H101 and H108), and two nests not used in the experiments that were excavated while we were present in the hatchery (H64 and H65).

Body Condition

We randomly selected 15 hatchlings from each of the eight experimental clutches (four at the beach site and four in the hatchery), weighed them, and measured Curved Carapace Length (CCL), Curved Carapace Width (CCW), Straight Carapace Length (SCL), and Straight Carapace Width (SCW).

These measurements were taken within the first two hours after the excavation of the nest. Because two of the hatchery nests that contained thermo dataloggers had very low hatchling successes (2.8 % in H93 and 6.4 % in H94), and contained fewer than 15 live hatchlings, we randomly selected two other nests excavated the same night in the hatchery (H101 and H108) to use for the body condition and locomotion performance experiments. Hatchlings from both sites were kept in a bucket in a cool dark place for approximately 24 hours and then weighed and measured again.

Locomotion Performance

From the 15 hatchlings previously weighed and measured from each nest, we randomly assigned six to be tested for running speed and six to be tested for swimming style. Running speed was assessed outside at night under natural light conditions. We used a 1-m long PVC gutter lined with sand and placed seaward on the beach with the natural beach slope. Hatchlings were placed at the upper part of the gutter and allowed to crawl to the lower end. A dim light was placed at the end of the gutter to stimulate hatchlings to crawl toward the sea. The time required by the hatchling to cover this distance was measured with a stop watch. We repeated the experiment three times with each hatchling, and calculated the average running speed for each individual. At the beginning of each test we recorded time and air temperature.

For the swimming style test, we used a transparent glass tank (30×30×55 cm) filled with 15 cm of sea water from the beach at Punta Ratón, and kept at ambient temperature. We fitted each hatchling with a 5-mm Velcro band around the widest part of the carapace, and attached it to a wood pole located on the top of the tank *via* a monofilament tether. The band did not touch any of the flippers during movement. The tether was adjusted in a manner such that the hatchling could swim freely, yet not reach the walls or the bottom of the tank. We videotaped 10 minutes of swimming for each hatchling, and counted the number of power strokes (synchronous movements of the frontal flippers), and the time to the nearest second performing dogpaddling swimming style (alternate movement of the four flippers), for 60-sec periods beginning at 1, 5 and 9 minutes. With these three values, we calculated the average number of power strokes per minute, and the average dogpaddling time per minute for each individual. Air and water temperature were recorded

at the beginning of each test. We repeated the running speed tests and the swimming style tests with the same hatchlings 24 h later.

Statistical Analyses

All statistical analyses were performed using IBM SPSS Statistics (Version 22; IBM Corporation 1989, 2011.). Alpha was set at 0.05 significance level. We used a two-way ANOVA with repeated measures to examine the effects of site (between-subjects factor) and incubation third (within-subjects factor) on incubation temperature. When a significant interaction was found, independent samples t-tests were conducted to compare beach and hatchery mean temperatures separately for each of the incubation thirds. To take into account sand temperature, we repeated the same analyses for metabolic heating (nest temperature minus ambient temperature). Because our low sample size (four nests per site) could jeopardize statistical significance, we also calculated effect size, represented by Pearson's correlation coefficient (r). Values corresponding to 0.1, 0.3, and 0.5 were considered small, medium, and large effects, respectively (Cohen 1988). Daily temperature ranges and hatchling success were compared using independent samples t-tests. When we could not assume homoscedasticity for any set of data, we used the adjusted t-value with reduced degrees of freedom provided by SPSS.

To test if hatching success was related to incubation temperatures, we performed correlation analyses among hatching success and seven temperature variables; mean temperature (MeanT), mean temperature for the first (MeanT1), second (MeanT2) and last (MeanT3) thirds of incubation, maximum nest temperature (MaxT), total of days with mean temperature over 35 °C (Days>35) and maximum number of consecutive days with mean temperatures over 35 °C (CDays>35). For the variables that showed correlation with

hatching success, we also performed linear regressions to assess which of them would be reliable predictors of hatching success.

To investigate the effects of site and retention time on hatchling body condition and locomotion performance (running speed and swimming style), we used linear mixed model analysis (Seltman 2014). As fixed effects, we entered site (beach *vs* hatchery), measurement (1st or 2nd), and the interaction between site and measurement. When the interaction had no significant effect, we removed it from the model. Because several hatchlings from each nest were used for body measurements and locomotion experiments, we included nest as a random effect. Again, because our low sample size (four nests in each site) could prevent statistical significance to be achieved, we repeated the mixed model analyses without including nest effects, and calculated effect size (r) when the differences were not significant. The variables tested in the mixed model analyses were body mass, CCL, running speed, power strokes per minute, and dogpaddling time per minute. We ran correlation analyses between the locomotion performance variables (running speed, power strokes per minute, and dogpaddling time per minute) and environmental temperatures (air and water temperatures). If a correlation was significant, environmental temperatures were included in the model as covariates.

Results

Thermal Regimes

Although all dataloggers deployed in actual nests were found when nests were excavated, we only recovered two dataloggers from pseudo-nests at the beach site, and only one from pseudo-nests at the hatchery site. The two dataloggers recovered from the beach site recorded different temperatures. CB2, the control pseudonest for nest B4, was buried

close to a brick wall and showed a thermal profile that did not always run parallel to the B4 thermal profile (Fig. 9C). Both CB2 and B4 recorded temperature values lower than the rest of the dataloggers at the beach. We interpreted that some external factor, such as water accumulation because of the wall presence, likely affected the temperature in that area. We thus discarded data from CB2 and use only data from datalogger CB1 as representative for sand temperature at the beach site.

Table 8 summarizes thermal data for the nests of the study. Thermal profiles for both nests and pseudo-nests are shown in Figures 9 (beach) and 10 (hatchery). A significant difference existed among incubation thirds ($F_{(2,12)} = 65.546$, $p < 0.001$) with mean (\pm SE) incubation temperatures progressively increasing across incubation thirds (32.2 ± 0.2 °C, 33.6 ± 0.2 °C and 34.8 ± 0.2 °C, respectively). Post hoc comparisons with Bonferroni corrections indicated that all temperature pairs were different, with $p < 0.001$ for MeanT1 vs MeanT2, and MeanT1 vs MeanT3, and $p = 0.014$ for MeanT2 vs MeanT3. No significant differences were found between sites for the whole incubation period or for any of the incubation thirds. However, the temperature difference during the second (1.2 °C) and last (0.4 °C) thirds were substantial, with effect sizes of $r = 0.67$ and $r = 0.35$, respectively (Fig. 11).

Table 8. Thermal data and hatching success for beach (B) and hatchery (H) nests. Pseudo-nest IDs start with the letter “C” (control). MeanT indicates mean temperatures \pm SE for the whole incubation period, whereas MeanT1, MeanT2, and MeanT3 indicate mean temperatures \pm SE for the first, second and last thirds of the incubation. MetHeat indicates mean metabolic heating \pm SE, whereas MetH1, MetH2 and MetH3 indicate mean metabolic heating \pm SE for the first, second and last thirds of incubation. Maximum temperatures (MaxT), total number of days over 35 °C (Days>35), and maximum number of consecutive days over 35°C (CDays>35) are also shown, as well as hatching success (HatSuc).

Nest	Site	MeanT	MeanT1	MeanT2	MeanT3	Max T	Met Heat	MetH1	MetH2	MetH3	Days >35	CDays >35	HatSuc(%)
B1	B	33.7 \pm 0.2	32.7 \pm 0.2	33.3 \pm 0.2	35.2 \pm 0.2	37.0	1.0 \pm 0.2	0.2 \pm 0.1	0.7 \pm 0.2	2.2 \pm 0.1	7	5	77.8
B2	B	33.4 \pm 0.2	32.6 \pm 0.3	33.2 \pm 0.2	34.4 \pm 0.3	36.5	0.7 \pm 0.1	0.1 \pm 0.0	0.7 \pm 0.1	1.4 \pm 0.1	6	6	80.8
B3	B	33.1 \pm 0.2	31.6 \pm 0.2	33.0 \pm 0.1	35.0 \pm 0.2	37.0	0.5 \pm 0.1	-0.5 \pm 0.1	0.5 \pm 0.2	1.6 \pm 0.0	11	7	93.3
B4	B	32.7 \pm 0.2	31.9 \pm 0.2	32.6 \pm 0.2	33.7 \pm 0.2	35.7	0.1 \pm 0.1	-0.3 \pm 0.1	0.1 \pm 0.1	0.4 \pm 0.1	1	1	92.9
H26	H	33.6 \pm 0.3	32.1 \pm 0.2	33.4 \pm 0.3	35.4 \pm 0.3	38.0	1.7 \pm 0.2	0.1 \pm 0.1	1.4 \pm 0.2	3.2 \pm 0.1	13	10	40.2
H67	H	33.3 \pm 0.2	31.6 \pm 0.2	33.4 \pm 0.2	34.7 \pm 0.3	37.5	1.4 \pm 0.2	-0.1 \pm 0.1	1.3 \pm 0.1	2.9 \pm 0.2	14	9	50.0
H93	H	34.0 \pm 0.2	32.4 \pm 0.3	34.7 \pm 0.3	34.7 \pm 0.3	38.2	2.2 \pm 1.8	0.8 \pm 0.2	2.5 \pm 0.2	3.2 \pm 0.1	13	9	2.8
H94	H	34.5 \pm 0.2	33.0 \pm 0.3	35.1 \pm 0.2	35.2 \pm 0.3	38.7	2.7 \pm 0.2	1.3 \pm 0.2	2.8 \pm 0.2	3.7 \pm 0.1	22	12	6.4
CB1	B	33.0 \pm 0.1	NA	NA	NA	35.5	NA	NA	NA	NA	NA	NA	NA
CB2	B	32.4 \pm 0.1	NA	NA	NA	35.5	NA	NA	NA	NA	NA	NA	NA
CH	H	31.8 \pm 0.1	NA	NA	NA	33.5	NA	NA	NA	NA	NA	NA	NA

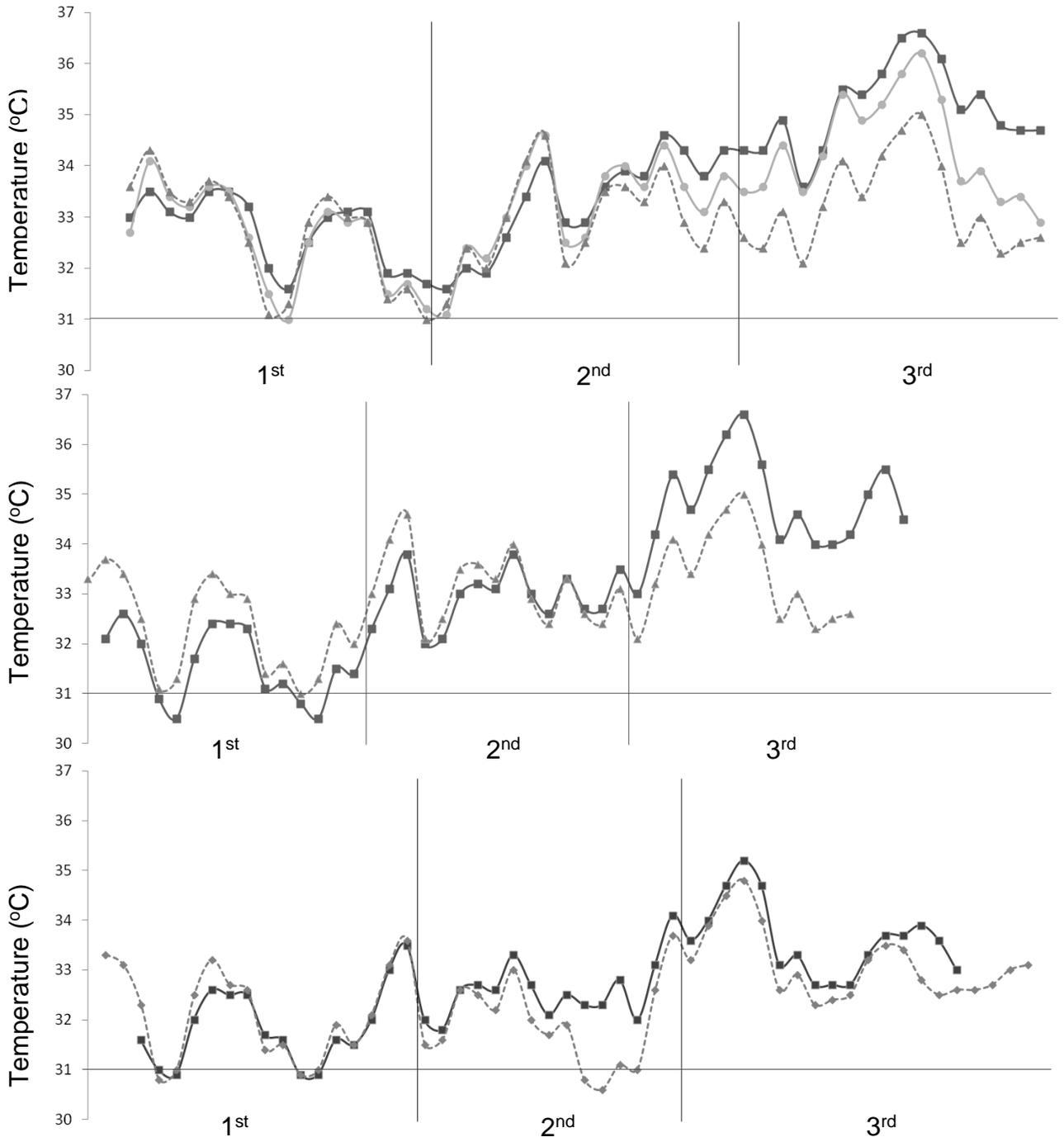


Figure 9. Thermal profiles for beach nests. A shows nests B1 (dark solid line, squares) and B2 (light line, circles), deposited the same night, along with pseudo-nest CB1 (dotted line, triangles). B shows nest B3 (solid line) and pseudo-nest CB1 (dotted line), and C shows nest B4 (solid line) and its corresponding pseudo-nest CB2 (dotted line). Vertical lines separate the thirds of the incubation period. Horizontal lines indicate the estimated pivotal temperature for sex determination. X axis corresponds to incubation time, with each datum representing consecutive days. Y axis indicates mean daily temperatures.

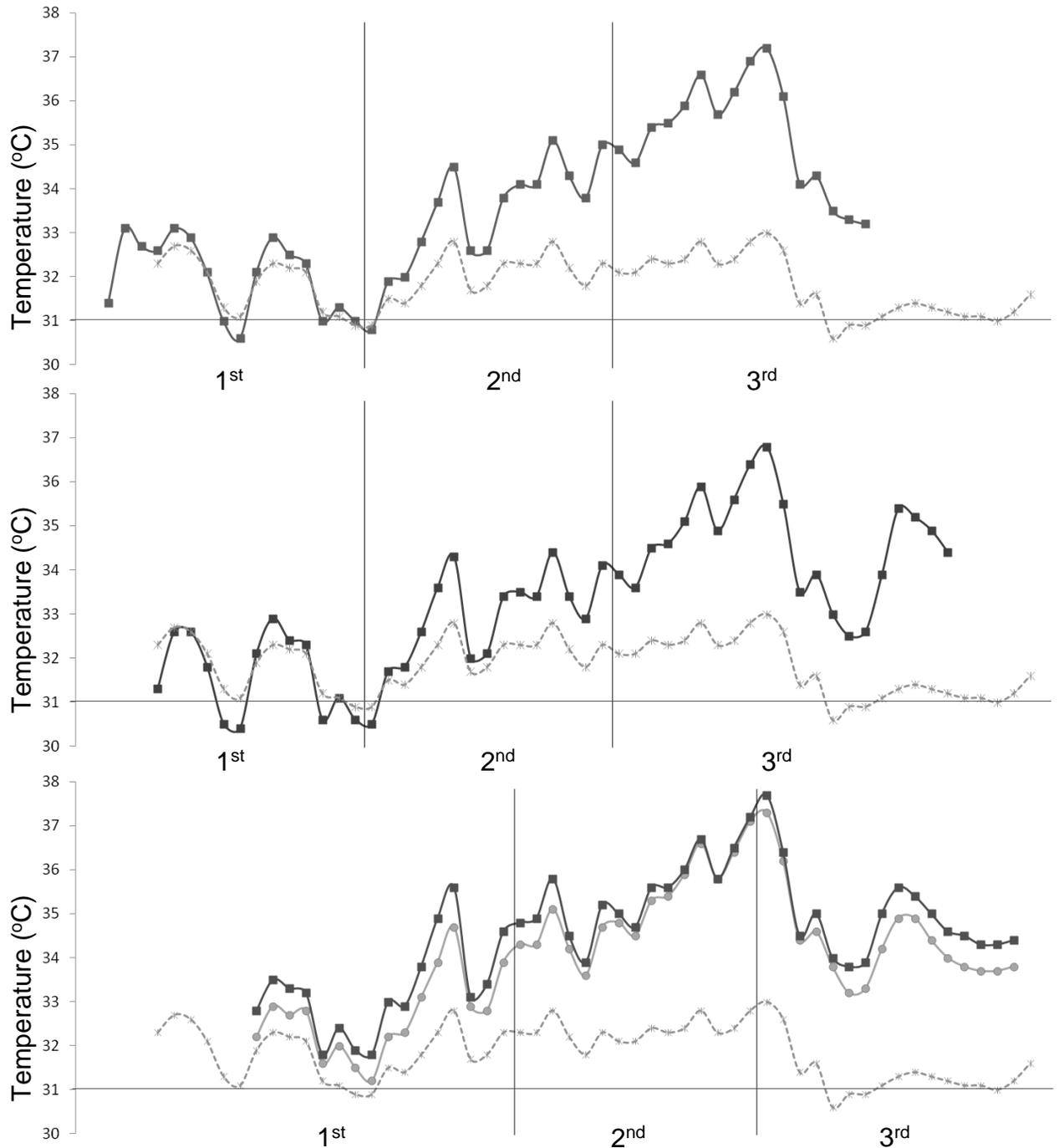


Figure 10. Thermal profiles for hatchery nests. Solid lines show nests H26 (A), H67 (B), H101 (C, squares) and H108 (C, circles). H101 and H108 are shown together because they were deposited the same night. Dotted lines show pseudo-nest CH. Vertical lines separate the thirds of the incubation period. Horizontal lines indicate the estimated pivotal temperature for sex determination. X axis corresponds to incubation time, with each datum representing consecutive days. Y axis indicates mean daily temperatures.

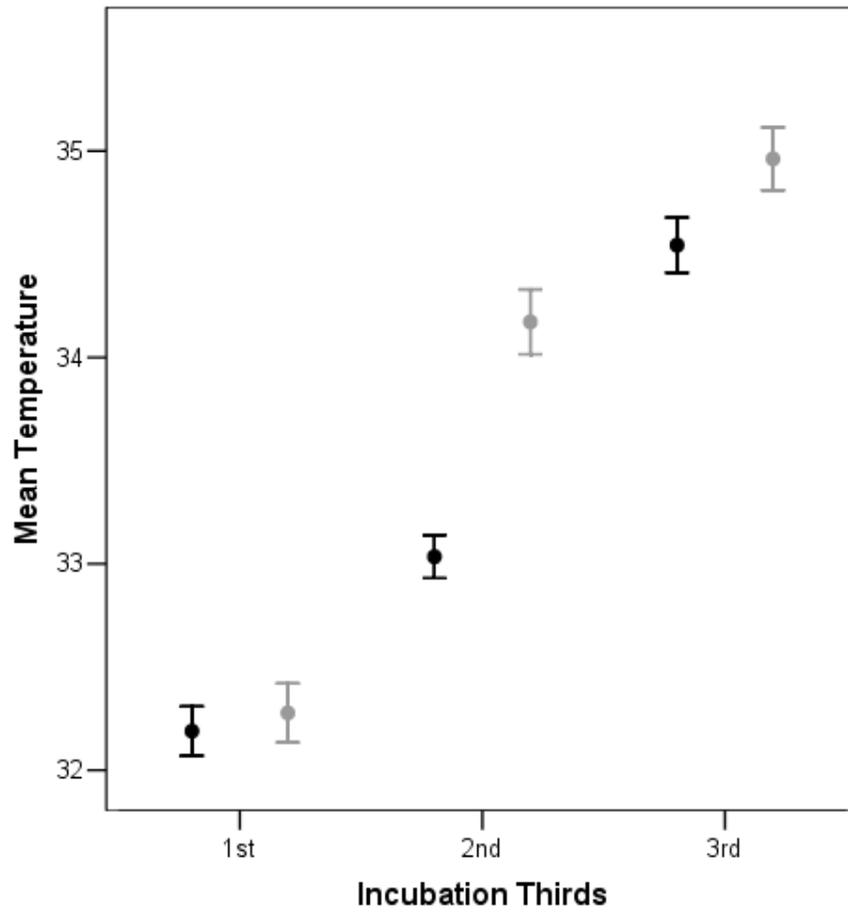


Figure 11. Mean incubation temperatures across incubation thirds. Black symbols indicate mean temperatures at the beach ($n = 4$), grey symbols indicate temperatures at the hatchery ($n = 4$). Error bars represent ± 1 SE.

