Instituto Tecnológico de Costa Rica
Universidad Nacional de Costa Rica
Universidad Estatal a Distancia

TEC Tecnológico de Costa Rica

UNA UNIVERSIDAD NACIONAL COSTA RICA

UNED UNIVERSIDAD ESTATAL A DISTANCIA
Institución Benemérita de la Educación y la Cultura

Lepidochelys olivacea status and its relationship with nest management on the eastern coast in the Pacifico of Guatemala

Trabajo sometido a consideración del Tribunal Evaluador como requisito para optar por el grado de Doctorado en Ciencias Naturales para el Desarrollo con énfasis en Gestión de los Recursos Naturales

Berta Alejandra Morales Mérida
Sustentante
Aprobado por los miembros del tribunal examinador

José Pablo Jiménez Madrigal, Ph.D.
Dirección Sistema de la Unidad de Posgrado

Giovanni Sáez Arce, Ph.D.
Coordinador General del DOCINADE

Marc Girondot, Ph.D.
Director de Tesis

Adriana A. Cortés Gómez, Ph.D.
Asesor de Tesis

Matthew H Godfrey

Matthew H. Godfrey, Ph.D.
Asesor de Tesis

Content

Declaration of Authenticity and Non Plagiarism

Through this document, I Berta Alejandra Morales Mérida, identified with Guatemalan identification document Nº 1929299681001, declare that the thesis presented with the title "*Lepidochelys olivacea* status and its relationship with nest management on the eastern coast in the Pacific of Guatemala," to opt for the Academic Degree of Doctor in Natural Sciences for Development with an emphasis on Natural Resource Management, it has been fully developed by the author who signs it, and I affirm that there is no plagiarism of any nature. Likewise, I note that the citations of other authors have been fully identified in the thesis, so the ideas issued by third parties have not been assumed as their own. Likewise, I affirm that I am responsible for all their content, and I assume, as the author, the consequences of any lack, error, or omission of references in the document. I know that this commitment to authenticity and not plagiarism can have ethical and legal overtones. Therefore, in the event of non-compliance with this statement, I submit to the provisions of the academic regulations issued by the Universities participating in this doctoral program.

Berta Alejandra Morales Mérida

Acknowledgments

I thank my country, which has suffered so much and has given me everything. To El Hawaii people, who inspire me to be a better professional. Overall, to my dearest Universidad de San Carlos de Guatemala and the Dirección General de Docencia, for funding my academic credits and other financial aid received to fulfill different phases of the doctoral process. For employing me to maintain an income to continue with the thesis process. And because, along with my family, my parents and husband, Marc, and the Ecology, Systematics, Evolution Laboratory of Paris Saclay University, different phases could not have been possible.

A special thanks to my doctoral advisors, professors, and the DOCINADE program for showing me a whole new meaning of development. To my doctoral peers, whose help and support were above asked. Especially to Aury, Bessie, Alejandro, Josué, Carol, Normita, Jovana, Maikol, Gaby, Fernando, and Astrid. To ARCAS ONG for allowing me to stay at the Park and use the installations. A kind thank you to the Director at that time, Alan Marroquín, for his support and openness. Also, I will always be grateful to you to the field personnel whose friendship and support are unmeasurable: Alex and Doña Mayra. And a special mention to those volunteers of ARCAS-Hawaii, who helped me and made a difference. To CONAP, for lending me, all the data used to analyze different processes. A special thanks to Airam López, whose help was always continuous and attentive.

A special thank you to my true and treasurable friends, to my advisees: Salomé, Jose Jorge, and Marisette. I will always be grateful for all your support and effort in the data collection, especially to María Renee. Her effort and help were unconditional and more valuable than she would ever imagine.

Dedication

I dedícate this thesis to my family, chosen and given. To my life partner Ricardo Gill, to my parents Barbara and Gustavo, because it would have been more difficult to continue and progress the way I did without all of their support, guide, and advice. To my sisters Babi (and Bob), Gabi, Ruthi, and especially to my Nephew Gustavo Alejandro and niece, Tábata Isabella. You inspire me to be a better person always. To all those I love and those who are not with us anymore, my grandparents, I'll always be grateful.

I dedicate this achievement to my country and my Universidad de San Carlos de Guatemala for giving me the sence of respect and the desire to make a difference, helping the environment and the communities.

A special thanks to my dearest friends, whose support was essential during each and every process: Reyna, Carlita, Pedro, Papita, Jorgito, Tefa, Aury, Jorge Luis, Pablo, Margarita, and all of those friends whose names I don't mention here, but are genuinely always present.

And last but not least, to my tutor Marc, whose guide and support is unquantifiable and constant. To Adriana, your friendship inspired me, but your guide makes me a better woman and scientist. And Matthew, your support and guide has always come at the best timing. I will always be grateful. The paths you showed me are now defining my way and how things can change, especially for sea turtles and the development of coastal communities in Guatemala.

Introducción (Introduction)

Las tortugas marinas están entre los grupos más primitivos de vertebrados, algunas especies terrestres migraron al océano y luego regresaron a la tierra hace más de 200 millones de años, dando origen a linajes extantes (Lockley et al., 2019). De acuerdo a los estudios filogenéticos, las tortugas marinas son un grupo monofilético del suborden Cryptodira, dentro del que se incluyen 2 familias: Cheloniidae y Dermochelydae. La primera contiene las especies *Caretta caretta, Chelonia mydas, Eretmochelys imbricata, Lepidochelys kempii, Lepidochelys olivacea* y *Natator depressus*, mientras que la segunda familia únicamente a la especie *Dermochelys coriacea* (Guillon et al., 2012). Su sobrevivencia puede atribuirse al desarrollo de adaptaciones al ambiente en que viven, siendo algunas: un cuerpo hidrodinámico, glándulas que excretan el excesos de sal, extremidades en forma de remos, sistemas especializados de buceo, receptores olfativos, entre otras (Hudgins et al., 2017).

El intercambio calórico lo realizan con el ambiente, como cualquier ectotermo, ocurriendo vía convección, radiación y conducción (Vitt & Caldwell, 2014). Es decir, dependen de los recursos ambientales para obtener calor corporal, el cual surge del metabolismo celular o mitocondrial (Merchant-Larios, 2000). Sin embargo, son capaces de autorregular su temperatura en rangos relativamente cortos, utilizando el sol y las superficies del ambiente que las rodea para la obtención del calor, así como la sombra, el agua y las superficies frías, para perderlo (Vitt & Caldwell, 2014). Las modificaciones anatómicas han sido resultado de su adaptación a la vida acuática, en donde permanecen casi durante toda su vida y como vestigios de su origen terrestre, regresan a las playas únicamente a desovar sus huevos (Hudgins et al., 2017).

Las tortugas marinas se caracterizan por poseer ciclos de vida complejos (Gane et al., 2020), en donde todas las fases están afectadas por la temperatura (Morales-Mérida, 2013). Son animales ovíparos sin cuidado parental, en donde las hembras desovan en la arena y luego regresan al mar a esperar la reanidación y al final de la temporada migran a zonas de alimentación a esperar dos años hasta la próxima época reproductiva. Algunos días después (dependiendo de la especie), los neonatos emergen del nido y se dirigen hacia el mar donde pasaran la mayor parte de su vida (Marco et al., 2017).

La temperatura también juega un rol importante en la incubación de los huevos, que dependen de las condiciones ambientales para tener un desarrollo exitoso (Mueller et al., 1994). En este sentido, afecta directamente al desarrollo embrionario, ya que los embriones son sensibles a ciertas temperaturas y esto se refleja en el éxito de eclosión semanas después (Bladow & Milton, 2019). Además del desarrollo, la temperatura es un factor clave en la determinación de sexo, debido a que son animales con determinación de sexo dependiente de la temperatura (TSD, por sus siglas en inglés) (Morales-Merida et al., 2018). Este mecanismo determina el sexo según la acumulación de temperaturas a las que son expuestas durante determinado período de su incubación (Girondot et al., 2018).

Las tortugas marinas anidan en vastas regiones del mundo, en donde las hembras regresan a desovar a la misma región de donde nacieron, fenómeno llamado filopatria (Martín-del-Campo & García-Gasca, 2019). Cada una de las playas donde desovan constituye un hábitat de anidación, el cual posee características propias que varían de una región a la otra. Por ejemplo, hay algunas playas de anidación en donde la vegetación es tan densa que algunas tortugas pueden anidar bajo sombra y otras directamente expuestas al sol (Mutalib & Fadzly, 2015). Las poblaciones que anidan en cada playa han desarrollado adaptaciones locales a las condiciones particulares de cada hábitat de anidación, de manera que es importante trabajar y ajustar las medidas de conservación a los lugares donde se implementan, utilizando datos propios de cada sitio (Morales-Mérida et al., 2015).

Comprender el hábitat de anidación es importante para hacer propuestas que permitan un mejor manejo de las tortugas marinas. Las áreas donde habitan y anidan, proveen información fundamental y algunas de las recomendaciones generales de los expertos, desde la propuesta original, son: documentar cuándo y dónde ocurren las anidaciones, determinar el éxito de eclosión, caracterizar la variabilidad genética, evaluar parámetros poblacionales e investigar temáticas de relevancia para la conservación, como el comportamiento de anidación y la sobrevivencia de embriones y neonatos (Richardson, 2000).

A pesar de los esfuerzos globales por conservar estas especies, las tortugas marinas, se consideran dentro de las especies más amenazadas de la costa marina (Darmawan et al., 2018). Las principales causas de la declinación de sus poblaciones son la explotación humana de sus huevos, carne y caparazón para subsistencia y comercio. Sin embargo, en las últimas décadas se reconoce también la captura incidental en pesquerías de palangre y camarón, la contaminación y la destrucción de sus hábitat (Blechschmidt et al., 2020; Panamá, 2017b). Tal situación ha llevado a que especialistas a nivel mundial busquen y tomen medidas especiales, en cuanto a su protección y conservación, considerando que su biología las hace depender de las playas para anidar o desovar en determinadas áreas costeras del mundo (Hudgins et al., 2017). Por tal razón, comprender la biología de la reproducción y anidación es indispensable para la recuperación y el manejo de las poblaciones tortugas marinas (Richardson, 2000).

En Guatemala, en sus 255 kilómetros de litoral Pacífico, anida principalmente *Lepidochelys olivacea* y esporádicamente *Dermochelys coriacea*, y en los 148 kilómetros de costa del Caribe, anida *Caretta Caretta, Chelonia mydas* y *Eretmochelys imbricata* (CONAP, 2015). Aunque no hay datos recientes, de las cinco especies que anidan, la parlama (*L. olivacea*) continúa siendo considerada la tortuga marina más abundante del Pacífico guatemalteco (Montes Osorio, 2004). En general, esta especie habita aguas tropicales, subtropicales y templadas. Es considerada, a nivel mundial, la especie de tortuga marina más pequeña y abundante de las siete especies. Puede crecer hasta 70 centímetros de largo y pesar 45 kilogramos. Además, alcanzan la madurez sexual de 10 a 15 años luego de su eclosión (Hudgins et al., 2017). Una vez alcanzada la madurez sexual, en la época de anidación (reproductiva), cada tortuga puede reanidar entre 2 y 3 veces, poniendo entre 95 y 120 huevos por nido, con incubación de alrededor de 45 días (CIT, 2011). En Guatemala se ha registrado, que dependiendo de las temperaturas generales de incubación, ésta puede abarcar desde los 40 días hasta los 54, aproximadamente (Morales Mérida, 2013).

En Guatemala se han protegido a las tortugas marinas a través de programas de conservación que se basan exclusivamente en el uso de recintos artificiales para incubación de huevos, llamados tortugarios, situados en las costas del país. En estos recintos, los huevos son reubicados y, al nacer, los neonatos son liberados al mar. En 1971 se estableció el primer tortugario en una comunidad pesquera del Pacífico de Guatemala (CONAP, 2009). Esto marcó el inicio de tortugarios en el país, como estrategia nacional de conservación de estas especies.

La incubación de huevos de tortugas marinas en tortugarios se justifica cuando la protección de huevos de tortugas marinas *in situ* es imposible o baja (Mutalib & Fadzly, 2015). Estos deben de estar ubicados tan cerca de la playa de anidación como sea posible, con el fin de minimizar el trauma físico que puedan sufrir los huevos durante el transporte y también para poder proporcionarle a los embriones y a los neonatos la oportunidad de realizar la impronta sobre la playa de anidación donde nacieron (Eckert et al., 2000). En Guatemala, desde entonces, se ha incrementado el número de tortugario en la costa Pacífica, variando entre 13 y 23, dependiendo de los recursos y patrocinadores disponibles (CONAP, 2009).

El sistema de conservación de tortugas marinas en Guatemala, se mantiene a través de un sistema de cuotas de conservación, en el que los colectores (localmente conocidos como parlameros) proporcionan el 20% de cada nido colectado, a un tortugario oficialmente registrado ante el Consejo Nacional de Áreas Protegidas (CONAP), organismo encargado de resguardar la diversidad biológica en Guatemala según el Decreto 4-89 de la Ley de Áreas Protegidas de Guatemala

(CONAP, 2009). La entrega de esta cuota le permite a los parlameros comercializar el restante 80% del nido. La comercialización de los huevos es exclusivo para *L. olivacea*, los huevos de las otras especies que anidan o han anidado en el país es ilegal (CONAP, 2018).

De acuerdo con lo establecido por la Convención Interamericana para la Protección y Conservación de Tortugas Marinas (CIT), el manejo en cada país debe ser local, fundamentándose en investigaciones científicas sobre la fisiología reproductiva, morfología y otros aspectos de las especies de tortugas marinas, *in situ* y *ex situ*, en las playas de anidación de cada país (CIT, 2011). En Guatemala no se contaba con dicha información, por tanto la estrategia de conservación se implementó sin adaptación o modificación local. Utilizó muchas de las recomendaciones planteadas por expertos de la UICN y de la CIT.

En 2018 se actualizó la Estrategia Nacional para el manejo y conservación de tortugas marinas en Guatemala. El propósito que se mantienen es el de “regular el uso, manejo y conservación de Tortugas Marinas” (CONAP, 2018 p.7). Sin embargo, esta no incentiva la permanencia de nidos naturales ni se adaptó a las condiciones locales. Por el contrario, normalizó el establecimiento de tortugarios, por tanto es importante hacer aportes al sistema de tortugarios que permitan maximizar la eficiencia de los mismos en pro de la conservación de las tortugas marinas.

En Guatemala pocos estudios, que generen información aplicable, han sido realizados (Martínez Mencos & Calderón Pérez, 2011; Montes Osorio, 2004; Morales Mérida, 2013; Rivas Chacón, 2002). Lo que evidenció la necesidad de generar más información para hacer mejoras al sistema de conservación vigente. En el Parque Nacional Alas Purwo, Java Oriental, en Indonesia, una investigación generó información y se utilizó para fortalecer el sistema de conservación de las tortugas marinas (Maulany et al., 2012a). En dicho estudio, los resultados permitieron hacer aportes y mejoras al manejo de los tortugarios de ese parque, pues se establecieron los límites de mortalidad térmica de la especie estudiada en esa región, conociendo cómo las temperaturas obtenidas disminuyeron el éxito de la emergencia y el desempeño locomotor de los neonatos.

El estudio de biología y fisiología más reciente en Guatemala, es la tesis de grado de Morales Mérida (2013), en donde se evidenció la producción del 100% de hembras en Guatemala, bajo condiciones controladas de tortugario y semi-naturales. Esto abrió las puertas a querer generar más información acerca de su biología, de su comportamiento y de su hábitat de anidación. De manera que sea posible mejorar y crear programas de conservación consecuentes con las poblaciones del país. De aquí surgió la necesidad de resolver cuestionamientos básicos y querer saber más sobre las características de los nidos naturales de *L. olivacea*, de su hábitat de anidación, y del desempeño de los neonatos como factor predatorio de supervivencia. Además, de querer saber sobre el desarrollo embrionario y su relación con los factores de manejo en tortugarios. Intentando probar así la hipótesis de que sostiene que la supervivencia de los neonatos de *L. olivacea* es amenazada por los factores del manejo en la costa oriental del Pacífico de Guatemala, y no por factores asociados al estrés ambiental en su desarrollo embrionario.

A nivel mundial, se ha evaluado el manejo de tortugas marinas en tortugarios, tomando en cuenta varios aspectos como proporción de sexos, éxito de eclosión, éxito de emergencia, entre otros, contemplando el impacto que puede tener en la conservación, en diversas escalas (Sari & Yakup, 2017). En el presente estudio doctoral se determinaron varios aspectos de el estado de conservación de las tortugas marinas *L. olivacea* que anidan en Guatemala. Se logró determinar que las poblaciones no se encuentran en declive, como se presumen, sino se mantienen en una tendencia normal que sube y baja a lo largo del tiempo. También se determinó cómo las condiciones ambientales pueden afectar el desempeño de los neonatos de *L. olivacea*. Tomando en cuenta condiciones de incubación como: temperatura, peso y talla, y factores que se consideran estresores para los embriones como el éxito de eclosión y la asimetría en los neonatos. Esto también permitió conocer cuáles son los efectos sobre el desarrollo embrionario y los factores asociados directamente con el manejo. El éxito de eclosión es un factor clave ya que permite conocer la adecuación del tortugario y la salud en general de la población de tortugas marinas que anida en el país (Miller, 2000).

Esta investigación podría considerarse como una de las primeras investigaciones científicas fundantes de información básica de la biología de anidación *L. olivacea* en Guatemala. Pues, aunque es información conocida en otros países, en Guatemala, contribuye al desarrollo ecológico, generando información útil y viable en futuros planes de manejo y estrategias de conservación de esta especie en el país. A nivel general pretendió conocer el estado de conservación de esta especie a través de la evaluación de los factores que afectan el desempeño de las crías de *L. olivacea* en nidos reubicados en la costa oriental del Pacífico de Guatemala. Y finalmente se generaron recomendaciones basadas en los resultados obtenidos.

Objectives

General Objective

Evaluate the factors that affect the performance of *Lepidochelys olivacea* hatchlings in nests relocated on the eastern coast of the Pacific of Guatemala

Specific Objectives

1. Characterize the nests and nesting habitat of *L. olivacea* on the Pacific coast of Guatemala
2. Determine the performance of *L. olivacea* hatchlings that emerged from relocated nests on the eastern coast of the Pacific of Guatemala
3. Generate recommendations based on the analysis of management factors that affect the performance of *L. olivacea* hatchlings in relocated nests on the Pacific coast of Guatemala

Synthesis

Marine turtles are reptiles that have lived in the Earth’s oceans and nested on the beaches starting several million years ago and also have coexisted with coastal communities since humans colonized beach areas across the world. In recent decades, with increased recognition of biodiversity loss and animal extinction, scientists have focused their research on different conservations strategies for various taxa. Marine turtles, with their global distribution and widespread occurrence in various regions, have also been the focus of conservation research and planning.

Marine turtles spend the majority of their lives in the oceans, except for brief periods associated with reproduction: when females emerge from the ocean to lay eggs in the sand on open beaches, and when successfully developed hatchlings emerge from their nests and scramble to the ocean. As beaches are important habitat for sea turtles, they often are protected globally, with different conservation methods, and strategies of nest protection have emerged. In Guatemala, nest depredation by people (also referred to as poaching) has been identified as a threat to sea turtles since the early 1970s. This problem has led the authorities to establish an exclusive conservation strategy that focused on protected beach enclosures called hatcheries, in which sea turtle eggs are reburied for protection and ensure a higher hatching success (CONAP, 2015). In this system, people collect eggs from freshly laid sea turtles on various beaches. The egg collectors are required to deliver a “conservation quota” of 20% of the eggs found in the nest to an officially registered hatchery. In exchange, they are allowed to keep or sell the remaining eggs for human consumption or other purposes (CONAP, 2018). This system applies only to the marine turtle *Lepidochelys olivacea*, considered the most abundant and constant nesting species in the Pacific of Guatemala. Commercialization of eggs of other species is illegal under federal law.

This thesis aims to investigate the current conservation strategy in Guatemala and develop new ways of conservation for marine turtles based on scientific evidence through basic biological research. The spark for this work came in 2011 when it became clear that there was limited biological information underpinning Guatemalan national policies for sea turtle management. The initial step was to understand and document what is being done at various hatcheries, including the use of full-shade hatcheries for incubating eggs and its potential impacts on the hatchling’s sex ratio. Based on initial surveys and data collection, there is a high probability that all Olive ridley hatchlings released along Guatemala’s Pacific Coast are females (Morales Mérida, 2013).

Further related research was needed to contextualize these results, and also basic biological and ecological information on this species in Guatemala was needed. These information gaps were the main reason why this doctoral thesis was conceived. The research results and conclusions are based on current events and data and form the basis for recommendations for improved conservation strategies across the Pacific coast of Guatemala.

The current doctoral thesis was proposed to provide more information to solve this conservation problem and provide scientific foundations to management policies. Its aims include an improved understanding of the latest management actions and their potential impacts on the offspring produced and released to the sea to preserve the species. A practical and valuable means to assess this impact was by monitoring locomotor performance as a fitness metric. Following this, efforts were made to engage stakeholders and collaborators to document as much information about the species in Guatemala as possible, including population density and reproductive effort.

During the process of data gathering and analysis of this thesis, many collaborations were made. At least six undergraduate research projects were completed concurrently during the completion of this thesis. Because three specific objectives drove this thesis, three chapters are presented. Three of the six articles were conceptualized from the beginning, specifically for this thesis. The other three chapters were written opportunistically, based on the data obtained and their subsequent analyses.

The first chapter contains three articles. The first article analyzes and presents the nesting activities of females, using historical data of nesting tracks and hatchery information of the total amount of eggs incubated during 17 years and 11 beaches, using statistical models of nesting phenology. The second article was initially conceived on the observations of dark-colored sands in Guatemala and the potential to generate a female bias in hatchling sex ratio due to temperature differences. This led to the idea of analyzing nest density and sand color with respect to overall hatchling sex ratio production. The final article from this chapter used relatively new movement dataloggers that made it possible to record the exact moment of pipping (breaking of the shell inside the nest), making it possible to generate a more accurate incubation period for this species, information that helpful when working with the thermal reaction norm and reproductive physiology of this species.

The second chapter has two articles. The first focuses on the locomotor performance of the hatchlings and how this can give us information about what is expected of the offspring in the long term (in terms of fitness and survival) and how the management conditions of the hatcheries may have an influence on it. The second article of this chapter was related to temperature-dependent sex determination mechanisms and the advances in its modeling. This work was published using models that showed the advantages that Metropolis-Hastings with Markov chain produced by a Monte Carlo had over the commonly used maximum likelihood, using published data of incubation experiments using eggs from *L. olivacea* of the Northeast Indian, East Pacific, and West Atlantic Regional Management Units.

The third and last chapter consists of an essay article, in which various components of this thesis are brought together in a cohesive and integral document, where global recommendations. The recommendations are based on the results obtained within the context of the current management and environmental conditions, with the objective to enable what can and should be done to make a significant effort to conserve this species in Guatemala. Ideally, decision-makers will use them to improve Guatemala’s marine turtle conservation.

The first chapter contains three articles. The first article analyzes and presents the nesting activities of females, using historical data of nesting tracks and hatchery information of the total amount of eggs incubated during 17 years and 11 beaches, using statistical models of nesting phenology. The second article was initially conceived on the observations of dark-colored sands in Guatemala and the potential to generate a female bias in hatchling sex ratio due to temperature differences. This led to the idea of analyzing nest density and sand color with respect to overall hatchling sex ratio production. The final article from this chapter used relatively new movement dataloggers that made it possible to record the exact moment of pipping (breaking of the shell inside the nest), making it possible to generate a more accurate incubation period for this species, helpful when working with the thermal reaction norm and reproductive physiology of this species.

The second chapter has two articles. The first focuses on the locomotor performance of the hatchlings and how this can give us information about what is expected of the offspring in the long term (in terms of fitness and survival) and how the management conditions of the hatcheries may have an influence on it. The second article of this chapter was related to temperature-dependent sex determination mechanisms and the advances in its modeling. This work was published using models that showed the advantages that Metropolis-Hastings with Markov chain produced by a Monte Carlo had over the commonly used maximum likelihood, using published data of incubation experiments using eggs from *L. olivacea* of the Northeast Indian, East Pacific, and West Atlantic Regional Management Units.

The third and last chapter consists of an essay article, in which various components of this thesis are brought together in a cohesive and integral document, where global recommendations. The recommendations are based on the results obtained within the context of the current management and environmental conditions, with the objective to enable what can and should be done to make a significant effort to conserve this species in Guatemala. Ideally, decision-makers will use them to improve Guatemala’s marine turtle conservation system.

Articles

CHAPTER 1. Nests and nesting habitat of *Lepidochelys olivacea* on the eastern coast of the Pacific of Guatemala

ARTICLE 1. Morales-Mérida BA, Muccio C, Girondot M. (2021, in review). Trend in olive ridleys Nest counts in Guatemala in the light of national hatchery protection strategy. **Submitted to Oryx—The International Journal of Conservation**

Trend in olive ridleys Nest counts in Guatemala in the light of national hatchery protection strategy

B. ALEJANDRA MORALES-MÉRIDA
Laboratoire Écologie, Systématique, Évolution, AgroParisTech, CNRS, Université Paris Saclay, 91405 Orsay, France ; Doctorado en Ciencias Naturales para el Desarrollo DOCINADE, Instituto Tecnológico de Costa Rica (TEC), Universidad Nacional (UNA), Universidad Estatal a Distancia (UNED), Costa Rica, and Facultad de Ciencias Químicas y Farmacia, Universidad de San Carlos de Guatemala (USAC), Guatemala.
COLUM MUCCIO
Asociacion Rescate y Conservacion de Vida Silvestre (ARCAS), San Lucas Sacatepequez, Guatemala.
MARC GIRONDOT (Corresponding author)
Laboratoire Écologie, Systématique, Évolution, AgroParisTech, CNRS, Université Paris Saclay, 91405 Orsay, France. E-mail : marc.girondot@universite-paris-saclay.fr
orcid.org/0000-0001-6645-8530

Word count: 7825

Abstract

Marine turtles are important species both culturally and ecologically in Guatemala. Due to their current endangered status, multiple efforts have been undertaken for their conservation around the world. The olive ridley sea turtle (*Lepidochelys olivacea*) is the most abundant marine turtle species in Guatemala, representing over 99% of all nests. Conservation efforts rely almost exclusively on an informal system whereby local residents can harvest olive ridley nests on the condition that they donate 20% of each nest to a hatchery, where the eggs are incubated and the hatchlings later released back into the Pacific Ocean. This system was initiated in the 1970s, and until recently, no effort has been made to determine its sustainability and impact on the overall population trend of the olive ridleys on the Pacific coast of Guatemala. In a 2020 publication based on a single beach track in Guatemala as well as 15 years of egg incubation data from Guatemalan hatcheries, it was concluded that the population had increased. To verify the validity of this conclusion, we analyzed data from 17 years and 11 beaches using statistical models of nesting phenology. We invalidated the hypothesis that olive ridley nesting activity is increasing. The total nesting activity for these 17 beaches revealed several peaks, but it was generally stable over this period. Due to uneven hatchery management and the financial dependency of inhabitants on the sale of eggs, incubated eggs cannot be used as an indicator of the population trend of olive ridleys in Guatemala.

Keywords *Lepidochelys olivacea*, Guatemala, beach, nests, hatchery, marine turtle.

Introduction

Marine turtles are classified as endangered or critically endangered according to the IUCN Red List, although their conservation status has been extensively debated (Mrosovsky, 1997; Mrosovsky, 2004; Seminoff & Shanker, 2008). For the past few decades, there has been a tendency to treat inference in a biased way by accepting any inference that a population is declining but demanding a high level of proof before accepting its increase (Mrosovsky, 2002; Mrosovsky, 2004). This bias was favored by the absence of a long time series of data, but in the past few years, a massive effort has been made to create an unbiased conservation status using data-guided evidence (Wallace et al., 2011) and the development of regional conservation status ratings (Wallace et al., 2010). Further, the IUCN general conservation status is based on the following criteria: the rate of population decline, the geographic range, whether the species has a small population size or lives in a restricted area, and whether the results of quantitative analysis indicate a high probability of extinction in the wild (IUCN, 2012). For marine turtles, the rate of population change is the main criteria used to infer population and thus conservation status (Seminoff et al., 2004; Wallace, 2019). Conservation status can also be determined at the national level (IUCN Species Survival Commission, 2012) to inform governments about the pertinence of the conservation measures implemented for certain species. The IUCN Red List is the most comprehensive global inventory of species conservation status (Godley et al., 2020). The most numerous species of marine turtle worldwide and also locally along the Pacific coast of Guatemala and Mexico is *Lepidochelys olivacea* (Hart et al., 2018). This species is characterized by its polymorphic nesting behaviors: the arribada or synchronous mass nesting event and solitary nesting behavior, which has made them of particular interest to scientists around the world. For the purpose of accuracy, the regional management units for this species have been separated according to nesting behavior, although the geographic boundaries are identical within a region (Wallace et al., 2010). Based on the management unit distribution, *L. olivacea* is found in the Eastern Pacific Ocean, the Central and Western Pacific Ocean, the Eastern Indian Ocean, the Western Indian Ocean, the Eastern Atlantic Ocean, and the Western Atlantic Ocean. The most recent assessment status on the IUCN Red List classifies this species as “vulnerable” (Abreu-Grobois & Plotkin, 2008), meaning that based on the available evidence, this species is facing a risk of extinction in the wild (IUCN, 2021). Regarding the status of *L. olivacea* in the East Pacific, Rguez-Baron et al. (2019) report that the main threats are coastal development and artificial illumination, egg harvesting, climate change and rising seas, introduced animals such as feral and domestic dogs and pigs, and removal of beach sand. Conservation efforts can and should therefore focus on managing these threats. In countries where arribadas occur, these arribada beaches are viewed as the priority, whereas solitary nesting beaches are used to reinforce tourism and education with hatchlings (Hart et al., 2018). In Guatemala, which only has solitary nesting beaches, all beaches and hatchlings are used for tourist purposes; although some have an educational aim, most are used for tourist and economic purposes.

In general, marine turtles are subject to widespread exploitation for subsistence use as well as for domestic and international trade. Seven species of marine turtles are present in all oceans and their coastal territories, leading to their interaction with coastal communities, probably since the beginning of human settlement; they have also become an important source of income for local inhabitants (Waylen et al., 2009). The conservation of the species therefore relies on the efforts of scientists and governments (Godley et al., 2020). Guatemala is of special interest because of its unique national conservation strategy. Hatcheries are at the core of this strategy, allowing egg collectors to deliver 20% of the nest to a hatchery in exchange for selling the rest of the nest (CONAP, 2018). In this context, nearly 100% of nests are collected, and no nests are left on the beaches, thus preventing natural selection and adaptation processes. Although this has been done for over 50 years, the question as to whether the system is sustainable remains unknown.

In 2020, an analysis of the number of recorded tracks along a 7.5 km stretch of El Hawaii nesting beach (longitude W90°41’95.19”, latitude N13°85’90.12”, WWGS 84) on the Guatemalan Pacific coast concluded that the activities of olive ridleys increased exponentially from 2003 to 2018 (Ariano-Sánchez et al., 2020). This information is especially important, since it was interpreted as the global trend at the scale of the country. However, we note several weaknesses in this study. For example, the number of recorded tracks in Table 1 of Ariano-Sánchez et al. (2020) is only partially consistent with the values reported in the original reports of the ONG ARCAS, an Association for wildlife rescue and conservation, (Muccio, 2017; Muccio, 2018; Muccio, 2019). Second, the authors define the nesting season based on civil years (January to December), whereas one nesting season spans two civil years from early July to February or March. Third, only counted tracks from the field were used, although the monitoring period was not precisely indicated for all the study years; thus, the sum of the tracks for each year was dependent on the monitoring effort, which was not detailed. Fourth, all the years were not considered, as the period from 1997 to 2001 was excluded. Fifth, the use of fixed start and end dates of the monitoring period supposed that the phenology was constant from year to year, but this was not tested. A decrease in the net change of eggs taken to the hatcheries occurred in the years following the two extreme El Niño-Southern oscillation events (one El Niño and one La Niña phase). El Niño-Southern oscillation is an irregular periodic variation in winds and sea surface temperatures over the tropical eastern Pacific Ocean, thus affecting the climate of much of the tropics and subtropics (Zhang et al., 2019). The warming phase of the sea temperature is known as El Niño and the cooling phase as La Niña. It should be noted, however, that the two extreme El Niño-Southern oscillation events occurred only once during this time series, thus making it impossible to conclude about the generality of this information. Furthermore, both El Niño and La Niña events produced the same effect on the number of nests in the hatcheries, which was unexpected. An effect of El Niño-Southern oscillation on the phenology of the nesting season could be expected, although this was not tested. Finally, data from a single 7.5 km beach were used, whereas nesting occurs along the entire 250 km Pacific coastline of Guatemala. For all the above reasons, we think that conclusion about "an upward trend in olive ridley nesting abundance on the Pacific coast of Guatemala" (Ariano-Sánchez et al., 2020) should be revisited with new modeling techniques.

Marine turtles exhibit philopatry behavior, meaning that they return to the regions where they were born (Clusa et al., 2018). Given that various threats and pressures exerted on marine turtles can affect their nesting, it is important to analyze a larger coastline than a 7.5 km stretch. Especially in Guatemala, cultural differences may differently affect the 250 km Pacific coastline where *L. olivacea* nests, and egg collecting is permitted. Using all the available field nesting data as well as the global information about hatcheries, this work aims to describe the national situation for olive ridleys along the Pacific coast of Guatemala.

Methods

Field data collection: Number of olive ridley tracks

Beach patrols were conducted by field workers hired by the ONG ARCAS during nesting seasons from 1997 to 2012 in Hawaii beach and from 2013 to 2018 in a total of seven beaches: El Chico, Churririn, El Paredon, Conacaste, Monterrico, Hawaii, and La Barrona (Fig. 1). Patrols were normally performed at dawn six days per week to count the number of tracks from the previous night. The distinction between successful and unsuccessful nesting attempts was sometimes difficult to determine, and for this reason, all tracks (successful and unsuccessful) were counted. We preferred using the number of tracks as a proxy of nesting activity rather than converting the tracks into nest numbers by applying a conversion factor. Despite sporadic nesting throughout the year, the main olive ridley nesting season spans from July to November, with peaks in August and September. Table 1 presents an overview of available data. For statistical modeling of the nesting season, the seasons are defined here from June 1 to May 31 in the following year.

The teams of field workers did not consistently report the 0 counts in the databases, as this could indicate either that they did not go out or that they did go out but did not find any tracks. For this reason, the 0 reported in the databases was removed, and a strategy using conditional likelihood was used to take the absence of 0 counts into account (Girondot, 2010a).

Hatchery data

Hatcheries have existed in Guatemala since early 1971. As governmental policy, the National Council of Protected Areas is the entity responsible for gathering and managing each hatchery and collecting its data. This means that each hatchery must provide information on an annual basis about the number of eggs incubated, hatchlings released, and percentage of hatching success.

On the Pacific coast of Guatemala, according to official information collected between 2003 and 2018, about 30 hatcheries were officially registered at the National Council. However, this number fluctuates from 9 to 25 hatcheries depending on the year. These hatcheries are located in different beaches of the Pacific coast of Guatemala and are mainly (but not strictly) named after the beach: La Barrona, Hawaii, El Rosario, El Conacaste, Monterrico, Barra del Jiote, El Garitón, El Banco, Candelaria, Las Lisas, El Chapetón, La Mañanitas, Tilapa, El Chico, Sipacate, El Naranjo, El Paredon, Tres Cruces, Madre Vieja, Ocos, AAK, La Barona, Tulate, Churirin, El Parlamar, Tahuexco, Bahía Tortuga, El Manchon, La Barrona, and Champerico. The location of these hatcheries is shown in Fig. 1.

Data on the number of eggs incubated in each hatchery per year from 2001 to 2018 were available from the official National Council documents as well as informal surveys conducted by the ONG ARCAS. As shown in Table 2, there is a lack of information for some years and hatcheries. Complete information is missing for the years prior to 2001 and from 2007 to 2011.

Model for nesting seasonality

Nesting seasonality was modeled following the Girondot phenological model (Girondot, 2010b; Girondot, 2017). This model can be applied to any proxy of nesting such as clutch, nest, or track counts (hereafter, only nest term will be used for the sake of simplicity). Assuming that t is an ordinal date (June 1 is 0 and May 31 in the following year is 364 or 365) and that Nt is the observed number of nests for this date, the number of nests deposited per night is modeled using the following set of equations (equation 1):

$$n_t = \begin{cases} \mathrm{t} < \mathrm{B} \rightarrow \mathrm{PMin} \times \ \mathrm{Max} \\ \mathrm{t} \in [\mathrm{B}, \mathrm{P} - \mathrm{F}[\rightarrow \left(\left(1 + \cos\left(\pi(\mathrm{P} - \mathrm{F} - \mathrm{t})(\mathrm{P} - \mathrm{F} - \mathrm{B})\right)\right)/2\right)(\mathrm{Max} - \mathrm{PMin} \times \ \mathrm{Max}) + \mathrm{PMin} \times \ \mathrm{Max} \\ \mathrm{t} \in [\mathrm{P} - \mathrm{F}, \mathrm{P} + \mathrm{F}[\rightarrow \mathrm{Max} \\ \mathrm{t} \in [\mathrm{P} + \mathrm{F}, \mathrm{E}] \rightarrow \left(\left(1 + \cos\left(\pi(\mathrm{t} - \mathrm{P} + \mathrm{F})(\mathrm{E} - \mathrm{P} + \mathrm{F})\right)\right)/2\right)(\mathrm{Max} - \mathrm{PMin} \times \ \mathrm{Max}) + \mathrm{PMin} \times \ \mathrm{Max} \\ \mathrm{t} > \mathrm{E} \rightarrow \mathrm{PMin} \times \ \mathrm{Max} \end{cases}$$

The model requires at most seven parameters, all of which have direct biological interpretations:

B and E are the ordinal dates for the start and end of the nesting season.

P is the ordinal date for the peak of the nesting season.

F is half of the number of days around P for which the curve flattens out.

Max is the mean number of nests at the peak of the nesting season.

PMin is the mean nightly nest numbers relative to Max before and after the nesting season.

The nesting season is described in segments, and all segments form one continuous function. The nesting season is defined as the interval [B, E]. If F is equal to 0, no flat portion is observed. Rather than fitting B and E, it is more convenient to fit

LengthB = P – B and LengthE = E – P with LengthB > 0 and LengthE > 0 to ensure that B < P < E. The parameters B, E, P, f, LengthB, and LengthE are hereafter defined as shape parameters, and PMin and Max as scale parameters.

In such a situation when several nesting seasons are analyzed, it is also possible to implement the year effect for peak (P) and/or for LengthB and LengthE. Four categories of models were then fitted depending on the year effects for Peak and/or for LengthB and LengthE. These are defined as the Peak-Global or Peak-Year and Length-Global or Length-Year.

Interannual spatial and temporal trends

A model is used to estimate the number of nests for a beach that is not patrolled based on the relative frequency of tracks on the different beaches and the total number of tracks for each year. Let the total theoretical number of tracks be Ti for year i in the entire region where K beaches were monitored during a range of Y years. Three different models can be used to describe Ti according to year i:

Constant number of nests Ti = T; one parameter, T

Exponential model being Ti = T0 er.i; two parameters, T0 and r

The distribution of the nests across the different beaches is defined by the proportion pj of Ti nests in the j beach. For a total of K beaches, K–1 parameters p are necessary due to the relation $\sum_{j=1}^{K} p_j = 1$. The values pj can be modeled as constant (K–1 parameters) or first (2.K–2 parameters) or second (3.K–3 parameters) order as a function of time to represent situations with changes in the relative use of the different nesting sites.

The expected number of nests for year i in the beach j is then Ei,j = Ti × pj.

Let Ni,j be an observed number of nests with a standard deviation of Si,j. The distribution of Ni,j may be close to a Gaussian distribution when the number of nests and monitoring coverage are high, but it can also be a positive skew when the number of nests or monitoring coverage is low. For this reason, a gamma distribution was used to model the data; the gamma distribution is always positive and can show a positive skew when the standard deviation is high compared to the mean. The fit of the parameters was then done using maximum likelihood with a gamma distribution. For the final estimate, the expected number of nests Ei,j was only used when no observation was available; in other situations, the number of nests fitted using the phenology model was preferred.

Strategy for parameter fitting

The strategy for the fitting parameters model is similar for the seasonality model and the interannual spatial and temporal trend model. First, the parameters are fitted using maximum likelihood (see below for details), and then the models are selected using the Akaike Information Criterion (AIC; Akaike, 1974) and Akaike weight (Burnham & Anderson, 2002). In short, AIC evaluates the quality of the fit that penalizes for overfitting too many parameters, while the Akaike weight gives the relative support of the different models: i.e., the probability for each model being the best one. Finally, the distribution of parameters was searched using the Metropolis-Hastings algorithm, which is a Markov chain Monte Carlo method for obtaining a sequence of random samples from a probability distribution (Metropolis et al., 1953; Hastings, 1970). The adaptive proposal distribution (Rosenthal, 2011) as implemented in R package HelpersMG (Girondot, 2021a) ensures that the acceptance rate was close to 0.234, which is the optimal acceptance rate (Roberts & Rosenthal, 2001). A total of 10,000 iterations was run. Priors were all uniform with a range of proposals large enough to ensure that it does not constrain the limits of the parameters. From the 10,000 sets of parameters, we calculated their respective mean and standard error.

For the seasonality model, parameter fitting was performed using maximum likelihood with negative binomial (NB) daily nest distribution with values produced by equation 1 (m=nt) as theoretical values and the observed counts (x=Nt) as observations. In ecology, NB distribution is used to describe the distribution of an organism while taking into account the mean number of individuals m and an aggregation parameter k (Taylor et al., 1979). The probability mass function of NB distribution is:

$$NB(x;m;k) = Pr(X = x) = \frac{\Gamma(k+x)}{x!\ \Gamma(k)}\left(\frac{m}{m+k}\right)^{x}\left(\frac{k}{m+k}\right)^{k}\ , m > 0, k > 0$$

For the situation when 0 nest counts were not reported, the zero-truncated negative binomial (ZTNB) is used with:
$Pr(X = x; x > 0) = NB(x; m; k)/\left(1 - NB(0; m; k)\right), m > 0, k > 0$ (Girondot, 2010a) or
$Pr(X = x; x > 0) = \frac{\Gamma(k+x)}{x!\,\Gamma(k)}\left(\frac{m}{m+k}\right)^{x}\left(\frac{k}{m+k}\right)^{k}\left(1-\left(\frac{k}{m+k}\right)^{k}\right)^{-1}, m > 0, k > 0$ (Arrabal et al., 2014).

These two equations are identical. When a time series is only composed with counts with a single observation, the maximum likelihood is obtained for $m \to 0$ $(m > 0)$. In such a situation, Max and Min for these series could not be fitted, and they were fixed to 10-4 and 10-6, respectively. Consequently, the standard error for these parameters was not available. When nt is equal to 0, it was replaced with 10-9 as the negative binomial model is not defined for m=0.

For the interannual spatial and temporal trend model, the -ln likelihood of the observations is simply the sum of the -ln likelihood for each observation Ni,j within the gamma model.

The adjustments were done using the R package *phenology* available in the Comprehensive R Archive Network (https://cran.r-project.org) that implements these models (Girondot, 2021b). Comparisons between the observed and modeled values were based on the adjusted coefficient of determination (1991).

Relationship between phenology parameters and El Niño-Southern oscillation

The relationship between the parameters of the phenology (*Peak*, *Begin*, *End*, *LengthB*, *LengthE*, *Length* being *LengthB* + *LengthE*) and the total number of nests (*Number*) was tested using the iconography of correlations method, a method of data aggregation that uses partial correlations. The iconography of correlations is a geometric method used to search for links when multiple variables are studied in a single dataset (Lesty & Buat-Ménard, 1982; Lesty, 1999). This method is based on the comparison of correlations between all couples of variables *Rxy* and partial correlations between all triplets of variables *Rxyz*. The correlation measures a link between two variables, whereas the partial correlation measures a link between two variables controlled for the influence of a third (or more). If a correlation is high between two variables but low in the case of a partial correlation between these two variables and a third one, this means that the correlation between the two initial variables was probably not direct but rather mediated by the effect of the third one. With the methodology of the iconography of correlations, a correlation is considered "remarkable" and materialized by a link between x and y if and only if the correlation coefficient *Rxy* and all the partial correlation coefficients *Rxyz* between the two variables x and y, for every variable *z*, are greater than a predetermined threshold and have the same sign as *Rxy*. This method has been shown to be particularly adapted to the analysis of small samples with a large number of variables (Lesty et al., 2004). The threshold used to retain a correlation or partial correlation was chosen to retain all the significant correlations with p=0.1% (Cortés-Gómez et al., 2021). This level was chosen based on the recent recommendations to revise statistical significance (Benjamin et al., 2018).

The relationships between phenology parameters and El Niño-Southern oscillation were also investigated. The bi-monthly Multivariate ENSO index (MEI.v2) uses five variables over the tropical Pacific: sea level pressure, sea surface temperature, zonal and meridional components of the surface wind, and outgoing longwave radiation (Zhang et al., 2019). The lagged effect of El Niño-Southern oscillation as well as its cumulative effect are not known. Partial Spearman correlations between phenology parameters and El Niño-Southern oscillation averaged for 1 to 37 months with a lag between 0 to 36 months were calculated.

Results

Seasonality of track counts and spatial and temporal trends for track counts

A total of four models were fitted and compared (Table 1). Models differed according to season-specific values for Peak and/or the couple (*LengthB; LengthE*). The selected model is the year-specific *Peak*, *LengthB*, and *LengthE* model. The second model by AIC rank order is the model with a common *Peak* but still with year-specific *LengthB* and *LengthE*. The ∆AIC of this second model is 30.23 with a probability of 0 that it could be the best model among the tested ones (Akaike weight). Thus, we retain only the selected model based on the minimum AIC and Akaike weight being equal to 1.

The observed and modeled seasonality of the track counts for Hawaii beach is shown in Fig. 2, while the limits of the nesting season (B and E parameters) as well as its peak (P) are shown in Fig. 3.

Model of spatial and temporal changes in nesting activities

The total number of nesting activities was available for 11 beaches and 20 years of data for a range over 22 years (1997-2018). The model selection for the four tested models (constant or exponential trend of nesting activity and constant or first-order model temporal change in the proportion of tracks) is shown in Table 2. The selected model is the constant number of nesting activities for the 11 beaches but with a first-order year change for the frequentation of using these beaches. The total number of nesting activities for the 11 beaches is shown in Fig. 4A, and the comparison between the observed and estimated number of nesting activities at the beach level is shown in Fig. 4B (adjusted R2=0.44). The proportion of nesting activities in the different beaches for these 22 years is shown in Fig. 4C.

Model of spatial and temporal change in the number of eggs incubated in hatcheries

The number of incubated eggs in 29 hatcheries on the Pacific coast of Guatemala was available for 13 years from 2001 to 2018 with a complete lack of data from 2007 to 2011. Among these 522 combinations of hatchery-year, data were available for 183 combinations (35%). The missing information was estimated using a model linking the identity of the hatchery to the year. This model assumes that the number of incubated eggs in a hatchery is related to the number of nests in the nearby beaches. The selected model is an exponential model with a constant temporal proportion of the number of eggs incubated in each hatchery (Table 3). The fitted model shows an instantaneous growth rate of 0.23 (95% credibility interval 0.22-0.24) (Fig. 5). The second model by the order of AIC and Akaike weight had a probability of 0.14 of being the best model. This model is also an exponential model but with a first-order time proportion of the number of eggs incubated in the hatcheries.

El Niño-Southern oscillation impact on level of nesting and phenology

The relationship between phenology parameters and number of nests per year was investigated using the iconography of correlations (Fig. 6). Phenology parameters are organized into two groups: (*Begin*, *LengthB*, *Peak*) and (*Length, End, LengthE*). The annual number of nests is not linked to the other parameters (Fig. 6B). Thus, the relationships between El Niño-Southern oscillation and phenology parameters were searched for the number of nests, Begin, and Length. These parameters were chosen as they are the easiest to link with the biology of marine turtles if a relationship is found. No clear signal between lagged cumulative El Niño-Southern oscillation and number of nests was detected (Fig. 7A). Nevertheless, a positive signal was found between the sum of 10 months of El Niño-Southern oscillation lagged by 18 months and the beginning of the nesting season (Fig. 7B). A positive signal was also found between the sum of 15 months of El Niño-Southern oscillation lagged by 12 months and the length of nesting season (Fig. 7C).

Discussion

The conservation status of sea turtles is often inferred from the trend in the number of nesting activities on beaches. The conservation status at the national scale can also be used to test whether the implemented conservation strategy is correct. Ariano-Sánchez et al. (2020) concluded about the existence of an exponential upward trend in nesting activity in the Hawaii beach in Guatemala (7.5 km), and based on this conclusion, they supposed that the conservation strategy in Guatemala was correct. However, our reading of this publication raised several issues that required further analysis.

In the publication, the nesting season was based on the civil year (January to December). In Guatemala, however, it is common to state that the nesting season starts in July and ends in February/March, with peaks in other months; nevertheless, the monitoring and hatchery activities only spanned from July to December or

January. In Fig. 2 in which all the nesting beaches were fitted to model the nesting trend, nesting season, and season peak, variations may be observed on an annual basis, which means that the information could be lost by only monitoring the same months of each year. Fig. 3 also shows that nesting peaks are not consistent between years: the nesting season spans from July to March but changes from year to year. When these data are included in the interannual analysis, we conclude that a constant trend is the best selected model (Fig. 4A) but not an increasing exponential trend (p=0, Table 2). Nevertheless, the proportion of nests in Hawaii beach compared to the total number of nesting activities in the 11 beaches along the Pacific coast of Guatemala shows an increasing trend (Fig. 4C). Thus, the increasing trend reported by Ariano-Sánchez et al. (2020) for Hawaii beach should not be interpreted as a national trend.

There is a contradictory issue in the findings and conclusions of Ariano-Sánchez et al. (2020) when considering the El Niño-Southern oscillation impact on nesting. They observed an increase in the nesting abundance of the population along the Pacific coast of Guatemala, which was resilient to El Niño-Southern oscillation variability, although they found no correlation between El Niño-Southern oscillation and the abundance. They also based their conclusion about the upward trend on the fact that the number of eggs incubated in the hatcheries increased in the second year after two extreme El Niño-Southern oscillation events, even though they found a decrease in 2010 and 2015, immediately after the extreme events (La Niña and El Niño, respectively). Using more precise analysis, we did not detect a relationship between the annual number of nests and the El Niño-Southern oscillation index. Nevertheless, we do detect a strong effect of El Niño-Southern oscillation lagged by 12 or 18 months in terms of both the beginning and the length of the nesting season (Fig. 7). The longer nesting seasons could result from two different phenomena: either larger clutch frequency (number of nests deposited by females during one nesting season) or larger polymorphism in the arrival date of females. A lack of information means that we are unable to confirm these hypotheses. The precise link between El Niño-Southern oscillation and nesting phenology deserves to be studied in greater detail.

The comparison between nest counts (Fig. 4A) and yearly incubated egg numbers in hatcheries (Fig. 5A) indicates that the number of eggs incubated in hatcheries cannot be safely used as a proxy for *L. olivacea* trends along the Pacific coast of Guatemala. The number of eggs purchased and incubated in the hatcheries has increased due to the popular demand of releasing hatchlings, which are paid by tourists visiting each hatchery. For instance, some hatcheries obtain funding from national or international organizations. Others are funded by individual sponsors from tourists who pay a fee to release one hatchling. This probably explains why many hatcheries are selling the rest of the nests (80%) to collectors or incubating the whole nest, although this can affect turtles and their habitats if not correctly performed (Katselidis et al., 2013). Thus, the exponential increase in the number of incubated eggs cannot be used as evidence for an increase in the number of nests deposited on the Pacific coast of Guatemala.

Our data invalidate the hypothesis that the nesting activities of Olive Ridleys increased from 2003 to 2018 (Ariano-Sánchez et al., 2020). The trend is rather stable with some peaks. The cultural and local context is rather related to the increase in the number of eggs incubated in each hatchery, as each hatchery has the independent means of purchasing eggs in each nesting season depending on their funding. Nevertheless, the evaluation of the population status should focus on the nesting phenology and the total number of nests deposited on the beach as opposed to the number of incubated eggs.

Author contributions Study design: BAM, MG; fieldwork coordination: CM; data analysis, writing: BAM, MG.

Acknowledgements The nesting data used in this study was part of the ONG ARCAS's sea turtle population monitoring program, which has been funded by the USAID, the National Conservation Fund of Guatemala, The USFWS, and Idea Wild. Special thanks to the local patrollers and park directors that helped in recording the nesting tracks at Hawaii beach. The hatcheries data was available and provided by the National Council of Protected Areas, and a special thanks to Airam López for her patience and effective response to provide such data. The authors acknowledge the support of the Virtual Data initiative, run by LABEX P2IO and supported by Université Paris-Saclay, for providing computing resources on its cloud infrastructure.

Conflicts of interest None.

Ethical standards Collection of data was performed with authorization of the government of Guatemala, track information was obtained under the authority and permissions obtained by ONG ARCAS. Hatchery information is provided from hatcheries to the National Council of Protected Areas (Guatemala’s governmental authority), for them to make an annual database. This database was officially provided for this publication.

References

Abreu-Grobois, A. & Plotkin, P. T. (2008) *Lepidochelys olivacea*. In The IUCN Red List of Threatened Species IUCN SSC Marine Turtle Specialist Group, Gland, Switzerland.

Akaike, H. (1974) A new look at the statistical model identification. IEEE Transactions on Automatic Control, 19, 716-723.

Ariano-Sánchez, D., Muccio, C., Rosell, F. & Reinhardt, S. (2020) Are trends in Olive Ridley sea turtle (*Lepidochelys olivacea*) nesting abundance affected by El Niño Southern Oscillation (ENSO) variability? Sixteen years of monitoring on the Pacific coast of northern Central America. Global Ecology and Conservation, 24.

Arrabal, C. T., dos Santos Silva, K. P. & Bandeira, L. N. (2014) Zero-truncated negative binomial applied to nonlinear data. JP Journal of Biostatistics, 11, 55-67.

Benjamin, D. J., Berger, J. O., Johannesson, M., Nosek, B. A., Wagenmakers, E.-J., Berk, R., Bollen, K. A., Brembs, B. r., Brown, L., Camerer, C., Cesarini, D., Chambers, C. D., Clyde, M., Cook, T. D., Boeck, P. D., Dienes, Z., Dreber, A., Easwaran, K., Efferson, C., Fehr, E., Fidler, F., Field, A. P., Forster, M., George, E. I., Gonzalez, R., Goodman, S., Green, E., Green, D. P., Greenwald, A., Hadfield, J. D., Hedges, L. V., Held, L., Ho, T. H., Hoijtink, H., Jones, J. H., Hruschka, D. J., Imai, K., Imbens, G., Ioannidis, J. P. A., Jeon, M., Kirchler, M., Laibson, D., List, J., Little, R., Lupia, A., Machery, E., Maxwell, S. E., McCarthy, M., Moore, D., Morgan, S. L., Munafó, M., Nakagawa, S., Nyhan, B., Parker, T. H., Pericchi, L., Perugini, M., Rouder, J., Rousseau, J., Savalei, V., Schönbrodt, F. D., Sellke, T., Sinclair, B., Tingley, D., Zandt, T. V., Vazire, S., Watts, D. J., Winship, C., Wolpert, R. L., Xie, Y., Young, C., Zinman, J. & Johnson, V. E. (2018) Redefine statistical significance. Human Nature Behavior, 2, 6-10.