Metabolic heating was significantly different across thirds ($F_{(2,12)} = 90.774$, $p < 0.001$), showing increasing values (0.2 ± 0.2 °C, 1.2 ± 0.2 °C, and 2.3 ± 0.2 °C, respectively). Post hoc comparisons with Bonferroni correction indicated significant differences among all pairs of data, with $p < 0.001$ for MeanT1 vs Mean T2 and MeanT1 vs MeanT3, and $p = 0.004$ for Mean T2 vs MeanT3. A significant difference also existed between sites ($F_1 = 14.193$, $p = 0.009$) with higher metabolic heating at the hatchery (1.9 ± 0.2 °C) than at the beach (0.6 ± 0.2 °C). However, the significant interaction between site and third ($F_{(2,12)} = 7.758$, $p = 0.007$) suggested that metabolic heating was higher at the hatchery than at the beach during the second ($t_{3,75} = -3.60$, $p = 0.025$) and last ($t_6 = -4.54$, $p = 0.004$) incubation thirds, but not during the first ($t_6 = -1.71$, $p = 0.138$) (Fig. 12).

Daily temperature ranges on the beach were significantly lower than in the hatchery ($t_{360,89} = -11.839$, $p < 0.001$), with mean values of 1.17 ± 0.04 °C for the beach and 1.82 ± 0.04 °C for the hatchery. Temperature ranges at the beach nests fluctuated an average of 0.6 °C less than the temperatures in the surrounding sand (Fig. 13). Maximum nest temperatures registered during incubation were 37 °C for the beach and 38.7 °C for the hatchery. Maximum sand temperatures were 35.5 °C for the beach and 33.5 °C for the hatchery.

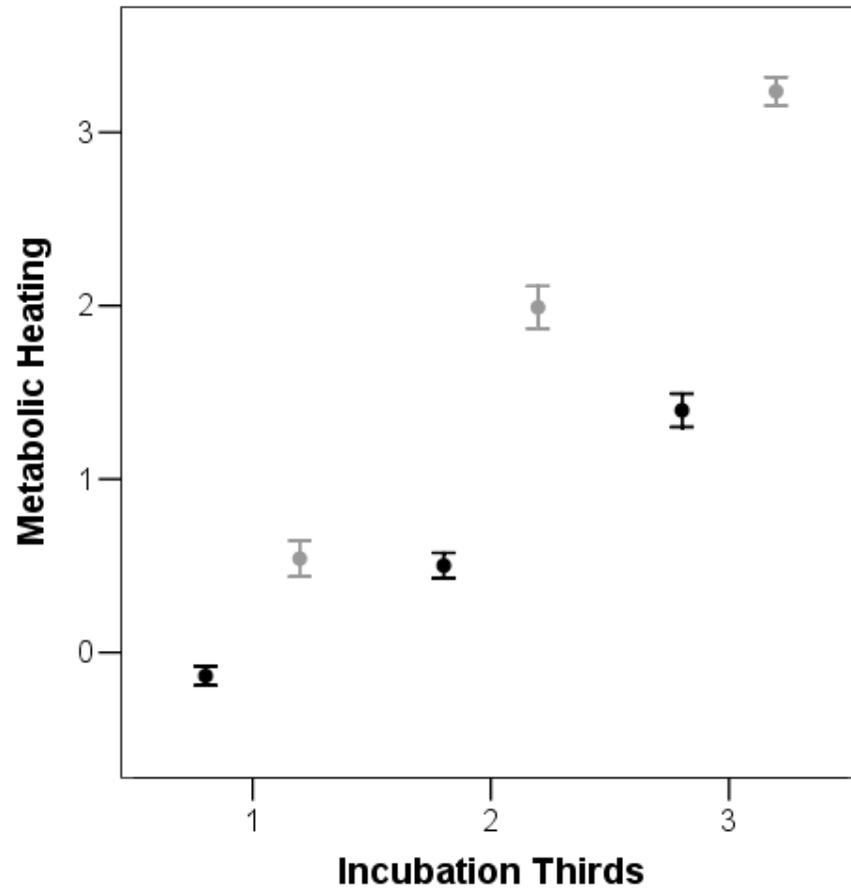


Figure 12. Mean metabolic heating (nest temperature minus ambient temperature) across incubation thirds. Black symbols indicate metabolic heating at the beach ($n = 4$), grey symbols indicate metabolic heating at the hatchery ($n = 4$). Error bars represent ± 1 SE.

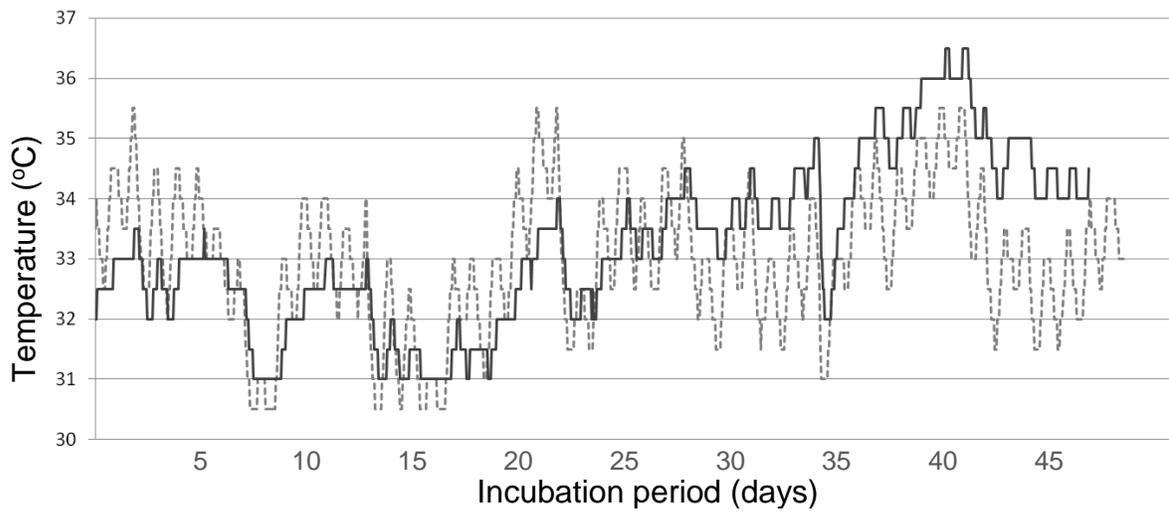


Figure 13. Example of beach thermal profile with daily temperature ranges. Lines indicate hourly temperature data for nest B1 (solid line) and pseudo-nest CB1 (dotted line) during the incubation period. X axis corresponds to incubation time, with each peak indicating one day. Y axis indicates temperature in °C.

Hatching Success

Mean clutch hatching success differed significantly between the nests at the beach site and the nests in the hatchery site (beach: $83.22\% \pm 4.04$ SE; hatchery: $24.08\% \pm 6.00$ SE, $t_{10} = 6.818$, $p < 0.001$) (Fig. 14). MeanT, MeanT2, MaxT, Days>35 and CDays>35 were correlated with hatching success and with each other (Table 9).

To avoid multicollinearity in the multiple regression analysis, we ran independent linear regressions for each of these variables with hatching success, and found that the best predictor for hatching success (highest r^2) was MeanT2. The regression equation was $\text{HatSuc} = 1389.084 - 39.704 * \text{MeanT2}$, $r^2 = 0.861$; $F_{(1, 6)} = 37.250$, $p < 0.001$.

Body Condition

Figure 15 shows mean body mass for hatchlings from the beach and the hatchery, both just after emergence and 24 hours later. Table 10 shows the linear mixed effect model estimates for fixed effects on hatchling body mass. When nest was included as a random factor, the analysis revealed significant differences in hatchling body mass between measurements ($p < 0.001$), but not between sites ($p < 0.353$). After 24 hours of retention, hatchling body mass decreased by an average of $0.93\text{g} \pm 0.05$ SE.

Although not statistically significant, the average body mass difference between the beach and the hatchery was substantial ($0.94\text{g} \pm 0.93$ SE, approximately 6.75%), with moderate effect sizes of $r = 0.35$ for the first measurement and $r = 0.32$ for the second. The interaction effect was significant ($p = 0.02$), with the average body mass lost during the first 24 hours $0.23\text{g} \pm 0.07$ SE higher at the beach than in the hatchery. When nest was not included, the model revealed significant effect also due to site ($p < 0.001$).

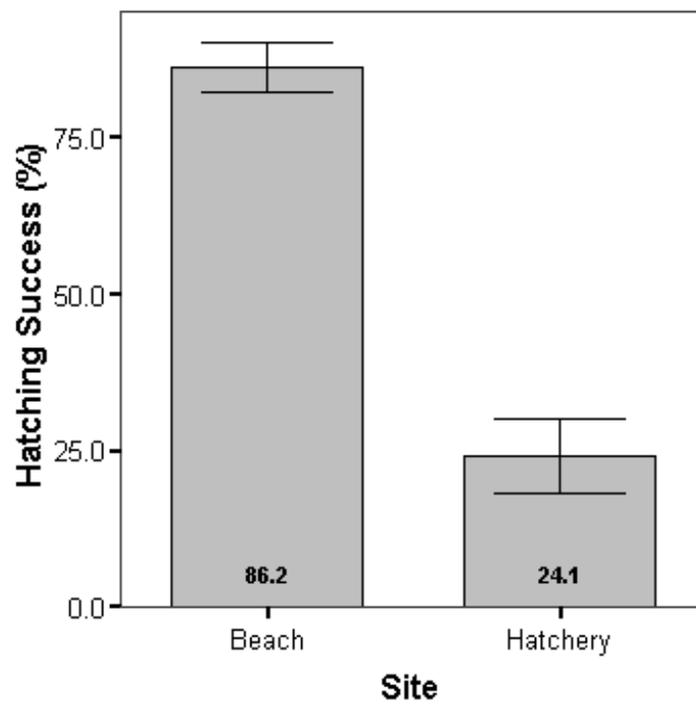


Figure 14. Mean (± 1 SE) hatching success of beach (N = 4) and hatchery nests (N = 8).

Table 9. Correlations among hatching success (HatSuc) and seven thermal variables included in the study: overall mean temperature (MeanT), mean temperatures for the first (MeanT1), second (MeanT2) and last (MeanT3) thirds of the incubation, maximum temperature (MaxT), total number of days over 35 °C (Days>35), and maximum number of consecutive days over 35°C (CDays>35).

	HatSuc	MeanT	MeanT1	MeanT2	MeanT3	MaxT	Days> 35	CDays >35
HatSuc								
MeanT	-0.839**							
MeanT1	-0.431	0.764*						
MeanT2	-0.928**	0.932**	0.612					
MeanT3	-0.410	0.637	0.277	0.428				
MaxT	-0.800*	0.820*	0.285	0.803*	0.810*			
Days>35	-0.791*	0.792*	0.271	0.815*	0.680	0.934**		
CDays>35	-0.800*	0.777*	0.256	0.766*	0.733*	0.958**	0.955**	

* p < 0.05, ** p < 0.01

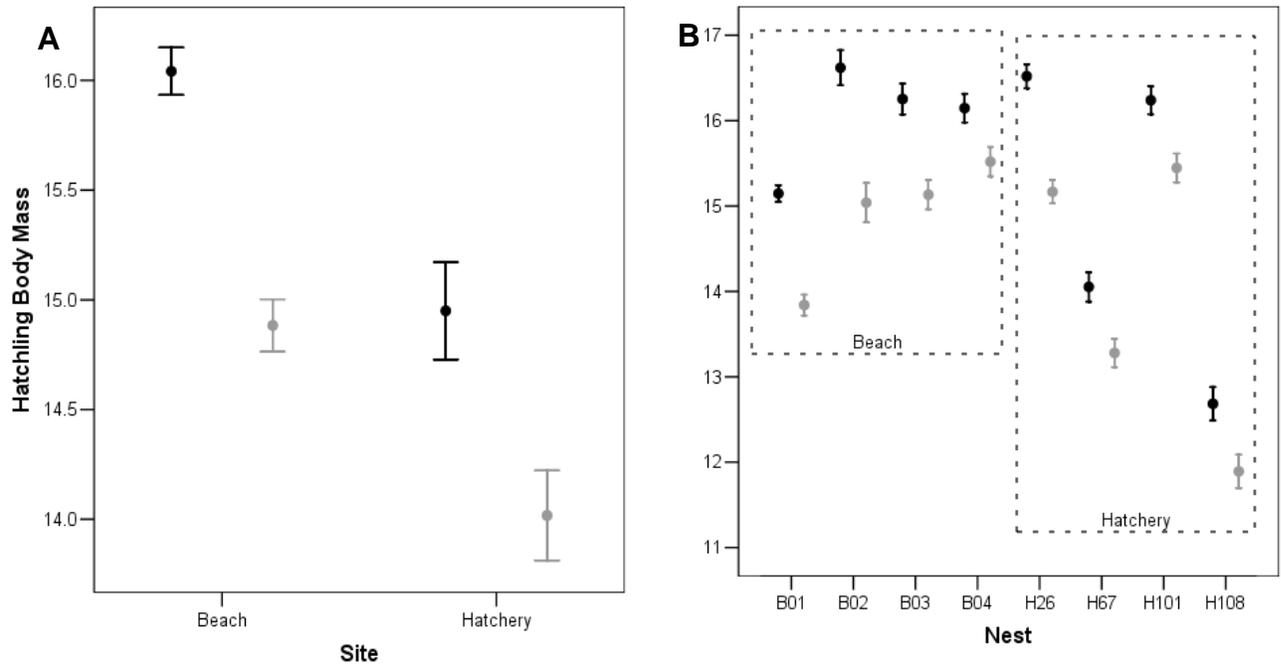


Figure 15. Comparison of mean hatchling body mass between the beach and the hatchery sites. A shows pooled data from all nests at each site ($n = 24$), B shows data for each independent nest ($n = 6$). First measurements are shown in black, second measurements (24 hours later) are shown in grey. Error bars represent ± 1 SE.

Table 10. Fixed effects for two models predicting hatchling body mass (g). Intercept indicates estimated mean hatchling mass; site indicates estimated difference between the two sites (beach and hatchery), measurement indicates estimated difference between the two measurements (emergence and 24 h later), and interaction indicates estimated difference between measures at the beach versus the hatchery.

Parameter	Random effect (Nest)		No Random Effect	
	Estimate	SE	Estimate	SE
Intercept	13.95**	(0.66)	14.02**	(0.17)
Site	0.94	(0.93)	0.87**	(0.24)
Measurement	0.93**	(0.05)	0.93**	(0.05)
Site x Measurement	0.23**	(0.07)	0.23**	(0.07)

* $p < 0.05$, ** $p < 0.01$

Mean CCLs for the beach and hatchery sites at first and second measurements are shown in Figure 16. CCL was not affected by measurement ($p = 0.291$) or site ($p = 0.332$), when nest was included in the model, but showed a significant effect of site ($p < 0.001$) when nest was not included (Table 11). Effect sizes for site were fairly small ($r = 0.25$ for the first measurement and $r = 0.22$ for the second).

Locomotion Performance

A summary of the data collected during the locomotion performance experiments is shown in Table 12. For running speed, estimates of fixed effects from the linear mixed model (Table 13) revealed significant differences between the two measurements separated by 24 h ($p < 0.001$). Site effects were not detected if nest was included as random effect in the model, although the result approached significance ($p = 0.065$), with a fairly small effect size ($r = 0.22$) for the first measurement and a moderate effect size ($r = 0.31$) for the second measurement. Running speed was significantly different between the beach and the hatchery hatchlings when the nest effect was not included ($p = 0.018$) (Table 13).

Regarding swimming style measurements, the number of power strokes per minute was significantly affected by both measurement ($p = 0.034$) and site ($p = 0.031$), even when the nest effect was included in the model (Table 14). Water temperature was correlated with dogpaddling time per minute ($r = 0.244$, $p = 0.02$): thus, we entered water temperature as a covariate in the linear mixed model. When nest was included as a random factor, significant differences were found for measurement ($p = 0.042$) but not for site ($p = 0.206$). Conversely, when nest was not included, site was found to significantly affect dogpaddling time per minute ($p = 0.044$), but measurement was not significant ($p = 0.061$) (Table 15).

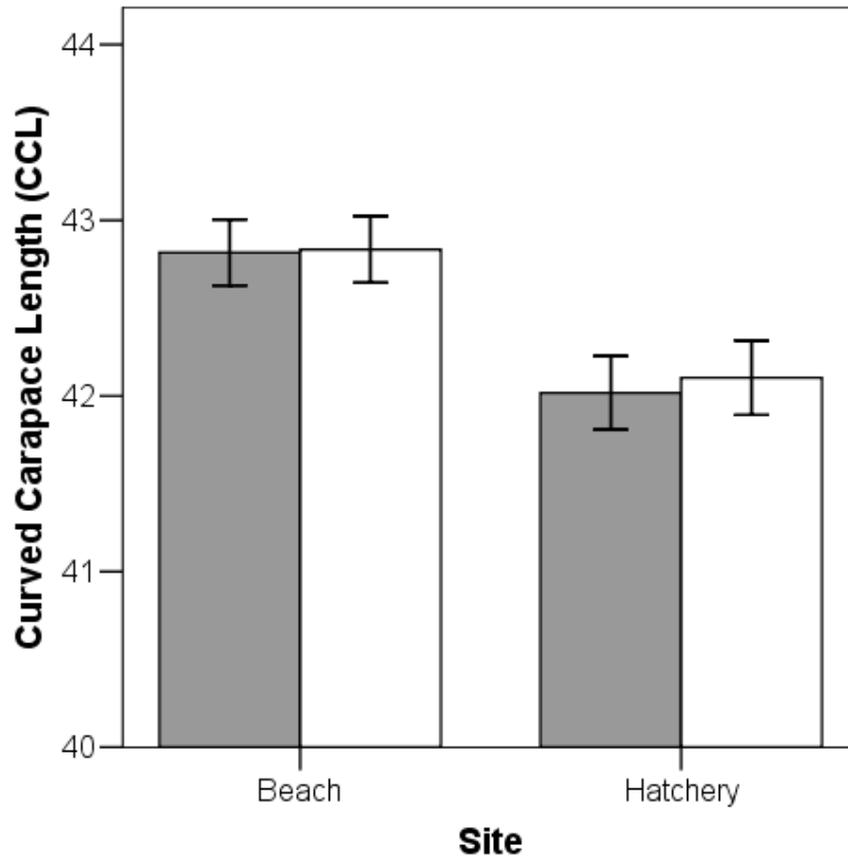


Figure 16. Mean CCL (mm) for first and second measurement at the two experimental sites. First measurement is shown in grey, second measurement (24 hours later) is showed in white. Error bars represent ± 1 SE.

Table 11. Fixed effects for two models predicting hatchling curved carapace length (CCL) (mm).). Intercept indicates estimated mean CCL, site indicates estimated difference between the two sites (beach and hatchery), and measurement indicates estimated difference between the two measurements (emergence and 24 h later).