Burnham, K. P. & Anderson, D. R. (2002) Model selection and multimodel inference: A practical information-theoretic approach, Springer-Verlag, New York.

Clusa, M., Carreras, C., Cardona, L., Demetropoulos, A., Margaritoulis, D., Rees, A. F., Hamza, A. A., Khalil, M., Levy, Y. & Turkozan, O. (2018) Philopatry in loggerhead turtles Caretta caretta: beyond the gender paradigm. Marine Ecology Progress Series, 588, 201-213.

CONAP (2018) Normativo sobre manejo y conservación de tortugas marinas consejo nacional de áreas protegidas. pp. 39. Consejo Nacional de Áreas Protegidas, Guatemala city, Guatemala.

Cortés-Gómez, A., Romero, D., Santos, J., Rivera-Hernández, J. R. & Girondot, M. (2021) Inorganic pollutants in live vs dead nesting Olive Ridley marine turtles in the Mexican Pacific: introducing a new way to make statistics in ecotoxicology. Science of the Total Environment, 761, 143249.

Girondot, M. (2010a) Editorial: The zero counts. Marine Turtle Newsletter, 129, 5-6.

Girondot, M. (2010b) Estimating density of animals during migratory waves: application to marine turtles at nesting site. Endangered Species Research, 12, 85-105.

Girondot, M. (2017) Optimizing sampling design to infer marine turtles seasonal nest number for low-and high-density nesting beach using convolution of negative binomial distribution. Ecological Indicators, 81, 83–89.

Girondot, M. (2021a) HelpersMG: Tools for Environmental Analyses, Ecotoxicology and Various R Functions. The Comprehensive R Archive Network.

Girondot, M. (2021b) phenology: Tools to manage a parametric function that describes phenology. The Comprehensive R Archive Network.

Godley, B. J., Broderick, A. C., Colman, L. P., Formia, A., Godfrey, M. H., Hamann, M., Nuno, A., Omeyer, L. C. M., Patrício, A. R., Phillott, A. D., Rees, A. F. & Shanker, K. (2020) Reflections on sea turtle conservation. Oryx, 54, 287-289.

Hart, C. E., Maldonado-Gasca, A., Ley-Quiñonez, C. P., Flores-Peregrina, M., de Jesús Romero-Villarruel, J., Aranda-Mena, O. S., Plata-Rosas, L. J., Tena-Espinoza, M., Llamas-González, I., Zavala-Norzagaray, A. A., Godley, B. J. & Abreu-Grobois, F. A. (2018) Status of olive ridley sea turtles (*Lepidochelys olivacea*) after 29 years of nesting rookery conservation in Nayarit and Bahía de Banderas, Mexico. Chelonian Conservation and Biology, 17, 27-36.

Hastings, W. K. (1970) Monte Carlo sampling methods using Markov chains and their applications. Biometrika, 57, 97-109.

IUCN (2012) UCN Red List Categories and Criteria: Version 3.1, IUCN, Gland, Switzerland and Cambridge, UK.

IUCN (2021) The IUCN Red List of Threatened Species. Version 2021-1. International Union for Conservation of Nature and Natural Resources, Gland, Suisse.

IUCN Species Survival Commission (2012) Guidelines for application of IUCN red list criteria at regional and national levels. version 4.0. pp. 46. IUCN, Gland, Switzerland and Cambridge, UK.

Katselidis, K. A., Schofield, G., Stamou, G., Dimopoulos, P. & Pantis, J. D. (2013) Evidence-based management to regulate the impact of tourism at a key marine turtle rookery on Zakynthos Island, Greece. Oryx, 47, 584-594.

Lesty, C., Pleau-Varet, J. & Kujas, M. (2004) Geometric method and generalized linear models: Two opposite multiparametric approaches illustrated on a sample of pituitary adenomas. Journal of Applied Statistics, 31, 191-213.

Lesty, M. (1999) Une nouvelle approche dans le choix des régresseurs de la régression multiple en présence d'intéractions et de colinearités. La revue de Modulad, 22, 41-77.

Lesty, M. & Buat-Ménard, P. (1982) La synthèse géométrique des corrélations multidimensionnelles. Les Cahiers de l'Analyse des données, VII, 355-370.

Metropolis, N., Rosenbluth, A. W., Rosenbluth, M. N., Teller, A. H. & Teller, E. (1953) Equations of state calculations by fast computing machines. Journal of Chemical Physics, 21, 1087-1092.

Mrosovsky, N. (1997) IUCN's credibility critically endangered. Nature, 389, 436.

Mrosovsky, N. (2002) Hype. Marine Turtle Newsletter, 96, 1-4.

Mrosovsky, N. (2004) Predicting Extinction: Fundamental Flaws in IUCN's Red List System, Exemplified by the Case of Sea Turtles, Department of Zoology, University of Toronto.

Muccio, C. (2017) Situational analysis of the conservation of the sea turtle in Guatemala. pp. 4. ARCAS Guatemala, Guatemala city, Guatemala.

Muccio, C. (2018) Analisis situacional de la conservación de tortugas marinas en Guatemala. pp. 18. ARCAS Guatemala, Guatemala city, Guatemala.

Muccio, C. (2019) Analisis situacional de la conservación de tortugas marinas en Guatemala. pp. 18. ARCAS Guatemala, Guatemala city, Guatemala.

Nagelkerke, N. J. D. (1991) A note on a general definition of the coefficient of determination. Biometrika, 78, 691-192.

Rguez-Baron, J. M., Kelez, S., Liles, M., Zavala-Norzagaray, A., Torres-Suárez, O. L., Amorocho, D. F. & Gaos, A. R. (eds.) (2019) Sea Turtles in the East Pacific Ocean Region, IUCN-SSC Marine Turtle Specialist Group.

Roberts, G. O. & Rosenthal, J. S. (2001) Optimal scaling for various Metropolis-Hastings algorithms. Statistical Science, 16, 351-367.

Rosenthal, J. S. (2011) Optimal proposal distributions and adaptive MCMC. In MCMC Handbook (eds S. Brooks, A. Gelman, G. Jones & X.-L. Meng), pp. 93-112. Chapman and Hall/CRC.

Seminoff, J. A., Crouse, D. & Pilcher, N. (2004) 2004 IUCN Red List of Threatened Species - Chelonia mydas. IUCN, Gand, Switzerland.

Seminoff, J. A. & Shanker, K. (2008) Marine turtles and IUCN Red Listing: a review of the process, the pitfalls, and novel assessment approaches. Journal of Experimental Marine Biology and Ecology, 356, 52-68.

Taylor, L. R., Woiwod, I. P. & Perry, N. (1979) The negative binomial as a dynamic ecological model for aggregation, and the density dependence of k. Journal of Animal Ecology, 48, 289-304.

Wallace, B. P. (2019) Re-evaluating the IUCN Red List status of Northwest Atlantic leatherbacks. In 2019 WIDECAST Annual General Meeting Paramaribo, Suriname.

Wallace, B. P., DiMatteo, A. D., Bolten, A. B., Chaloupka, M. Y., Hutchinson, B. J., Abreu-Grobois, F. A., Mortimer, J. A., Seminoff, J. A., Amorocho, D., Bjorndal, K. A., Bourjea, J., Bowen, B. W., Dueñas, R. B., Casale, P., Choudhury, B. C., Costa, A., Dutton, P. H., Fallabrino, A., Finkbeiner, E. M., Girard, A., Girondot, M., Hamann, M., Hurley, B. J., Lopez-Mendilaharsu, M., Marcovaldi, M. A., Musick, J. A., Nel, R., Pilcher, N. J., Troëng, S., Witherington, B. & Mast, R. B. (2011) Global conservation priorities for marine turtles. PLoS One, 6, e24510.

Wallace, B. P., DiMatteo, A. D., Hurley, B. J., Finkbeiner, E. M., Bolten, A. B., Chaloupka, M. Y., Hutchinson, B. J., Abreu-Grobois, F. A., Amorocho, D., Bjorndal, K. A., Bourjea, J., Bowen, B. W., Dueñas, R. B., Casale, P., Choudhury, B. C., Costa, A., Dutton, P. H., Fallabrino, A., Girard, A., Girondot, M., Godfrey, M. H., Hamann, M., López-Mendilaharsu, M., Marcovaldi, M. A., Mortimer, J. A., Musick, J. A., Nel, R., Seminoff, J. A., Troëng, S., Witherington, B. & Mast, R. B. (2010) Regional management units for marine turtles: a novel framework for prioritizing conservation and research across multiple scales. PLoS One, 5, e15465.

Waylen, K. A., McGowan, P. J., Milner-Gulland, E. & Group, P. S. (2009) Ecotourism positively affects awareness and attitudes but not conservation behaviours: a case study at Grande Riviere, Trinidad. Oryx, 43, 343-351.

Zhang, T., Hoell, A., Perlwitz, J., Eischeid, J., Murray, D., Hoerling, M. & Hamill, T. M. (2019) Towards probabilistic multivariate ENSO monitoring. Geophys. Res. Lett., 46, 10532-10540.

TABLE 1 Model selection for nesting seasonality. The selected model is shown in bold. For all the models, the estimated nest number was set at 10^{-9} when it was 0. *Max* and *Min* parameter for Churirin in 2014-2015 and El Chico in 2016-2017 (both times series have only 1-counts reported) were fixed at 10^{-4} and 10^{-6}, respectively.

Parameters	-Ln L	AIC	ΔAIC	Akaike weight
Peak-global Length-global				
68	17323.20	34782.39	415.32	0.00
Peak-year Length-global				
87	17160.55	34495.10	128.03	0.00
Peak-global Length-year				
106	17092.65	34397.30	30.23	0.00
Peak-year Length-year				
125	**17058.53**	**34367.06**	**0.00**	**1.00**

TABLE 2 Model selection of the spatial and temporal change in nesting activities on the Pacific coast of Guatemala. The selected model is shown in bold.

Trend	Beach use	AIC	ΔAIC	Akaike weight
Constant	Constant	976.60	16.90	0.00
Constant	**1st order time change**	**959.71**	**0.00**	**1.00**
Exponential	Constant	976.75	17.04	0.00
Exponential	1st order time change	998.11	38.41	0.00

TABLE 3 Model selection of the spatial and temporal change in the number of eggs in hatcheries on the Pacific coast of Guatemala. The selected model is shown in bold.

Trend	Hatchery use	AIC	∆AIC	Akaike weight
Constant	Constant	4221.47	325.88	0.00
Constant	1st order time change	4154.50	258.91	0.00
Exponential	**Constant**	**3895.59**	**0.00**	**0.86**
Exponential	1st order time change	3899.15	3.56	0.14

FIG. 1 (A) General view of the north of Central America: 1 Mexico, 2 Guatemala, 3 Belize, 4 Salvador, 5 Honduras, and 6 Nicaragua. (B) Pacific coast map of Guatemala with monitored beaches and hatcheries.

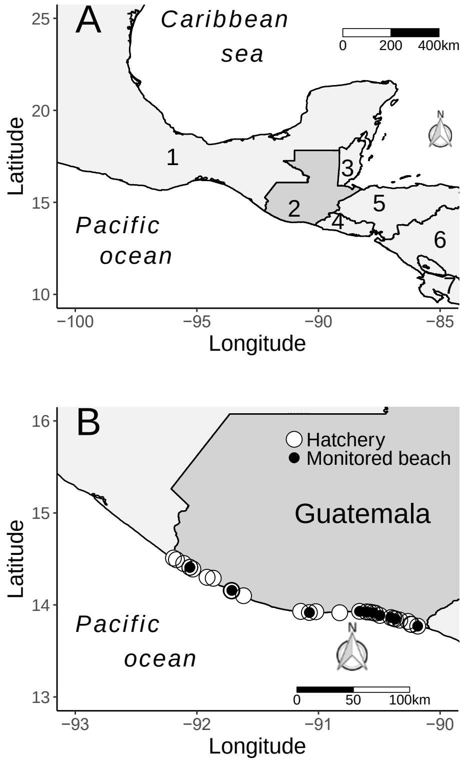
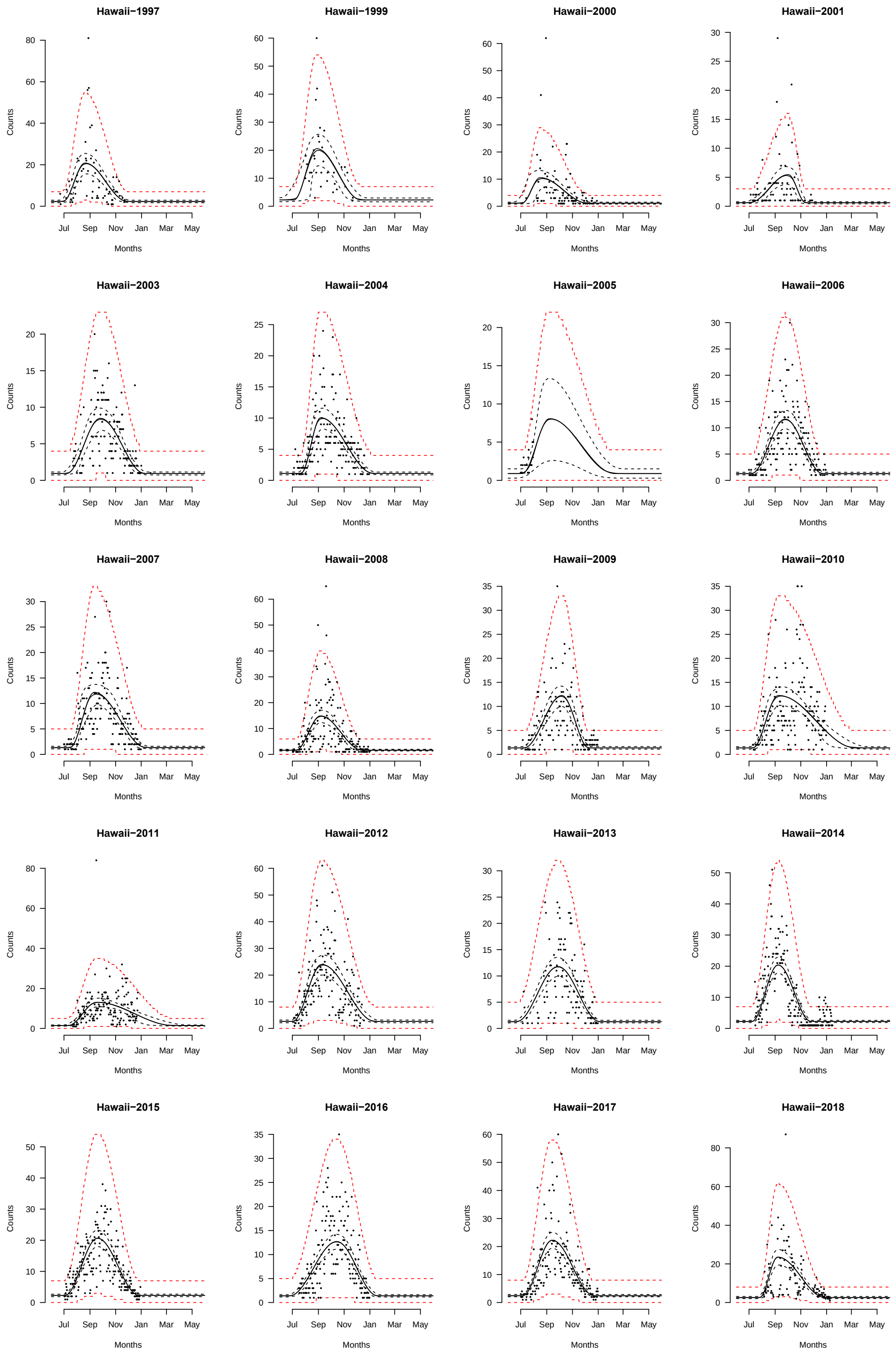


FIG. 2 Seasonality of nesting activity measured on Hawaii beach. The selected model is with year effect on *Peak*, *LengthB*, and *LengthE* parameters (see Table 1).



Hawaii−1997
Hawaii−1999
Hawaii−2000
Hawaii−2001
Hawaii−2003
Hawaii−2004
Hawaii−2005
Hawaii−2006
Hawaii−2007
Hawaii−2008
Hawaii−2009
Hawaii−2010
Hawaii−2011
Hawaii−2012
Hawaii−2013
Hawaii−2014
Hawaii−2015
Hawaii−2016
Hawaii−2017
Hawaii−2018

FIG. 3 Synthetic view of nesting season from the beginning at the bottom to the end at the top of each line. The date of the peak is shown as a black dot.

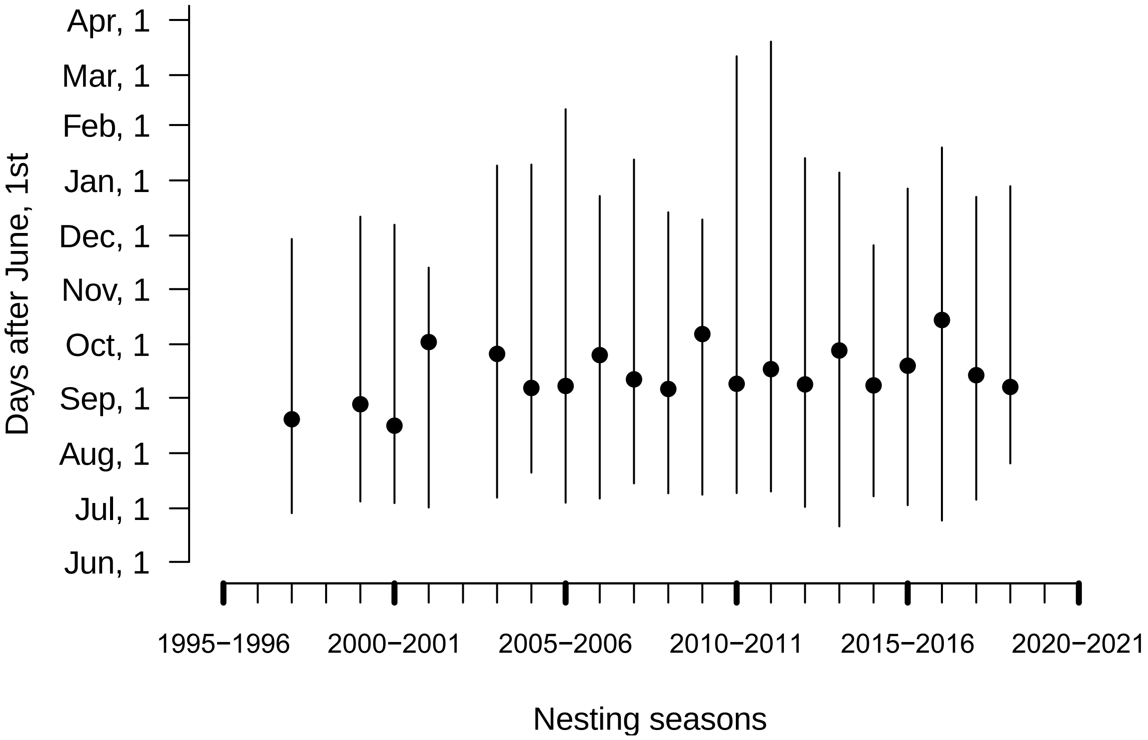


FIG. 4 Spatial and temporal distribution of the nesting activities on the Pacific Coast of Guatemala. (A) Total nesting activities and 95% confidence interval for 11 beaches; (B) relationship between the recorded number of nesting activities and the modeled number to estimate the spatial and temporal trend (65 observations and 23 parameters); and (C) temporal distribution of the proportion of nesting activities along the coast. The beaches are organized from south-east to north-west.

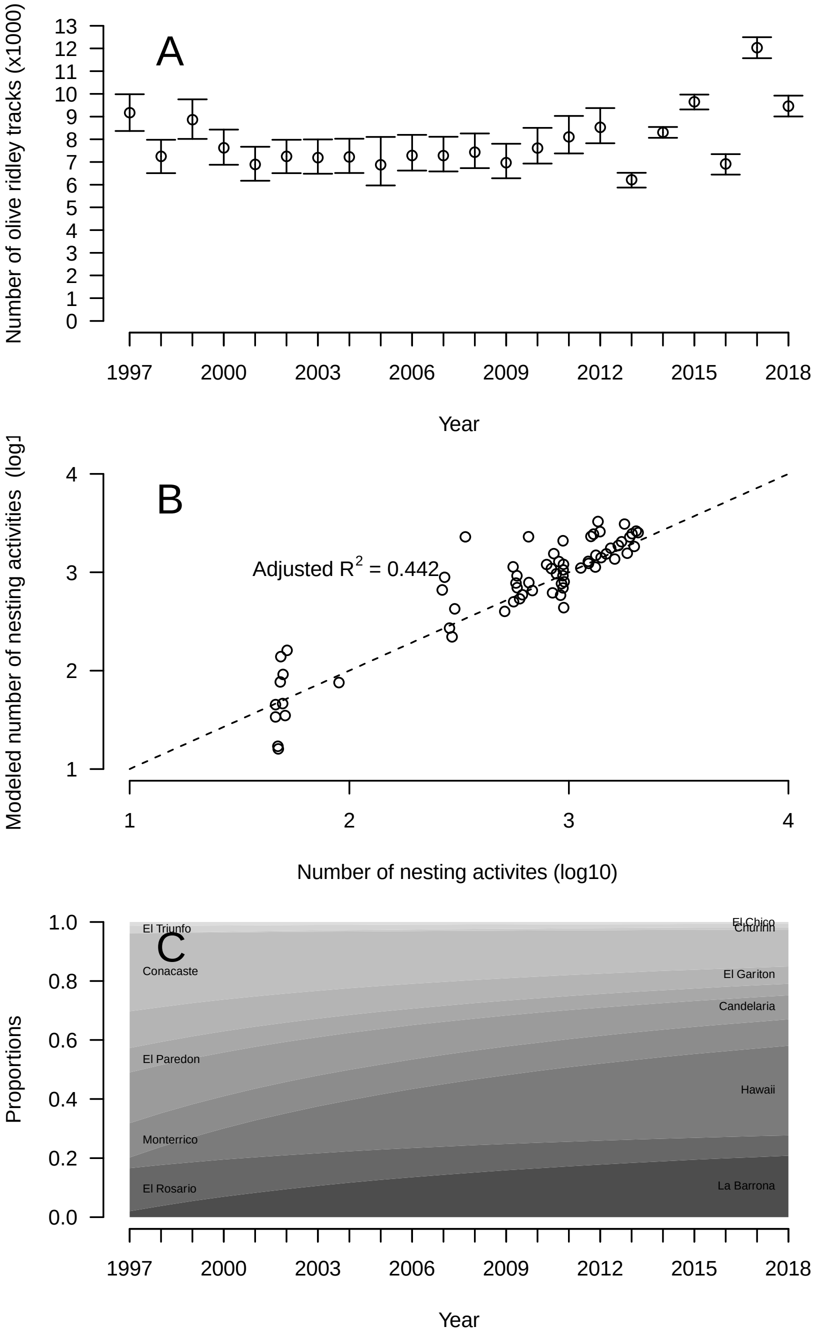


FIG. 5 (A) Total number of eggs incubated in hatcheries and 95% confidence interval for 29 hatcheries on the Pacific coast of Guatemala. (B) Relationship between the recorded number of eggs in hatcheries and the modeled number to estimate the spatial and temporal trend (183 observations and 31 parameters).

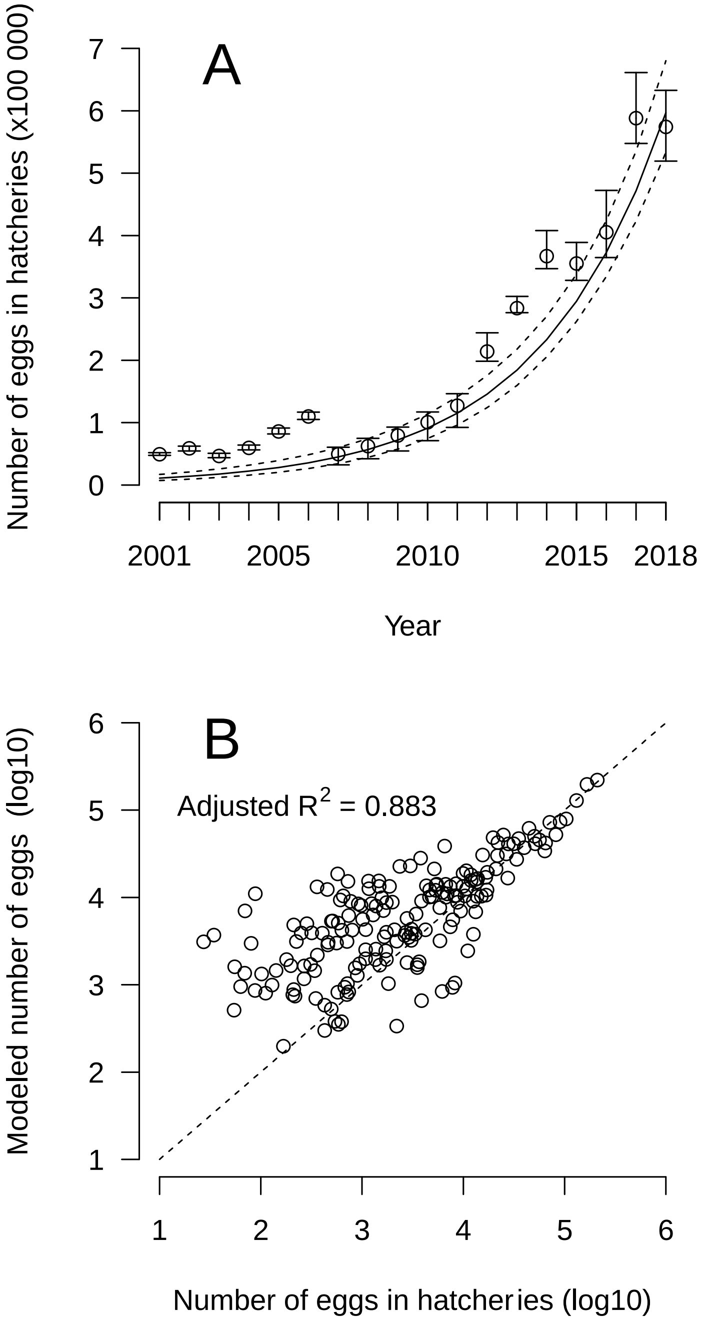


Fig. 6 (A) Matrix of correlations for phenology parameters. For each pair, the confidence interval of the correlation coefficient is shown. (B) Relationship between parameters based on their partial Spearman correlation coefficients using the iconography of correlations. Plain and dashed lines represent positive and negative relationships, respectively. The thicker the line, the stronger the relationship is.