Parameter	Random effect (Nest)		No Random Effect	
	Estimate	SE	Estimate	SE
Intercept	42.04**	(0.54)	42.09**	(0.20)
Site	0.81	(0.77)	0.76**	(0.28)
Measurement	-0.05	(0.48)	-0.05	(0.05)

* $p < 0.05$, ** $p < 0.01$

Table 12. Locomotion performance results. Means and SE for running speed (RunSpeed), number of power strokes (PS) per minute, and time per minute swimming dogpaddling style (DPTIME), are shown for hatchlings from the two experimental sites.

	1st measurement			2nd measurement (after 24hours)		
	RunSpeed (cm/s)	Number PS per min	DPTIME per min (s)	RunSpeed (cm/s)	Number PS per min	DPTIME per min (s)
Beach	1.48 ± 0.11	38.9 ± 5.5	19.2 ± 1.7	1.06 ± 0.13	31.7 ± 4.8	13.5 ± 1.3
Hatchery	1.23 ± 0.15	25.7 ± 3.1	14.4 ± 1.1	0.68 ± 0.11	20.4 ± 3.8	13.4 ± 1.5

Table 13. Fixed effects for two models predicting hatchling running speed (cm/s). Intercept indicates estimated mean running speed, site indicates estimated difference between the two sites (beach and hatchery) and measurement indicates estimated difference between the two measurements (emergence and 24 h later).

Parameter	Random effect (Nest)		No Random Effect	
	Estimate	SE	Estimate	SE
Intercept	0.71**	(0.12)	0.71**	(0.11)
Site	0.32 [†]	(0.14)	0.32*	(0.13)
Measurement	0.49**	(0.12)	0.49**	(0.13)

[†] $p < 0.1$, * $p < 0.05$, ** $p < 0.01$

Table 14. Fixed effects for model predicting hatchling number of power strokes per minute. Intercept indicates estimated mean number of power strokes, site indicates estimated difference between the two sites (beach and hatchery), and measurement indicates estimated difference between the two measurements (emergence and 24 h later).

Parameter	Random effect (Nest)	
	Estimate	SE
Intercept	19.90**	(4.14)
Site	12.26*	(5.49)
Measurement	6.27*	(2.87)

* $p < 0.05$, ** $p < 0.01$

Table 15. Fixed effects for two models predicting time hatchlings spent swimming dogpaddling style per minute (s). Intercept indicates estimated mean dogpaddling time, site indicates estimated difference between the two sites (beach and hatchery), and measurement indicates estimated difference between the two measurements (emergence and 24 h later).

Parameter	Random effect (Nest)		No Random Effect	
	Estimate	SE	Estimate	SE
Intercept	12.46**	(1.59)	12.57**	(1.27)
Site	3.01	(2.07)	3.23*	(1.56)
Measurement	2.84*	(1.36)	2.62 [†]	(1.36)

[†] p < 0.1, * p < 0.05, ** p < 0.01

Effect size for the difference between sites was moderate for the first measurement ($r = 0.33$) but negligible for the second measurement ($r = 0.00$).

Discussion

Thermal Regimes and Hatching Success

This study has demonstrated that, under the current conditions of hatchery management in Punta Ratón, Honduras, olive ridley nests in the hatchery incubated at higher temperatures, especially during the second third of incubation ($34.2\text{ }^{\circ}\text{C}$ vs $33.0\text{ }^{\circ}\text{C}$), experienced higher metabolic heating ($1.9\text{ }^{\circ}\text{C}$ vs $0.6\text{ }^{\circ}\text{C}$), and showed lower hatching success (24.08% vs 83.22%) than semi-natural nests buried by female turtles on the upper part of the beach and kept *in situ*.

The high incubation temperatures observed at the hatchery were likely the result of metabolic heating in individual nests affecting neighboring nests due to the close proximity of nests to each other. Localized increases in temperature caused by hatchery nests placed less than 1 m apart have been previously documented in olive ridley nests from Alas Purwo, Indonesia (Maulany et al. 2012).

Metabolic heating is produced by the metabolism of the embryos within the eggs and has been reported to affect nest temperatures mostly during the last third of incubation (Godley et al. 2001; Hewavisenthi and Parmenter 2002), although several studies also recorded metabolic heating during the second third (Broderick et al. 2001; DeGregorio and Williard 2011; Damazo 2014). In our beach nests, we detected moderately increased nest temperatures relative to sand temperatures ($+ 0.5\text{ }^{\circ}\text{C}$) during the second third, and a higher increase ($+ 1.4\text{ }^{\circ}\text{C}$) during the last third of incubation, corresponding to the common pattern found in previous studies on different species of sea turtles (Howard et al. 2014). In

our hatchery nests, however, we found significant metabolic heating starting in the first third (+ 0.5 °C), progressively increasing to 2.0 and 3.2 °C for the second and last thirds, respectively. The fact that we detected increased temperatures in the first third of incubation suggests that these nests were likely being affected by other nearby nests with embryos in a more advanced state of development. The high values obtained for these nests during second and last thirds also suggest a cumulative effect during incubation that likely caused the extremely high incubation temperatures observed in the hatchery.

High incubation temperatures are known to affect hatching success. Sea turtle embryos develop within a specific temperature range, and excessively high temperatures may have lethal effects on them, reducing hatching success or even causing complete clutch failure (Valverde *et al.* 2010). Several studies have estimated thermal tolerances of developing sea turtle embryos and proposed the upper limit between 33 – 35 °C (Ackerman 1997; Miller 1997). However, many examples exist of successful hatching after experiencing maximum temperatures over 35 °C, for greens (Broderick *et al.* 2001), loggerheads (Matsuzawa *et al.* 2002; Read *et al.* 2013), flatbacks (*Natator depressus*) (Hewavisenthi *et al.* 2002), leatherbacks (Wallace *et al.* 2004), hawksbills (*Eretmochelys imbricata*) (Dobbs *et al.* 2010) and olive ridleys (Valverde *et al.* 2010). Mean temperatures over the entire incubation period have been shown to be better estimates for assessing thermal embryonic tolerances than maximum incubation temperatures (Valverde *et al.* 2010; Howard *et al.* 2014). In a study on Costa Rican olive ridleys, Valverde *et al.* (2010) found that mean incubation temperatures over 35 °C caused clutch failure, although embryos survived sporadic temperatures higher than 37 °C and still hatched. Furthermore, Valverde *et al.* (2010) in Costa Rica, and Maulany *et al.* (2012b) in Indonesia reported that

the number of days with incubation temperatures over a specific threshold (35 °C for the Costa Rican population and 34 °C for the Indonesian population) affected hatching success negatively. In our study, although none of our nests experienced mean incubation temperatures higher than 35 °C, we also observed that the nests with the highest mean temperatures and which experienced more days above 35 °C were those with lower hatching success. Valverde's study (Valverde *et al.* 2010) was conducted during the dry season and the vast majority of nests (86.5 %) were lost due to the high temperatures. Only five nests, with mean incubation temperatures below 35 °C, yielded live hatchlings, but their hatching success was lower than what we observed in our beach nests for similar mean temperatures. For mean temperatures between 33.1 and 33.7 °C, Valverde's nests showed hatching successes from 5.3 to 34 %, whereas in the current study, hatching success for beach nests with similar mean temperatures ranged from 77.8 to 93.3 %. Our hatchery nests, however, showed hatching success similar to Valverde's (2.8 – 50.0 %). These differences suggest that mean temperature alone may not be the best predictor of hatching success. In our case, we found that mean temperature during the second third of incubation had a higher influence on hatching success than the overall mean incubation temperature. Nests H93 and H94 showed MeanT2 around 35 °C, and their hatching success was very low. Most embryos likely died during this second third due to high incubation temperatures and thus, because they did not continue contributing to metabolic heating, these nests did not experience typical temperature increases during the last third of the incubation.

Experiencing high nest temperatures just before hatching is common for several sea turtle species. Binkley (1998) reported that leatherback nests incubated at a constant

temperature of 33 °C yielded 0% hatching success, but also that nests may exceed 38 °C towards the end of the incubation and still produce viable hatchlings. This and similar reports for greens (Ischer *et al.* 2009) and flatbacks (Hewavisenthi *et al.* 2002) suggest that thermal tolerances may change as the embryo develops, with embryos in the first and second thirds of the incubation period being more sensitive to high temperatures than in the third (Howard *et al.* 2014). Maulany *et al.* (2012b) found that olive ridley hatchlings are clearly more tolerant to high temperatures during the last third of incubation. Our results support this idea, and highlight the importance of the second third. According to our study, although MeanT3 was close to or above 35 °C for several nests, it was not correlated with hatching success, whereas MeanT2 was highly correlated, and was the best predictor for hatching success.

Alterations of natural thermal regimes, especially during the second third of incubation, are of concern not only regarding thermal tolerances but also because they impact sex ratios. In sea turtles, sex is determined by the temperatures experienced by the developing embryos during the second third of the incubation (Merchant-Larios *et al.* 1989). The incubation temperature that produces 50% males and 50% females is called the “pivotal temperature”. Cooler temperatures will produce a higher proportion of males and warmer temperatures will produce a higher proportion of females. Pivotal temperatures for sea turtles range between 27.7 °C and 31°C, and vary not only among sea turtle species, but also among populations of the same species (Wibbels 2007). The pivotal temperature for the Honduran olive ridley population has not been assessed, but if we use the reported pivotal temperature for this species in Pacific Costa Rica (30.5 – 31 °C) (Wibbels *et al.* 1998) to calculate sex ratios for Punta Ratón during the 2013 nesting season, we may

conclude that all hatchlings, both at the hatchery and at the beach, would be females. Mean temperatures during the second third of the incubation were several degrees higher than 31 °C for all nests in both sites, implying 100 % females, unless the pivotal temperature in Punta Ratón is actually higher than in Costa Rica. Significant differences in pivotal temperatures are known among different olive ridley populations. The pivotal temperature for the Indian population at Gahirmatha is less than 29 °C (Dimond and Mohanty-Hejmadi 1983), at least 1.5 degrees lower than the pivotal temperature at Nancite beach in Costa Rica. Incubation durations also differ between these two populations, so that the incubation period is close to 60 days in India (Silas *et al.* 1985; Subba Rao and Raja Sekhar 1997), yet ranges between 46 and 60 days in Costa Rica (Clusella Trullas and Paladino 2007; Honarvar *et al.* 2008; Valverde *et al.* 2010). The incubation period at Punta Raton lasts approximately 45 days, and appears to be one of the shortest durations reported in the literature. Incubation durations are known to be inversely related with incubation temperatures (Mrosovsky *et al.* 1999). Therefore, the Honduran olive ridley population may be experiencing among the highest incubation temperatures for the species. If olive ridleys in Honduras are naturally adapted to higher incubation temperatures than other populations, they may likewise have a higher pivotal temperature, although determining this was beyond the scope of the current study. In any case, our results suggest that current incubation temperatures in Punta Ratón are highly female-biased and close to the upper tolerance limit for the population.

Body Condition and Locomotion Performance

Although statistically significant differences were not demonstrated in all cases, our results suggest that nests at the hatchery produced smaller and less fit hatchlings than the

nests at the beach. Hatchlings from the beach averaged 0.9 (6.5 %) g larger, ran 0.3 cm/s (42 %) faster, and swam using 12 (61%) more power strokes per minute than hatchlings from the hatchery. Although actual values of the differences were high, statistical significance was not detected when we included nest as a random factor, most likely due to our low sample size (only four nests in each site) and to high variances among the hatchery nests compared with nests from the beach. To illustrate this, mean body mass values for hatchlings from beach nests only ranged from 15.1 g \pm 0.1 SE to 16.6 g \pm 0.2 SE, whereas hatchery nests had quite different mean hatchling body masses. Hatchlings from nest H108, for example, were very small (12.7 g \pm 0.2 SE) yet hatchlings from nest H26 were large, similar to the largest ones from the beach (16.5 g \pm 0.1 SE) (Fig 15B). Higher incubation temperatures are known to produce smaller hatchlings (Booth *et al.* 2004; Burgess *et al.* 2006; Booth and Evans 2011), and if excessively high, may reduce locomotion performance. In a study on hatchling greens from Heron Island, Australia, Booth and Evans (2011) found that hatchlings incubated at higher temperatures had lower size index (SCL·SCW) scores and produced less thrust per power stroke than hatchlings incubated at lower temperatures. Although relocating nests to a hatchery may have negative effects independent of temperature (Pintus *et al.* 2009), in our case the observed effects on hatchling size, running speed, and swimming performance were more likely a result of the high incubation temperatures experienced by nests in the hatchery. We suggest that these excessively high temperatures were a consequence of the metabolic heating of nests affecting near neighbor nests. According to this hypothesis, the temperatures at each nest would have differed depending on the specific position of the nest, how many nests were nearby, the state of development of embryos in those nests, and the distances separating

them. This could explain the data showing large differences in body size and performance among hatchery nests. A correlation analysis between incubation temperature and body mass, or between incubation temperature and locomotion performance for the nests in the hatchery would have been ideal to test this hypothesis, but we could not perform those analyses because two of the four nests with thermo dataloggers in the hatchery yielded insufficient hatchlings.

Retention Time

Results of this study clearly show that retaining hatchlings for 24h after emergence or nest excavation may be detrimental. The effects of retention were similar for hatchlings from both the beach and the hatchery. After 24 h of retention, hatchlings had lost an average of 0.9 g (6.5 % of their body mass), and they ran much slower (0.2 vs 0.7 cm/s), and swam fewer (13.6 vs 19.9 power strokes per minute and 9.5 vs 12.5 s using dogpaddling style) than just after emergence. Negative effects of retention time on running speed, swimming speed, and swimming style have been reported in previous studies, even for much shorter periods of retention. Pilcher and Enderby (2001) registered more than a 12 % decrease in swimming speed after retaining green hatchlings for 6 h, along with a change in swimming style from the more efficient power stroke to the less efficient dogpaddling. In our case, however, both the power stroke number and the dogpaddle time decreased, meaning that, after 24h, hatchlings spent less time actively swimming and more time performing slow flipper movements or not moving at all. In another study by van der Merwe et al. (2013), the running speed of green turtle hatchlings from Malaysia progressively decreased with 1, 3, and 6 h of retention, slowing them to approximately half of their original speed when they were kept for 6 h. In the same study, hatchlings

experienced a significant body mass loss after 3 and 6 h of retention, mostly due to water loss. High dehydration levels likely affect hatchling locomotion performance (van der Merwe *et al.* 2013), slowing down their running and swimming abilities and impairing hatchlings during the most dangerous phase of life (Gyuris 1994; Stewart and Wyneken 2004). Recently emerged hatchlings run as fast as possible from the beach to the surf to avoid terrestrial predators, and once in the water, they begin a period of continuous fast swimming known as *the frenzy*, in order to leave the shallow, predator-rich, coastal waters as quickly as possible (Carr 1962; Salmon and Wyneken 1987; Wyneken and Salmon 1992). Our data indicates that retaining hatchlings for long periods of time after emergence, or removal from the nest, prevents them from optimally performing these natural behaviors, and threatens their survival by extending the time spent on land and in shallow waters.

Conclusion

Sea turtle conservation practices consisting of relocating eggs laid on the beach to hatcheries may have detrimental results for embryos and hatchlings when these hatcheries are not appropriately built and managed. Hatchery nests in this study experienced shared metabolic heating and reached excessively high temperatures during the second third of incubation, likely causing much lower hatching success, as well as smaller and less fit hatchlings than those nests incubated on the beach. We thus recommend nests to be kept *in situ* as much as possible, even if it is necessary to use creative solutions, such as moving the incoming females to safe parts of the beach to nest. In cases where hatcheries are the only viable option, Garcia *et al.* (2003) have shown that appropriate beach management and consistent hatchery practices can minimize the detrimental effects of egg relocation.

Careful minimum distances between nests should be established to prevent metabolic heating from affecting near-neighboring nests. Sand temperatures should be controlled periodically and shade or moisture (watering) provided, if necessary. Regarding retention times, hatchlings should not be kept for long periods of time, and should be released as soon as possible after emergence.

Beyond practical applications, this study has provided further information about the thermal characteristics of olive ridley incubation. We have confirmed that metabolic heating affects nest temperatures during the second third of the incubation, and thus it could potentially impact sex ratios in some populations. We also found that the mean temperature reached during the second third of the incubation is a good predictor of hatchling success. Further research is needed to accurately assess embryological thermal tolerances and pivotal temperature for this population, which may be adapted to higher incubation temperatures than other olive ridley populations.

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CHAPTER FIVE
DIFFERENCES IN DIURNAL AND NOCTURNAL
SWIMMING PATTERNS OF OLIVE RIDLEY HATCHLINGS
IN THE GULF OF FONSECA, HONDURAS

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Abstract

Sea turtle hatchlings from Honduran beaches along the Pacific coast must swim more than 30 km through the shallow, presumably predator-rich waters of the Gulf of Fonseca before reaching the open ocean. We tracked 25 olive ridley hatchlings from Punta Ratón, Honduras, during the first two hours of their offshore migration to assess aquatic predation rates, and observed no predation events. The absence of rocky bottom areas and reef structures where predators can find refuge, and a decline in the number of predators due to overfishing are two possible reasons for this unexpected result. We also compared diurnal and nocturnal swimming patterns of recently emerged olive ridley hatchlings with regards to their position in the water column while swimming. We discovered that hatchlings at night swam near the surface more than 97% of the time, with only sporadic brief dives. During daytime, however, hatchlings spent at least 78% of the time swimming at depth, going back to the surface for brief periods to breathe. Due to the high turbidity of the Gulf of Fonseca waters, this daytime behavior may serve to keep hatchlings out of sight of predatory seabirds. We propose that this newly described differential swimming behavior has adaptive significance in avoiding aerial predation in the specific conditions of the Gulf of Fonseca.

Keywords: *Lepidochelys olivacea*, sea turtle, predation, anti-predatory behavior, off-shore migration, swimming patterns

Introduction

In the hours after they enter the water and swim offshore, sea turtle hatchlings can suffer high mortality from fish and avian predators (Burger and Gochfeld, 2014; Frick, 1976; Gyuris, 1994; Pilcher et al., 2000). They are therefore generally believed to swim offshore as quickly as possible (Whelan and Wyneken, 2007) and then, once in deeper offshore water, they drift passively (Bolten and Balazs, 1995; Carr, 1987). Several studies have quantified the rate of aquatic predation suffered by sea turtle hatchlings in the nearshore environment (Witherington and Salmon 1992; Gyuris 1994; Wyneken et al. 1997; Pilcher et al. 2000; Stewart and Wyneken 2004; Whelan and Wyneken 2007). Although recorded predation rates vary greatly among sites, from 4.6 % for loggerhead (*Caretta caretta*) hatchlings in South Florida (Whelan and Wyneken 2007) to 85 % for green (*Chelonia mydas*) hatchlings at Heron Island, Australia (Gyuris 1994), most authors identify nearshore waters as highly threatening for hatchling turtles. Taking into account that observation periods of these studies have typically consisted of approximately 10 - 15 minutes, even the lowest observed rates (4.6 % by Whelan and Wyneken, 2007; 5 % by Stewart and Wyneken, 2004; 7 % by Wyneken et al., 1997) are likely to exact heavy losses if hatchlings remain in nearshore environments for long periods of time (Whelan and Wyneken 2007). Several factors, such as depth, bottom-structures, and release protocols have been shown to affect predation risk, which is especially high in shallow (<10 m) waters (Witherington and Salmon 1992; Pilcher et al. 2000), and when hatchlings cross reef structures (Frick 1976; Witherington and Salmon 1992; Gyuris 1994; Pilcher et al. 2000). Because high hatchling densities attract aquatic predators (Wyneken et al. 2000), hatchlings released *en masse* from hatchery sites suffer from 1.5 (Pilcher et al. 2000) to ten times (Wyneken et al. 2000) higher predation rates than those released from natural sites

with a low density of nests. Other factors that may affect predation rates are tidal and moon phases (Gyuris 1994; Harewood and Horrocks 2008), water clarity, coast-specific predator assemblages, and fish movement patterns (Whelan and Wyneken 2007).