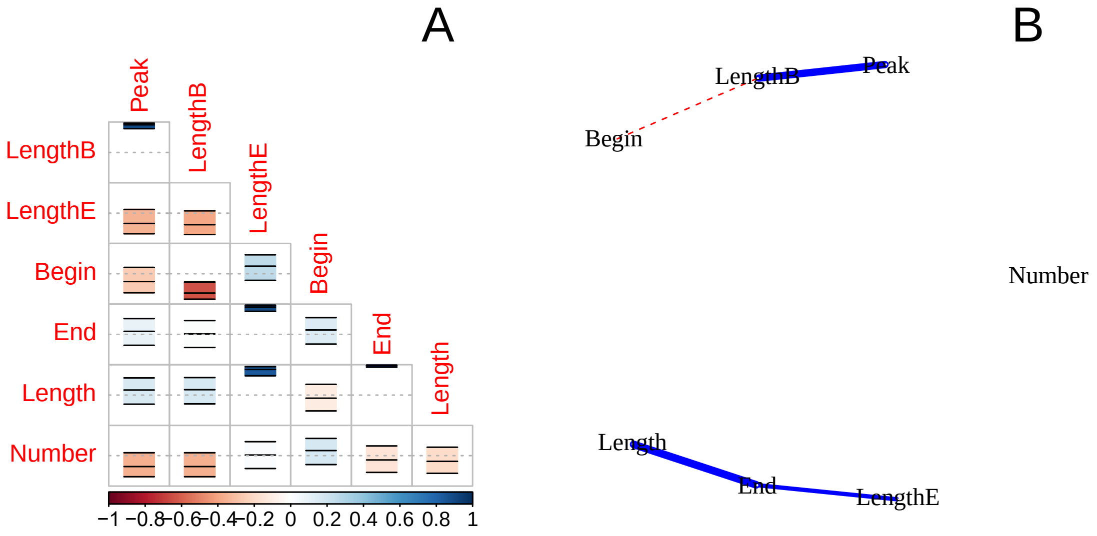
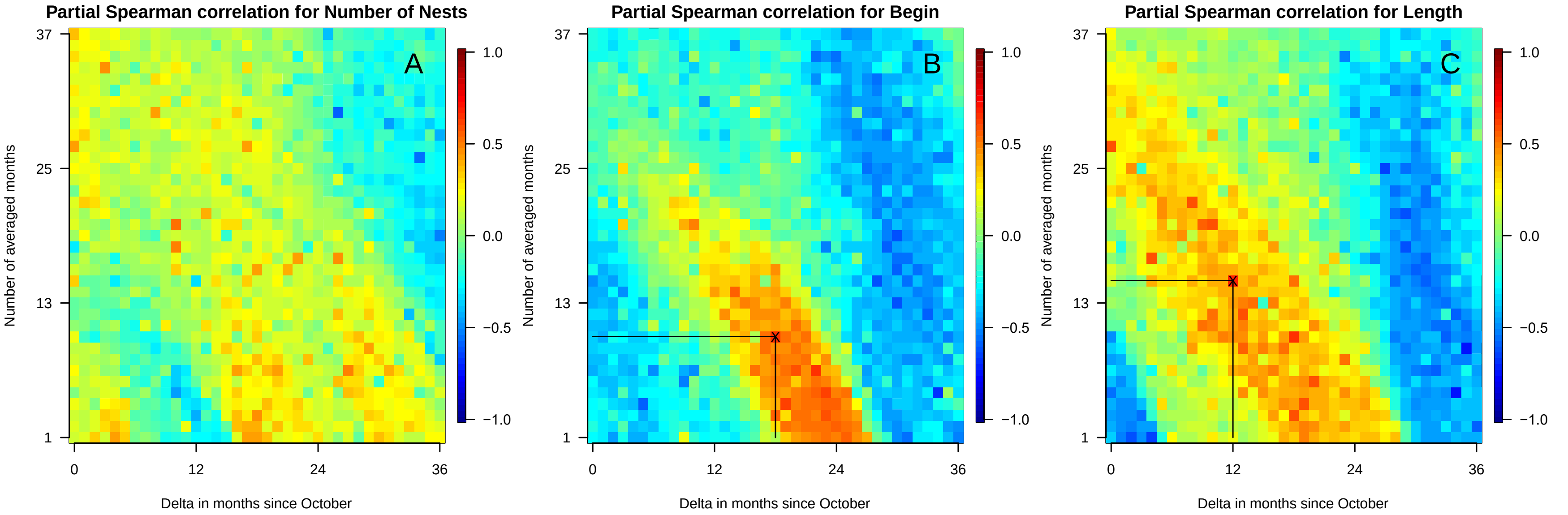


FIG. 7 Relationship between cumulative El Niño-Southern oscillation effect and (A) number of nests, (B) beginning of the nesting season, and (C) length of the nesting season. The x- and y-axes represent the lag in months and the number of months of the El Niño-Southern oscillation effect, respectively.



ARTICLE 2. Morales-Mérida BA., Helier A., Cortés-Gómez A, Girondot, M. (2021, accepted). Hatching Success Rather than Temperature-Dependent Sex Determination as the Main Driver of Olive Ridley (*Lepidochelys olivacea*) Nesting Activity in the Pacific Coast of Central America. **Accepted in Animals**

Hatching Success Rather than Temperature-Dependent Sex Determination as the Main Driver of Olive Ridley (*Lepidochelys olivacea*) Nesting Activity in the Pacific Coast of Central America

Alejandra Morales Mérida 1,2,3, Aude Helier 1, Adriana A. Cortés-Gómez 1, and Marc Girondot 1,*

1 Laboratoire Écologie, Systématique, Évolution, Université Paris Saclay, AgroParisTech, Centre National de la Recherche Scientifique, 91405 Orsay, France; alemoralesmerida@gmail.com (A.M.M.); aude.helier@hotmail.fr (A.H.); adriana.niobe@gmail.com (A.A.C-G.) ; jean-michel.guillon@universite-paris-saclay.fr (J.-M.G)

2 Doctorado en Ciencias Naturales para el Desarrollo (DOCINADE), Instituto Tecnológico de Costa Rica (TEC), Universidad Nacional (UNA), Universidad Estatal a Distancia (UNED), Costa Rica.

3 Facultad de Ciencias Químicas y Farmacia. Universidad de San Carlos de Guatemala (USAC). Guatemala, Guatemala

* Correspondence: marc.girondot@universite-paris-saclay.fr; Tel.: +33 6 20 18 22 16

Simple Summary: In marine turtles, sex is determined during a precise period during incubation: males are produced at lower temperatures and females at higher temperatures, a phenomenon called temperature-dependent sex determination. Most predictions about the long-term persistence of sea turtle populations in the face of climate change have focused on the effect of incubation temperature on sex ratios. In Central America, alternance of dark sand beaches (hotter sand) and light sand beach (cooler sand) are observed. Due to the higher production of females at high temperature and natal homing phenomenon in marine turtles, the largest proportion of nests in dark sand beach was expected. However, the inverse has been observed. We hypothesize that high beach temperatures, being seen in darker sand, increased female-biased primary sex ratios but reduced output of female hatchlings due to embryo thermal lethality at high temperature. Our study reveals that when we think about sea turtle population dynamics, we should consider a va-riety of factors and not only sex ratio.

Abstract: In marine turtles, sex is determined during a precise period during incubation: males are produced at lower temperatures and females at higher temperatures, a phenomenon called temperature-dependent sex determination. Nest temperature depends on many factors, including solar radiation. Albedo is the measure of the proportion of reflected solar radiation, and in terms of sand color, black sand absorbs the most energy, while white sand reflects more solar radiation. Based on this observation, darker sand beaches with higher temperatures should produce more females. As marine turtles show a high degree of philopatry, including natal homing, dark beaches should also produce more female hatchlings that return to nest when mature. When sand color is heterogeneous in a region, we hypothesize that darker beaches would have the most nests. Nevertheless, the high incubation temperature in beaches with a low albedo may result in low hatching success. Using Google Earth images and the SWOT database of nesting olive ridleys (*Lepidochelys olivacea*) in the Pacific coast of Mexico and Central America, we modeled sand color and nesting activity to test the hypothesis that darker beaches host larger concentrations of females because of feminization in darker beaches and female philopatry. We found the opposite result: the lower hatching success at beaches with a lower albedo could be the main driver of nesting activity heterogeneity for olive ridleys in Central America.

Keywords: Temperature-dependent sex determination; Hatching success; Albedo; *Lepidochelys olivacea*; Olive ridley; Sea turtle

1. Introduction

Marine turtles are present on many tropical sandy beaches during their nesting periods. However, not all beaches in intertropical regions receive the same number of marine turtle nests: some beaches host high densities of nests, whereas others, sometimes located in the same region, may have very few nests [1]. The origin of this difference in nesting at nearby beaches may relate to several factors: (i) accessibility of the beach from the sea [2], (ii) social facilitation for finding a nesting beach [3,4], and (iii) female philopatry with past heterogeneous nesting activity [5]. Philopatry is the tendency of an organism to stay in or habitually return to a particular area [6]. Marine turtles show natal philopatry [7] and nesting philopatry both among available nesting beaches and within the same nesting beach [2]. Thus, the spatial heterogeneity of nest-ing density could result from differences in female production at beaches. Indeed, if more juvenile females are produced at one beach, then due to natal homing, this beach would be expected to receive more female adults in the next generation, and this phenomenon will increase beach heterogeneity from year to year.

Two opposite phenomena relating to nest temperature can drive female production at beaches [8]. First, in marine turtles, the sexual phenotype of embryos is determined by incubation during the middle third of development (middle-third of incubation at constant temperature): males are produced at lower temperatures and females at higher temperatures. This phenomenon is known as temperature-dependent sex determination. Second, embryo development can be hampered if incubation temperatures are too high or too low. The temperature range for development is between 25-35 °C for marine turtles [9]. In turn, nest temperature depends on many factors such as the depth of the nest, vegetation cover [10], the sea temperature [11], and the soil ab-sorbed incident solar radiation [12]. Albedo is the measure of the diffuse reflection of solar radiation out of the total incident solar radiation and is measured on a scale from 0, corresponding to a black body that absorbs all incident radiation, to 1, corresponding to a body that reflects all incident radiation. Beach sand is comprised of different materials of diverse origins. Consequently, sand can have a range of different colors from white sand (of coral origin, for example) to black sand (of volcanic origin). Soil color can be used to predict albedo [13,14]. The effect of sand color on nest temperature has been demonstrated: nests deposited in black sand are warmer than those in white sand [12]. As a result, more females should be produced in black sand beaches that have higher temperatures if the nests are not too hot to produce hatchlings. When this observation is linked to female philopatry, we should expect a higher nesting activity in beaches with dark sand.

Central America is a relatively recent geological formation (< 3.5 million years) with many active volcanos [15]. This history is visible on its beaches: dark sand results from the erosion of recent volcanic eruptions, whereas lighter sand is due to the aggregation of organic material from the sea. Four marine turtle species nest on the Pa-cific beaches of Central America every year (hawksbills - *Eretmochelys imbricata*, olive ridleys - *Lepidochelys olivacea*, leatherbacks - *Dermochelys coriacea*, and green turtles - *Chelonia mydas*). *L. olivacea* employs two nesting strategies depending on aggregation density: 1) solitary nesting like other species, and 2) the group or mass nesting (arribada) behavior in which several thousand females simultaneously nest on the same beach [16]. In Central America, beaches have been monitored for several decades, and density maps of olive ridley crawls, nests, and nesting females are available in both the scientific and gray literature.

Thus, the alternating darker and lighter sand in Pacific Central America and the presence of beaches with varying densities of marine turtle nests represent an ideal situation to test the hypothesis that female production combined with natal homing is the driver behind the heterogeneity of nesting activity. The aim of this study was to test whether a correlation exists between sand albedo and the nesting activity and discriminate between the hypotheses that population dynamics are linked to temperature-dependent sex determination or to the deleterious effects of high temperature on incubation success.

2. Materials and Methods

2.1. *Datasets*

Beach images were searched for using Google Earth Pro V 7.3.2.5776 by visually examining the whole Central American Pacific coastline. A beach was defined as a continuous stretch of sand visible in satellite photography available from Google Earth. The Pacific coasts of Mexico, Guatemala, El Salvador, Costa Rica, and Panama were examined to identify the presence of beaches, resulting in a total of 291 beaches (Figure 1). Only most recent images were used. For each beach, the coordinates of both ends, the image date, and the standardized color of the sand in the middle of the beach (see below for description) were recorded. Length of the beaches was calculated using harversine distance between both ends coordinates. View altitude was always chosen to display the image of the entire beach in a 15” monitor. Volcano longitude and latitude were retrieved from the Smithsonian Institution’s online database of Holocene Volcanos [17]. For marine turtle density, data on crawls, nests, and nesting females along the Central American Pacific coast was retrieved from the State of the World's Sea Turtles database online (SWOT; March 2020 version) [18] along with any relevant literature using the database information.

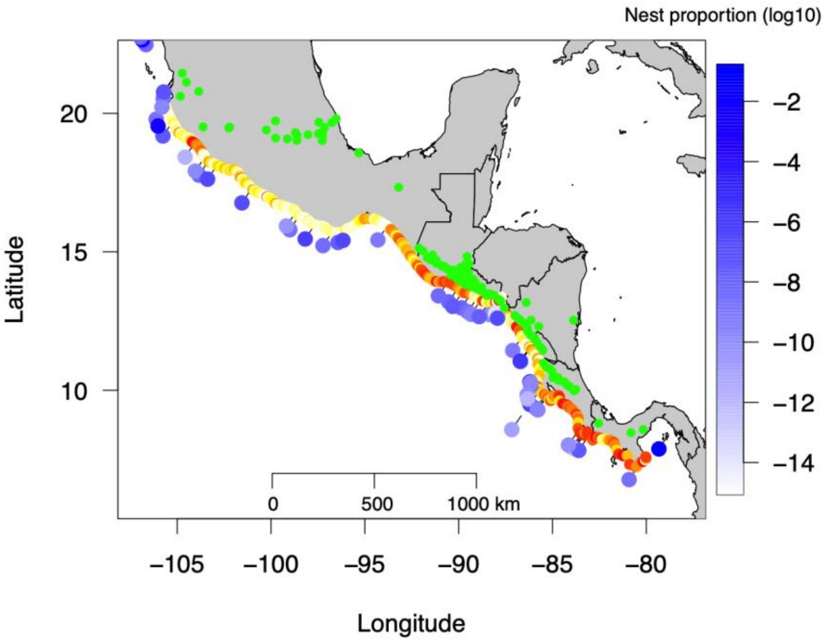


Figure 1. Map of Central America showing the recorded beaches with their estimated darkness (light yellow dots correspond to white sand with a higher albedo and red dots to darker sand with a lower albedo). Green triangles indicate the position of Holocene volcanos. Blue dots indicate the log10 proportion of olive ridley nests.

Data from different studies, from both dark and light beaches, that incubated eggs at constant temperatures were extracted from the literature and are available in the "DatabaseTSD" file, as part of the R package embryogrowth [19]. The database (2021-09-16 version) included 1,456 records for 59 species. Only data from eggs incubated in temperature-regulated chambers were used. Regional management units (RMUs) for olive ridley sea turtles of the East Pacific and Atlantic West were retrieved [20-30]. RMUs were inferred from the marine turtle biogeography, including nesting sites, population abundances and trends, population genetics, and satellite telemetry [31]. The following variables were retrieved from the database: incubation temperature, total number of incubated eggs, number of hatched eggs, number of sexed turtles, and number of males and females.

2.2. *Beach color*

The beach images captured on Google Earth (1024 × 768 pixels) were processed using the software Photofiltre (version 7.2.1, http://www.photofiltre.com). Color histograms for the red, green, and blue (RGB) components of the pixels for a portion of the image can be extracted with this software.

A square of 85 × 85 pixels (7285 pixels) located at the center of each beach was analyzed. This square was chosen in the middle of the transect from the sea to the vegetation line of the beach so as to include only sand. The modal value for the color of the pixels in each square was calculated. The use of the center was justified by the observation that it is generally the zone with highest density of nests [32]. The lightest and darkest zones of the image that included the entire beach were then selected to represent the color endpoints to standardize color variability across beaches. The Euclidean distance between the RGB modes of the lightest and darkest zones of the image and the RGB modes of the center of the beach were calculated. The two resulting values were standardized to obtain a final value between 0 and 1 (0 being the value for a white range and 1 for a black range). These values are inversely related to the albedo of the sand. Colors were individually standardized for each image: thus, this methodology corrects for the time of day and the cloud cover when each photograph was taken.

Two tests were performed to evaluate the accuracy of the color estimation from GoogleEarth pictures. First, it was checked that color of beach sand estimated from GoogleEarth pictures shows a spatial structure. Second, it was tested whether dark beaches are located closer to volcanos than lighter beaches, as expected because basalt material from volcanic origin is darker than material of non-volcanic origin. Color estimation was cross-checked with personal observations of the authors for some of these beaches (AMM: Guatemala, MG: Mexico, Guatemala, Costa Rica, AACG: Mexico), and with a survey of the literature.

The relationship between the estimated darkness of the beach and the closest volcano was estimated using the Mantel test, which is a statistical test of correlation between two matrices. It is based on a linear correlation and thus subject to the same assumptions as the Pearson correlation. Because of this limitation, permutation methods are used for significance testing when assumptions of independence are not met. This is the case for spatially distributed information that is linked by their process of formation. For example, basalt material of volcano origin can be present on beach and thus beach material and volcano presence are not independent. The Mantel test was performed using 9,999 permutations with the function mantel of the R Community Ecology Package vegan 2.5-6 [33].

2.3. *Standardizing Nesting Activity*

Quantitative nesting information was available for 90 beaches in Central America, Pacific coast for the years ranging from 1997 to 2014 (1620 year-beach combinations). The number of nests was available for 169 combinations. To obtain an index of nesting for each nesting beach, a model of temporal and spatial nesting patterns in the region was built to estimate the proportion of nests for a beach based on the relative frequency of nests at the different beaches and the total number of nests for each year. The aim of this model was to define an index of the nesting activity for each beach in that region when the years with data are not the same for all the beaches.

Considering the total number of nests Ti for year i in the entire region (SWOT database) where K beaches were monitored for Y years, three different models can be used to describe Ti according to year i:

- Constant number of nests: Ti = T; one parameter, T;
- Exponential model: Ti = T0 er.i; two parameters, T0 and r being the number of nests at time 0 and the growth rate respectively;
- Year-specific number of nests: Ti; Y parameters, T1 to TY.

The distribution of nests across the different beaches is defined by the proportion pj of Ti nests in j beach. It should be noted that the pj are constrained to be constant over time thus the index of nesting on each beach is the same for any year. For a total of K beaches, a total number of K–1 parameters p is necessary due to the relation$\sum_{j=1}^{K} p_j = 1$. Using one model, the expected number of nests for year i in beach j is thus Ei,j = Ti × pj. The time-constant constraint about pj is made necessary by the scarce information that was available (169 beach-year data points), which prevent a more complex model to be fitted.

Let Ni,j be the observed number of nests. During the fit, the standard deviation was modelled as a linear estimate of the observed number of nests: Si,j = a Ni,j + b with a and b > 0 (two parameters) and a Gaussian distribution model was used. For the final estimate, the expected number of nests Ei,j was only used when no observation was available; in other situations, the number of observed nests Ni,j was preferred.

The -ln likelihood of the observations within the model is simply the sum of the -ln likelihood for each observation Ni,j within the Gaussian model N(E_(i,j),S_(i,j)). The best-fitting model for each dataset was selected based on the maximum likelihood. Model selection was performed based on the minimum Akaike information criterion (AIC) [34]. AIC measures the quality of the fit, which is simultaneously penalized for the number of parameters in the model. It facilitates the selection of the best compromise between fit quality and over-parametrization from a set of models. When a set of models is compared, it is possible to estimate the relative probability that each model is the best among those tested using the Akaike Weight [35]. Maximum likelihood fitting of parameters was made using the R package phenology that implements this model [36].

2.4. *Relationship between sand color and nesting activity*

A linear model has been used to test for the relationship between index of nesting activity for each beach and the beach sand color and beach length. We used the log10 proportion of nests in different beaches to normalize data and then a Gaussian distribution was used.

2.5. *Thermal reaction norm for hatching success and sex ratio*

Due to albedo change, incubation temperatures in dark sand beaches are supposed to be higher than in white sand beach. Incubation temperature influences both hatching success and sex ratio. The fitting of the sex ratio thermal reaction norm for the East Pacific RMU was published in Abreu-Grobois, et al. [37]. The methodology is recalled here briefly. Data on the number of males and females produced for incubations at 17 constant temperatures were used. The relationship between constant incubation temperature and sex ratio was fitted using the logistic equation [38], and the credible interval was fitted using the Metropolis-Hastings algorithm with a Monte-Carlo Markov chain in Bayesian analyses with uniform priors [see 37 for more details].

Data on the number of hatchlings produced at constant incubation temperatures are available for the Pacific East RMU (20 constant incubation temperatures from Costa Rica, Panama, and Mexico) and Atlantic West RMU (13 constant incubation temperatures from Brazil). These data were fitted using the scaled product of two logistic equations to model the observation that hatching success (HS) according to constant incubation temperature (t) is null at low and high temperatures.

$$HS = MaxHS \times \frac{1}{1+e^{4\,(P_{low}-t)/S_{low}}} \times \frac{1}{1+e^{4\,(P_{low}+\Delta P-t)/S_{high}}} \quad (1)$$

Plow and Slow refer to the transition from 0 to MaxHS at lower temperatures, whereas Plow + ∆P (with ∆P > 0) and Shigh refer to the transition from MaxHS to 0 at higher temperatures. In these equations, P is the temperature at which hatching success is 0.5 and S is the slope at P. The fitting was made using binomial distribution and maximum likelihood. The credible interval was fitted using the Metropolis-Hastings algorithm with a Monte-Carlo Markov chain in Bayesian analyses with uniform priors $P_{low} \sim \mathcal{U}(20;40)$, $S_{low} \sim \mathcal{U}(0;5)$, $MaxHS \sim \mathcal{U}(0;1)$, $\Delta P \sim \mathcal{U}(0;10)$, and $S_{high} \sim \mathcal{U}(-5;0)$; [see 25 for more details of the statistical methodology]. Maximum likelihood and Bayesian estimates were made using the R package embryogrowth that implements these models [19].

3. Results

3.1. *Beach albedo from satellite images*

A Mantel test using matrices of distances and albedo differences among beaches shows a significant spatial organization of beach albedo (Mantel test, p=0.02), indicating that two nearby beaches are more similar in albedo than expected from a random distribution.

The relationship between beach color and distance to the closest Holocene volcano is very strong (∆AIC = 25.50, Akaike weight > 0.9999): sand albedo increases with the proximity of the nearest volcano.

3.2. Temporal olive ridley nest abundance in Pacific Central America

Different temporal models were tested. The Year-Specific (YS) model is the selected model (Table 1) with an Akaike weight of 0.94 indicating a strong support. The temporal and spatial results are shown in Figure 2, and the estimated proportion of nests (log10 scale) for the 90 nesting beaches with information is shown in Figure 1.

3.3. *Relationship between beach albedo and olive ridley nest number and density*

We only used olive ridley nest counts from the SWOT database, as this species has the larger amount of data, both temporally and spatially. We used the log10 proportion of nests in different beaches to normalize data. The relationship between the log10 proportion of nests number and density of nests per km and the sand darkness index was negative (Figure 3A and B): darker beaches tend to have less nesting activity than lighter beaches.

3.4. *Thermal reaction norm for sex ratio and hatching success*

The pivotal temperature (theoretical temperature that produced both sexes in equal proportion) for East Pacific olive ridleys is 30.24 °C (95% credible interval 30.04-30.50 °C), while the transitional range of temperatures 5% (TRT 5%, temperature range producing 5% to 95% of both sexes) is 3.84 °C (95% CI 3.08-4.72 °C) (Figure 4A). The lower and upper limits of TRT 5% are, respectively, 28.33 °C (95% CI 27.80-28.76 °C) and 32.16 °C (95% CI 31.71-32.68 °C).

The fitted thermal reaction norm for hatching success is shown in Figure 4B. It shows two abrupt declines below Plow = 24.83 °C (95% CI 23.19-24.98 °C) and above Plow + ΔP = 33.57 °C (95% CI 33.08-34.28 °C).

Table 1: Model selection for temporal distribution of olive ridley nesting activity in Central America.

Temporal model	AIC	ΔAIC	Akaike weight
Constant	2254.397	12.53	0.002
Exponential	2247.399	5.53	0.06
Year-specific	2241.867	0.00	0.94

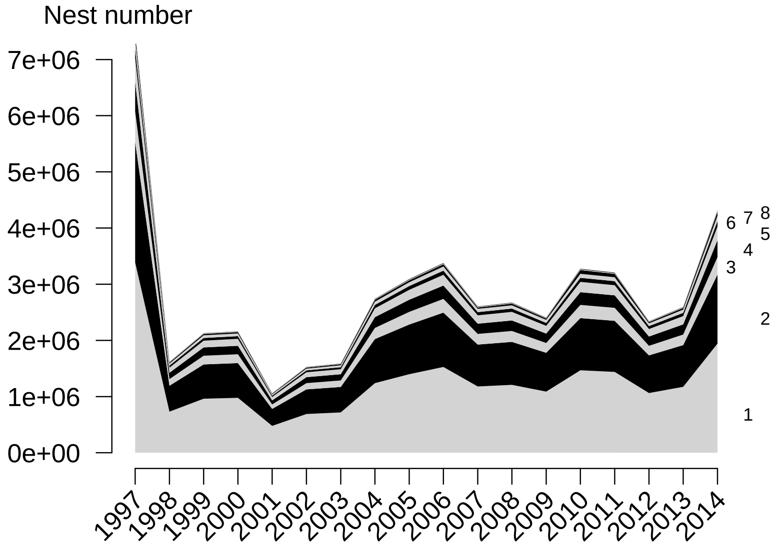


Figure 2. Temporal and spatial distribution of the number of olive ridley nests in Central America. Only eight major beaches are named here from among the 90 beaches used in the present study because the number of nests per

year for other ones is too small to be visualized on the figure. Beaches: 1: Santuario Playa de Escobilla; 2: Marinera; 3: Morro Ayuta; 4: La Flor, Carazo; 5: Ixtapilla; 6: RVS Río Escalante-Chacocente; 7: Chacocente; 8: Nancite. They represent 96.7% of nesting of the analyzed beaches.

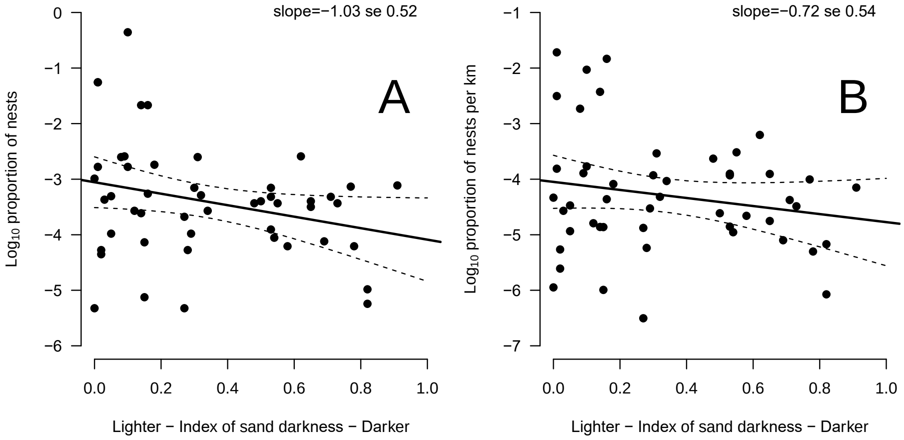


Figure 3. Relationship between sand darkness and (A) the proportion of olive ridley nests and (B) the proportion of olive ridley nests divided by beach length in Central American beaches. Note that the proportion of nests is log-transformed.

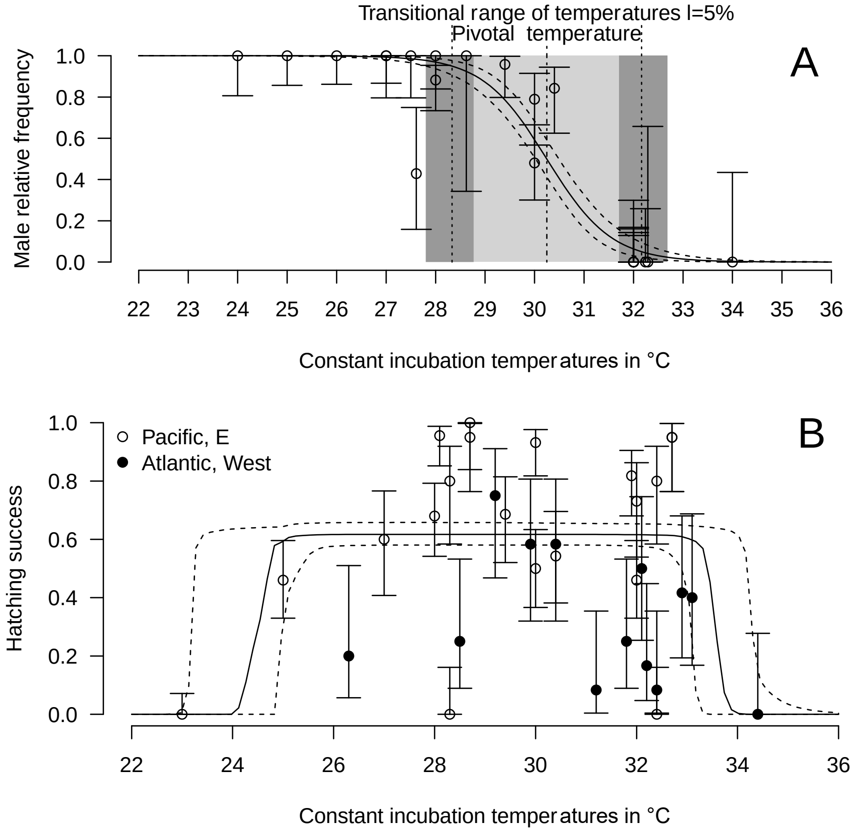


Figure 4. Sex ratio and hatching success at constant incubation temperatures. The thermal reaction norms for (A) sex ratio and (B) hatching success are shown in solid lines. Light gray temperatures in (A)

are the range of temperatures that produced a sex ratio from 5% to 95% (transitional range of temperature 5%), and dark gray are the 95% credible region for limits of the transitional range of temperature 5%. The temperature that produced 50% of each sex (pivotal temperature) is shown by the interrupted vertical line. In both graphs, the 95% credible regions of the thermal reaction norm are shown with dashed lines. Each point represents a set of eggs from the same origin at a specific constant temperature.

4. Discussion

Google Earth images have already been used in science for numerous applications [39]. We expand this use to the study of beach geomorphology. We identify a total of 291 beaches across 3000 km of Pacific coast in Central America and evaluate sand darkness for each beach. The quality of these data was cross-checked with independent information: (i) field observations made on some of these beaches (AMM: Guatemala, MG: Mexico, Guatemala, Costa Rica, AACG: Mexico), and (ii) a survey of the literature, e.g. “This dark sand beach is located within the Ostional Wildlife Refuge and measures 3.9 km in length.” [28]. We find other evidence indicating that the images carry valuable information: (i) we detect a significant spatial pattern, since two proximate beaches are more similar than randomly expected and (ii) as expected from geology, we detect a positive relationship of sand lightness with distance to the closest volcano Yet, a few light sand beaches are observed in proximity with a volcano. Two processes could explain this pattern: (i) the geological signal could be attenuated when the volcano’s last eruption was ancient, and (ii) flowing and erosion could have transported volcanic material in other directions than the beach.

We find a pivotal temperature of 30.24 °C for temperature-dependent sex determination at constant temperatures (95% credible interval 30.04-30.50 °C) and an upper limit of transitional range of temperatures 5% at 32.16 °C (95% credible interval 31.70-32.68 °C), relatively low values compared to average incubation temperatures recorded in nests in this region, which can exceed 33 °C by large, especially on dark sand beaches [40-42]. Hatching success dramatically drops to 0 when constant incubation temperatures are over 33.57 °C (Figure 4B). This result is consistent with the observation that hatching success in inversely related to the number of hours spent above 35°C in olive ridley nests on Playa Coyote, Costa Rica [43]. Similarly, another study reported that hatching success of L. olivacea decreased as incubation temperatures increased above 31°C in Costa Rica [44].

We find a negative relationship between the proportion of olive ridley nests among the different beaches of Central America and the darkness of the sand (Figure 3). This means that nesting activity was more intense on beaches with lighter sand, higher albedo and most likely cooler incubation temperatures. According to the natal philopatry hypothesis, production of females on light sand – low temperature beaches would thus be higher overall than on dark sand – high temperature beaches. This conclusion is concordant with previous observations for leatherback turtles in Playa Grande beach, Costa Rica, in the same region [45] and experiments conducted on the freshwater turtle Chrysemys picta [46]. These studies showed that hotter beaches yielded female-biased primary sex ratios but reduced the total output of female hatchlings. Thus, among our two competing hypotheses, our results support the hypothesis that frequentation of beaches in the region studied is related to differences in hatching success, rather than differences in nest sex ratios resulting from temperature sex determination. This pattern could be a specificity of the East Pacific Central American Coast, where feminizing conditions are often associated with lethal incubation temperatures. It is worth mentioning that we hypothesized that no microhabitat selection for temperature-dependent sex determination pattern and lethality has occurred, as demonstrated for green turtles at Ascension Island [47]. However, further work could reveal the same pattern in other nesting areas where thermal limits for viable embryo development might be exceeded (e.g. Arabian Peninsula). Such studies in other regions and other sea turtle species are warranted to help assessing future prospects of actual rookeries in the context of climatic change [48,49].

5. Conclusions

After the surprising discovery of the mechanism of temperature-dependent sex determination in turtles [50,51], most predictions about the long-term persistence of sea turtle populations in the face of climate change have focused on the effect of incubation temperature on sex ratios. Other factors involved in population dynamics such as the actual number of juveniles produced in nesting beaches have often been overlooked. Our study reveals that when we think about sea turtle population dynamics, we should consider a variety of factors and not only sex ratio.

Author Contributions: Conceptualization, M.G..; methodology, A.M.M., A.H., A.A.C.G., M.G.; software, M.G.; validation, M.G.; writing—original draft preparation, A.H.; writing—review and editing, A.M.M., A.H., A.A.C.G., M.G.; supervision, M.G. All authors have read and agreed to the published version of the manuscript.

Funding: This study received regular funding from the Centre National de la Recherche Scien-tifique, AgroParisTech and Université Paris Saclay.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Public databases Google Earth Pro V 7.3.2.5776, DatabaseTSD from R package embryogrowth and SWOT database (https://www.seaturtlestatus.org) were used in this study.

Acknowledgments: Dr Victoria Grace (www.english-publications.com) is thanked for her careful reading of the manuscript and correction of the English. All the organizations that provided data to the Central American Pacific SWOT database have been contacted by email to obtain the authorization that we used their data for this work. They are all thanked as well as SWOT organization to have provided the data that were used in this work. We thank referees for their constructive remarks that help to clarify the manuscript. This manuscript has benefited from the expertise of Dr Jean-Michel Guillon (CNRS).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Kamrowski, R.L.; Limpus, C.; Jones, R.; Anderson, S.; Hamann, M. Temporal changes in artificial light exposure of ma-rine turtle nesting areas. Glob Chang Biol 2014, 20, 2437-2449, doi:10.1111/gcb.12503.
2. Girondot, M.; Godfrey, M.H.; Ponge, L.; Rivalan, P. Modeling approaches to quantify leatherback nesting trends in French Guiana and Suriname. Chelonian Conservation and Biology 2007, 6, 37-46.

3. Freedberg, S.; Wade, M.J. Cultural inheritance as a mechanism for population sex-ratio bias in reptiles. Evolution 2001, 55, 1049-1055.
4. Escalona, T.; Valenzuela, N.; Adams, D.C. Do local environmental factors and lunar cycle influence timing and syn-chrony of oviposition of a turtle with strict nocturnal nesting? Diversity 2019, 11, doi:10.3390/d11050078.
5. Lee, P.L.M.; Luschi, P.; Hays, G.C. Detecting female precise natal philopatry in green turtles using assignment methods. Molecular Ecology 2007, 16, 61-74.
6. Lawrence, E., (Ed.) Henderson's dictionary of biological terms. 12th edition ed.; Prentice Hall: 2000.
7. Meylan, A.B.; Bowen, B.W.; Avise, J.C. A genetic test of the natal homing versus social facilitation models for green turtle migration. Science 1990, 248, 724-727.
8. Hays, G.C.; Mazaris, A.D.; Schofield, G.; Laloë, J.O. Population viability at extreme sex-ratio skews produced by temper-ature-dependent sex determination. Proc Biol Sci 2017, 284, doi:10.1098/rspb.2016.2576.
9. Ackerman, R.A. The nest environment and the embryonic development of sea turtles. In The Biology of Sea Turtles, Lutz, P.L., Musick, J.A., Eds.; CRC Press: New York, USA, 1997; pp. 83-106.
10. Kamel, S.J. Vegetation cover predicts temperature in nests of the hawksbill sea turtle: implications for beach management and offspring sex ratios. Endangered Species Research 2013, 20, 41-48, doi:10.3354/esr00489.
11. Girondot, M.; Kaska, Y. Nest temperatures in a loggerhead-nesting beach in Turkey is more determined by sea surface temperature than air temperature. Journal of Thermal Biology 2015, 47, 13-18, doi:10.1016/j.jtherbio.2014.10.008.
12. Hays, G.C.; Ashworth, J.S.; Barnsley, M.J.; Broderick, A.C.; Emery, D.R.; Godley, B.J.; Henwood, A.; Jones, E.L. The im-portance of sand albedo for the thermal conditions on sea turtle nesting beaches. Oikos 2001, 93, 87-94.
13. Fontes, A.F. Soil albedo in relation to soil color, moisture and roughness. The University of Arizona, 1996.
14. Post, D.F.; Fimbres, A.; Matthias, A.D.; Sano, E.E.; Accioly, L.; Batchily, A.K.; Ferreira, L.G. Predicting soil albedo from soil color and spectral reflectance data. Soil Science Society of America Journal 2000, 64, 1027-1034, doi:10.2136/sssaj2000.6431027x.

15. Coates, A.G.; Jackson, J.B.C.; Collins, L.S.; Cronin, T.M.; Dowsett, H.J.; Bybell, L.M.; Jung, P.; Obando, J.A. Closure of the Isthmus of Panama: The near-shore marine record of Costa Rica and western Panama. Geological Society of America Bulletin 1992, 104, 814-828, doi:10.1130/0016-7606(1992)104<0814:COTIOP>2.3.CO;2.

16. Shanker, K.; Abreu-Grobois, A.; Bezy, V.; Briseño, R.; Colman, L.; Girard, A.; Girondot, M.; Jensen, M.; Manoharakrish-nan, M.; Rguez-Baron, J.M.; et al. Olive ridleys. The quirky turtles that conquered the world. SWOT 2021, 16.

17. Siebert, L.; Simkin, T. Volcanoes of the world: An illustrated catalog of Holocene volcanoes and their eruptions. Availa-ble online: http://www.volcano.si.edu (accessed on 2019-06-01).

18. DiMatteo, A.; Fujioka, E.; Wallace, B.; Hutchinson, B.; Cleary, J.; Halpin, P. SWOT Database Online. Available online: http://seamap.env.duke.edu/swot (accessed on 10th July 2011).

19. Girondot, M. embryogrowth: Tools to analyze the thermal reaction norm of embryo growth, 8.1; The Comprehensive R Archive Network: 2021.

20. Castheloge, V.D.; de D. dos Santos, M.R.; de Castilhos, J.C.; de J. Filho, P.R.; de C. Gomes, L.; Clemente-Carvalho, R.B.G.; Ferreira, P.D. Pivotal temperature and hatchling sex ratio of olive ridley sea turtles Lepidochelys olivacea from the South Atlantic coast of Brazil. Herpetological Conservation and Biology 2018, 13, 488-496.

21. McCoy, C.J.; Vogt, R.C.; Censky, E.J. Temperature-controlled sex determination in the sea turtle Lepidochelys olivacea. Journal of Herpetology 1983, 17, 404-406.

22. Wibbels, T.; Rostal, D.C.; Byles, R. High pivotal temperature in the sex determination of the olive ridley sea turtle, Lepidochelys olivacea, from Playa Nancite, Costa Rica. Copeia 1998, 1998, 1086-1088.

23. Michel-Morfin, J.E.; Gómez Muñoz, V.M.; Navarro Rodríguez, C. Morphometric model for sex assessment in hatchling olive ridley sea turtles. Chelonian Conservation and Biology 2001, 40, 53-58.

24. Navarro Sánchez, E.J. Efecto de la temperatura de incubación y la diferenciación sexual sobre la morfología de crías de tortuga marina Lepidochelys

olivacea. Tesis de Maestría, Universidad Nacional Autónoma de México, México, D.F., 2015.

25. Ruiz García, N.A. Efectos de la temperatura sobre el desarrollo embrionario y el desempeño de crías de la tortuga golfina, Lepidochelys olivacea. Tesis Nivel Maestria, 2014.

26. Merchant-Larios, H.; Díaz-Hernández, V.; Marmolejo-Valencia, A. Gonadal morphogenesis and gene expression in rep-tiles with temperature-dependent sex determination. Sexual Development 2010, 4, 50-61, doi:10.1159/000276768.

27. Merchant-Larios, H.; Ruiz-Ramirez, S.; Moreno-Mendoza, N.; Marmolejo-Valencia, A. Correlation among thermosensi-tive period, estradiol response, and gonad differentiation in the sea turtle Lepidochelys olivacea. General and Comparative Endocrinology 1997, 107, 373-385.

28. Merchant-Larios, H.; Villalpando-Fierro, I.; Centeno-Urruiza, B. Gonadal morphogenesis under controlled temperature in the sea turtle Lepidochelys olivacea. Herpetol. Monographs 1989, 3, 43-61.

29. Sandoval Espinoza, S. Proporción sexual en crías de tortuga Lepidochelys olivacea en corrales de incubación del Pacífico mexicano. PhD dissertation, Instituto Politécnico Nacional, La Paz, BCS, México, 2012.

30. López Correa, J.Y. Diferenciación gonádica en crias de Lepidochelys olivacea (Eschscholtz, 1829) (Testudinata: Cheloni-idae). PhD dissertation, Instituto Politécnico Nacional, Centro Interdisciplinaria de Ciencias Marinas, La Paz, BCS, 2010.

31. Wallace, B.P.; DiMatteo, A.D.; Hurley, B.J.; Finkbeiner, E.M.; Bolten, A.B.; Chaloupka, M.Y.; Hutchinson, B.J.; Abreu-Grobois, F.A.; Amorocho, D.; Bjorndal, K.A.; et al. Regional management units for marine turtles: a novel frame-work for prioritizing conservation and research across multiple scales. PLoS One 2010, 5, e15465.

32. Tiwari, M.; Bjorndal, K.A.; Bolten, A.B.; Bolker, B.M. Intraspecific application of the mid-domain effect model: spatial and temporal nest distributions of green turtles, Chelonia mydas, at Tortuguero, Costa Rica. Ecology Letters 2005, 8, 918-924.

33. Oksanen, J.; Blanchet, F.G.; Friendly, M.; Kindt, R.; Legendre, P.; McGlinn, D.; Minchin, P.R.; O'Hara, R.B.; Simpson, G.L.; Solymos, P.; et al. vegan: Community Ecology Package, 2.5-6; The Comprehensive R Archive Network, 2019.

34. Akaike, H. A new look at the statistical model identification. IEEE Transactions on Automatic Control 1974, 19, 716-723.

35. Burnham, K.P.; Anderson, D.R. Model selection and multimodel inference: A practical information-theoretic approach; Springer-Verlag: New York, 2002; p. 488.

36. Girondot, M. phenology: Tools to manage a parametric function that describes phenology, 7.8; The Comprehensive R Archive Network: 2021.

37. Abreu-Grobois, F.A.; Morales-Mérida, B.A.; Hart, C.E.; Guillon, J.-M.; Godfrey, M.H.; Navarro, E.; Girondot, M. Recent advances on the estimation of the thermal reaction norm for sex ratios. PeerJ 2020, 8, e8451, doi:10.7717/peerj.8451.

38. Hulin, V.; Delmas, V.; Girondot, M.; Godfrey, M.H.; Guillon, J.-M. Temperature-dependent sex determination and global change: Are some species at greater risk? Oecologia 2009, 160, 493-506.

39. Yu, L.; Gong, P. Google Earth as a virtual globe tool for Earth science applications at the global scale: progress and per-spectives. International Journal of Remote Sensing 2012, 33, 3966-3986, doi:10.1080/01431161.2011.636081.

40. Valverde, R.A.; Wingard, S.; Gómez, F.; Tordoir, M.T.; Orrego, C.M. Field lethal incubation temperature of olive ridley sea turtle Lepidochelys olivacea embryos at a mass nesting rookery. Endangered Species Research 2010, 12, 77-86, doi:10.3354/esr00296.

41. Morales-Merida, B.A.; Bustamante, D.M.; Monsinjon, J.; Girondot, M. Reaction norm of embryo growth rate dependent on incubation temperature in the Olive Ridley sea turtle, Lepidochelys olivacea, from Pacific Central America. Journal of Embryology 2018, 1, 12-24.

42. Morales-Mérida, B.A.; Contreras-Mérida, M.R.; Girondot, M. Pipping dynamics in marine turtle Lepidochelys olivacea nests. Trends in Developmental Biology 2019, 12, 23-30.

43. Binhammer, M.R.; Beange, M.; Arauz, R. Sand temperature, sex ratios, and nest success in olive ridley sea turtles. Marine Turtle Newsletter 2019, 159, 5-9.

44. Dornfeld, T.C.; Robinson, N.J.; Tomillo, P.S.; Paladino, F.V. Ecology of solitary nesting olive ridley sea turtles at Playa Grande, Costa Rica. Marine Biology 2014, 162, 123-139, doi:10.1007/s00227-014-2583-7.

45. Santidrián Tomillo, P.; Oro, D.; Paladino, F.V.; Piedra, R.; Sieg, A.E.; Spotila, J.R. High beach temperatures increased fe-male-biased primary sex ratios but

reduced output of female hatchlings in the leatherback turtle. Biological Conservation 2014, 176, 71-79, doi:10.1016/j.biocon.2014.05.011.

46. Valenzuela, N.; Literman, R.; Neuwald, J.L.; Mizoguchi, B.; Iverson, J.B.; Riley, J.L.; Litzgus, J.D. Extreme thermal fluc-tuations from climate change unexpectedly accelerate demographic collapse of vertebrates with temperature-dependent sex determination. Scientific Reports 2019, 9, 4254, doi:10.1038/s41598-019-40597-4.
47. Tilley, D.; Ball, S.; Ellick, J.; Godley, B.J.; Weber, N.; Weber, S.B.; Broderick, A.C. No evidence of fine scale thermal adap-tation in green turtles. Journal of Experimental Marine Biology and Ecology 2019, 514-515, 110-117, doi:10.1016/j.jembe.2019.04.001.
48. Monsinjon, J.R.; Wyneken, J.; Rusenko, K.; López-Mendilaharsu, M.; Lara, P.; Santos, A.; dei Marcovaldi, M.A.G.; Fuentes, M.M.P.B.; Kaska, Y.; Tucek, J.; et al. The climatic debt of loggerhead sea turtle populations in a warming world. Ecological Indicators 2019, 107, 1-13.
49. Patrício, A.R.; Hawkes, L.A.; Monsinjon, J.R.; Godley, B.J.; Fuentes, M. Climate change and marine turtles: recent ad-vances and future directions. Endangered Species Research 2021, 44, 363-395, doi:10.3354/esr01110.
50. Pieau, C. Sur la proportion sexuelle chez les embryons de deux Chéloniens (Testudo graeca L. et Emys orbicularis L.) issus d'oeufs incubés artificiellement. Comptes Rendus de l'Académie des Sciences Paris Série D 1971, 272, 3071-3074.
51. Pieau, C. Effets de la température sur le développement des glandes génitales chez les embryons de deux Chéloniens, Emys orbicularis L. et Testudo graeca L. Comptes Rendus de l'Académie des Sciences Paris Série D 1972, 274, 719-722.

ARTICLE 3. Morales-Mérida BA, Contreras-Mérida MR, Girondot M. (2019). Pipping dynamics in marine turtle *Lepidochelys olivacea* nests. ***Trends in Developmental Biology***, **12: 23-30**

Pipping dynamics in marine turtle *Lepidochelys olivacea* nests

B. Alejandra Morales-Mérida[1,2,3], María Renee Contreras-Mérida[2], and Marc Girondot[1]

1 - Laboratoire Écologie, Systématique, Évolution, Université Paris-Sud, AgroParisTech, CNRS, Université Paris Saclay, 91405 Orsay, France
2 - Facultad de Ciencias Químicas y Farmacia, Universidad de San Carlos de Guatemala (USAC), San Carlos, Guatemala
3 - Doctorado en Ciencias Naturales para el Desarrollo (DOCINADE), Instituto Tecnológico de Costa Rica (TEC), Universidad Nacional (UNA), Universidad Estatal a Distancia (UNED), Costa Rica

Corresponding author: Marc Girondot
Laboratoire Écologie, Systématique, Évolution
91405 Orsay cedex
France
Email: marc.girondot@u-psud.fr

Running title: Pipping dynamics in olive ridley
Journal: Trends in Developmental Biology

Abstract

In marine turtles, incubation begins when females lay eggs on sandy beaches. Emergence of hatchlings from the sand marks the conclusion of a much longer process. At the end of incubation, the amnion ruptures inside the egg, and the chorioallantois moves posterior to the embryo to reveal the head and forelimbs, thus freeing the embryo to pip the shell. The juveniles then slowly move to the surface of the sand. The interval between egg pipping and emergence from the nest is not firmly established. We therefore used a motion detector and temperature dataloggers in five Olive ridley sea turtle nests to evaluate the time between pipping and emergence. The peak of movement is detected between 2 and 3.7 days before emergence, but the first signs of movements are detected as early as 6.5 days prior to this. No thermal signature of pipping was detected. The proximal and ultimate mechanisms relating to emergence synchrony are discussed in the light of these results.

KEYWORDS: olive ridley, pipping time, incubation, movement, embryo, *Lepidochelys olivacea*

Introduction

The spectacle of marine turtle hatchlings emerging from tropical or temperate beaches is an unforgettable time for any visitor of nesting beaches. However, emergence from the sand marks the conclusion of a much longer process that ends incubation. In turtles, oxygen consumption increases to a peak several days before hatching [1]. The amnion then ruptures inside the egg, and the chorioallantois moves posterior to the embryo to reveal the head and forelimbs, thus freeing the embryo to pip the shell [2]. This is an important stage for hatchlings, as it gives them time to close and straighten the plastron. Indeed, the residual yolk is metabolized by hatchlings while still in the nest [3]. At this time, the first breaths are taken, and lung secretions are maximal [4]. The hatchlings then emerge from the shell and move toward the surface. The interval between egg pipping and emergence from the nest is not firmly established [5].The variability of this time between species, beaches, nests, or individuals is also unknown. Understanding the interval between hatching and emergence has conservation and management implications. For instance, in some types of sand, marine turtles may have more difficulty in digging to the surface after pipping. In the case of beach nourishment, a common technique used to stem beach erosion, the introduced sand sometimes differs from the naturally occurring sand, as it may be more compacted [6]. This would lead to an increased emergence time, not only because the hatchlings must work harder to reach the surface, but also because the greater exertion produces higher amounts of lactate in the body. Consequently, the high lactate levels would require a longer resting period for hatchlings just beneath the surface to allow the degradation of lactate [7].

Previous studies estimating the hatching-emergence interval often manipulated the nest: for example, digging into the nests prior to emergence to see if the eggs had hatched, or placing a glass pane on one side of the nests [5]. An alternative indirect method compared the sex ratio measured at constant temperature and the sex ratio from natural nests [5]. Here, the estimated hatching-emergence interval is estimated by calculating the interval between egg hatching in the laboratory and hatchling emergence in the field. The development rate was standardized using similar sex ratios, since the sex ratio and development rate are correlated to incubation temperature [8, 9]. However, this method necessitates killing the embryos, as sex can only be identified after dissection, and thus it cannot be recommended as a general practice. It was previously proposed that pipping commenced when nest temperatures did not react predictably to changes in ambient soil temperatures at similar depths [10]. However, this method has not been validated experimentally.

In this paper, we attempt to evidence the time interval between egg pipping and crawling up to the surface from the nest. A total of five Olive ridley sea turtle nests were monitored using temperature and accelerometer dataloggers. When plotting the temperature and movement series, it was found that temperatures do not abruptly increase with the pipping event, as assumed by some authors in the past. Furthermore, the movement dynamics detected in the nest showed a period of increased movement lasting approximately 2-3 hours before a peak of movement.

Materials and Methods

Field data

Night patrols searching for nesting *Lepidochelys olivacea* were made over 8 km of the “Área de Usos Múltiples Hawaii” beach located in the eastern Pacific coast of Guatemala. After finding a nest, the eggs were collected, and the original shape of the nests was measured. Given the very high levels of legal human removals of this species, natural incubation is impossible to monitor. The eggs were relocated to the hatchery within 2 hours of egg deposition to minimize the potential disturbance of the developing embryos. The hatchery nests were built to respect the same depth and width of the original nests. A total of five nests were monitored between August 11 and October 2 during the 2019 nesting season. Two dataloggers were placed in the center of the clutch. First, HOBO Pendant G dataloggers (Onset Computer) were set to record every half hour to measure movement inside the nest. The HOBO Pendant G Acceleration datalogger is three-channel logger with 8-bit resolution that uses an internal three-axis accelerometer with a range of ± 3 g. The software displayed acceleration in units of *g*-force, with the returned value being dependent on the spatial position of the datalogger. Second, temperatures were measured hourly using HOBO Temp Pro dataloggers (Onset Computer), which had a resolution of 0.01°C. The temperature dataloggers were calibrated prior to use and were guaranteed to fall within an accuracy range of ±0.18 °C.

Nests were monitored nightly after the 40th day of incubation to check for hatchling emergence. Between 4 and 6 days after the last emergence, each nest was exhumed to characterize the remaining eggs and calculate the hatching success and number of emerging hatchlings. Dataloggers were removed from the nest when no juveniles hatched for 3 days.

Data analysis

For each nest, both temperature and movement data series were truncated so as to retain only records between the beginning of incubation and the emergence from the sand that occurred 45 to 46 days later (Fig. 1). Acceleration data were converted into their first-order derivative using an R script to measure the displacement of the datalogger within the nest. The total movement is the sum of movement on the x, y, and z axes. During the first 30 days, the embryos are too small to generate movements that can be recorded. The standard deviations of the recorded movement during these first 30 days were calculated. When recorded movement deviated from mean ± *k* SD, it was counted as a period of movement in the nest. Then we searched for the *k* factors (one per nest) that ensured that no false positive movement was detected during the early incubation. An R function, movement is provided to analyze the recorded data using the HOBO Pendant G Acceleration datalogger in the nest. It is available in the R package embryogrowth version 7.7 and higher (https://CRAN.R-project.org/package=embryogrowth).