Sea turtle hatchlings have no active defenses against predators (Gyuris 1994; Stewart and Wyneken 2004; Whelan and Wyneken 2007), and thus their main options for avoiding predation are fleeing or hiding (Bolles 1970). One general strategy used by green, loggerhead, and leatherback (*Dermochelys coriacea*) hatchlings is to leave predator-rich nearshore waters as quickly as possible by maintaining a vigorous offshore swimming frenzy during the first 24 h (Carr 1962; Salmon and Wyneken 1987; Wyneken and Salmon 1992). In contrast, hawksbill (*Eretmochelys imbricata*) hatchlings do not show a frenzy period of hyperactive swimming. Instead, they swim for only 6 h a day, employing slow, drag-based gaits, and spend most of the time floating motionless in a “tuck” position, helping them remain inconspicuous to predators that use movement for prey detection (Chung et al. 2009; Chung et al. 2009). When facing attack or imminent predation threat, behavioral strategies also differ among species. A series of simulated predation experiments by Mellgren et al (2003) demonstrated that loggerhead and hawksbill hatchlings tended to remain immobile, whereas green hatchlings responded by actively fleeing via vigorous swimming and diving. Field observations by Hasbún (2002) confirmed that hawksbill hatchlings fold their front flippers along the top of the carapace and remain motionless when approached or attacked by fish. Reactions to an aerial predation threat are similar for both green (Frick 1976) and loggerhead hatchlings (Witherington and Salmon 1992), which quickly dive in response to birds overhead.

Besides sporadic dives that usually happen in response to disturbance (Frick 1976; Martin 2003) or flying objects (Frick 1976; Witherington and Salmon 1992; Witherington et al. 1995), sea turtle hatchlings tend to swim near the water surface during their offshore migration, both during nighttime and daylight hours, likely due to their positive buoyancy (Carr 1982; Davenport and Clough 1986). Abe et al (2000) and Frick (1976) followed green hatchlings during daytime and recorded them swimming at 10 and 20 cm depths, respectively. Liew and Chan (1995) tracked leatherback hatchlings with subminiature radiotransmitters for more than 30 consecutive hours, and described them swimming 5 - 10 cm just below the surface. Similar results were reported by Witherington et al. (1995) on loggerhead hatchlings and by Hasbún (2002) on hawksbill hatchlings.

To our knowledge, no previous studies on hatchling offshore swimming, in-water predation, or antipredator behavior have focused on olive ridley (*Lepidochelys olivacea*) sea turtles. In Honduras, this species nests in the eastern end of the Gulf of Fonseca, a shallow inlet of the Pacific Ocean with coast shared by El Salvador and Nicaragua (Fig. 17). Olive ridleys have been partially protected in Honduras since 1975, when the government established a yearly period during which commercial egg collection is forbidden and the eggs are relocated to hatcheries (Minarik 1985).

Currently there are four working hatcheries along the South coast of Honduras located on the beaches at Punta Ratón, El Venado, Boca del Río Viejo, and Cedeño. Punta Ratón (13.26570N, 87.51228W), the field site for this study, is the main nesting beach in the country, with an estimated 400 – 500 nests per season. Hatchlings released from Honduran beaches must swim across more than 30 km of shallow waters before reaching the open sea.

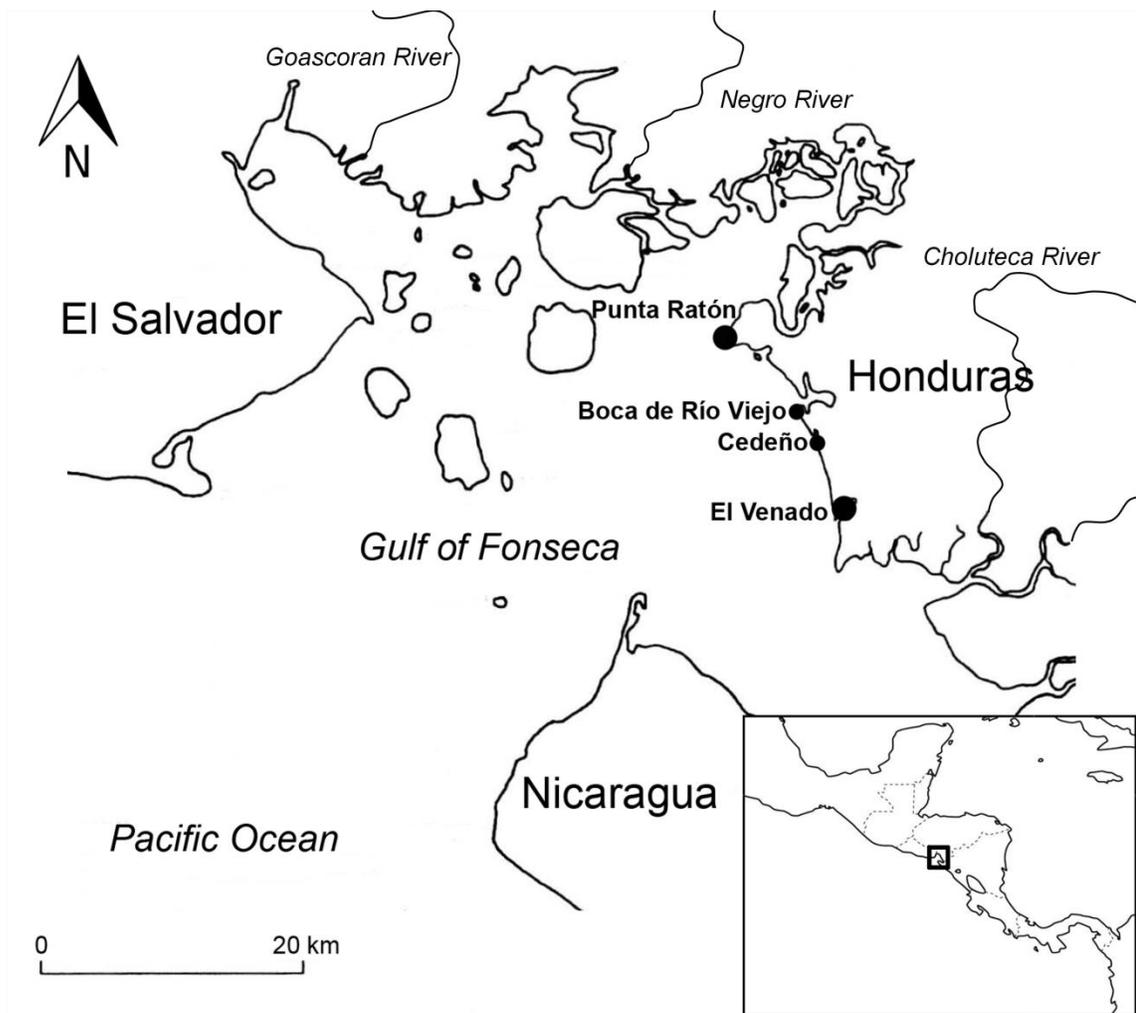


Figure 17. The Gulf of Fonseca. Black circles indicate the four main nesting beaches for *L. olivacea* on the South coast of Honduras. Inset shows a regional view of Central America.

The Gulf of Fonseca is an important fishing area for the country and several genera of fishes known to prey on sea turtle hatchlings – *Caranx* sp., *Haemulon* sp., *Lutjanus* sp., *Epinephelus* sp. (Gyuris 1994; Wyneken et al. 1997; Vose and Shank 2003; Stewart and Wyneken 2004; Whelan and Wyneken 2007) – inhabit its waters (Box and Bonilla 2009). The Gulf of Fonseca is also home to several species of potential avian predators, such as black vultures (*Coragyps atratus*), turkey vultures (*Cathartes aura*), magnificent frigatebirds (*Frigata magnificens*), Caspian terns (*Sterna caspia*), Forster's terns (*Sterna forsteri*), laughing gulls (*Larus atricilla*), and brown pelicans (*Pelecanus occidentalis*) (Gallardo 2014). Black and turkey vultures have been reported feeding on olive ridley hatchlings at the Ostional (Costa Rica) mass nesting beach (Burger and Gochfeld 2014), and frigatebirds, gulls and terns are known to capture hatchlings from the water (Frick 1976; Stancyk 1982; Gyuris 1994; Martin 2003; Burger and Gochfeld 2014).

The original goal of this study was to quantify in-water predation rates suffered by olive ridley hatchlings from Punta Ratón, Honduras, during the first hours after hatchlings are released. Taking into account that the mean depth of the Gulf of Fonseca is 15 m, and the beaches in South Honduras are contiguous with several kilometers of waters less than 5 m deep, we hypothesized high levels of hatchling in-water predation.

Although abundant work has been done on tracking adult and juvenile sea turtles (Godley et al., 2008), and investigating their swimming patterns and diving behavior (Eckert et al., 1989; Hays et al., 2000; Houghton et al., 2008; Minamikawa et al., 1997), little is known about hatchling migration paths and swimming patterns. Laboratory experiments have yielded valuable information on hatchling orientation (Lohmann et al., 2012), and activity patterns during the frenzy swimming (Booth, 2009; Jones et al., 2007),

while particle tracking models have been used to infer hatchling passive drift (Hays et al., 2010). However, direct observation of hatchlings migrating in their natural environment, such in the current study, may disclose previously undetected behavioral traits and their adaptive significance.

The second goal of the study was to describe swimming patterns of the Honduran olive ridley hatchlings with regards to their position in the water column during the first hours of their offshore migration, and to assess potential differences between diurnal and nocturnal swimming patterns. Due to high turbidity of the waters in the Gulf of Fonseca, any animal swimming at depths greater than 25-30 cm cannot be seen from the air, and, therefore we hypothesized that differences in swimming depth between day and night hours may suggest local strategies of predator avoidance.

Methods

Study Site

Punta Ratón (13.26570N, 87.51228W) comprises the main nesting beach for *L.olivacea* in Honduras. This beach is located at the eastern end of the Gulf of Fonseca, a shallow 1,600 km² inlet of the Pacific Ocean, approximately 50 km wide (NE-SW) and 80 km long (NW-SE) (Fig. 17). The mean depth of the gulf is 15 m, and the coastal areas are bounded by several kilometers of waters with depths less 5 m, although some channels of 10 to 12 m exist, allowing navigation by deep sea vessels. In the area near Punta Ratón the bottom is sandy or silty (Vergne et al. 1993), lacking any hard-substrate structures. During the hatching season (October-November), waters are very turbid due to the high river discharge originating from the Choluteca River.

Predation Study

This study was carried out during October and November, 2011. We assessed predation rates using 25 hatchlings from the hatchery at Punta Ratón. Hatchlings were collected from the hatchery as soon as they emerged from nests, and kept in dark containers until nightfall. Hatchlings selected for the experiments were weighed and measured. A “Witherington float” (Witherington and Salmon 1992; Lorne and Salmon 2007; Whelan and Wyneken 2007) (Fig. 18B), consisting of a small black balsa wood boat (71mm long, 16 mm high and 12 mm wide) with an embedded 3.8 cm green glowstick (GlowProducts, BC, Canada, suppliers), was attached to the animal via a 1.5-m sewing thread. The weight of the float in air (4 g) was approximately 25 % of the hatchling weight. These floats may reduce swimming speed by ~15%, but do not attract predators or impede the normal swimming-diving behavior of the hatchlings (Stewart and Wyneken 2004). Following the usual release protocol in the hatchery at Punta Ratón, we conducted most of the observations at night, although we also tracked three hatchlings during daytime at the end of the season. Hatchlings were released directly into the water, 5 - 10 m from the shore. We followed the animals in a small fishing skiff, keeping a distance of at least 10 m to avoid interfering with normal behavior, and recorded GPS positions every 5 minutes. Observations continued for 2 hours unless the animal was predated or lost sight of. We chose this observation period, much longer than the 10 - 15 min period typical of most hatchling predation studies, because in the Gulf of Fonseca hatchlings remain in shallow, presumably predator-rich waters for several hours. After two hours swimming away from the coast at the normal swimming speed of approximately 1.3 km/h (Salmon and Wyneken 1987), water depth was still less than 5 m.

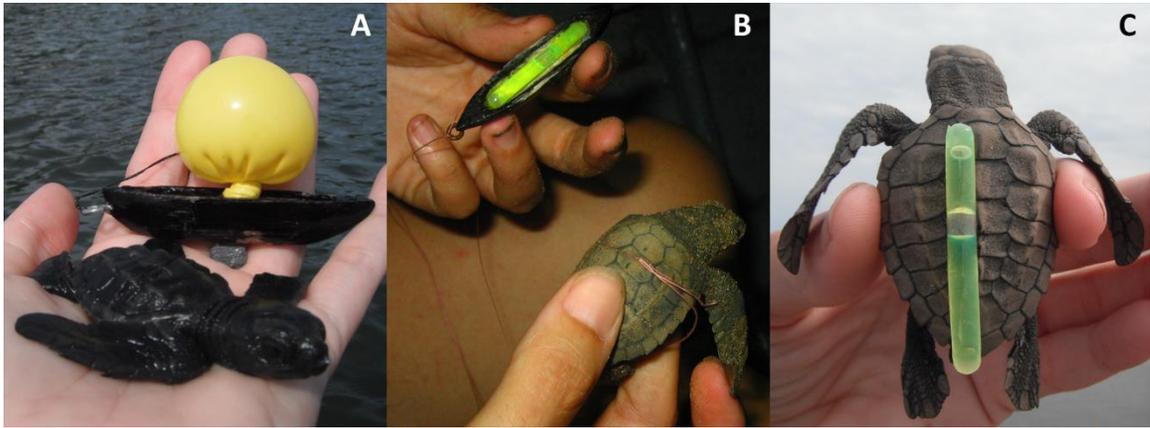


Figure 18. Floats and identification devices. Witherington floats used for (A) diurnal, and (B) nocturnal observations. (C) Hatchling with a glowstick directly attached to its carapace, used during nocturnal and control diurnal observations.

Features used by others to identify predation events were sudden disappearance of the hatchling with submersion of the float, or recording of the float travelling faster than hatchlings are capable of swimming (Whelan and Wyneken 2007). After each set of observations, the hatchling was recaptured, the tether and float removed, and the hatchling re-released into the water. We performed 22 trackings at night, with 11 during decreasing tides and 11 during increasing tides. We also performed 3 trackings during daytime. Due to the shallow depth of the Gulf, tides are extreme, and large sandy areas become exposed during low tide periods. We thus avoided starting trackings at low tide.

Swimming Pattern Study

This study was undertaken during the months of October and November of 2012 and 2013. In 2012, we investigated the diurnal and nocturnal swimming patterns of 32 hatchlings through direct observations during their offshore migrations. We collected hatchlings from the hatchery at Punta Ratón as they emerged from the nests and kept them in dark containers until starting observations. The maximum retention time was 12 h. After measuring and weighing the treatment animals, a Witherington float was attached to each via a 1.5 m sewing thread. These floats were identical to the floats used for the 2011 predation study, yet we replaced the glowstick with a small yellow balloon during diurnal observations (Fig 18A). We released the hatchlings directly into the water 50 m from the shore, and followed them in a small fishing skiff, keeping a distance of 3-5 m. After a 5-minute acclimation period, we began observing the hatchlings for 25 minutes and recorded the time the hatchling swam near the surface and at depth. We considered time swimming “near the surface” when the animal was swimming at the surface or just 10-20 cm below it. Although water visibility was low, hatchlings were clearly visible from the boat when they

were swimming near the surface during the day, down to approximately 25-30 cm deep. We considered time swimming “at depth” when we could not see the animal from the surface. Although we did not measure the exact position of the animals when they were swimming at depth, we could see the upper part of the 1.5 m thread connecting the turtle to the float, which remained tight and almost perpendicular to the surface. We thus estimated that hatchlings swimming at depth were located between 1 and 1.5 m deep. To see the position of the hatchlings during night observations we attached a 3.8 cm green glowstick directly to the carapace of the hatchling (Fig. 18C). To assess if this glowstick affected hatchling swimming behavior, we also attached exhausted glowsticks to the carapace of a group of hatchlings during daytime observations. We thus had three experimental groups: 13 hatchlings observed during the day with no attached glowsticks, 11 hatchlings observed at night with glowsticks attached to their carapaces, and a control group of 8 hatchlings observed during the day with attached glowsticks. Glowsticks were removed at the end of all observations and hatchlings were released directly into the water.

In 2013, we conducted a repeated measures experiment using seven hatchlings from the hatchery at Punta Ratón. Taking advantage of a study that involved following hatchlings during 12 h to assess the influence of tidal currents on their offshore migration movement (Duran and Dunbar, *In prep*; Duran and Dunbar, *In review*), we recorded the swimming pattern of the same individuals during both day and night. The methods for this study were the same as in the 2012 study. The animals were attached to a Witherington float and had a glowstick attached to their carapaces during both observation periods. Each observation period was for 10 minutes, and we recorded time near the surface and time at

depth. The first observation was done in the first hour of the tracking, and the second one between 3 and 6 hours later. In all but one case, night observations were done first.

Data Analysis

For the predation study, we plotted hatchling tracks in a geographic information system (GIS), superimposed them on a map containing bathymetric data for the Gulf of Fonseca, and calculated the depths for the observation area. Because we did not observe any predation events, no calculations or further analysis were done on the frequency of predation.

For the swimming pattern study we calculated the mean percent of time near the surface and compared it among groups. Because our data were not normally distributed even after several transformations, we analyzed them with non-parametric tests. In the 2012 study with independent samples, we applied a Kruskal-Wallis analysis of variance to compare the percent time near the surface for the three experimental groups. We made post-hoc comparisons of groups using Mann-Whitney *U* tests with Bonferroni corrections to find which group percent differed significantly. In the 2013 repeated measures experiment, we used a Wilcoxon signed-rank test to compare day and night percent time near the surface. We set α level at 0.05 for all tests.

Results

Predation Study

We collected a total of 461 data points on hatchling positions, 90.02% (415) of which were in waters less than 5 meters in depth and 79.18% (365) of which were in waters of less than 2 meters in depth. Twenty-nine observation points (6.29%) were located in

waters between 5 and 10 meters in depth and only 17 (3.69%) were in areas deeper than 10 meters (Fig. 19). Hatchling trajectories during experimental observations were mostly parallel to the coast.

Mean weight for the 22 hatchlings tracked at night was $16.1 \text{ g} \pm 0.3 \text{ SE}$ (range 13 – 18 g), and the average curved carapace length (CCL) was $44.1 \text{ mm} \pm 0.3 \text{ SE}$ (range 40.0 – 46.0 mm) (Table 16). Observation times ranged from 18 to 133 minutes, with an average of $91.8 \text{ min} \pm 8.2 \text{ SE}$. We did not observe any predation events during this study. Thirteen animals completed the 2-hour observation period, and were released at the end. The remaining nine hatchlings were lost during the trial when the thread detached from the turtle or broke from the float due to friction. Still, we saw no evidence of predation in any of these cases, such as floats quickly pulled or suddenly submerged.

The three hatchlings followed during daytime had weights ranging from 16 to 17 g and CCLs ranging from 43 to 46 mm. These hatchlings were tracked for 120, 128 and 114 minutes, respectively, and none of them was predated. However, we observed a different swimming behavior in these three hatchlings compared to those followed at night. During the night, the animals swam just beneath the surface, with a few sporadic dives, whereas the hatchlings followed during the daytime spent most of the time swimming at depth, and went up to the surface only for short periods to breathe. Because our sample size ($N=3$) was too small to draw valid conclusions, we considered these as preliminary results and did not include data from these animals in the statistical calculations for this study. We decided to broaden the goals of our predation study and to investigate the diurnal and nocturnal swimming patterns of the Honduran hatchlings during the subsequent seasons.