Temperatures in the five nests were also plotted using their calendar date as a reference to check whether pipping events could be detected based on temperature records.

Hatching success was tested using the depth and number of eggs as cofactors based on a generalized linear model with binomial distribution and logit link.

Results

The laying date and time for each nest is shown in Table 1, along with the total depth of the nest, the number of eggs laid in each nest, the date and time of emergence, the number of emerged hatchlings, and the hatching success. Hatching success is linked to both the depth and number of eggs in the nest (ΔAIC=39, Akaike weight=0), but this result should be used cautiously, because only five nests were analyzed. For the five nests, all juveniles emerged at the same time.

All dataloggers emitted the expected signals except the HOBO Pendant G datalogger deployed in nest A (Fig. 1A). This nest was not further analyzed for this characteristic. Movement was detectable at the end of incubation for the remaining four nests (Fig 1B-E). The peak of movement was observed 2.73, 2.11, 3.67, and 2.52 days before emergence from the sand. When a standard deviation $k = 4$ was chosen, only four false positive movements were detected (two for nest B and two for nest D) (Fig. 1) for a total of around 8,000 measurements (excluding nest A). The rate of false positive is therefore around 5.10^{-4}. The first signs of movement detected in the nests were between 6.5 and 0.5 days before the peak of movement (Table 2). No relationship can be detected between the start of movements and the interval between the peak of movement and emergence.

Temperatures and movements during the incubation period until hatchling emergence are plotted in Figure 1 for each nest. To facilitate the comparison, temperatures are plotted alongside the calendar dates in Figure 2. No distinct thermal pattern emerged for the incubation period, pipping, hatching, or emergence.

Discussion

Incubation duration is not clearly defined in the scientific literature. Indeed, opinions differ regarding the end of the incubation period: pipping, when the hatchling is half or completely out of the shell, or when it emerges from the sand. A clear definition and understanding of incubation duration are important for biological and ecological purposes in order to model embryonic growth. It is also important in terms of the management of nests in natural or hatchery conditions. Anticipating the incubation duration is crucial for hatcheries where eggs are incubated for conservation purposes [11], because the knowledge of emergence timing can help these programs be prepared to collect the hatchlings and avoid predation. The pattern of high intra-nest synchronicity in emergence was shown to be beneficial as an anti-predator strategy for marine turtles [12]. Synchronous hatching may therefore be an adaptive “bet-hedging” strategy, with different mechanisms used across turtle species [12-15].

Synchrony is a common phenomenon in marine turtle embryos. First, embryos are maintained in a pre-ovipositional embryonic arrest in the oviduct (developmental arrest), so that they all synchronize and continue their growth simultaneously after being laid by nesting females [16]. The rate of embryonic growth depends on temperature [11]. Although the intra-nest temperature variations experienced by embryos can be as large as 6 °C, synchronous emergence generally occurs. Synchrony can occur because embryos delay hatching until stimulated by an environmental cue [14, 17, 18], or because less developed embryos either adjust their developmental rate through metabolic compensation [15] or hatch at an earlier developmental stage [13, 19].

Our results show that the first movements are detected from 6 to 0.5 days before the peak of movement (Table 2). The precise dynamics of pipping in different eggs are not well understood, but some wait up to 6 days in the egg chamber before moving toward the surface and emerging. The time between the peak of movement and emergence is also variable, ranging from 4 to 2 days. One factor to take into account is the respiration process of hatchlings. On their way to the surface, their respiration process is essentially composed of a single breath followed by a long respiratory pause with a slow frequency and high metabolic rate, which is similar to diving adults [20]. Nevertheless, it was found that the timing of emergence may be influenced by non-physiological factors, including thermoregulatory constraints [12].

Conclusion

Embryo growth and temperature modeling is a valuable tool in studies aiming to develop embryo behavior and related models. For instance, the embryo growth model was based on the period of incubation from the beginning of incubation until emergence depending on the size of the hatchling. Knowing when embryos pipped may lead to more exact calculations in embryo growth, considering that hatchlings may still grow between pipping and emergence. Indeed, during this period, they not only improve behavioral synchrony but also close and straighten their plastron and absorb the remnants of the yolk sac [5]. In terms of energetic cost, the ascension to the surface represents between 11 and 68% of the energy contained in the residual yolk at hatching [21], which means that the timing may depend on the type of sand, the energetic component of each individual, or even the respiration process. We demonstrate here that movement can be detected within the nest using 3D accelerometer datalogger located within the nest and pipping time can be estimated.

Acknowledgments

We thank the hatchery of the Área de Usos Múltiples Hawaii as well as the Guatemalan authorities for authorizing our use of the eggs collected for this experiment. We would like to thank Victoria Grace (http://www.english-publications.com) who helped us in correcting our English text.

Conflict of Interest Statement

The authors declare no conflicts of interest.

References

1 Booth, D.T. and Astill, K. 2001, Aust. J. Zool., 49, 389-396.
2 Ewert, M.A. 1985, Biology of the reptilia, Gans, C., Billett, F. and Maderson, P., (Eds), John Wiley & Sons, New York, NY, 75-268.
3 Kraemer, J.E. and Bennett, S.H. 1981, Copeia, 1981, 406-411.
4 Johnston, S.D., Daniels, C.B., and Booth, D.T. 2001, Respir. Physiol., 126, 75-84.
5 Godfrey, M.H. and Mrosovsky, N. 1997, Chelonian Conserv. Biol., 2, 581-585.
6 Brock, K.A., Reece, J.S., and Ehrhart, L.M. 2009, Restor. Ecol., 17, 297-307.
7 Dial, B.E. 1987, Herpetologica, 43, 307-315.
8 Mrosovsky, N. 1988, Can. J. Zool.-Rev. Can. Zool., 66, 661-669.
9 Mrosovsky, N. and Yntema, C.L. 1980, Biol. Conserv., 18, 271-280.
10 Neville, A., Webster, W.D., and Brooks, W.B. 1989, Journal, 131-133.
11 Girondot, M. and Kaska, Y. 2014, J. Therm. Biol., 45, 96-102.
12 Santos, R.G., Pinheiro, H.T., Martins, A.S., Riul, P., Bruno, S.C., Janzen, F.J., and Ioannou, C.C. 2016, Proc. R. Soc. Lond. Ser. B-Biol. Sci., 283, 20160697.
13 Colbert, P.L., Spencer, R.J., and Janzen, F.J. 2010, Funct. Ecol., 24, 112-121.
14 Doody, J.S., Georges, A., Young, J.E., Pauza, M.D., Pepper, A.L., Alderman, R.L., and Welsh, M.A. 2001, Can. J. Zool.-Rev. Can. Zool., 79, 1062-1072.
15 McGlashan, J.K., Spencer, R.J., and Old, J.M. 2012, Proc. R. Soc. Lond. Ser. B-Biol. Sci., 279, 1709-1715.
16 Rafferty, A.R. and Reina, R.D. 2012, Proc. R. Soc. Lond. Ser. B-Biol. Sci., 279, 2299-2308.
17 Doody, J.S., Stewart, B., Camacho, C., and Christian, K. 2012, Anim. Behav., 83, 645-651.
18 Webb, G.J.W., Choquenot, D., and Whitehead, P.J. 1986, J. Zool., 1, 521–550.

19 Spencer, R.J. and Janzen, F.J. 2011, Integr. Comp. Biol., 51, 100-110.

20 Price, E.R., Paladino, F.V., Strohl, K.P., Santidrian, T.P., Klann, K., and Spotila, J.R. 2007, Comp. Biochem. Physiol. A-Mol. Integr. Physiol., 146, 422-428.

21 Rusli, M.U., Joseph, J., Liew, H.-C., and Bachok, Z. 2015, Sains Malays., 44, 49-55.

Annex

Table 1. Data for the five nests collected in natural conditions and relocated to the hatchery. Dates are shown as MM-DD-YYYY format and times as HH:MM format.

Nest	Date/time of female nesting	Total nest depth (cm)	Number of eggs	Date/time of emergence	Number of emerged hatchlings	Nest hatching success
A	11-08-2019 / 20:40	26	83	26-09-2019 / 22:00	73	89.2%
B	12-08-2019 / 21:20	29	78	27-09-2019 / 23:00	74	93.6%
C	16-08-2019 / 20:36	35	112	30-09-2019 / 23:00	72	64.3%
D	16-08-2019 / 21:55	34	95	02-10-2019 / 00:00	60	66.7%
E	17-08-2019 / 21:53	33	112	02-10-2019 / 23:00	67	59.8%

Table 2. Incubation timing events. The acceleration datalogger deployed in nest A supposedly failed.

Nest	Total incubation period (until emergence)	Time from the first movement to the peak of movement	Time from the peak of movement to emergence
A	46 days	na	na
B	46 days	5 days	2.73 days

C	45 days	6 days	2.11 days
D	46 days	2.5 days	3.67 days
E	46 days	0.5 days	2.52 days

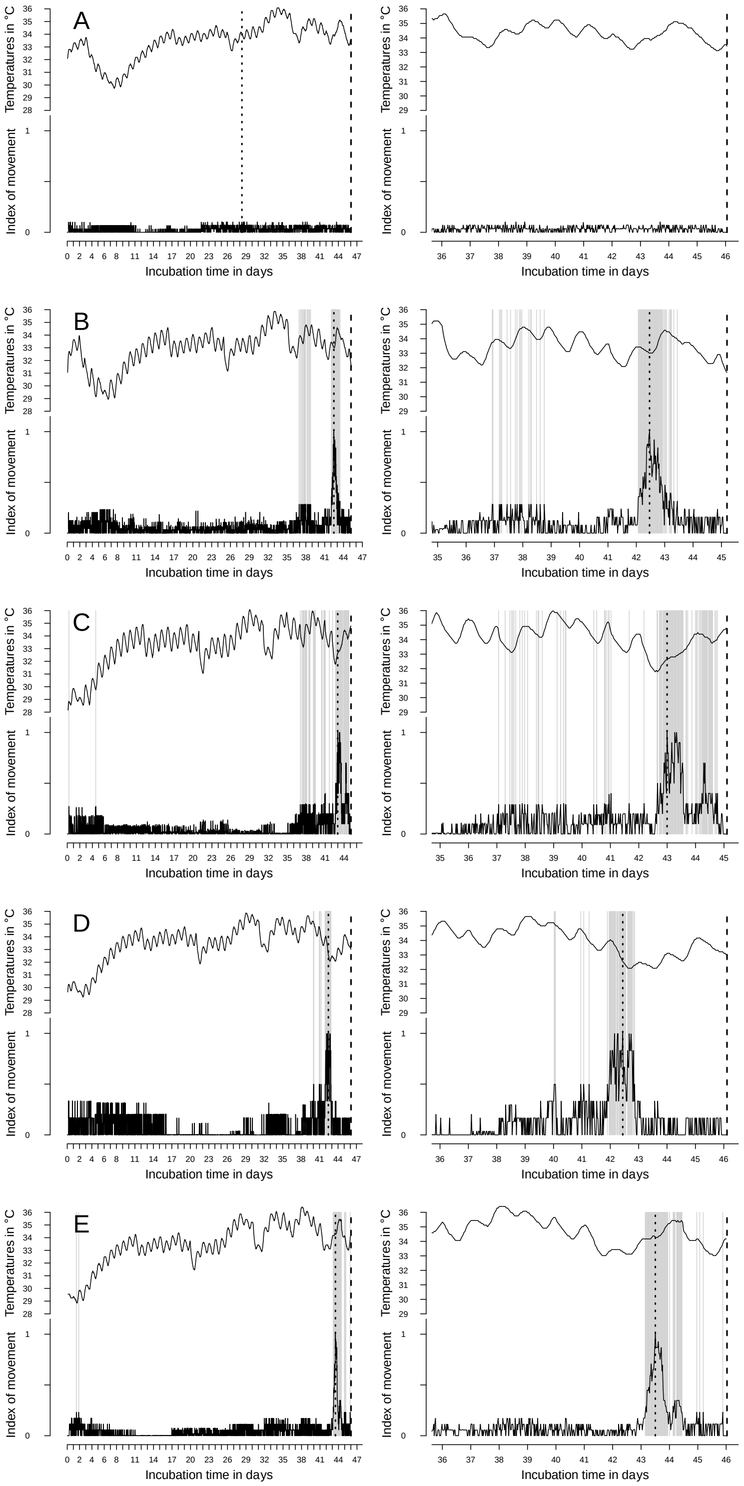


Figure 1: Temperatures (top graph) and index of movements (bottom graph) for the five nests (one per row, A-E) monitored from egg laying (day 0) to emergence from the sand (dashed line at the right of the graphs). The left panels include the entire incubation period, while the right panels focus on the last 10 days. The periods with significant movements are shown as gray rectangles. The time with the highest detected movement is marked with a dotted line. The acceleration datalogger deployed in nest A supposedly failed.

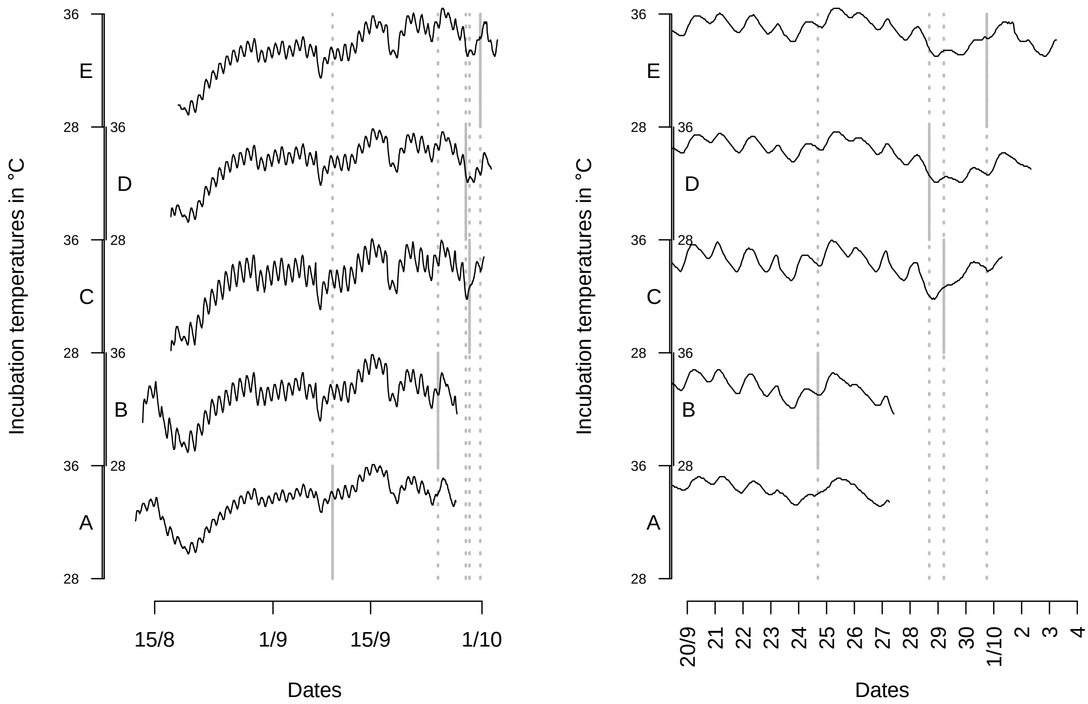


Figure 2: Temperatures recorded in the five nests (A-E) during the entire incubation period (left) and at the end of incubation (right). The dotted vertical lines indicate the periods with the highest movement for at least one of the nests. The solid gray vertical line shows the periods with the highest movement for each nest. The acceleration datalogger deployed in nest A supposedly failed.

CHAPTER 2. Performance of *Lepidochelys olivacea* hatchlings from relocated nests on the eastern coast of the Pacific of Guatemala

ARTICLE 4. Morales-Mérida BA, Contreras-Merida MR, Cortés-Gómez A, Godfrey MH, Girondot, M. (2021, in review). Performance of the sea turtle *Lepidochelys olivacea* hatchlings from a hatchery on the Pacific coast of Guatemala. **Submitted to Basic and Applied Herpetology**

Performance of the sea turtle *Lepidochelys olivacea* hatchlings from a hatchery on the Pacific coast of Guatemala

B. Alejandra Morales-Mérida* (alemoralesmerida@gmail.com / Phone: (+502) 5306 0229)[1,2,3], María Renee Contreras-Mérida (ma.reneecont@gmail.com)[3], Adriana Cortés-Gómez (adriana.cortes@u-psud.fr)[2], Matthew H. Godfrey (mgodfrey@seaturtle.org)[4,5,6], Marc Girondot (marc.girondot@universite-paris-saclay.fr)[2]

1-Doctorado en Ciencias Naturales para el Desarrollo (DOCINADE), Instituto Tecnológico de Costa Rica (TEC), Universidad Nacional (UNA), Universidad Estatal a Distancia (UNED), Costa Rica. Orcid ID: https://orcid.org/0000-0001-6724-9028.
2-Laboratoire Écologie, Systématique, Évolution, Université Paris-Sud, AgroParisTech, CNRS, Université Paris Saclay, 91405 Orsay, France.
3-Facultad de Ciencias Químicas y Farmacia. Universidad de San Carlos de Guatemala (USAC). Guatemala, Guatemala.
4-North Carolina Wildlife Resources Commission, Beaufort, NC 28516, USA.
5-Duke University Marine Laboratory, Nicholas School of the Environment, Duke University, Beaufort, NC, 28516, USA.
6-Department of Clinical Sciences and Center for Marine Sciences and Technology, College of Veterinary Medicine, North Carolina State University, Morehead City, NC, 28557, USA.

*Corresponding author.

Summary statement

When hatcheries are the only sea turtle conservation strategy, and their well-functioning is vital to achieve this purpose, performance can provide information of hatchlings' fitness in response to management conditions.

Abstract

Sea turtles are marine species that are generally in danger of extinction. The conservation strategies in the different countries are attempting to preserve these species and should be constantly updating their policies according to research results taking place on site. The most abundant and frequent species of sea turtle that nest in the Pacific Coast of Guatemala is *Lepidochelys olivacea* (Eschscholtz, 1829), therefore human predation has been historically high. The solution to this predation, since the 1970s, as a conservation strategy was to place eggs in enclosed protected spaces called hatcheries, where collectors must give 20% of the nest as a conservation quota. Since this program leads to no natural nests (in situ) remaining on the beaches, the good functioning of the hatcheries plays a fundamental role in the conservation process to work. To understand and predict the fitness of the hatchlings being produced in Guatemalan hatcheries, crawling performance and self-righting performance were measured in 210 hatchlings of the Multiple Uses Area of Hawaii, in the Pacific Coast of Guatemala. The results of the performance were contrasted with incubation conditions to provide an insight into how the management may influence it. We found that self-righting may be a more meaningful measure of variable behavior than crawling performance, showing that there was little variation due to the homogeneous environment of the hatcheries. We can conclude that a greater number of eggs result in faster self-righting, while deeper nests produce hatchlings with slower rates of self-righting.

Key words: Performance, conservation strategy, fitness, hatchery, sea turtle, Guatemala.

Introduction

Temperature plays an important role in the development of sea turtle embryos that incubate inside eggs placed on nesting beaches. In particular, much attention has been focused on the impacts of temperature on hatchling sex ratios, because of temperature-dependent sexual differentiation or TSD (Flores-Aguirre, Díaz-Hernández, Ugarte, Caballero, & Cruz, 2020), and on hatching success, because of thermal limits to successful incubation (Howard, Bell, & Pike, 2014). Furthermore, projected warming of nesting beaches related to climate change has increased concern about skewed sex ratios and reduced rates of hatching for sea turtles (Hamann, Fuentes, Ban, & Mocellin, 2013; Jensen et al., 2018). In addition to these impacts, incubation temperatures have been shown to impact the behavior and performance of sea turtle hatchlings, which likely has impacts on overall fitness and thus population dynamcis (David T Booth, 2018).

Performance of hatchlings has been studied to understand and predict the survival of the hatchlings, thus, it can be linked to different factors, depending on the focus of the research (D. T. Booth, Burgess, McCosker, & Lanyon, 2004; David T. Booth & Evans, 2011; David T. Booth, Feeney, & Shibata, 2013; Burgess, Booth, & Lanyon, 2006; Gatto & Reina, 2020a, 2020b; Ischer, Ireland, & Booth, 2009; Mueller, Ruiz-García, García-Gasca, & Abreu-Grobois, 2019; E. L. Sim, Booth, & Limpus, 2014; E. L. Sim, Booth, Limpus, & Guinea, 2014). The most common performance measure studied has been swimming locomotion, in which the activity rate of hatchlings is measured and contrasted with certain factors that are expected to influence the time, such as temperature (Mueller et al., 2019). Another performance factor that has been studied is locomotion, which can be measured by different metrics, including crawling speed, which is measured as the time taken for a hatchling to traverse a set distance, and self righting responses, in which the time required for a hatchling to return to normal position after being placed in a recumbent position (Rivas, Esteban, & Marco, 2019).

Performance studies in sea turtles have most commonly focused on hatchlings of Chelonia mydas, Natator depressus, and *Lepidochelys olivacea* (D. T. Booth et al., 2004; David T. Booth & Evans, 2011; David T. Booth et al., 2013; Burgess et al., 2006; Gatto & Reina, 2020a, 2020b; Ischer et al., 2009; Mueller et al., 2019; E. L. Sim, Booth, & Limpus, 2014; E. L. Sim, Booth, Limpus, et al., 2014). In addition, there are some research also done with Caretta caretta, and even with freshwater turtles (D. T. Booth et al., 2004; Du & Ji, 2003; Elnitsky & Claussen, 2006; E. L. Sim, Booth, & Limpus, 2014, 2015). Swimming locomotion is assumed to provide the most reliable results because hatchlings engage in swimming immediately after entering the ocean from the beach where were produced. The rate of predation of hatchlings can be high in coastal waters, as they move towards their feeding areas in more open waters (Gyuris 1993). Measurements of swimming performance of sea turtle hatchlings have been typically collected during the first 24 hours after emergence, but various other factors can affect swim speeds, including incubation conditions and light exposure after hatching (Gatto and Reina (2020a).

In addition to looking at impacts of incubation temperature, locomotor and self righting have been measured in C. mydas, N. depressus, and *L. olivacea* hatchlings in relation to other incubation factors such as moisture (Gatto & Reina, 2020a). In the latter case, it was shown for C. mydas, that moisture had no effect on self righting, while for *L. olivacea* and N. depressus hatchlings, increased levels of moisture (from 4-8%) during incubation led to slower self-righting times. In terms of crawl speeds, for *L. olivacea* hatchlings, their crawling was slower at 4% moisture, while there was no relationship between moisture levels of incubation and crawl speeds for C. mydas and N. depressus hatchlings. It is likely that other factors, including those related to incubation that may influence embryo behavior and performance.

The survival of sea turtle embryos depends on the interaction of several factors during incubation, including salinity, humidity, temperature, gas exchange, rain, storm surge, erosion, and predation (Kaska & Downie, 1999). In locations where natural incubation is unlikely to be successful (e.g., because of threat of erosion, predation or collection by people), a common strategy is to move eggs to areas that can be protected until hatchlings are produced (Garcia et al. 2003). For sea turtles eggs relocated for conservation purposes, eggs should be incubated in conditions similar to those of natural nests (Mutalib & Fadzly, 2015), in order to minimize potential impacts, including those on hatchling performance (Maulany et al. 2012).

The most common nesting species of sea turtle in Pacific Guatemala is the olive ridley (*Lepidochelys olivacea*), and egg relocation is universally used for all known sea turtle nests found on beaches along the Pacific coast. Since the early 1970s, the authorities established a conservation system using protected hatcheries, in which sea turtle eggs are reburied for incubation. Under this conservation system, egg collectors deliver 20% of the eggs from each nest and in exchange, they are allowed to sell the rest of the eggs (CONAP, 2018), either for consumption at a local marketplace or for sale to hatcheries where the excess eggs will be incubated. This study aimed to evaluate the performance of hatchling turtles, in relation to various conditions measured at the Hatchery of Multiple Uses Area of El Hawaii (AUMH), during the nesting season of 2019.

Materials and Methods

The research was executed under Guatemalan research license number DRSO 001/2019, and data consisted in olive ridley (*L. olivacea*) hatchlings produced from eggs obtained from nests collected from the east coast of Pacific Guatemala, specifically in the area surrounding AUMH. More than 60 nighttime patrols were conducted along the beaches from Monterrico to El Hawaii, during July and August of 2019, to encounter nesting female olive ridley turtles. Once a nesting female was found, we took photos of the turtle for later assessment of asymmetry (see below), and after oviposition, we carefully removed the eggs from the nest cavity, and noted depth and width in order to replicate those conditions in the hatchery. Eggs from each nest were relocated in the hatchery, and placed in a constructed nest cavity with similar dimensions as the original nest. We also inserted a HOBO®Pendant datalogger in the middle of the egg clutch, to record temperatures every hour during the entire incubation period. After 40 days of incubation, a metal mesh cylinder was placed over the nest to retain emergent hatchlings so they could be linked to a specific nest.

Five nests were intended initially, nevertheless, data of the hatchlings (DIx and morphometric data) was able to measure at the time of their emergence, and so performance information of these hatchlings was obtained, this nest was a regular nest from the hatchery and therefore no natural nest information and temperatures were recoded. From the six nests, we assessed 210 hatchlings. The amount of hatchlings measured from each nest corresponded to all the hatchlings that emerged at the same time, thus, the amount measured hatchlings varies from 10 to 55. They were put aside and started measuring one by one. When the remaining hatchlings of each nest emerged (from 12 to 24 hours after the first group emerged), they were released as part of the usual management process, by the park rangers of volunteers.

For each hatchling assessed, we collected morphometric data including mass, with a digital scale, curved carapace length (CCL) and curved carapace width (CCW) with a flexible measuring tape, and straight carapace length (SCL) with calipers. The lengths of both front flippers were measured with a measuring tape. We also took an overhead photo of the hatchling, to calculate asymmetry based on the developmental instability index –DIx- (see below).

For performance two parameters were used: time of self righting and time of crawling from one point to another, based on the methodologies proposed by E. L. Sim, Booth, Limpus, et al. (2014). Each hatchling was measured first for the self righting test, in which the hatchling was placed in a recumbent position on a surface of smoothed sand, and we recorded the time needed for the turtle to return to an upright position. For the cases when the hatchling did not successfully right itself within 10 seconds, the hatchling was manually placed in its normal position until it became active again (generally within one or 12 seconds). Subsequently, it was tested again for self righting. If a turtle failed to self-right during three trials, it was not tested again.

After the self righting test, crawling performance was measured using a “race track,” which was a 50 cm long plastic gutter filled with moist, flattened, and compact beach sand, and placed at a flat angle. Each hatchling was placed at one end and we recorded the time taken for the turtle reach the other end of the gutter, all hatchlings were measured one time in which the end was reached. Both performance measurements were made as soon as the hatchlings started to emerge from the nests (whatever hour was that happened, mainly after midnight), with data collected from one hatchlings at a time, while the other turtles were kept in an open bin apart from the measuring area. Hatchlings were retained for performance measurements no more than four hours after emergence from the nest. After the performance tests were completed, pictures were taken and morphometric measurements were collected, and subsequently all hatchlings were released into the sea. All tests were conducted in the presence of red light, to allow data collection while avoiding impacts to turtle behavior. Unfortunately, the first group of hatchlings that emerged from the fifth nest were not observed on time, and could not be used for the study, although, ten hatchlings form another emerging group of this nest were measured as soon as emerged, (the next day after the first group emerged). This lack of hatchlings was compensated with the measurement of 43 hatchling of a sixth nest.