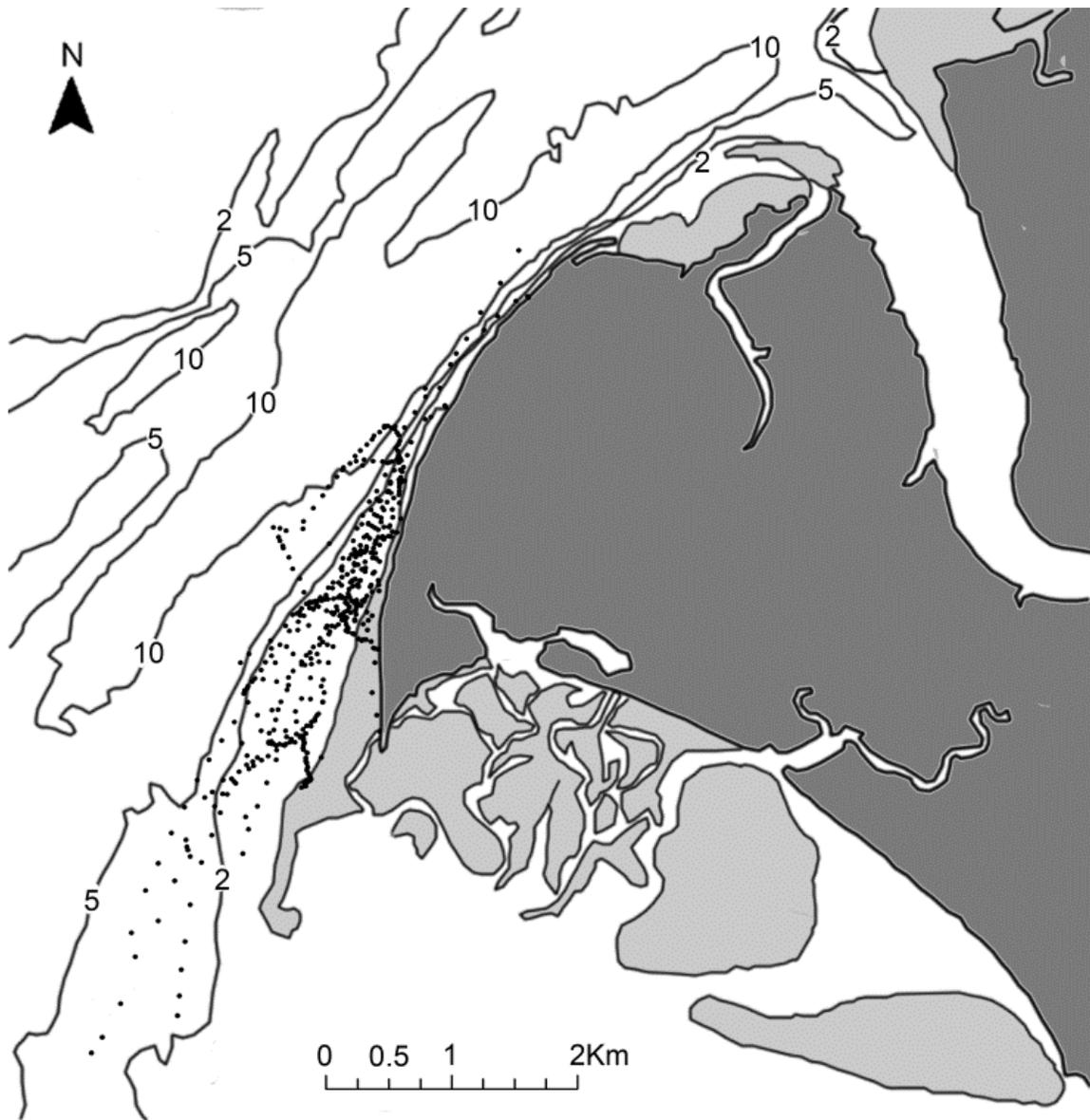


Figure 19. Location of the observations for the predation study. Dots represent the 461 individual observation points from the 22 hatchlings tracked at night. Land area is colored dark grey, light grey zones represent very shallow areas exposed during low tide. White areas represent water, with isobaths indicating depths in meters.

Table 16. Overview of hatchling measurements and results for all three studies

Study	Mean Weight (g)	Mean CCL (mm)	Mean % surface time DAY	Mean % surface time NIGHT
Night predation study (N=22)	16.1 ± 0.3	44.1 ± 0.3	---	---
Independent samples swimming study (N=32)	15.9 ± 0.3	44.7 ± 0.3	18.29 ± 6.59 ^a 14.14 ± 2.03 ^b	99.3 ± 0.53 ^c
Repeated measures swimming study (N=7)	15.3 ± 0.6	43.7 ± 0.3	21.85 ± 8.01	97.54 ± 2.46

^a Hatchlings with no glowstick attached to their carapaces (N=13)

^b Control hatchlings with glowstick attached to their carapaces (N=11)

^c Hatchlings observed at night (N=8)

Swimming Pattern Study

The 32 hatchlings used for the first part of the study (2012) had an average weight of $15.9 \text{ g} \pm 0.3 \text{ SE}$, and an average CCL of $44.7 \text{ mm} \pm 0.3 \text{ SE}$ (Table 16). The percentage of time spent swimming near the surface differed significantly among the three experimental groups (Kruskal-Wallis test: $H = 21.10$, $df = 2$, $p < 0.001$) (Fig. 20, Table 16). Post hoc Mann-Whitney U tests results indicated no significant difference between the two groups of hatchlings followed during the day (without and with glowsticks attached to their carapaces), with average times swimming at the surface of $18.29 \% \pm 6.59 \text{ SE}$ and $14.14 \% \pm 2.03 \text{ SE}$, respectively (Mann-Whitney $U = 51.0$, $p = 0.942$, Fig. 20). However, hatchlings swimming during the night spent much more time near the surface (average $99.37\% \pm 0.53 \text{ SE}$) than both groups observed swimming during the day (Mann-Whitney $U = 1.0$, $p < 0.001$, for comparison of hatchlings without glowstick and $U = 0.0$, $p < 0.001$, for comparison of hatchlings with attached glowstick, Fig. 20). Although the typical pattern for diurnal swimming was long periods of deep swimming separated by short periods at the surface (Fig. 21A), one hatchling (#25, Figs. 20 and 21B) observed during the day remained near the surface for 1420 out of 1500 seconds (95%). Because there was no difference between the two groups observed during the day, we pooled both groups (with and without glowstick) and found that the average duration of the dives for each hatchling ranged from 20 s to 140.44 s, with a mean of $63.45 \text{ s} \pm 5.59 \text{ SE}$. The longest recorded dive lasted 221 s. During the night, 9 out of 11 hatchlings (81.8 %) swam near the surface during the whole observation time (1500 sec) (Fig. 22A). For the remaining two hatchlings, the average dive duration during the night was $1.75 \text{ s} \pm 1.18 \text{ SE}$ and the longest dive lasted 19 s (Fig. 22B).

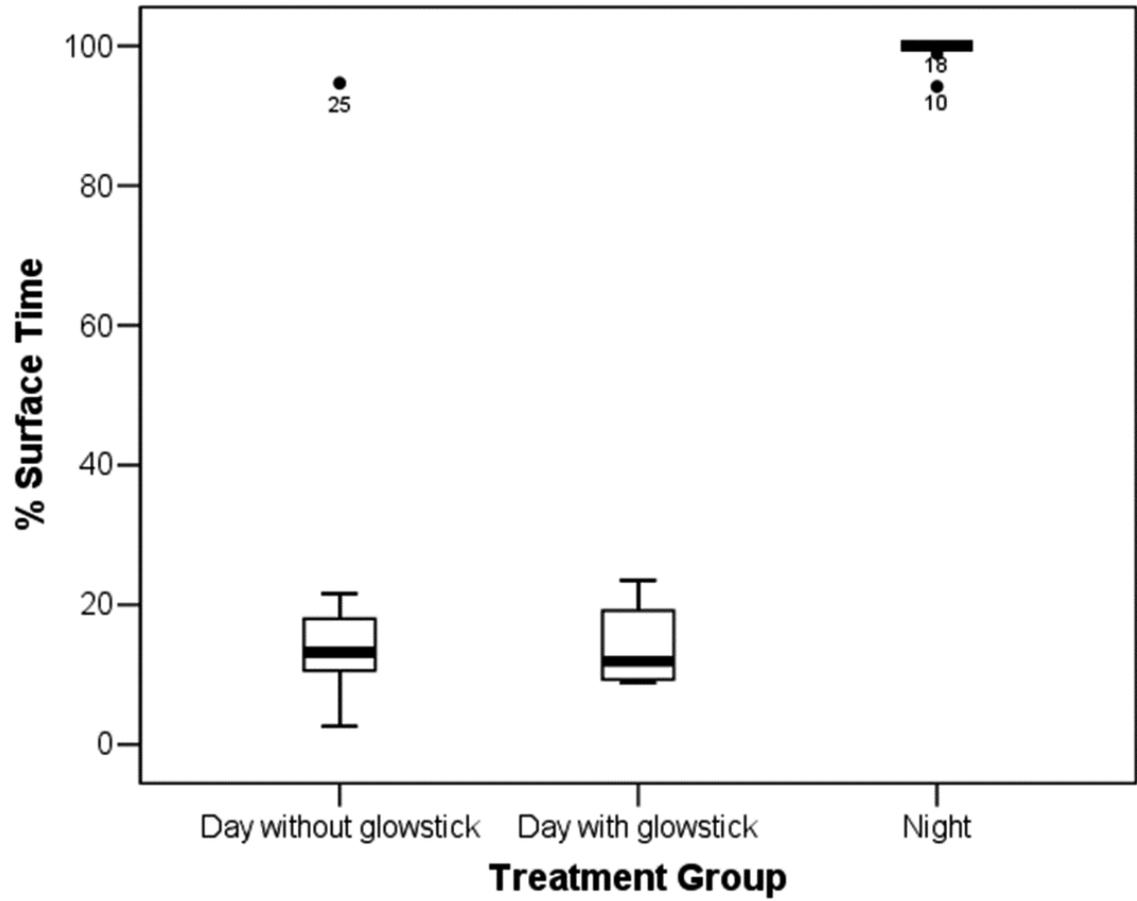


Figure 20. Box and whisker plots comparing the percent time swimming near the surface by the three experimental groups.

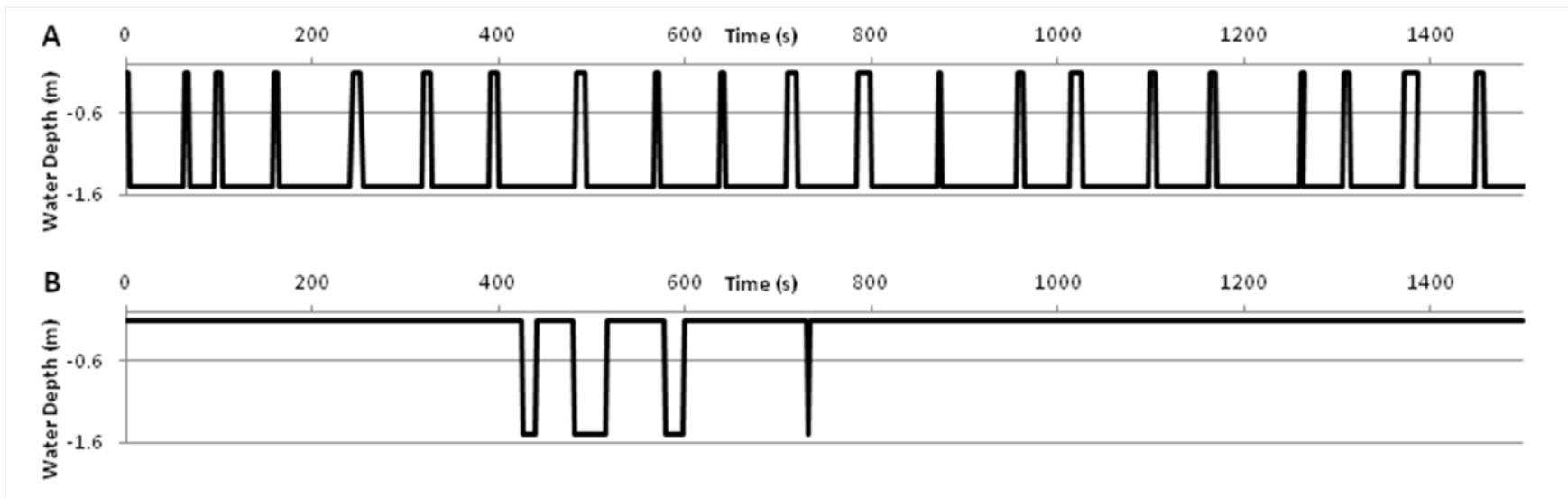


Figure 21. Diurnal swimming profiles. A shows the swimming profile of hatchling H12, representative of the typical diurnal swimming profile for olive ridley hatchlings at Punta Ratón, B is an anomalous behavior shown by only one hatchling (H25), likely due to low energy reserves or disorientation. X axis represents time in seconds and Y axis represents water depth in meters. The upper horizontal line represents the water surface. Solid black lines represent the time each of the hatchlings spent swimming close to the surface and at depth.

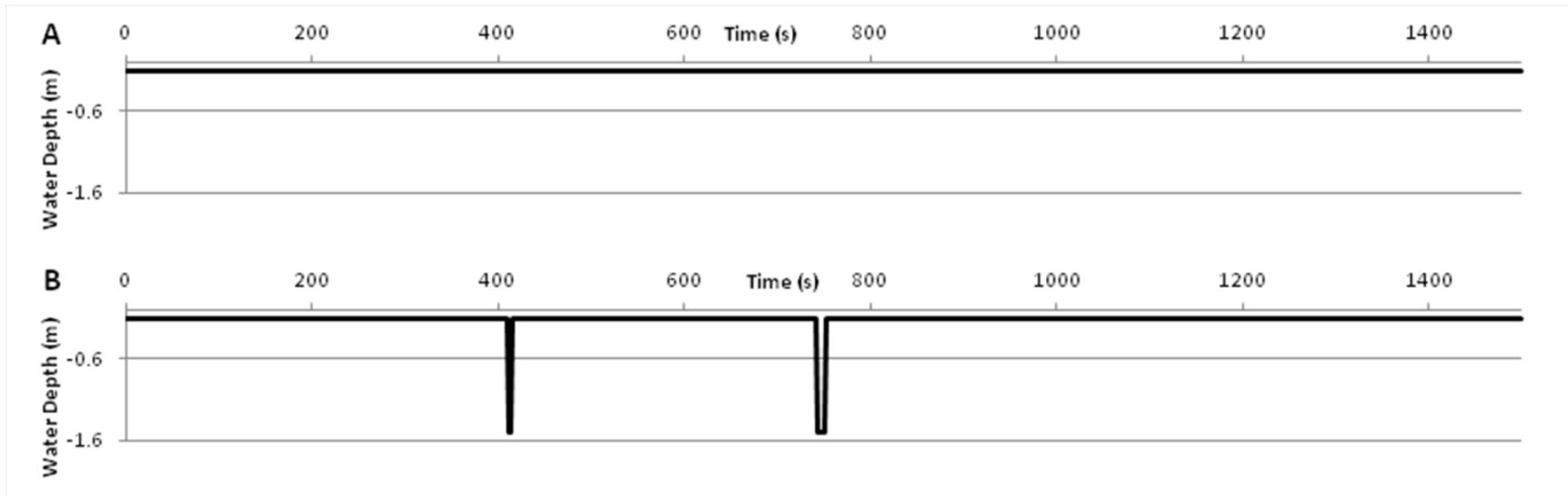


Figure 22. Nocturnal swimming profiles. A shows the most common profile, displayed by 9 out of 11 hatchlings, which remained near the surface the entire observation time. B shows hatchling H18, which performed a few short dives. X axis represents time in seconds and Y axis represents water depth in meters. The upper horizontal line represents the water surface. Solid black lines represent the time each of the hatchlings spent swimming close to the surface and at depth.

The seven hatchlings used for the repeated measures experiment (2013) weighed an average of $15.3 \text{ g} \pm 0.6 \text{ SE}$ and had an average CCL of $43.7 \text{ mm} \pm 0.3 \text{ SE}$ (Table 16). Time between the two observations of each hatchling ranged between 3.00 and 5.97 hours, with a mean of $5.07 \text{ h} \pm 0.40 \text{ SE}$. Results of the Wilcoxon signed-rank test showed that hatchlings spent significantly more time swimming near the surface during the night ($97.54 \% \pm 2.46 \text{ SE}$) than during the day ($21.85\% \pm 8.01 \text{ SE}$) ($Z=-2.37$, $p=0.018$) (Table 16). These hatchlings exhibited diurnal and nocturnal swimming patterns similar to those shown by the hatchlings used in the independent samples 2012 experiments (Fig. 23).

Regarding predation by birds, while we did not recorded predation events or bird attacks on hatchlings during the 22.3 hours of diurnal experiments (total for both studies), we did observe laughing gulls attacking and capturing hatchlings from the water twice, when groups of 4-6 hatchlings were released at the end of the daily experiments. These hatchlings remained at the water surface, motionless or dog-paddling for several minutes, likely making themselves more conspicuous to the birds. On one occasion we observed a laughing gull taking one of the hatchlings, which fell from its beak a few seconds later, but was not recaptured. On another occasion, several laughing gulls and terns attacked the group of hatchlings, and at least one was taken by a gull.

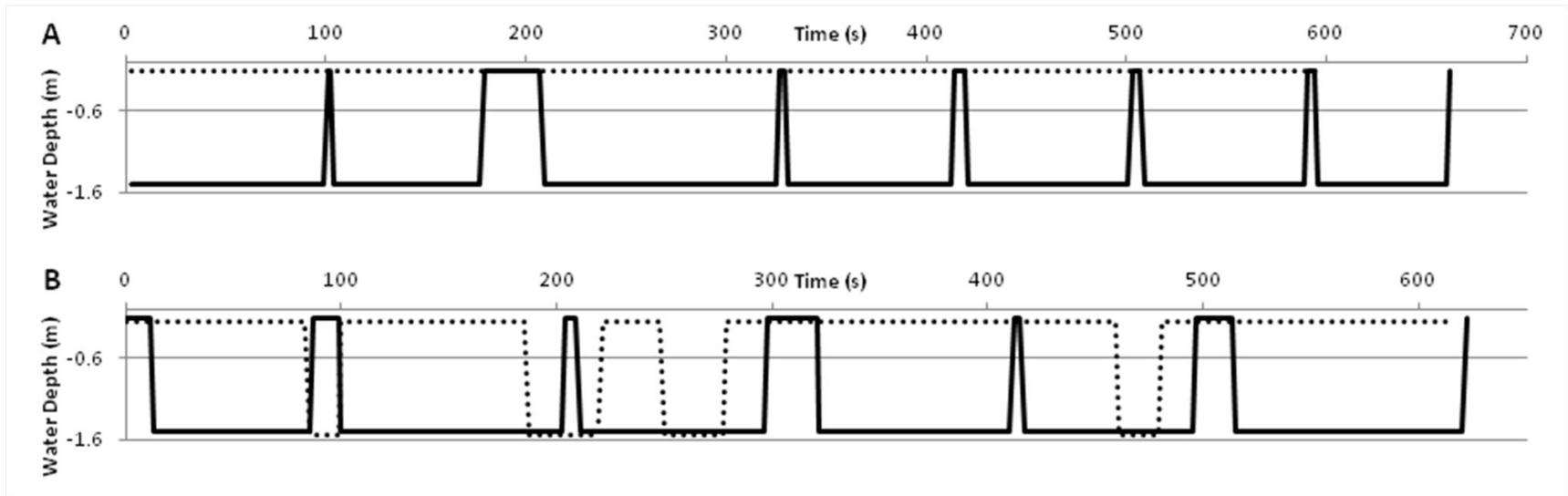


Figure 23. Example of nocturnal and diurnal profiles of hatchlings H107 (A) and H 105 (B) during the repeated measures experiment. X axis represents time in seconds and Y axis represents water depth in meters. The upper horizontal line represents the water surface. Solid black lines represent diurnal profiles, while dotted lines represent nocturnal profiles.