After 4 to 6 days of the first emergence, the hatching success of each nest was based on inventory of the nest six days after first hatchling emergence, when the contents of the nest were characterized (Mutalib & Fadzly, 2015). The remains of each nest were classified as number of shells, number of hatchlings found alive within the nest, number of dead hatchlings in the nest, number of unhatched eggs, including those without apparent development and those with unhatched term embryos. Calculation of hatching success followed Miller (2000).

Data analysis

To estimate the asymmetry of the carapace of both hatchling and mother, as part of the developmental instability, the DIx was used. This index, rather than size, contemplates relative proportions, which it achieves by integrating the analysis of the diversity of scutes by means of an averaged geometric analysis of Shannon H entropy and the difference in the costal scutes of both sides obtained through Edward's angular distance analysis (Cortés-Gómes, Romero, & Girondot, 2018). To do this, using photographs, the costal scutes of the left and the right side were counted and measured. In order to obtain the inter-side differences, the shields of both sides were counted, while to obtain the intra-side differences. Inkscape Software 0.92.4 was used to measure each scale with a ruler. The measurements were tabulated in the program Microsoft Excel® and analyzed with the HelpersMG package (≥ 1.7) and DIx, in the Software statistical package R 4.0.2.

Using the Software statistical package R 4.0.2, we used General Linear Models (GLM) to analyze the interactions among incubation conditions, hatchling and maternal development instability, and hatchling sizes (IDx, IDxMother, minT, maxT, MeanT, MeanF, LF, RF, Eggs, HatchSuccess, NDepth, Mass, SCL, CCL, and CCW, where T = temperature, F=flipper length), and the performance of the hatchlings, in terms of times of self righting and crawling.

Results

Nesting data, success and hatchling measurements (Table 1) were obtained from relocated nests in the hatchery of the AUMH. Complete data from nest 1 to 5 is available, unlike nest 6 which lacks data for DIx of the mother (DIxMother), number of eggs (Eggs) and nest depth (NDepth), temperatures (High, Low, Mean), and hatching success of the nest). Therefore, analyses for DIxMother, NDepth, Eggs, temperatures (High, Low, Mean), and Hatching success was for nests 1-5 only (Table 2 and Table 3).

Table 1. Data of the hatchlings measured from nests relocated to the hatchery of the AUMH.

Nest	Total depth of the nest	Number of eggs	Hatching success of the nest	Number of turtles assessed	Mean crawling time	Mean self righting time	Mean Mass	Mean CCL	Mean CCW	Mean SCL
1	26 cm	83	89.2 %	34	103.8 s	6.82 s	0.019	4.42	4.45	4.19
2	29 cm	78	93.6 %	20	96 s	3.12 s	0.017	4.09	4.26	3.99
3	35 cm	112	64.3 %	55	122.4 s	4.64 s	0.017	4.37	4.31	4.15
4	34 cm	95	66.7 %	48	132.6 s	6.85 s	0.016	4.25	4.16	4.1
5	33 cm	112	59.8 %	10	146.4 s	9.76 s	0.015	4.46	4.28	4.18
6	NA	NA	NA	43	109.8 s	3.53 s	0.017	4.34	4.40	4.32

We collected performance measurements on 210 hatchlings overall. Intrinsic and extrinsic factors were not found strongly significant to be related to the crawling performance, as shown in Table 2, where only the hatchlings DIx has a 0.05 significance.

Table 2. GLM results of the crawling performance and intrinsic and extrinsic factors.

Factor	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**	**Signif.**
DIx	1.0079	0.4221	2.388	0.017	0.05
DIxMother	-5.43514	8.41459	-0.646	0.5183	1
SCL	0.97729	0.84242	1.160	0. 2460	1
CCL	-0.96787	0.54372	-1.780	0.0751	0.1
CCW	0.13960	0.60771	0.230	0.8183	1
Mass	10.23300	89.29932	-0.115	0.9088	1
NDepth	-0.05325	0.07092	-0.751	0.4527	1
Eggs	0.02282	0.02395	0.953	0.3406	1
Flipper size	NA	NA	NA	NA	NA
High temperatures	NA	NA	NA	NA	NA
Low temperatures	NA	NA	NA	NA	NA
Mean temperatures	NA	NA	NA	NA	NA
Hatching success	NA	NA	NA	NA	NA

Of all intrinsic and extrinsic factors, only the DIx of the hatchlings had a relationship with the crawling performance (p=0.05), so the focus of the results and discussion is on the self righting performance data. As shown in Table 3, the nest depth and the amount of eggs in the nest are significantly related to the self righting performance. Specifically, there is an inverse relationship between the nest depth and the self righting time, whereas the number of eggs is positively related to the amount of time it took each hatchling to flip.

Table 3. GLM results of the self righting performance and intrinsic and extrinsic factors.

Factor	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**	**Signif.**
DIx	0.12745	0.25981	0.491	0.62374	1
DIxMother	- 10.02495	3.07344	-3.262	0.001107	0.01
SCL	-0.13457	0.45141	-0.298	0.76562	1
CCL	0.09370	0.29614	0.316	0.75170	1
CCW	0.66654	0.29863	2.232	0.025616	0.05
Mass	49.57048	52.50818	0.944	0.34514	1
NDepth	-0.223092	0.031394	-7.106	1.19e-12	0.001
Eggs	0.056774	0.008635	6.575	4.88e-11	0.001
Flipper size	NA	NA	NA	NA	NA
High temperatures	NA	NA	NA	NA	NA
Low temperatures	NA	NA	NA	NA	NA
Mean temperatures	NA	NA	NA	NA	NA

Hatching success	NA	NA	NA	NA	NA

Significance was also found in the mother's DIx (DIxMother), and in the size of the hatchling, in terms of the CCW, and the self righting time.

Figure 1. Influence of the nest depth and the number of eggs in the nest had a significant influence over the self righting performance of the 210 hatchlings measured.

Figure 2 shows the thermal progression of nests one to five, every hour during the whole incubation period. There was no datalogger on nest six, because is a nest for which hatchlings' morphometric and DIx data was obtained later one, therefore, no temperature information was collected.

Figure 2. Clutch temperature, recorded every hour, in each nest of the 6 nests monitored. Starting at the nest burial until the first hatchling emerged. Note initial cold-pulse on first day when eggs were relocated to the hatchery.

Discussion

The hatchlings phenotype can be a reflection of the genetic components or maternal origins (intrinsic factors), but also can be a reflection of the incubation conditions or nest effects (extrinsic factors) (David T. Booth et al., 2013). There are different intrinsic and extrinsic factors or conditions that can affect the performance of a hatchling, as we have shown. The intrinsic factors that are commonly studied are the characteristics directly related to the biology and physiology of the hatchlings, including maternal and genetic effects. In this study, we used the DIx metric of hatchlings and mothers, hatchlings size and mass, and flipper lengths. In addition, we considered extrinsic factors, including nest depth, clutch size, nest temperature, and hatching success (Table 1).

For marine turtles, hatchling performance measures collected soon after emergence from the nest have been used as indices of fitness (David T. Booth et al., 2013; Fisher, Godfrey, & Owens, 2014; Read, Booth, & Limpus, 2012). The performance is thought to correlate with survival because it influences the length of time a hatchling will spend on the beach, exposed to land-based predators such as ghost crabs, and some nocturnal mammals and birds (David T. Booth et al., 2013; Ischer et al., 2009; F.J. Janzen, Tucker, & Paukstis, 2007; Paitz, Gould, Holgersson, & Bowden, 2010; Pankaew & Milton, 2017; Read et al., 2012). Freedberg, Stumpf, Ewert, and Nelson (2004), found that conditions experienced during development can affect the self righting response of older juveniles, indicating that the environment that the embryos experience during the incubation period can have a long term effect on its phenotype.

In this study, we found that only the DIx of hatchlings (Table 2) was significantly related to the crawling speed, despite the findings of other studies. For instance, it has been shown that hatchlings from nests with a three-day-maximum temperature below 34°C could have a faster crawling, than hatchlings with nests with three-day-maximum above 34°C (Maulany, Booth, & Baxter, 2012). Ischer et al. (2009), also found that hatchlings from cooler nests were faster crawlers. In addition, E. L. Sim et al. (2015) found that that larger hatchlings tend to be produced in cooler nests and as a result are faster crawlers, which would be expected because of their longer limbs and consequent greater stride length (Ischer et al., 2009). Interestingly, Le Gouvello, Nel, and Cloete (2020), found no relationship between crawling speed and any of the hatchling's attributes (different body size measures), similar to the our study, although the relationship we found was not strong.

We also looked at self righting as an indicator of performance of the hatchlings (Table 3). Contrary to what we found, previous reports have found that hatchlings from high incubation temperature nests took more time to self-right than hatchlings from cooler nests (Ashmore & Janzen, 2003; Fleming, 2019; Ischer et al., 2009; Maulany et al., 2012; Read et al., 2012; Wood, Booth, & Limpus, 2014). We found no effect of incubation temperature on self righting performance of hatchlings (Table 3).

We found that factors such as the nest depth and the amount of eggs in the nest have a significant influence over the self righting performance, where a higher number of eggs increases the time of self righting, and deeper nests produce hatchlings with faster flipping response (Figure 1). This suggests that the management decisions at the hatchery can affect hatchling performance, and thus fitness, which should be explored with more data in the future. For example, greater investigation of performance measurements can be explored, including possibly increasing the distance for crawl measurements, investigating swimming, and even reversing the order types of tests administered to hatchlings. Other studies have found differences in performance data measured in the same groups of turtles. For example, E. L. Sim et al. (2015), looking at C. caretta, found that the self righting performance was not informative while crawling performance was. Additionally, Gatto and Reina (2020b), using *L. olivacea*, found a positive relationship between the crawling performance and the self righting performance, although they also reported species-specific differences in performance measurements.

There have been studies that reported how hatchlings that had incubation temperatures above 30°C needed longer time to self righting than those incubated at lower temperatures (Fisher et al., 2014; Fleming, 2019; Maulany et al., 2012). We had no hatchlings produced at incubation temperatures below 30°C, which may have obscured a significant relationship between incubation temperature and performance in this sea turtle population. Moreover, in our study, all nest temperatures at some point surpassed the upper limit of embryo tolerance (34°C), with values of 35.8-36.4°C towards the end of the incubation period (Figure 2). During the development of the embryos, when almost fully developed, high temperatures can cause uncoordinated movement in emergent hatchlings (E. L. Sim et al., 2015). Future studies should include hatchlings produced at a wider range of incubation temperatures, to better understand the relationship between incubation conditions and hatchling performance.

Studies propose that incubation temperatures induce differences in muscle fiber composition and number within limb muscles which in turn affects locomotion performance (Ischer et al., 2009). Hatchlings that are upside down on the beach and cannot successfully self right, are more likely to die from predation, dehydration, or desiccation (Elizabeth Louise Sim, 2014). Although not measured in this study, it has been reported that hatchlings use their head and neck to lift the carapace off the sand to flip-over (Read et al., 2012; Elizabeth Louise Sim, 2014), which would be an interesting to study in order to understand the self righting time in hatchlings. Marine turtles have two stable balance points: the carapace and the plastron (Domokos & Várkonyi, 2008). This creates a high energy barrier between the two stable equilibria, which has to be overcome by primary biomechanical forces resulting from vertical pushing with the head against the substrate with the hyperextension of the neck (Domokos & Várkonyi, 2008). This can make neck length an important trait to consider in self righting studies (Read et al., 2012; Elizabeth Louise Sim, 2014).

Other studies have reported a negative correlation between carapace size and hatchling righting time (David T. Booth et al., 2013), with larger hatchlings reported to have a greater chance of survival because they express greater locomotor ability and ability to escape from gap-limited predators (David T. Booth et al., 2013; F. J. Janzen, Tucker, & Paukstis, 2000). However, warmer incubation temperatures produce hatchlings that are smaller in size, due to a shortened period of incubation in which they have less time to convert yolk into tissue before hatching from the egg (Stewart, Booth, & Rusli, 2019). Based on this, it is possible that there is a relationship between the amount of yolk metabolized during a short incubation period and the self righting time of the hatchling.

Our data on performance of hatchlings was related to a variety of intrinsic and extrinsic incubation variables contribute to a growing number of studies on this subject (Booth 2018). We found that there is a wide range of approaches and measures used in various studies, which makes comparisons more challenging. We recommend that future studies consider following standardized approaches, including protocols for testing, analyzing and presenting data. This would greatly help facilitate comparisons across studies and improve our understanding of the impacts of incubation conditions on sea turtle fitness.

The initial objective of this study was to analyze the performance of the hatchlings in relation to the different environmental (extrinsic) and biological (intrinsic) factors experienced by developing olive ridley embryos in the hatchery environment that has been used for sea turtle egg incubation for over 50 years. However, it is likely that the hatchery conditions were not variable enough to illuminate all the potential relationships among the measures we collected. Nevertheless, based on the data collected, it appears that nest depth and number of eggs in each nest have some influence on hatchling performance, and deserve more study, in the context of informing management decisions that may improve hatchling fitness. We also suggest that some olive ridley nests are allowed to remain in place for natural incubation, in order to facilitate comparisons to hatchery-based incubation.

Conclusions

We provide the results of the first study to assess the impacts of incubation condition on hatchling performance for olive ridley sea turtles in Guatemala. We found that self righting provided a more meaningful measure of variable behavior in response to different incubation variables than crawling speed. However, we also found there was limited variation in some incubation variables, such as temperature, due to the homogeneous environment of the hatcheries. Our results suggest that nest depth and amount of eggs in each nest can have an effect on self righting of hatchlings, with greater number of eggs result in faster flipping, while deeper nests produce slower rates of flipping by hatchlings. We recommend more studies be done on crawling performance, perhaps with a wider variety of incubation conditions and possibly longer runways, and we recognize that standardized protocols for hatchling fitness studies would facilitate better comparisons of datasets across studies.

Competing interests

The authors received no funding for this work, as well as declare that there are no competing interests.

Acknowledgements

We thank the Multiple Uses Area of Hawaii and ARCAS for supporting the realization of this research, as well as the volunteers that help with the data gathering. We especially would like to thank Alex García, Ricardo Gill, José Jorge Ubico, Salomé Hernández, Marisette Quiñónez, Doña Mayra, and Allan Marroquín for all their valuable help on site. Finally, we would also like to acknowledge that this research was executed under the permit no. DRSO 001-2019, granted by the Guatemalan National Council of Protected Areas (CONAP).

References

Ashmore, G. M., & Janzen, F. J. (2003). Phenotypic variation in smooth softshell turtles (Apalone mutica) from eggs incubated in constant versus fluctuating temperatures. Oecologia, 134(2), 182-188. doi: 10.1007/s00442-002-1109-z

Booth, D. T. (2018). Incubation temperature induced phenotypic plasticity in oviparous reptiles: Where to next? Journal of Experimental Zoology Part A: Ecological and Integrative Physiology, 329(6-7), 343-350.

Booth, D. T., Burgess, E., McCosker, J., & Lanyon, J. M. (2004). The influence of incubation temperature on post-hatching fitness characteristics of turtles. Paper presented at the Animals and environments: proceedings of the Third International Conference of Comparative Physiology and Biochemistry KwaZulu-Natal, South Africa.

Booth, D. T., & Evans, A. (2011). Warm water and cool nests are best. How global warming might influence hatchling green turtle swimming performance. PLoS One, 6(8), e23162. doi: 10.1371/journal.pone.0023162.g001

Booth, D. T., Feeney, R., & Shibata, Y. (2013). Nest and maternal origin can influence morphology and locomotor performance of hatchling green turtles (Chelonia mydas) incubated in field nests. Marine Biology, 160, 127-137.

Burgess, E. A., Booth, D. T., & Lanyon, J. M. (2006). Swimming performance of hatchling green turtles is affected by incubation temperature. Coral Reefs, 25(3), 341-349.

CONAP. (2018). Normativo para el Manejo y Conservación de Tortugas Marinas Documento Técnico No. 36-2017. (Documento Técnico No. 36-2017). Guatemala.

Cortés-Gómes, A. A., Romero, D., & Girondot, M. (2018). Carapace asymmetry: A possible biomarker for accumulation in adult Olive Ridley marine turtles? Marine Pollution Bulletin, 129, 92-101.

Domokos, G., & Várkonyi, P. (2008). Geometry and self-righting of turtles. Proceedings of the Royal Society A-Mathematical Physical and Engineering Sciences, 275, 11-17.

Du, W.-G., & Ji, X. (2003). The effects of incubation thermal environments on size, locomotor performance and early growth of hatchling soft-shelled turtles, Pelodiscus sinensis. Journal of Thermal Biology, 28(4), 279-286.

Elnitsky, M. A., & Claussen, D. L. (2006). The effects of temperature and inter-individual variation on the locomotor performance of juvenile turtles. Journal of Comparative Physiology B, 176(6), 497-504.

Fisher, L. R., Godfrey, M. H., & Owens, D. W. (2014). Incubation temperature effects on hatchling performance in the loggerhead sea turtle (Caretta caretta). PLoS One, 9(12), e114880. doi: 10.1371/journal.pone.0114880

Fleming, K. A. (2019). Effects of In Situ Incubation Temperatures on Hatchling Loggerhead Sea Turtle (Caretta caretta) Morphology, Health Indices, and Locomotor Performance. (Master Master of Science), University of South Florida St. Petersburg, https://digital.stpetersburg.usf.edu/masterstheses/179/. (179)

Flores-Aguirre, C. D., Díaz-Hernández, V., Ugarte, I. H. S., Caballero, L. E. S., & Cruz, F. R. M. d. l. (2020). Feminization tendency of Hawksbill Turtles (Eretmochelys imbricata) in the western Yucatán Peninsula, Mexico. . Amphibian & Reptile Conservation, 14, 190–202.

Freedberg, S., Stumpf, A. L., Ewert, M. A., & Nelson, C. E. (2004). Developmental environment has long-lasting effects on behavioural performance in two turtles with environmental sex determination. Evolutionary Ecology Research, 6, 739-747.

Gatto, C. R., & Reina, R. D. (2020a). The ontogeny of sea turtle hatchling swimming performance. Biological Journal of the Linnean Society, 131(1), 172-182.

Gatto, C. R., & Reina, R. D. (2020b). Sea turtle hatchling locomotor performance: incubation moisture effects, ontogeny and species-specific patterns. Journal of Comparative Physiology B, 190(6), 779-793.

Hamann, M., Fuentes, M., Ban, N. C., & Mocellin, V. (2013). 13 climate change and marine turtles. The biology of sea turtles, 3, 353.

Howard, R., Bell, I., & Pike, D. A. (2014). Thermal tolerances of sea turtle embryos: current understanding and future directions. Endangered Species Research, 26, 75-86. doi: 10.3354/esr00636

Ischer, T., Ireland, K., & Booth, D. T. (2009). Locomotion performance of green turtle hatchlings from the Heron Island Rookery, Great Barrier Reef. Marine Biology, 156(7), 1399–1409.

Janzen, F. J., Tucker, J., & Paukstis, J. (2007). Experimental analysis of life-history stage: direct or indirect selection on body size of hatchling turtles. Functional Ecology, 21(1), 162-170.

Janzen, F. J., Tucker, J. K., & Paukstis, G. L. (2000). Experimental analysis of an early life-history stage: Selection on size of hatchling turtles. Ecology, 81(8), 2290-2304.

Jensen, M. P., Allen, C. D., Eguchi, T., Bell, I. P., LaCasella, E. L., Hilton, W. A., . . . Dutton, P. H. (2018). Environmental warming and feminization of one of the largest sea turtle populations in the world. Curr Biol, 28(1), 154-159 e154. doi: 10.1016/j.cub.2017.11.057

Kaska, Y., & Downie, R. (1999). Embryological development of sea turtles (Chelonia mydas, Caretta caretta) in the Mediterranean. Zoology in the Middle East, 19, 55-69.

Le Gouvello, D. Z. M., Nel, R., & Cloete, A. E. (2020). The influence of individual size on clutch size and hatchling fitness traits in sea turtles., . Journal of Experimental Marine Biology and Ecology, 527, 151372.

Maulany, R. I., Booth, D. T., & Baxter, G. S. (2012). The effect of incubation temperature on hatchling quality in the olive ridley turtle, *Lepidochelys olivacea*, from Alas Purwo National Park, East Java, Indonesia: implications for hatchery management. Marine Biology, 159(12), 2651-2661. doi: 10.1007/s00227-012-2022-6

Miller, J. D. (2000). Determinación del Tamaño de la Nidada y el Éxito de la Eclosión. In K. L. Eckert, K. A. Bjorndal, F. A. Abreu-Grobois, & M. Donnelly (Eds.), Técnicas de Investigación y Manejo para la Conservación de las Tortugas Marinas (pp. 143-149). Washington, DC: Grupo Especialista en Tortugas Marinas UICN/CSE.

Mueller, M. S., Ruiz-García, N. A., García-Gasca, A., & Abreu-Grobois, F. A. (2019). Best swimmers hatch from intermediate temperatures: Effect of incubation temperature on swimming performance of olive ridley sea turtle hatchlings.

Journal of Experimental Marine Biology and Ecology, 519. doi: https://doi.org/10.1016/j.jembe.2019.151186

Mutalib, A. H. A., & Fadzly, N. (2015). Assessing hatchery management as a conservation tool for sea turtles: A case study in Setiu, Terengganu. Ocean & Coastal Management, 113, 47-53. doi: http://dx.doi.org/10.1016/j.ocecoaman.2015.05.010

Paitz, R. T., Gould, A. C., Holgersson, M. C. N., & Bowden, R. M. (2010). Temperature, phenotype, and the evolution of temperature-dependent sex determination: how do natural incubations compare to laboratory incubations? Journal of Experimental Zoology Part B: Molecular and Developmental Evolution, 314B(1), 86-93.

Pankaew, K., & Milton, S. (2017). The effects of extended crawling on the physiology and swim performance of loggerhead and green sea turtle hatchlings. . Journal of Experimental Biology.

Read, T., Booth, D. T., & Limpus, C. J. (2012). Effect of nest temperature on hatchling phenotype of loggerhead turtles (Caretta caretta) from two South Pacific rookeries, Mon Repos and La Roche Percée. Australian Journal of Zoology, 60(6). doi: 10.1071/zo12079

Rivas, M. L., Esteban, N., & Marco, A. (2019). Potential male leatherback hatchlings exhibit higher fitness which might balance sea turtle sex ratios in the face of climate change. Climatic Change. doi: 10.1007/s10584-019-02462-1

Sim, E. L. (2014). Nest environment and hatchling fitness correlates in the sea turtles Caretta caretta and Natator depressus. (PhD Thesis), University of Queensland.

Sim, E. L., Booth, D. T., & Limpus, C. J. (2014). Non-modal scute patterns, morphology, and locomotor performance of loggerhead (Caretta caretta) and flatback (Natator depressus) turtle hatchlings. Copeia, 2014(1), 63-69.

Sim, E. L., Booth, D. T., & Limpus, C. J. (2015). Incubation temperature, morphology and performance in loggerhead (Caretta caretta) turtle hatchlings from Mon Repos, Queensland, Australia. Biology Open, 4(6), 685-692. doi: 10.1242/bio.20148995

Sim, E. L., Booth, D. T., Limpus, C. J., & Guinea, M. L. (2014). A comparison of hatchling locomotor performance and scute pattern variation between two rookeries of the flatback turtle (Natator depressus). Copeia, 2014(2), 339-344.

Stewart, T. A., Booth, D. T., & Rusli, M. U. (2019). Influence of sand grain size and nest microenvironment on incubation success, hatchling morphology and locomotion performance of green turtles (Chelonia mydas) at the Chagar Hutang Turtle Sanctuary, Redang Island, Malaysia. Australian Journal of Zoology, 66(6), 356-368.

Wood, A., Booth, D. T., & Limpus, C. J. (2014). Sun exposure, nest temperature and loggerhead turtle hatchlings: Implications for beach shading management strategies at sea turtle rookeries. Journal of Experimental Marine Biology and Ecology, 451, 105-114. doi: 10.1016/j.jembe.2013.11.005

ARTICLE 5. Abreu-Grobois FA, Morales-Mérida BA, Hart CE, Guillon J-M, Godfrey MH, Navarro E, Girondot M. (2020). Recent advances on the estimation of the thermal reaction norm for sex ratios. ***PeerJ* 8: e8451**

Recent advances on the estimation of the thermal reaction norm for sex ratios

Short title: TSD pattern estimation

F. Alberto Abreu-Grobois[1], B. Alejandra Morales-Mérida [2, 3, 4], Catherine E. Hart [5,6], Jean-Michel Guillon [2], Matthew H. Godfrey [7,8,9], Erik Navarro [1], Marc Girondot [2]

1 - Laboratorio de Genética, Unidad Académica Mazatlán, Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Mazatlán, Sinaloa 82000, Mexico.

2 - Laboratoire Écologie, Systématique, Évolution, Université Paris-Sud, AgroParisTech, CNRS, Université Paris Saclay, 91405 Orsay, France

3 - Facultad de Ciencias Químicas y Farmacia. Universidad de San Carlos de Guatemala (USAC).

4 - Doctorado en Ciencias Naturales para el Desarrollo (DOCINADE), Instituto Tecnológico de Costa Rica (TEC), Universidad Nacional (UNA), Universidad Estatal a Distancia (UNED), Costa Rica.

5 - Grupo Tortuguero de las Californias A.C., La Paz, Baja California Sur, Mexico.

6 - Investigación, Capacitación y Soluciones Ambientas y Sociales A.C. Tepic, Nayarit, Mexico.

7 - North Carolina Wildlife Resources Commission, Beaufort, NC 28516, USA.

8 - Duke Marine Laboratory, Nicholas School of the Environment, Duke University, Beaufort, NC 28516

9 - Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27607

Corresponding author: Marc Girondot

e-mail: marc.girondot@u-psud.fr

Abstract

Temperature-dependent sex determination, or TSD, is a widespread phenomenon in reptiles. The shape of the relationship between constant incubation temperature and sex ratio defines the TSD pattern. The TSD pattern is considered a life-history parameter important for conservation because the wider the range of temperatures producing both sexes, the more resilient the species is to climate change impacts. We review the different published equations and methodologies that have been used to model TSD patterns. We describe a new flexible model that allows for an asymmetrical pattern around the pivotal temperature, which is the constant temperature producing both sexes in equal proportions. We show that Metropolis-Hastings with Markov chain produced by a Monte Carlo process has many advantages compared to maximum likelihood and should be preferred. Finally, we apply the models to results from incubation experiments using eggs from the marine turtle *Lepidochelys olivacea* originating in Northeast Indian, East Pacific, and West Atlantic Regional Management Units (RMUs) and find large differences in pivotal temperatures but not in transitional range of temperatures.

Keywords: Temperature-dependent sex determination, sex ratio, TSD, Reaction norm, Marine turtles, Incubation, Eggs, Olive ridley, *Lepidochelys olivacea*.

Introduction

Sex determination is the biological process whereby an embryo can become male or female. Temperature-dependent sex determination (TSD), a special case of environmental sex determination (ESD), is widespread in animal kingdom (Korpelainen 1990) and is frequent in reptiles: all crocodilians have TSD, as do many turtles and some lepidosaurians (Valenzuela 2004). In this system, the sexual phenotype of the embryo is defined by the temperature of the incubation occurring during a part of development termed the thermosensitive period (TSP) (Girondot et al. 2018b).

In oviparous reptiles, three patterns of TSD have been described, according to the changes in sex ratios as a function of different constant incubation temperatures (Bull 1983; Ewert et al. 1994; Lang & Andrews 1994). In the TSD Ia pattern, present in some turtles, low temperatures produce males and high temperatures produce females. The opposite occurs in some lizards (TSD Ib pattern). In the TSD II pattern, present in crocodile species, some turtles and some lizards, females are produced at low and high temperatures and more males are produced at intermediate temperatures. Temperature-dependent sex determination can be described as the reaction norm of the resulting sex ratio of embryos incubated at a range of constant temperatures. By definition, a reaction norm describes the pattern of the variation in phenotypic expression of a single genotype across a range of environments (Lewontin 2000).