Discussion

The absence of predation by fish during the initial phase of the offshore migration of olive ridley hatchlings in the Gulf of Fonseca diverges from the findings of previous studies on other sea turtle species in different regions, which always found predation, although in variable rates (60 - 76 % in Gyuris (1994); 40.7 – 61.9 % in Pilcher et al. (2000); 5 % in Stewart and Wyneken (2004); 1 – 9 % in Whelan and Wyneken (2007); 6.8 % in Witherington and Salmon (1992); and 7 – 34 % in Wyneken et al. (1997)). Due to the shallow depth of the Gulf waters, even as far as several kilometers away from the beach, we originally expected high losses from in-water predation, based on calculations from Whelan and Wyneken (2007). However, none of the 25 hatchlings in the predation study were taken by fish during the experiments. Likewise none of the 39 hatchlings in the swimming pattern study were predated over the period of our observations.

Although our sample sizes are lower those in other predation studies, our observation time (2 hours) exceeded most (10 min by Gyuris, 1994; 15 minutes by Wyneken and Salmon, 1997, Stewart and Wyneken, 2004, and Whelan and Wyneken, 2007). The total observation time in the current study was 33 hours for the predation study and 15 hours for the swimming pattern study. The fact that no predation events were recorded during 48 hours of observation suggests that in-water predation rates in the Gulf of Fonseca may be extremely low. Several factors may potentially contribute to these findings. First, high hatchling predation rates tend to be associated with shallow waters, but also with the presence of reef or reef-like structures (Witherington and Salmon 1992; Gyuris 1994), which provide shelter for predatory fish (Gyuris 1994). These two factors characterize many sea turtle nesting sites, yet on the Honduran coast at Punta Ratón, although depth is low, the bottom is sandy and silty and hatchlings do not cross over rocky

areas or reef structures. Therefore, we suggest that the type of bottom structures present in shallow waters may be more important in determining predation rates than depth alone. Results from the study of Whelan and Wyneken (2007) on loggerhead hatchlings from South Florida support this idea. They assessed hatchling predation by fish in three different beaches, and found very low rates (1 %) in one of them, the beach at Naples. The characteristics of this beach are quite similar to those present in the Gulf of Fonseca: turbid waters with visibility lower than 0.5 m, and a sandy bottom. Only one species of predatory fish was recorded in snorkeling surveys in that study, and the authors suggested that the absence of bottom structures that allow fish to congregate could explain the low predation rates. Second, it is possible that, for some reason, fish from the Gulf of Fonseca do not feed on olive ridley hatchlings. We hypothesized that predation by fish would occur in the Gulf of Fonseca based on fish assemblages known to inhabit its waters. However, to date we have no evidence of Gulf fishes feeding on olive ridley hatchlings. During the three years of our study, we interacted regularly with the fishermen from Punta Ratón, who never reported finding sea turtle hatchling remains when eviscerating captured fish. On only one occasion did we observed a fish feeding on a hatchling, which was dead prior to the incident. In that case, we were using a hatchling carcass attached to a Witherington float as a control experiment for a study on the influence of tidal currents on hatchling offshore migration (Duran and Dunbar 2013), when the hatchling was taken by a large fish we were unable to identify. Finally, predation rates may have been higher in the past, but may now have decreased to minimum levels due to a decline in predator numbers. In a study on the fishing activity at the Gulf of Fonseca from 2004 to 2010, Soto (2012) suggested that fish populations in the Gulf have been threatened by recent fishing efforts. Over-fishing and

declining fishing stocks are global trends (Jackson et al. 2001; Pauly et al. 2002) that have previously been suggested as one explanation for a decrease in nearshore predation pressure on sea turtle hatchlings (Whelan and Wyneken 2007). We suggest that such declines in the Gulf of Fonseca may also contribute to low predation risks to hatchlings in this area.

Another explanation for the absence of predation found in our study is that such absence is actually an artifact of the study methods, because the presence of our boat, or the sound of its engine, could have caused flight reactions in fish predators. Following hatchlings with a kayak from an approximate distance of 10 m is the usual method for studies on hatchling nearshore predation (Stewart and Wyneken 2004; Whelan and Wyneken 2007). In our case an engine boat was required for safety reasons because of the substantial currents and the sudden, violent electrical storms common in the area. Still, we think it unlikely that the use of the engine distorted the results of the study, for two reasons. First, we had the engine turned off most of the time, using it only for short periods of time to approach the hatchling when it had moved too far away to be observed from our position. Second, the boat we used was a local fishing skiff, similar to most boats that work in the Gulf of Fonseca during both night and day. Thus, fishes in the Gulf of Fonseca are likely accustomed to the presence of these types of boats and their sounds, which constitute a common disturbance in their environment. However, we did observe hatchling behavior changes in response to the engine sound, though only when in close proximity. Hatchlings followed at night tended to dive when they approached the engine of the boat. Frick (1976) reported a similar behavior in Costa Rican green turtle hatchlings, whose dives were sometimes caused by the approach of a boat. Some recent studies have avoided the use of a

boat when tracking sea turtle hatchlings in nearshore waters, by equipping the hatchlings with miniature acoustic-coded transmitters, and deploying an array of receivers in the surf zone (Thums et al., 2013). Although very useful, this technique does not allow continuous monitoring or direct observations of each individual hatchling, and thus, visual tracking was preferred for the current study.

We are aware of no research studies to date that have focused on assessing rates of bird predation on sea turtle hatchlings once they have reached the water. Although not a primary goal of the current investigation, our study on diurnal and nocturnal swimming patterns provided some insights regarding bird predation pressure on sea turtle hatchlings in the Gulf of Fonseca waters, as well as about potential predator avoiding strategies employed by hatchlings.

In many sites, the risk of aerial predation on sea turtle hatchlings is relatively low. Hatchlings usually emerge soon after dusk in response to cooling surface sand (Hays et al. 1992), implying that the initial phase of their migration tends to be at night when most seabirds remain inactive. After several hours of swimming offshore and as daylight approaches, hatchlings are usually far enough from land to be out of reach of most birds. In southern Honduras, however, this is not the case. Seabirds are abundant in the Gulf of Fonseca. During the day, magnificent frigatebirds, brown pelicans, terns, and gulls are frequently observed over the entire Gulf. To reach open water, olive ridley hatchlings from Punta Ratón must swim more than 30 km across the Gulf waters, and thus are exposed to attacks by seabirds. These hatchlings swim at an average speed of 1.2 km/h (Duran and Dunbar *In prep*), which is a normal swimming speed for hatchlings during the frenzy period. Loggerhead hatchlings swim at 1.10 to 1.37 km/h (Salmon and Wyneken 1987) and

green hatchlings reach speeds of up to 1.62 km/h (Frick 1976; Abe et al. 2000). Swimming continuously at 1.2 km/h in a straight line from Punta Ratón would require more than 25 hours for the hatchlings to reach the mouth of the Gulf of Fonseca. However, the observation of hatchling trajectories being parallel to the coast rather than directly offshore, along with recent data from Duran and Dunbar (2013; *In prep*) suggest that these hatchlings are being pulled back and forth by tidal currents during their offshore migration, potentially extending their stay in the Gulf, and prolonging their exposure to bird predation, for up to several days.

In this scenario, to adopt an antipredator strategy specific for birds may have adaptive significance for Honduran olive ridley hatchlings. The diurnal swimming pattern observed in this study has not been previously described in the literature, suggesting it may be a localized behavior of this population. Previous studies have recorded differences in the nocturnal activity of loggerhead hatchlings from different populations (Wyneken et al. 2008; Scott et al. 2014), suggesting that local oceanic conditions drive the evolution of innate swimming behaviors (Scott et al. 2014). Wyneken et al. (2008) showed that hatchlings from SE Florida beaches, much closer to their target current than those of SW Florida beaches, were more inactive at night after the first 24 hours of frenzy swimming. Scott et al (2014) found a similar result studying hatchlings from Cape Verde, which by the third night were essentially inactive. He suggested that this behavior helps hatchlings to minimize predation risks from crepuscular and nocturnal aquatic predators. In the case of Honduran olive ridley hatchlings, we found no difference in the amount of nocturnal swimming activity, but instead found a difference in diurnal swimming depth. In both cases, the observed differences may be adaptations to improve effectiveness of the offshore

migration behavior under specific local conditions. Given the high turbidity of the Gulf of Fonseca waters, our data suggest that swimming at depth during the daytime may serve as a predator avoidance strategy to reduce detection of hatchlings by aerial predators. Our observations of sporadic birds attacking and capturing hatchlings that remained conspicuous at the water surface during day time support the effectiveness of swimming at depth as an antipredatory measure.

In the Gulf of Fonseca, due to its abundance of sea birds and very turbid waters, it is clearly advantageous for the hatchlings to swim at depth as much of the time as possible during the day, but this strategy may represent high energetic costs for the animals. Swimming 10-20 cm from the water surface, with only occasional dives, is the normal behavior for sea turtle hatchlings (Frick 1976; Davenport et al. 1984; Liew and Chan 1995; Witherington et al. 1995; Abe et al. 2000; Hasbún 2002), and appears to be the most efficient form of swimming. Swimming just at the water surface, or very close to it, reduces performance due to the creation and propagation of surface waves (Webb et al. 1991). Total drag due to these types of waves becomes minimal at a depth of at least 2.5-3 times the animal's body thickness (Hertel 1966; Hertel 1969), coinciding with the approximate depth at which sea turtle hatchlings (Martin 2003), as well as sea turtle adults (Hays et al., 2001), swim.

While drag avoidance may be the reason why sea turtle hatchlings do not swim at the water surface, it does little to explain why they do not usually swim deeper than 20 cm. One likely reason is that swimming at depth would imply spending more time moving to and from the surface for breathing, thus, hatchlings would spend excess time and energy in vertical movements when they should maximize horizontal offshore movements. An

additional important reason is that hatchlings are positively buoyant (Carr 1982; Davenport and Clough 1986). Studies on loggerhead and green turtles showed that the diving abilities of hatchlings are poor until they are several months old, because buoyancy control is undeveloped (Milsom 1975; Davenport et al. 1984; Davenport and Clough 1986). Still, several studies have shown that hatchlings are able to dive to depths of more than 1 meter, yet do so only sporadically (Davenport and Clough 1986; Witherington et al. 1995; Abe et al. 2000; Hasbún 2002; Martin 2003). These dives usually happen when hatchlings are disturbed (Frick 1976; Martin 2003), or in response to a bird or other object appearing overhead (Frick 1976; Witherington and Salmon 1992; Witherington et al. 1995). When hatchlings are threatened from the air, they dive almost vertically, and remain underwater for up to two minutes (Frick 1976; Witherington and Salmon 1992). To keep themselves at depth implies a great effort on the part of the hatchlings, which appear to be highly buoyant. Davenport et al. (1986) observed that loggerhead hatchlings beat their foreflippers vigorously in order to dive down to 1 m, and rapidly bobbed to the surface as soon as they stopped swimming. To remain submerged, hatchlings needed to counteract the tendency to float by holding the body 45° to the horizontal plain with the head down and performing specific flipper movements.

At night hatchlings from the Gulf of Fonseca, typically swam near the surface with only a few deep dives, however, during the day, their swimming pattern was the opposite. Swimming at depth during the daytime did not appear to be a reaction to overhead disturbances, but instead appeared to be the normal behavior for these hatchlings. After spending a few seconds at the surface, Honduran hatchlings went straight down from the surface and continued swimming at 1-1.5 m deep for more than one minute, after which

they returned to the surface to breath. This cycle was repeated uninterruptedly during our diurnal observations. Because of the energy investment required, an animal with low energy reserves could not perform deep dives for long. This could explain the inconsistent behavior of hatchling number 25, which spent 95% of the time at the surface during day time (Fig. 21C). This hatchling also happened to be one of the smallest hatchlings in the study, with a weight of only 14 g.

We propose that diurnal diving behavior shown by olive ridley hatchlings in this study has adaptive significance in avoiding aerial predation in the specific conditions of the Gulf of Fonseca, where turbid waters obscure hatchlings swimming more than 25 cm from the surface. Although there are no specific studies on the swimming and diving abilities of olive ridley hatchlings, we assume that, similar to the hatchlings of other sea turtle species, they are positively buoyant. Thus, this behavior implies an energetic investment that is compensated by the advantages it confers on hatchlings. This diurnal strategy of deep swimming appears to be successful, since we observed no predation events by birds on single hatchlings at any time while employing this pattern.

Further research is needed to determine whether this behavior is characteristic of olive ridley sea turtles in other areas or if it is a local adaptation for enhancing survival under the specific conditions of the Gulf of Fonseca. In any case, it would be of interest to perform laboratory studies to assess the actual energy investment this behavior requires of the animals, and compare it with the amount of energy used by hatchlings of other species normally swimming near the surface and during diving in response to the presence of aerial threats. In the case that this behavior appears to exist only in the Honduran population of the Gulf of Fonseca, it would be worth investigating whether hatchlings of other sea turtle

species nesting in the area, such as hawksbill and green turtles, also show a similar behavior during offshore migration from beaches of Pacific Honduras.

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CHAPTER SIX
RIDING THE TIDES: OFFSHORE MIGRATIONS OF OLIVE RIDLEY
HATCHLINGS ARE INFLUENCED BY TIDAL CURRENTS IN PACIFIC
HONDURAS

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Abstract

Sea turtle nesting beaches located in shallow bays and estuaries are often influenced by tidal variations in water elevation and currents. Due to the small size of sea turtle neonates, flood tidal currents may hamper the offshore migration of hatchlings by pulling them back to shore. We assessed the effect of tidal currents on the movements of olive ridley hatchlings released at Punta Ratón, Honduras. This beach is located on the eastern end of the Gulf of Fonseca, a 3,000 km² estuary of the Pacific Ocean. Hatchlings released at Punta Ratón must swim approximately 38 km to reach open waters, and their offshore migration is shown to be greatly affected by tidal currents present in the Gulf. We followed 10 hatchlings for up to 12 hours, covering both outgoing and incoming tidal periods. During outgoing tides, hatchlings moved toward the mouth of the Gulf, while during incoming tides they were pulled back to the shore. Net and effective distances covered during the first tidal cycle differed depending on the time of release of the hatchlings. Hatchlings released just after high tide covered larger net distances (7.30 vs 1.51 km) and advanced more toward open waters (4.55 vs 0.90 km) than hatchlings released at mid outgoing tide. Our results suggest that release of hatchery-raised hatchlings at the beginning of the tidal cycle provided them more time to move away from the shore during the first outgoing tide, reducing the reversal effect of the following incoming tide. This study provides insight on potential negative effects of flood tidal currents on hatchling movements in sites with tidal currents of high magnitude. We have also demonstrated that appropriate conservation management decisions, such as time of hatchling release, may help counter such negative effects and improve success of hatchling offshore migrations, in conditions similar to those present in the Gulf.

KEYWORDS: *Lepidochelys olivacea*, sea turtles, shallow waters, estuaries, tides, tidal-oriented movement

Introduction

Sea turtles spend most of their life in the ocean, yet females periodically return to land to lay their eggs on sandy beaches. When neonates emerge from the nest, they crawl down the beach, reach the surf, and begin the journey toward deep oceanic waters. Due to the abundance of predators both on land and in nearshore waters, the first hours in the life of a sea turtle hatchling are very dangerous (Ireland et al. 1978; Salmon and Wyneken 1987; Gyuris 1994; Stewart and Wyneken 2004). Upon reaching the water, hatchling sea turtles of most species engage in a continuous rapid swimming period (the *frenzy*) that lasts approximately 24 hours and has the function of moving hatchlings away from these hazardous habitats as soon as possible (Wyneken and Salmon 1992). Remaining in shallow waters close to the shore for long periods of time could have disastrous consequences for the hatchlings. In an aquatic predation study on loggerhead (*Caretta caretta*) hatchlings in Florida, Whelan and Wyneken (2007) estimated that every 15 minutes in shallow waters (< 3 m) implied a 5% loss due to predation, with 100% loss if they remained in the nearshore environment for more than 20 hours.

Hatchlings locate the water using visual cues (Limpus 1971; Mrosovsky 1978), and initially swim against the waves (Lohmann et al. 1990; Lohmann and Lohmann 1992). Because waves are usually near-perpendicular to the coast, this innate behavior ensures they move directly towards deep, open waters. Once a swimming direction has been established, hatchlings maintain it even if the direction of the waves changes, using magnetic orientation (Lohmann and Lohmann 1996). However, the direction of swimming

does not guarantee the direction of hatchling movement. Sea turtle hatchlings are small, weighing from 13 to 40 g depending on the species, and their offshore migration trajectories are strongly affected by currents. Several studies on green (*Chelonia mydas*), hawksbill (*Eretmochelys imbricata*), loggerhead, and leatherback (*Dermochelys coriacea*) sea turtle hatchlings have recorded that both the paths followed by the neonates and their swimming speeds depend on ocean surface currents (Frick 1976; Witherington 1991; Liew and Chan 1995; Witherington et al. 1995; Abe et al. 2000; Hasbún 2002; Okuyama et al. 2009).

The “current,” or total movement of the water, in a specific coastal site is the result of a combination of forcing from winds, density gradients (from temperature gradients, salinity gradients, and river discharge), and tides. Tidal currents are the horizontal movement of water that accompanies the rise and fall of tides, generated by mutual attraction forces between Earth, moon, and sun (Roos 1997; Bowditch 2002). In rivers, estuaries, and other locations where the water flow is somehow restricted, tidal currents periodically reverse, flowing in approximately opposite directions during flood and ebb, with a short period of little or no current in between, called slack water. Current speed, which is zero during slack water, increases and decreases progressively, reaching a maximum about midway between slacks (Bowditch 2002). The maximum speed of the current in a particular location depends on the heights reached by the high and low tides, which in turn depend on the positions of the moon and the sun at that moment, latitude, shape of the water basin, water depth, and atmospheric conditions (Bowditch 2002; NOAA's National Ocean Service 2010).

Tides and tidal currents tend to be magnified in shallow bays and estuaries with specific shapes (NOAA's National Ocean Service 2010), and in coastal sites with shallow continental shelves, such the coast of the Kimberley region of Western Australia, with tidal ranges up to 11 meters during spring tides, and tidal currents that can reach 2 to 5 m/s (7.4 - 18.52 km/h) (Cresswell and Badcock 2000; Purcell 2002). Strong currents and rapid shifts present challenges for animals that inhabit these types of environments, and if present in sea turtle nesting areas, these could likely affect both females approaching, and hatchlings departing, from the beach. However, a larger impact may be expected on the hatchlings due to their much smaller size and reduced strength. While other studies have investigated the movements of juvenile and adult turtles with respect to tidal currents in shallow coastal foraging areas, to our knowledge, no previous studies have focused on the specific influence of tidal currents on hatchling movements during the first hours of offshore migration. This study focuses on just such an influence in an estuary, the Gulf of Fonseca, through which olive ridley hatchlings must swim to reach the open Pacific.

The Gulf of Fonseca is an estuarine embayment of the Pacific Ocean, bordered by the countries of El Salvador, Honduras, and Nicaragua (Fig. 24), approximately 80 km long and 50 km wide. The Gulf connection with the Pacific Ocean is 30 km wide and only 20 m deep (Ward 2000). The entire area of the Gulf has an average depth of only 15 m (Valle-Levinson and Bosley 2003), although some deep channels exist that allow navigation by large commercial vessels (Ward 2000).

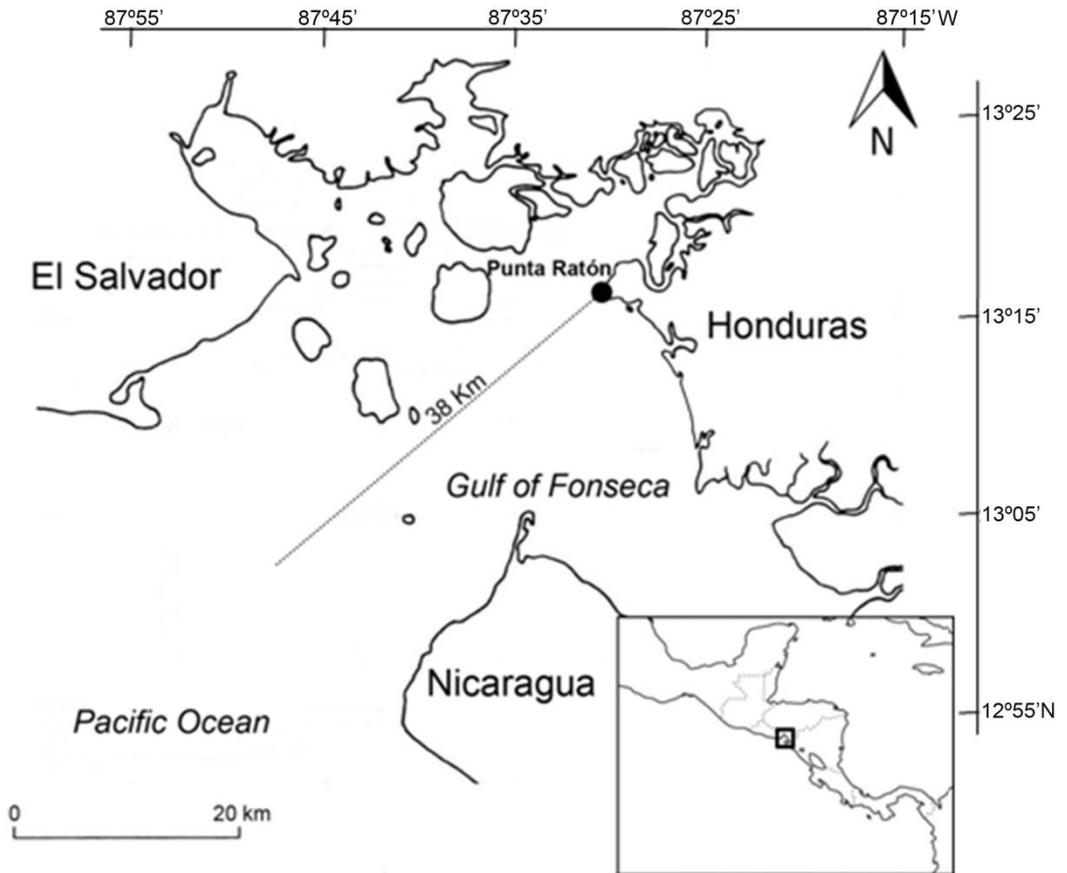


Figure 24. The Gulf of Fonseca. The circle indicates Punta Ratón, the study site, located approximately 38 km away from the mouth of the Gulf.

The hydrography of the Gulf is clearly affected by the tides. The tidal cycle is predominantly semidiurnal, with a period of 12.4 h (Vergne *et al.* 1993). Tidal amplitudes reach over 3 m in many Gulf coastal inlets and channel estuaries (Ward 2000). Water movement of flood and ebb tides is also substantial, reaching speeds over 1.6 m/s (5.6 km/h) (Admiralty Hydrographic Office 1951).

Along the Honduran coast, in the eastern end of the Gulf, there are several olive ridley (*Lepidochelys olivacea*) nesting beaches, where an estimated total of 1,000 nests are laid per year (Duran *et al.* 2015). The conservation program for olive ridleys in Honduras includes a yearly protected period, enforced by the government, when the collection of eggs for commerce and consumption is prohibited. During this period, encompassing the first 25 days of September, beaches are patrolled in search of nesting females, and eggs are transported to hatcheries managed by local communities. Hatchlings emerge after approximately 45 days of incubation and are released on the same beaches where the hatcheries are located. Punta Ratón is the main olive ridley nesting beach in Honduras (Fig. 24), with more than 200 nests per season. Hatchlings released from Punta Ratón must swim approximately 38 km across the Gulf to reach the open ocean. During this journey neonates face specific challenges related with the site, such as high risks of bird predation (Duran and Dunbar 2013), and strong tidal currents.

The first goal of this study was to quantify the effect of the tidal currents on the offshore migrations of olive ridley hatchlings released from the beach at Punta Ratón, Honduras. We hypothesized hatchlings would move towards the mouth of the Gulf during outgoing tides but their movement would be hampered during incoming tides.

Second, we assessed the effect of time of release respective to the tidal cycle on the final outcome. We hypothesized that hatchlings released just after high tide would have more time to distance themselves from the coast during the first outgoing tide than those released at mid-outgoing tide, and thus, movement towards the mouth of the Gulf would be more efficient for the former than for the later.

Methods

We conducted this study during the months of October and November of 2012 and 2013. Hatchlings were collected from the hatchery at Punta Ratón as soon as they emerged from the nests, measured and weighed, and kept in dark containers until the beginning of the tracking trials, with a maximum retention time of 12 h. We released the hatchlings from a small skiff located 50 to 100 m from the shore, similar to the release protocol used at the hatchery. To reduce the potential for hatchlings to immediately be swept back to the beach by the waves, local hatchery managers release the turtles directly into the water, rather than placing them on the beach and allowing them to reach the surf by themselves. Releases take place from fishing boats if available, or a person may carry the hatchlings out approximately 50 m into the water and release them from there.

We attached a modified “Witherington float” to each hatchling by means of 1.5 m of sewing thread tied around the carapace. These small canoe-shaped floats measured 71×16×12 mm and weighed 4 g. To ensure they could be seen from the skiff, the floats contained a 38.1 mm green, cold-chemical glowstick (GlowProducts, Victoria, BC, Canada, suppliers) during the night, and a small yellow balloon during the day. We followed the hatchlings from an approximate distance of 10 m and took GPS positions every 30 minutes, using a hand-held Garmin eTrex® Venture HC GPS.

We followed a total of 10 hatchlings for times ranging between 3.5 and 12 h. In order to assess the effects of equivalent outgoing and incoming tidal currents, trackings started during outgoing tide and lasted until we had observed the same amount of time in both tidal current directions. However, several trials were halted before the intended time because the tether thread broke and the hatchling was lost, or due to inclement weather. We removed trackings that covered only outgoing tides from the analyses. Data from trackings that covered both outgoing and incoming tides, but with different durations, were adjusted using low tide time as the reference and considering the same amount of observation time before and after low tide.

To assess the influence of time of release respective to the tidal cycle in hatchling movement, we divided the hatchlings in two experimental groups. The first group was released during mid-outgoing tide (2-4 hours after high tide). The mid-outgoing tide (MOT) group comprised 5 hatchlings, which were observed for less than 8 hours. The second group was released just after high tide (first two hours of outgoing tide). The after high tide (AHT) group included 5 hatchlings observed for 8 to 12 h.

To assess the effect of the floats on hatchling movements in the specific conditions of our study, we performed three control trackings with unattached hatchlings. We followed these hatchlings during the night and attached a small chemical glowstick to their carapaces in order to see them from the boat. Besides the effect on swimming speed, our main concern was that the float, pulled backwards by the flood tidal current, could cause reverse movements of the hatchlings towards the shore. For this reason we performed control trials with hatchlings with no float attached. We followed 2 hatchlings (C-1 and C-2) for one hour during incoming tides, taking GPS positions every 5 minutes. We also

followed one unattached hatchling (H111-C3) for 7.58 hours, covering both outgoing and incoming tides.

To assess the influence of tidal currents in the absence of hatchling movement, we did one trial with a dead hatchling. We attached the carcass to the float used for the rest of the experiments and followed it for 4.9 h.

We plotted our data in a geographic information system (GIS), using ArcMap 10.1 (ESRI, Redlands, CA, supplier) and calculated mean swimming speeds, total distances of the hatchling trajectories, net distances, main directions during outgoing and incoming tide, and distances from the starting and ending positions of the hatchlings to the mouth of the Gulf of Fonseca. We defined net distance as the straight distance between the initial and final position of each hatchling. We calculated the effective distance covered by a hatchling as follows: for each tracking, we took the distance from the initial position to the mouth of the Gulf and subtracted the distance from the ending position to the mouth of the Gulf. We conducted independent-samples t tests to compare total distances, net distances, and effective distances between the two experimental groups, and one way ANOVA tests to compare weights, CCLs, and swimming speeds among the two experimental groups and the group of control hatchlings. We set the α level to 0.05 for all analyses.

Results

Fourteen different hatchlings were used for this study: five in the MOT experimental group, tracked for less than 8 hours; five in the AHT experimental group, tracked between 8 and 12 h; 3 controls that were tracked without pulling a float between 1 and 7 h; and one dead hatchling that was tracked for almost 5 hours. No significant differences were found in weight or curve carapace length (CCL) among the groups. Mean

weight for the fourteen hatchlings was 15.6 g (SE = 1.4), and mean CCL was 43.3 mm (SE = 1.6).

We observed the five hatchlings released at mid-outgoing tide (MOT group) for an average of 6.00 h (SE = 0.68; range 3.28 - 7.38 h). All hatchlings from this group moved SW during outgoing tide, and four out of five moved in the opposite direction (NE) (Fig. 25A - D) during the following incoming tide, whereas hatchling #H24 moved directly N (Table 17, Fig. 25E). The total length of their trajectories averaged 7.48 km (SE = 1.16), with a range of 3.25 - 9.65 km. Net distances ranged from 0.16 km to 4.56 km, with a mean of 1.51 km (SE = 0.79). At the end of the trial, these hatchlings were located, on average, 0.90 km (SE = 1.02) closer to the mouth of the Gulf of Fonseca than when they started (Table 17).

For the five hatchlings released just after high tide (AHT group), times of observation ranged from 8.08 h to 12.00 h, with an average of 9.42 h (SE = 0.73). These hatchlings moved to the SW during outgoing tides, but the direction of their trajectories during incoming tides varied, with one moving SE (Table 17, Fig. 26A), two moving NE (Fig. 26B, D), and two moving E (Fig. 26C, E). Total distances covered by hatchlings from the AHT group ranged from 8.25 to 15.27 km, with a mean of 11.31 km (SE = 1.36). Net distances averaged 7.30 km (SE = 1.59), with a range from 3.65 to 12.72 km. The location of the AHT group hatchlings at the end of the experiments was on average, 4.55 km (SE = 1.05) closer to the mouth of the Gulf than the starting location (Table 17).

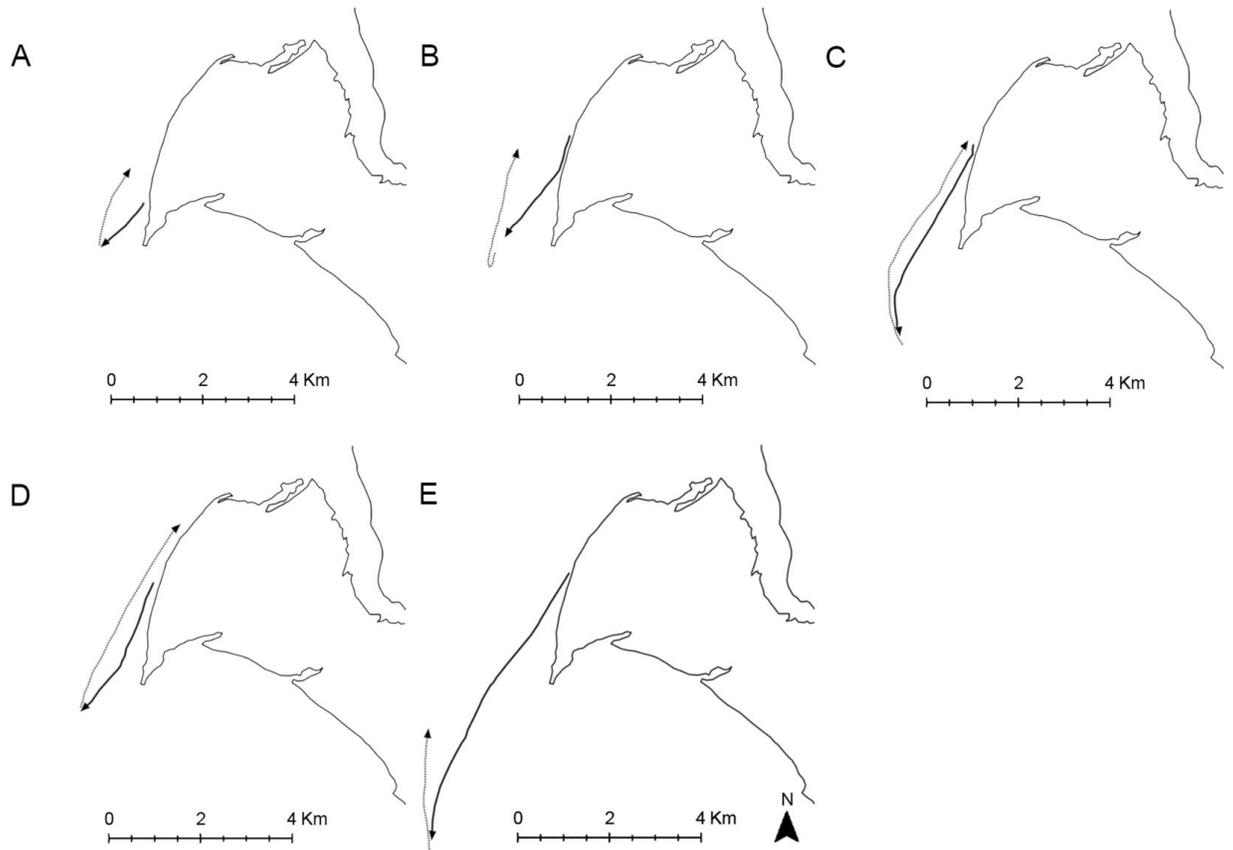


Figure 25. Trajectories of MOT group hatchlings, released at mid-outgoing tide: H1(A), H21(B), H22(C), H23(D) and H24(E). Solid arrows indicate movement during outgoing tide, dotted arrows indicate movement during incoming tide. Total tracking time ranged between 3.47 and 7.58 h. Observation time during outgoing tide was equal to observation time during incoming tide for all hatchlings.

Table 17. Results summary. Tracking time, total and net distances covered, average speed, main direction during decreasing (outgoing) and increasing (incoming) tides, and effective distance are shown for each of the 14 hatchlings used for the study. Hatchlings from experimental MOT group (H1-H24) were released at mid outgoing tide and followed for less than 8 hours. Hatchlings from experimental AHT group (H31-H110) were released within the first two hours after high tide and followed for 8 to 12 h. Control hatchlings (C) had no float attached and D corresponds to a dead hatchling.

	Group	Time (h)	Total Distance (km)	Net Distance (km)	Average Speed (km/h)	Dec. Tide Direction	Inc. Tide Direction	Effective Distance ^a (km)
H1	MOT	3.47	3.25	0.81	0.94	226° (SW)	68° (NE)	-0.360
H21	MOT	7.13	7.15	0.68	1.00	238° (SW)	77° (NE)	1.059
H22	MOT	6.15	9.65	0.16	1.57	249° (SW)	72° (NE)	0.058
H23	MOT	5.92	7.86	1.34	1.33	241° (SW)	62° (NE)	-1.008
H24	MOT	7.38	9.50	4.56	1.29	243° (SW)	91° (N)	4.763
H31	AHT	12.00	15.27	12.72	1.27	263° (S)	326° (SE)	6.019
H101	AHT	8.23	12.99	3.65	1.60	258° (SW)	44° (NE)	0.393
H105	AHT	8.08	11.75	8.78	1.45	254° (SW)	353° (E)	5.808
H107	AHT	8.70	8.29	5.25	1.01	248° (SW)	58° (NE)	5.117
H110	AHT	10.07	8.25	6.10	0.82	247° (SW)	359° (E)	5.402
C1	C	1.00	1.47	1.39	1.47		61° (NE)	
C2	C	1.00	0.87	0.81	0.87		72° (NE)	
H111(C3)	C	7.58	6.56	2.65	0.88	274° (S)	100° (N)	1.916
D1	D	4.90	3.77	0.11	0.77	246° (SW)	64° (NE)	-0.112

^a Effective distance is defined as the difference between the distances of the starting and ending points of each hatchling tracking to the mouth of the Gulf of Fonseca.

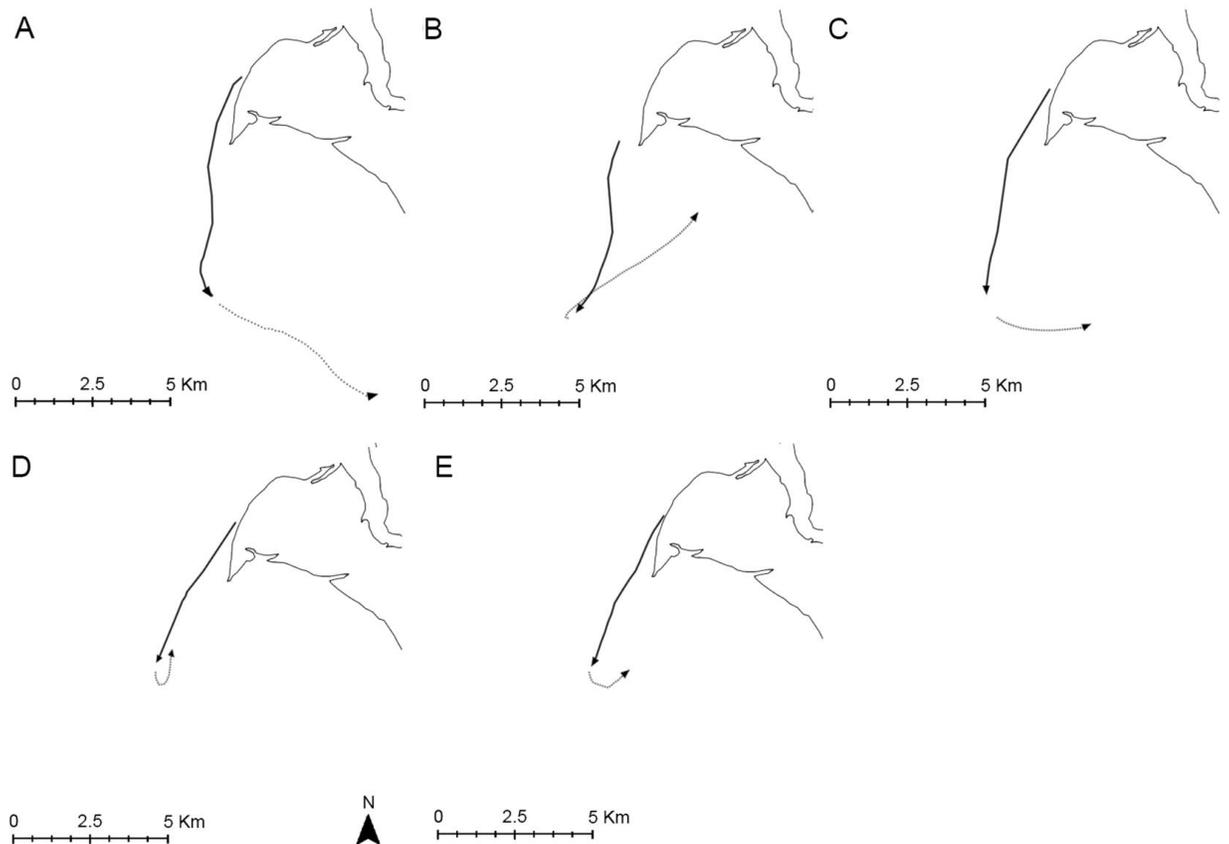


Figure 26. Trajectories of AHT group hatchlings, released within the first two hours after high tide: H31(A), H105(B), H101(C), H110(D) and H107(E). Solid arrows indicate movement during outgoing tide, dotted arrows indicate movement during incoming tide. Total tracking time ranged between 8.08 and 12.00 h. Observation time during outgoing tide was equal to observation time during incoming tide for all hatchlings.

Although the AHT group hatchlings swam, on average, 3.83 km more than the MOT group hatchlings, this difference in mean total distance was not significant ($t_8 = -2.14$, $p = 0.065$). However, net distances did differ significantly between the two experimental groups: AHT hatchlings covered, on average, 5.79 km more than MOT hatchlings ($t_8 = -3.27$, $p = -0.011$). We also found a significant difference in the effective distance ($t_8 = -2.49$, $p = 0.038$), which average was 3.65 km greater in the AHT group than in the MOT group hatchlings.

Control hatchlings C-1 and C-2, tracked for 1 hour during incoming tide, moved NE with average speeds of 1.47 and 0.87 km/h, respectively (Table 17, Fig. 27A). Control hatchling H111 (C3) moved S during outgoing tide and almost in the opposite direction during incoming tide, with an average speed of 0.88 km/h. Observation time for H111 (7.58 h) was in the range of times for the MOT group. Total distance (6.56 km), net distance (2.65 km), and effective distance relative to the mouth of the Gulf (1.92 km) all fell within the ranges of the MOT group (Table 17, Fig. 27B).

The dead hatchling (D-1) moved parallel to the coast of Punta Ratón, SW during the outgoing tide and NE during the following incoming tide (Table 17, Fig. 27C). The observation time for this hatchling (4.9 h) was comparable with the observation times for MOT hatchlings, and values for total distance, net distance, and average speed also fell close to the values for hatchlings in this group (Table 17). D-1 covered a total distance of 3.77 km, corresponding to the lower range for MOT group. Net distance for D-1 (0.11 km) was the lowest observed in the study, as was the average speed (0.77 km/h), yet this speed was not much lower than the lower values from the other groups (0.82 km/h for H110, 0.87 km/h for C-2, 0.88 km/h for H111, 0.94 km/h for H1).

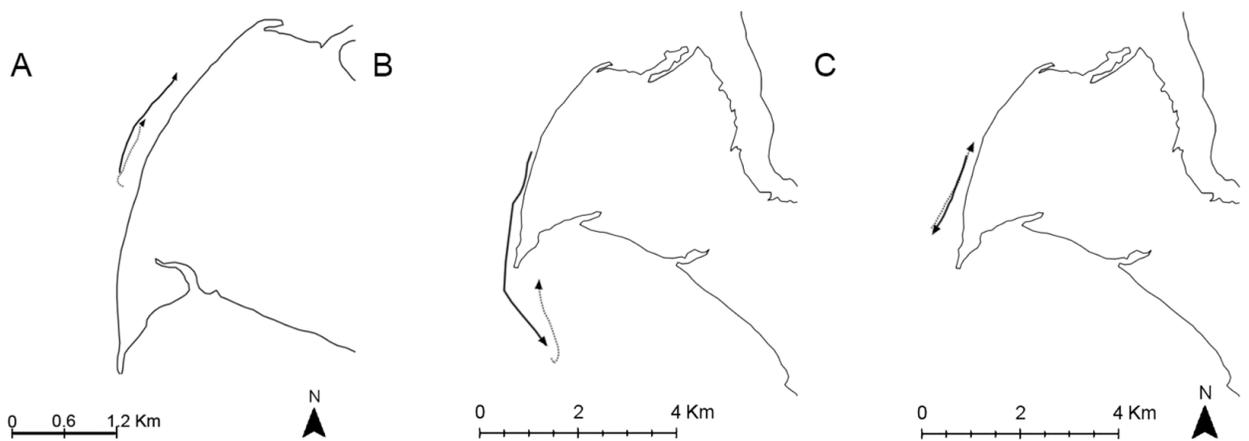


Figure 27. Trajectories of control hatchlings. Unattached hatchlings C1 (A, solid arrow) and C2 (A, dotted arrow), tracked for 1 hour each during incoming tides, and H111-C3 (B), tracked during both outgoing (solid arrow) and incoming (dotted arrow) tides for a total of 7.6 h. Dead hatchling (C), tracked during outgoing (solid arrow) and incoming (dotted arrow) tides for a total of 4.9 h.

A one-way ANOVA yielded no significant differences regarding average speed ($F_{(2,10)} = 0.305$, $p = 0.744$) between the MOT group (N=5, 1.23 km/h (SE = 0.11)), the AHT group (N=5, 1.23 km/h (SE = 0.14)), and control hatchlings with no float attached (N=3, 1.07 km/h (SE = 0.20)).

Discussion

Our study has demonstrated that flood tidal currents of the Gulf of Fonseca pull olive ridley hatchlings back to the shore, hampering their offshore migration and extending the time they remain in shallow waters. When hatchlings were released following the usual protocol of the local hatchery (the MOT group), they covered short net distances (average 1.51 km) and advanced little towards the mouth of the Gulf. After 6.00 h of swimming, the average effective distance travelled was only 0.9 km, and some ended up farther from the mouth of the Gulf than where they started (H1 and H23, Table 17, Fig. 25A, D). Taking into account that the amount of energy hatchlings have to support their offshore migration is limited (Kraemer and Bennett 1981; Jones et al. 2007), this delay in leaving nearshore waters could have disastrous consequences for them (Whelan and Wyneken 2007). Shallow waters are known to be especially dangerous for sea turtle hatchlings due to an abundance of predators (Witherington and Salmon 1992; Pilcher et al. 2000). For this reason, hatchlings of most sea turtle species swim continuously for the first 24 h to leave nearshore shallow waters as quickly as possible (Carr 1962; Salmon and Wyneken 1987; Wyneken and Salmon 1992). After this frenzy swimming period they continue swimming towards oceanic currents, doing so mostly during the day (Wyneken *et al.* 2008; Chung *et al.* 2009) and showing different degrees of nocturnal swimming activity, depending on the beaches they emerged from and the distance to target currents (Wyneken *et al.* 2008; Scott *et al.*

2014). Once hatchlings reach oceanic current systems, they drift within them for several years while they mature and grow to juvenile sizes (Carr 1986; Carr 1987; Bolten and Balazs 1995). These currents provide hatchlings with food and shelter, and a mechanism of transport to potential foraging and recruiting grounds (Carr and Meylan 1980; Bowen et al. 1995; Witherington 2002). The importance of these currents is such that they likely affect both the location of nesting beaches (Putman *et al.* 2010) and feeding areas (Hays *et al.* 2010). The amount of time that a hatchling requires to reach a main current depends on the species and the particular geographic location, but is usually estimated to be less than 1 week (Putman *et al.* 2012). If Honduran hatchlings released during mid outgoing tide at Punta Ratón continued swimming at the same pace, and suffering the same effects from tidal currents as they did during the first tidal cycle, we calculate it would take them more than forty days to reach the mouth of the Gulf of Fonseca and encounter the open Pacific. In calculating this rough estimate, we fully recognize that hatchling swimming patterns are known to change after the frenzy, and the effect of the tidal currents will also vary when they are swimming farther from the coast. In any case, the time in which hatchlings are entrained in the Gulf of Fonseca is likely to be greatly extended when hatchlings are released under a MOT type regime, and covering a distance of 38 km, which could be done in 1 or 2 days at the normal swimming speed, may actually take significantly longer.

According to our results, the change in the release time made a significant difference. Hatchlings released within two hours after high tide (the AHT group) had more time swimming with the outgoing tide, partly countering the reversal effect of the following incoming tide. Although hatchlings from the AHT group also experienced reversed movement away from the mouth of the Gulf, net distances were significantly

higher than the MOT group distances (average of 7.30 vs 1.51 km, respectively) and they advanced an average of 4.55 km towards the mouth of the Gulf in one tidal cycle. If hatchlings from the AHT group maintained these swimming rates, they could potentially reach the open Pacific in approximately 8 days. Although these calculated travel times should be considered with caution, our study demonstrated that a small change in hatchery management may result in a significant difference in the effective distance hatchlings cover during the first hours of their offshore migration.

Obtaining reliable field observations of hatchlings is complicated due to the challenge of locating the animals from a distance, which often requires of artificial identifying devices. In the current study, we used different types of controls to ensure that results were minimally affected by our methodology. Witherington floats used for this study have been previously used in several hatchling predation studies (Witherington and Salmon 1992; Lorne and Salmon 2007; Whelan and Wyneken 2007; Duran and Dunbar 2013). These floats have not been shown to affect hatchling orientation or swimming depth (Stewart and Wyneken 2004), although they may lower swimming speed by 10-20 % (Witherington and Salmon 1992; Witherington et al. 1995). Because swimming speed was important for our study, we compared swimming speeds of unattached hatchlings with both experimental groups, and found no significant differences. These unattached controls also demonstrated that Witherington floats are not likely affecting the direction of movement. C-1 and C-2 moved NW, the same direction as the majority of the treatment hatchlings during incoming tide, and H111(C3), followed the same pattern as the MOT group hatchlings, going away from the shore during outgoing tide and returning back to the shore during incoming tide. These results suggest that the reversal movement observed and

described in this study is not an artifact of our methodology, but an actual phenomenon caused by tidal currents in the particular conditions of the Gulf of Fonseca. The trajectory of the dead hatchling, similar to the trajectories of the MOT hatchlings, along with its average speed of 0.77 km/h, lower than, yet close to the speeds of live hatchlings, suggests that hatchling swimming has minimal effect on movement, and that tidal currents are the primary factor determining both direction and speed of hatchlings in the initial phases of their offshore migration.

Although hatchling movement associated with ebb and flood tidal currents has not been previously reported, it is known that juvenile and adult sea turtles may use tidal currents for energy-saving transportation. Tidal-oriented movements have been observed for juvenile loggerheads, juvenile and adult greens, and juvenile Kemp's ridleys (*Lepidochelys kempi*) in shallow feeding areas (Byles 1988; Limpus *et al.* 1994; Schmid *et al.* 2002; Brooks *et al.* 2009; Senko *et al.* 2010), where animals move in opposite directions with alternating floods and ebbs (Brooks *et al.* 2004), taking advantage of different feeding areas that become available in specific moments of the tidal cycle (Limpus *et al.* 1994; Limpus and Limpus 2000). The main difference between these tidal-oriented movements and those observed in the Honduran olive ridley hatchlings is that the former are considered advantageous for the animals performing them (Forward Jr. and Tankersley 2001). Moving with the tides appears to be beneficial as energy conservation measures for juvenile and adult sea turtles in shallow foraging areas (Brooks *et al.* 2004; Brooks *et al.* 2009; Senko *et al.* 2010), yet may potentially be harmful for hatchlings migrating offshore.

Although this study has focused on the effects of tidal movement, surface currents also depend on other factors that may have additional effects on hatchling movement. In

the Gulf of Fonseca, Valle-Levinson and Bosley (2003) demonstrated that rain seasonality affects water circulation. During the wet season (from May to October), water circulation at the entrance of the Gulf corresponds to a typical estuary, with outflow of surface waters and inflow of deep waters. However, during the dry season (from November to April) the pattern reverses and the Gulf resembles an inverse estuary, with surface waters entering the bay and deep waters leaving. The recorded speed of these surface currents, 0.15 m/s (0.54 km/h) (Valle-Levinson and Bosley 2003), is approximately 50 % of hatchling swimming speed and could also have some additional effects on hatchling movement. Outflow surface currents might aid hatchlings leaving the Gulf in the wet season, whereas inflow surface currents in the dry season are likely to negatively affect them. The natural nesting season for olive ridleys in Honduras lasts from July to November, but within the current conservation program, nests are only protected during 25 days in September. Natural hatching would fall mostly during the wet season, when the surface currents flow towards the ocean, but currently all Honduran hatchlings only emerge and are released from approximately October 15th to November 10th, the period coinciding with the transition from wet to dry season, and the change from outflow to inflow surface currents. More research is needed to assess the actual influence of these currents on hatchling offshore migration and movements, and whether a change in the dates of the protected period might positively impact the effectiveness of hatchling migration toward the mouth of the Gulf.

To our knowledge, no previous research has focused on potential negative effects that tidal currents may have on sea turtle hatchling offshore migrations. This study has shown that in geographical locations where tidal currents are reversing and are of the same order or greater than swimming speeds, these currents may hamper hatchling offshore

migration by pulling neonates back to the shore during incoming tides. Sea turtle nesting beaches located inside shallow gulfs and estuaries (Schulz 1975; Fretey and Girondot 1989; Meylan et al. 1996; Gaos and Urteaga 2010) or in coastal areas with shallow shelves (Prince 1993) may be especially prone to these types of effects. It is, however, necessary to study the specific conditions of each location, such as other currents present in the area, the distance hatchlings must cover to escape the negative influence of flood tidal currents, and whether nests are located *in situ* and emerge naturally, or are managed in hatcheries. We have also demonstrated that conservation management decisions, such as changes in the protected periods for egg collection, and hatchling release times respective to the tidal cycle, may be pivotal for hatchling survival, successful migration to open oceanic waters, and improved conservation of the species.

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CHAPTER SEVEN

CONCLUSIONS

In this dissertation, I investigated the reproductive ecology and the hatchling behavior of the olive ridley (*Lepidochelys olivacea*) sea turtle population that nests on the Pacific coast of Honduras. My work includes the first field studies on hatchlings of this species during their offshore migration, the first genetic analysis performed on the Honduran olive ridley population, and the first scientific assessment of the Honduran government sea turtle conservation program.

In this chapter, I summarize the main conclusions from my four empirical research projects (Chapters 3 to 6), and discuss their conservation implications.

1. The olive ridley population nesting in southern Honduras showed evidence of multiple paternity in 75% of the nests sampled, with 2 or 3 fathers per clutch. This level of multiple paternity is higher than expected for a solitary population estimated to be less than 2,000 nesting females, suggesting that some of these females may be coming from proximal Nicaraguan *arribada* nesting beaches.
2. The hatchery nests at Punta Ratón incubated at higher temperatures, and experienced higher metabolic heating than the nests left *in situ* on the beach; a likely consequence of nests being in close proximity to each other. The largest difference in mean temperature (1.2 °C) between the beach and the hatchery was recorded during the second third of incubation.
3. Hatching success was much lower for hatchery nests than for beach nests (24.1 vs 83.2 %), likely due to excessively high mean temperatures in the hatchery during

the second third of incubation, which approached lethal values (35 °C) in some of the nests.

4. The mean temperature during the second third of incubation was found to be the best predictor of hatching success among the thermal variables included in the study, according to the following regression equation: $\text{HatSuc} = 1389.084 - 39.704 * \text{MeanT2}$, $r^2 = 0.861$; $F_{(1, 6)} = 37.250$, $p < 0.001$.
5. Hatchlings from hatchery nests appeared to be of smaller size, and to show poorer locomotion performances than hatchlings from beach nests. Statistical significance occurred in some, but not all cases, likely due to the small sample size of my study. Therefore, these results should be considered with caution.
6. Retaining hatchlings for 24 hours after emergence significantly reduced their weight, running speed, and active swimming time.
7. Despite the low depths typical of the Gulf of Fonseca waters, aquatic predation rates on hatchlings migrating off-shore from Punta Ratón were very low. This was likely due to the sand and silt bottom, which lacks rocky and reef structures where predatory fishes typically aggregate.
8. Olive ridley hatchlings swimming offshore from southern Honduras performed different swimming patterns during the night than during the day, in relation to their position in the water column. During the night, hatchlings swam near the water surface with a few sporadic dives, whereas during the day, hatchlings spent most of the time (78%) swimming at depth, going back to the surface for brief periods to breathe. I suggest this daytime swimming behavior has adaptive significance in avoiding bird predation.

9. Hatchlings released from Punta Ratón beach during mid-outgoing tide experienced backward movements during the following incoming tide, which considerably reduced the effective distances they covered during the first tidal cycle of their offshore migration to an average of 0.90 km.
10. Changing the release time of hatchlings from mid-outgoing tide to just after high tide increased the mean effective distances covered by the hatchlings during the first tidal cycle from 0.90 to 4.55 km, thereby reducing the reversal effect of the incoming tide, and likely the time required for them to exit the Gulf of Fonseca waters and reach the open sea.

Results from this research will enrich current scientific knowledge of olive ridley reproduction. I have described several features not previously reported in the literature, such as the mean temperature during the second third of incubation being a good predictor for hatching success, the differential diurnal/nocturnal swimming behavior of the hatchlings, and the potential negative effects of tidal currents on hatchling movements in sites with tidal currents of high magnitude.

This research will also inform management decisions for conservation programs on olive ridley nesting beaches in general and, specifically, for the government-sponsored conservation program currently operating at Punta Ratón. Based on results of the research studies of this dissertation, I provide the following recommendations for the current hatchery protocols:

- On the beach at Punta Ratón, the possibility of moving incoming females to nest in suitable places along the beach, where nests would be kept *in situ*, should be explored as an alternative to the hatchery. For this strategy to be effective, several

large spaces should be selected along the upper part of the beach, above the high tide line, where females would be transported to nest. These sites should be cleaned, enclosed in some way to prevent livestock from walking over the nests, and monitored to prevent predation by wild animals and human poaching. Nests should be protected to prevent nesting turtles from digging up previous nests. At the end of the incubation period, natural emergence and unobstructed entrance to the sea should be allowed.

In case this strategy is not adopted, some measures should be taken to correct the two main problems currently affecting hatchery eggs and hatchlings; these being the excessively high incubation temperatures due to shared metabolic heating, and the long post-hatching retention times before hatchlings are released at sea.

- To prevent metabolic heating from individual nests affecting those in close proximity, nests should be separated by at least 1 m from each other. This implies that a larger space is required to build the hatchery. Sand temperatures should be controlled periodically and shade or moisture (fresh watering) provided if necessary.
- Hatchlings should not be kept for long periods, as they should be released as soon as possible after emergence. However, in the specific oceanic conditions at Punta Ratón, it is not recommended to release hatchlings during mid-late outgoing tides or during incoming tides.
- Hatchlings appear to protect themselves from bird predation by swimming at depth during the day, and, therefore releases during daytime should not be detrimental for

them. Therefore, I recommend releasing hatchlings at the beginning of the outgoing tide following emergence, even if this part of the tidal cycle happens during the day.

- If hatchlings must remain in the hatchery for several hours before being released, they should be kept in dark containers lined with moist sand to minimize locomotion activity and water loss. They should not be placed or held in water, as this stimulates them to swim, depleting the vitelline storage required for the swimming frenzy.

The adoption of these changes would likely improve hatching success and hatchling quality at the hatchery. They would also reduce the time hatchlings spend in the Gulf of Fonseca before reaching open Pacific waters, likely increasing their survival. These suggested changes are not difficult to implement and do not involve additional financial investment. All the necessary tasks for replacing the hatchery with protected zones on the beach where females are transported to nest could be performed by community members that currently participate in hatchery construction, beach patrolling, and hatchery management.

Future Directions

This research has raised many interesting questions that merit further investigation. I have suggested that some olive ridley females nesting in the south coast of Honduras may be coming from Nicaraguan *arribada* beaches, but this hypothesis has yet to be confirmed. In case future satellite tracking or genetic studies actually support these inter-beach movements, it would be interesting to accurately study some reproductive features of the Honduran turtle females, such as clutch size or internesting period, to assess if they correspond to *arribada*, solitary, or intermediate values. These types of studies would

provide valuable information on the life cycle and evolution of *arribadas*, which are still unresolved issues (Bernardo and Plotkin, 2007). Future studies should also investigate sex ratios and assess the pivotal temperature for Honduran olive ridleys. My observations suggest that the nests of this population experience some of the highest incubation temperatures for the species along its range, and it would be of interest to know if the pivotal temperature is also higher than that of other populations, with the possibility of reducing the extreme female bias currently assumed. Wibbels (2007) suggested that assessing and comparing pivotal temperature of olive ridleys from many nesting beaches worldwide could provide vital insight into the evolution of pivotal temperatures in sea turtles. Another intriguing question worth further exploration is the scope of the differential diurnal/nocturnal swimming pattern shown by Honduran olive ridley hatchlings. Is this behavior only performed by this local population, or is it also present in other olive ridley populations? Is the deep swimming pattern triggered by the turbidity of the Gulf waters, or would these hatchlings show the same pattern while swimming during the day in clear waters? Do hatchlings of other sea turtle species nesting in the Gulf of Fonseca, such as hawksbills or greens, show the same swimming patterns?

Several fruitful investigations may arise from this dissertation, enhancing the body of knowledge on the biology of sea turtles and aiding in their conservation.

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