The mathematical relationship between incubation temperature and sex (or more specifically, the sex ratios produced by a suite of constant incubation temperatures), referred to as "thermal reaction norm", is commonly characterized by two parameters: (1) the pivotal temperature (P), which is the constant temperature at which both sexes are produced in equal proportions (sex ratio = 1:1), and (2) the transitional range of temperatures (TRT), which is the range of constant temperatures that yields both sexes in variable proportions (Mrosovsky & Pieau 1991). Note that there may be two values of *P* and *TRT* when considering the TSD II pattern.

The correct description of the thermal reaction norm for sex ratios (hereafter named TSD pattern) is not merely a game for biostatisticians. Indeed, variation in the TSD pattern can have profound implications for the conservation of TSD species, particularly in a world affected by climate change. For example, species with a greater TRT should be more likely to evolve in response to new thermal conditions, thus putting them at lower risk to global change (Hulin et al. 2009; Hulin et al. 2008). When within- and among-population variation in the TSD patterns of 12 populations of painted turtles (*Chrysemys picta*) was studied, among-population variation in pivotal temperature could not be explained by geography or local thermal conditions, but the TRT was wider at lower latitudes, suggesting responsiveness to local incubation conditions (Carter et al. 2019). These results indicate that variation in TSD patterns among populations is not an artifact of incubation at constant temperatures and can provide insight into the ecology and evolution of temperature-dependent sex determination.

However, proper statistical tools are needed for robust analyses of the TSD patterns and for identifying the characteristics of interest. Although detailed methods for this have been developed and are freely available (Girondot 2019a), their application has remained challenging, hampering authors with empirical data from fully capitalizing their importance. Thus, the goal of this study is to provide a step-by-step workflow on how to analyze TSD patterns, focusing on the TSD Ia pattern, which is the most common TSD pattern found in turtles. We describe the most advanced statistical models to analyze the thermal reaction norm for the sex ratios produced by constant-temperature incubations, using data from published and unpublished research on the olive ridley marine turtle, *Lepidochelys olivacea*, as a test case. We choose this species for several reasons. It has a worldwide distribution, it is classified as Vulnerable by IUCN (Abreu-Grobois & Plotkin 2008), and is amply subjected to egg protection in beach hatcheries as a management strategy. Providing conservationists with adequate analytic tools to evaluate the sex ratios of resulting hatchlings (e.g. Dutton et al. 1985) is critical to reliably monitor the effect of temperature managements required to counter climate warming. Furthermore, with a recent study indicating contrasting embryonic responses to incubation temperature from two rookeries (Mexico and Costa Rica) within the same Regional Management Unit (RMU) (Morales Mérida et al. 2015), analyses of TSD patterns is required to verify if regional differences exist.

Materials and methods

Biological sources of the data

Data from different studies that incubated eggs at constant temperatures were extracted from publications (available in the “databaseTSD” file, as part of the R package *embryogrowth* (Girondot 2019a). New data from our own unpublished studies (Navarro Sánchez 2015) were also included (Table 1).

The version of the database available in the package can be obtained using the R command DatabaseTSD$Version[1]. The version used here (from *embryogrowth* ver. 7.6.7) is the 2019-11-19 version with 670 records for 25 species or subspecies.

Lepidochelys olivacea data including geographic origin, incubation temperatures and their amplitude were retrieved from this file. The Regional Management Units (RMUs) of olive ridley marine turtles as defined in Wallace et al. (2010) were inferred from the geographic origins. Whenever available, a temperature correction factor (difference between the measured incubator temperature and true internal egg temperature) was also obtained from the database. This correction factor has been reported in some publications (e.g., Godfrey & Mrosovsky 2006) and has been shown to be relevant particularly when the substrate contains humidity (Tezak et al. 2018). However, as few papers measure or report this parameter, it can be ignored when comparing studies where some, or all, do not report it. An alternative is to use an average correction factor based on all studies, but doing so will not contribute statistical information and will only shift fitted *P* values proportional to the correction factor. In the current analysis, the correction factor is not used and we used only sex ratio data from eggs incubated in temperature-regulated chambers. Incubation.temperature.Amplitude and 2ndThird.Incubation.temperature.Amplitude columns from databaseTSD refer to a measure of the variability of temperatures during the whole experimental incubation and the middle-third of the incubation, respectively. Mrosovsky & Pieau (1991) define the thermosensitive period (TSP) for sex determination as the interval of time when change of incubation temperature results in a change in resultant sex ratio. The TSP begins with the formation of the genital ridge at stage 21 (sensu Miller 1985), which corresponds to the onset of the gonad formation, and ends at stage 26 (sensu Miller 1985), when the gonadal formation is nearly completed. The TSP occurs during the middle-third of the incubation period when incubation temperature is constant. When incubation temperature fluctuates during development, the TSP can shift, and the exact delimitation of TSP must be inferred by taking into account the thermal reaction norm for growth of embryo (Girondot & Kaska 2014). As a consequence, under naturally variable incubation conditions, the TSP is not exactly located at the middle-third of the incubation period (Girondot et al. 2018b). For example, if the incubation temperature at the beginning of incubation is low, embryos will not grow until the temperature increases. Subsequently, the growth of the embryo will start, and the TSP will be shifted towards the end of the incubation, in some cases even after the middle-third of the incubation period. We recommend excluding incubation data from analysis if the recorded temperatures exhibit an amplitude > 2 °C. Indeed, even short daily bursts of high temperatures during egg incubation can lead to an abnormally high proportion of females, as compared to the expected sex ratio based on the average temperature (Georges 1989; Georges et al. 1994). Because egg incubation in natural conditions can expose eggs to wide daily fluctuations of temperature, especially for relatively shallow nests laid by the olive ridley marine turtle, field collected data should not be used in this analysis. Indeed, by definition, the TSD pattern is defined by data collected from constant temperature incubations, thus the TSP is assumed to occur during the middle third of incubation without taking into account thermal reaction norm of embryonic growth. Overall, the use of mean incubation temperatures obtained from nests in field conditions to feed a TSD pattern model has been shown to produce inaccurate results (Fuentes et al. 2017).

A further consideration relates to the difference among the resolution, accuracy, and uncertainty for temperature data loggers. Resolution refers to the data logger's level of specificity for temperature in its memory. For example, a resolution of 0.5 °C indicates that temperatures will be recorded and reported in 0.5 °C bins, even if the electronic chips can internally read temperatures with a better resolution. Accuracy is represented in the logger's technical datasheet as a range (±x °C), with x representing how close an individual recorded data point is to the true value. The uncertainty is a measure of the quality of the data logger temperature recordings, considering the accuracy, the resolution, and the sampling rate. Data logger uncertainty is then defined by the 95% confidence interval of the average temperature during a specific time, recorded during set sampling period by a data logger with known accuracy and resolution (Girondot et al. 2018a). The uncertainty of the mean temperature recorded every hour for 10 days is much lower than the accuracy and resolution of the logger. For example, using typical field conditions, an iButton DS1921G-F5# with accuracy=1 °C and resolution=0.5 °C has an uncertainty value of 0.15 °C (Girondot et al. 2018a). Overall, while the amplitude of temperatures during incubation should be minimized as much as possible, the uncertainty of the average temperature is a less important issue.

The data for this study originated from eggs collected in 6 nesting beaches (Pirambu, Brazil; Orissa, India; Playa Nancite, Costa Rica; El Verde Camacho, La Escobilla, and Playa La Destiladeras, Mexico) (Table 1, Fig. 1), belonging to 3 RMUs (West Atlantic, Northeast Indian, East Pacific). Though olive ridleys nesting on beaches in Pacific Mexico and Pacific Costa Rica belong to the same East Pacific RMU (Wallace et al. 2010), we analyzed these data separately because embryo growth dynamics from the two areas respond differently under a range of incubation temperatures (Morales Mérida et al. 2015).

Data included 40 incubations at various constant temperatures with a total of 464 sexed embryos (277 males, 168 females, and 19 intersexes). The 19 intersexes were reported from only 2 studies out of 8, from incubation temperatures ranging from 27.6 to 32.3 °C. An intersex is a transient state during development when the gonads are ovotestes that exhibit characteristics of both testes and ovaries (Pieau & Dorizzi 2004). After hatching, ovotestes generally evolve as normal testes (Pieau et al. 1998). Some adult gonads retain traces of intersexual characters when some oocytes may persist at the surface of testis in some species, whereas for others, no signs of intersex at the adult stage are observable. Because the criteria to define a gonad as an ovotestis are not entirely objective, we excluded data from hatchlings that were classified as intersex (n=19 values, or 3.9% of all sexed turtles, Table 1).

The East Pacific RMU was overrepresented in the database (384 embryos, 190 from Mexico and 197 from Costa Rica) as compared to Northeast India (31 embryos) and West Atlantic (50 embryos). The number of incubation temperatures that produced mixed sex ratios was 2 for West Atlantic, 1 for Northeast Indian, 4 for East Pacific (Costa Rica), and 1 for East Pacific (Mexico).

Where stated, amplitudes of incubation temperatures were mostly <1 °C. Only two incubation temperatures in the West Atlantic RMU had amplitude > 1.5 °C, but both included only 1 embryo and so are unlikely to have biased the results.

Thermal reaction norm for sex ratio

Several models have been published to describe the mathematical relationship between constant incubation temperatures and sex ratios. We enlist the most useful, with relevant comments:

- *Logistic* model. It is based on an equation originally developed to model population growth (Verhulst 1838; Verhulst 1846). This was the first model applied to constant incubation data (Girondot 1999) and fitted using maximum likelihood with software that is no longer recommended. The *logistic* model uses 2 parameters: P is the pivotal temperature, and S is one fourth of the inverse of the slope at P. The *TRT* can be easily calculated as $TRT_l = |S \cdot K_l|$ with K_l being a constant equal to $[2 \cdot \ln(l/1 - l)]$ with l being the limits to define the *TRT*. Girondot (1999) used $l = 0.05$ and then TRT was defined as the range of incubation temperatures with resulting sex ratios from 5% to 95%.
- Hill model. This model is used in biochemistry and pharmacology to reflect the binding of ligands to macromolecules as a function of the ligand concentration (Hill 1910). From a mathematical point of view it is similar to a *logistic* equation with the natural logarithms of temperatures on the x-axis. The Hill model is

therefore asymmetrical and uses 2 parameters. Control of the asymmetry in the shape is not possible. The Hill model was used previously to describe the TSD pattern but subsequently discarded for lack of sufficient flexibility (Godfrey et al. 2003).

- *A-logistic* model (*A*- for *Asymmetrical*) was specially developed for TSD pattern analysis in Godfrey et al. (2003). It is based on the *logistic* equation with an additional parameter named K that controls the asymmetry. This K parameter is not the same as the K_l parameter used to calculate TRT for the *logistic* model (a mistake made by Carter et al. 2019). Godfrey et al. (2003) also provided an equation to calculate the *TRT*. This model is asymmetrical, but the transitions towards the lower and upper asymptotes are not independent since they are controlled by a single parameter, K. The model has three parameters.
- Hulin model. Hulin et al. (2009), recognizing that the *A-logistic* model was insufficiently flexible in the transitions toward lower and upper asymptotes, introduced a modification to K, making it a linear function of temperature: $K = K_1 t + K_2$. Four parameters were therefore fitted, and TRT can only be calculated numerically. Unfortunately, this model is challenging to fit and often hangs on local minima because K can become very large during the search for maximum likelihood. In such situations, likelihood becomes insensitive to change in K_1 or K_2, and a local minimum is reached.

Additionally, it is noteworthy that the original description of TRT used the range of temperatures corresponding to sex ratios from 5% to 95%, which is unusual. Generally, the range is defined as occurring between 2.5% and 97.5%, thus encompassing a statistically meaningful 95% of the data. Sandoval et al. (2017) questioned the use of the 5-95% limits, arguing instead that the TRT limit should be proportional to the number of eggs. This, however, is a misconception of the role of models in biology. A model is not a means to replace data but rather to obtain a generalized description of a biological phenomenon using a mathematical formula. In this case it needs to be framed within meaningful sex ratio limits. Thus, we recommend maintaining 5%-95% sex ratio as the limits of the TRT.

The lack of an ideal sigmoid model to describe TSD patterns (i.e., asymmetrical in the transitions toward lower and upper asymptotes) prompted us to develop a new, more versatile sigmoid function (see supplementary material 1) called flexible-*logistic* or *flexit* model:

$$\begin{cases} \boldsymbol{x < P} \quad \boldsymbol{S_1 = \dfrac{2^{K_1-1}\,S\,K_1}{2^{K_1}-1}} \quad \boldsymbol{f(x) = \left(1 + (2^{K_1}-1)e^{4\,S_1\,(P-x)}\right)^{-1/K_1}} \\ \boldsymbol{x \geq P} \quad \boldsymbol{S_2 = \dfrac{2^{K_2-1}\,S\,K_2}{2^{K_2}-1}} \quad \boldsymbol{f(x) = 1 - \left(1 + (2^{K_2}-1)e^{4\,S_2\,(x-P)}\right)^{-1/K_2}} \end{cases}$$

$\boldsymbol{P}$ is the pivotal temperature and $\boldsymbol{S}$ is the slope (first-order derivative) at $\boldsymbol{P}$. $\boldsymbol{K_1}$ and $\boldsymbol{K_2}$ control the lower and upper asymptotes respectively (acute for positive values and obtuse for negative values).

TRT can be calculated exactly:

$$\boldsymbol{TRT = \frac{1}{4\,S_2} ln \frac{(1/(1-l))^{K_2}-1}{2^{K_2}-1} + \frac{1}{4\,S_1} ln \frac{(1/(1-l))^{K_1}-1}{2^{K_1}-1}}$$

A flexit model uses 4 parameters, a special case being $\boldsymbol{K_1 = K_2 = 1}$, which is the *logistic* model. The model is not defined for $\boldsymbol{K_1 = 0}$ or $\boldsymbol{K_2 = 0}$. In this scenario, the corresponding value is replaced by $\boldsymbol{10^{-9}}$.

The *flexit* model is included as a function in the *HelpersMG* R package (version 3.7 and higher) (Girondot 2019b) and is included in the tsd() function of the *embryogrowth* R package (version 7.5 and higher) (Girondot 2019a).

Overall, only two models are acceptable for our purpose: *logistic* and *flexit*. If an asymmetrical model is required, the *Hill*, *A-logistic* or *Hulin* models are not flexible enough when compared to a *flexit* version.

Fitting a model to the data: maximum likelihood

The fit of parameters (2 for *logistic* and 4 for *flexit* models) can be performed using the maximum likelihood methodology. The likelihood function (simply “likelihood”) expresses how probable a given set of observations is for different values of mathematical parameters. In the context of a model of TSD pattern, the observations are counts of categories of embryos. In most cases, two categories are found, males or females, and then a binomial distribution is used to estimate likelihood.

The likelihood of a set of *M* males and *F* females observed after an incubation *t* that produced a theoretical sex ratio of *flexit*(*t*; *P*, *S*, K_1, K_2) is named *L*:

$$\boldsymbol{L = \binom{M+F}{M} flexit(t;\ P, S, K1, K2)^{M} (1 - flexit(t;\ P, S, K1, K2)\,)^{F}}$$

with $\boldsymbol{\binom{M+F}{M}} = \frac{(M+F)!}{M!F!}$ being a constant for the set of observations.

An alternative that is described in some papers (e.g. Sandoval et al. 2017) is to fit the proportions with the hypothesis that proportions or their angular transformation are normally distributed. We do not recommend this method because it produced biased results: temperatures with the fewer eggs incubated will have a larger than expected influence. Furthermore, even one sexed embryo at one incubation temperature provides useful information and should be included in the analysis.

It should be noted that sex ratio is etymologically referred to as *M*/*F* or *F*/*M*, but this definition is not practical in statistics. For practical purposes, we use sex ratio in terms of the relative frequency of males or females. The choice to work with male or female relative frequency has no importance and depends on the researcher's preference. Here we use sex ratio as being relative male frequency, to be compatible with previous publications (Girondot 1999; Godfrey et al. 2003; Hulin et al. 2009).

A possible alternative would be to incorporate the data on intersex hatchlings into the model, which tend to be more frequent at intermediate temperatures, and use a multinomial distribution. This possible solution has not yet been developed but could be an interesting avenue to explore in future studies for some species.

The likelihood value is often presented as its inverse natural logarithm ($-\ln L$) for practical reasons: likelihoods are generally small numbers, thus $-\ln L$ will be positive numbers that are easier to manipulate.

The likelihood of a dataset of several incubation temperatures t_i with M_i and F_i within a model is simply the product of the likelihoods for each temperature, L_i, or the sum of the $-\ln L_i$.

To be able to fit the sex ratio thermal reaction norm using maximum likelihood, at least one incubation temperature producing a mixed sex ratio should be present in the study dataset. A rule of thumb is that fitting a model with p parameters necessitates at least p temperatures with mixed sex ratios. For example, if a *logistic* model (2 parameters) is fitted for a dataset with only one temperature that produced a mixed-sex ratio, an infinite number of combinations of P and S will share the same likelihood. It is still possible to fit a *logistic* model to a dataset with only one temperature producing a mixed sex ratio, but standard error of parameters will be generally high. If a dataset has no mixed sex ratio, it is still possible to use a Bayesian model to describe the credibility interval of the parameters and the outputs (see below).

The estimate of parameters for TSD patterns using maximum likelihood serves various purposes: (i) It allows an estimate of the confidence interval of the outputs (see below), (ii) it provides an estimate of the quality of fit (see below), (iii) it facilitates the comparison across datasets because their fitted parameters can be compared even if incubation temperatures were different, (iv) it can be used as a prior for Bayesian analysis (see below), and (v) it can be used as a starting point for iterations using a Metropolis-Hastings algorithm with a Monte-Carlo Markov chain in Bayesian analyses (see below).

Standard error and confidence interval

The standard error is an important indicator of the precision of an estimate of a sample statistic for a population parameter. Its calculation is based on the Hessian matrix, which is the matrix of second-order partial derivatives of the likelihood for all pairwise parameters. The second-order derivative of a function at its maximum measures a more or less acute form of the function around the maximum. If a parameter is slightly shifted from its value at maximum likelihood and the likelihood changes drastically, this denotes a robust parameter estimate. At the same time, its standard error, which measures how well the parameter is known, will be low. On the contrary, if the likelihood is insensitive to changes of a parameter, it means the data do not provide information to fit this parameter and its standard error will be high. The inverse of the Hessian matrix is an estimator of the asymptotic covariance matrix. Hence, the square roots of the diagonal elements of a covariance matrix are estimators of the standard errors. A parameter obtained using maximum likelihood is normally distributed asymptotically.

This mathematical definition is sometimes problematic when the standard error is large and the effect of the parameter on the function changes drastically at some value. An example is the case for the *S* parameters because the TSD pattern is completely the reverse for -*S* (females at lower temperatures rather than high). Thus, if the standard error of *S* is large, at the ends of the *S* distribution the model will become completely reversed, and male production will be predicted at feminizing temperatures. The coefficient of variation for one parameter estimate is a standardized measure of dispersion with $CV = SE/mean$. The larger the coefficient of variation, the worse the estimate of the parameter in the analysis.

The confidence interval for a parameter can be obtained directly from its point estimate and its standard error, assuming that it is normally distributed asymptotically. The confidence interval for a combination of parameters (for example, TRT) requires a more complicated calculation. Two main strategies are available to calculate the confidence intervals for a combination of parameters: the delta method and parameter resampling. The delta method uses the approximate probability distribution for a function of an asymptotically normal statistical estimator from knowledge of the limiting variance of that estimator. In short, if the standard error of the maximum likelihood parameters are known, the delta method permits an estimate of the distribution of any function of these parameters. The alternative is to generate many random numbers for the variance and covariance matrix of the estimators using the Cholesky decomposition (Tanabe & Sagae 1992). The function of interest is then applied to each set of random numbers. The advantage of the delta method is its rapidity. However, the assumption of a normal distribution for an estimator is important. It precludes the use of the delta method on a truncated distribution such as the *S* distribution of a TSD pattern. If for example, *S* changes sign, the likelihood degrades so much that it would be considered a truncated distribution. The advantage of the generation of many random numbers from the Hessian matrix is that it is possible to check each set of numbers for coherence and discard some if necessary. In the case of models for the TSD pattern, when the confidence interval of sex ratio according to temperature or TRT is estimated, we ensure that S, $K_1 - 1$, and $K_2 - 1$ do not change sign during resampling. However, this method will artificially reduce the confidence interval because the highly divergent values are removed. Using a Bayesian Metropolis-Hastings MCMC procedure solves this problem (see below).

Quality of fit

Often the quality of fit is measured by the determination coefficient R^2, which is derived from comparisons between observations and predictions. Whereas the determination coefficient has meaning when the distribution of the dependent variable is normally distributed, in the case of univariate regression, it has no meaningful statistical properties when used with proportions. Thus, R^2 should not be used in most of these cases and particularly here where we need to measure the fit of the model to sex ratio data. Instead, deviance can be used as a goodness-of-fit measure for a statistical model. It is twice the difference between the logarithm of likelihood of the saturated model ($\ln L_S$). In a saturated model, the fitted sex ratio is replaced by the observed sex ratio and the model fits the data perfectly, and the logarithm of maximum likelihood of the fitted model ($\ln L_M$) is:

$$D = 2\,(\ln L_S - \ln L_M)$$

Deviance has an asymptotic χ^2 distribution with the degrees of freedom calculated from the difference of the number of parameters in the saturated and the fitted model. Note, however, that if there are few observations (which is often the case), the distribution of deviance can deviate substantially from a χ^2 distribution and the test result could be wrong. For this reason, we developed an additional deviance test by randomly generating null deviance distributions with the same characteristics as those from the observations (the same number of incubation temperatures and the same number of eggs per incubation temperature). The probability that the observed deviance is obtained with the experimental design is then calculated by comparing the observed deviance and the distribution of deviances under the null hypothesis.

Comparison of models: Akaike Information Criterion and Akaike weight

When several models are fitted to the same datasets of observations, the comparison of the performance of the different models can be assayed using AIC (Akaike Information Criterion) (Akaike 1974). AIC is a measure of the quality of the fit, while simultaneously penalizes for the number of parameters in the model. It facilitates the selection from a set of models the best compromise between fit quality and over-parametrization.

$$AIC_j = -2\ln L_j + 2\,p_j$$

with L_j being the likelihood of the model and p_j the number of parameters of the model j.

When a set of *k* models are tested, the model with lowest AIC is considered to be the best non-overparametrized fit. It is important to note that AIC value itself is not strictly informative in terms of absolute model fit.

A corrected version of *AIC* for small sample sizes, named *AICc*, has been proposed when the model is univariate and linear with normal residuals (Hurvich & Tsai 1995):

$$AICc = AIC + \frac{2\,p\,(p\ +1)}{n - p - 1}$$

The formula can be difficult to determine when these conditions are not met, in which case the previous formula could be used (Burnham & Anderson 2002). In general, the

AICc should always be used instead of *AIC* (Burnham & Anderson 2002), especially for datasets comprised of small sample sizes, which is typical for sex ratio studies, and particularly when the study targets protected species.

When a set of models is compared, it is possible to estimate the relative probability that each model is the best among those tested using the Akaike weight (Burnham & Anderson 2002):

$$Akaike\ weight_j = \frac{e^{\frac{1}{2}AICc_j - min(AICc)}}{\sum_{i=1}^{k} e^{\frac{1}{2}AICc_i - min(AICc)}}$$

We use both *AICc* and *Akaike weight* to compare the fitted *logistic* and *flexit* models for our datasets.

The utility of model selection can be further extended to test for potential differences in the results from two or more datasets. In this case, the complete data are split into several subsets with each individual dataset being represented once and only once. The test question is: can the collection of datasets be modeled with a single set of parameters or must each dataset be modeled with its own set? In this situation, Bayesian Information Criterion (or *BIC*) should be used instead of *AIC* or *AICc*, because the true model is obviously among the tested alternatives:

$$BIC = -2\ln L + p\ln n$$

When the BIC statistic is used, all the priors of the models tested are assumed to be identical. It is also possible to estimate *BIC* weights by replacing *AICc* with *BIC* in the Akaike weight formula. The *w*-value has been defined as the probability that several datasets can be correctly modeled by grouping instead of independently (Girondot & Guillon 2018).

In our example, a model will be fitted first to the combined datasets and *BIC*(combined) will be estimated with *p* parameters. Then each dataset will be fitted separately and a set of *k* *BIC*(separate) values will be generated, each with *p* parameters, thus using a total of *k.p* parameters. This is similar to the case of fitted models with a dataset effect. The global *-ln* likelihood for the separate fits is simply the sum of the *-ln* likelihoods. Then the *BIC* weights will provide a statistically sound method of choosing between the hypotheses that a single model is sufficient for all datasets or that each dataset is best fitted with a different model. We named *w*-value the BIC weight and we propose this statistic as a replacement for the contentious *p*-value (Girondot & Guillon 2018).

Bayesian approach using Metropolis-Hastings with Monte-Carlo Markov chain

The Metropolis–Hastings algorithm is a Markov chain Monte Carlo (MCMC) method for obtaining a sequence of random samples from a probability distribution (Hastings 1970; Metropolis et al. 1953). This method is now widely used as it offers a high-performance tool to fit a model. To run a Bayesian analysis with this algorithm, several parameters must be defined for each estimator in the model. The following terms are those used in the function tsd_MHmcmc() of the R package ***embryogrowth***:

1. Density: The R function for density distribution used for the prior. Generally, uniform or Gaussian distribution are used with *dunif* or *dnorm*, respectively.
2. Prior1 and Prior2: The parameters describing the prior distribution. For *dunif*, Prior1 and Prior2 are respectively the minimum and maximum of the distribution, and for *dnorm*, they are the mean and standard deviation of the distribution;
3. SDProp: The standard deviation for each new parameter;
4. Min and Max: The range of possible values defined by the minimum and maximum;
5. Init: An initial starting point for the Markov chain.

It is beyond the scope of this paper to fully explore the fine details of this algorithm, and rather we focus on how to use it.

The choice of the prior is not straightforward (Lemoine 2019) and can be critical if only a few observations are available. A uniform distribution for priors indicates that all values within a range are equally probable, whereas a Gaussian distribution can use a mean and standard deviation obtained from a previous analysis with the same or other species.

At the end of the analysis, it is essential to evaluate the distribution of posteriors. If they are the same as the distribution of priors, it generally means that the observations did not help inform the fit for this parameter. In this case, it implies that the results are dependent on the priors and not on the data and, therefore, the results should not be used, or used with caution.

During the iteration process, a Markov Chain is constructed using the actual parameter values π_t on which a new proposal random function defined by its standard deviation (*s)* is applied, $\pi_{t+1} = \mathcal{N}(\pi_t, s)$. This is the Monte-Carlo process. The standard deviation (*s)* for a new proposal is a compromise between the two constraints: if the values are too high, the new values could yield results far from the optimal solution, while if they are too low, the model can become stuck in local minima. The adaptive proposal distribution (Rosenthal 2011) as implemented in R package ***HelpersMG*** (Girondot 2019b) will ensure that the acceptance rate is close to 0.234, which is the optimal acceptance rate (Roberts & Rosenthal 2001). The burn-in value is the number of iterations necessary to stabilize the likelihood. It can be low (around 10) when the starting values are the maximum likelihood estimators. The total number of iterations required is defined after an initial run of 10,000 iterations (Raftery & Lewis 1992). The result of the MCMC analysis is a table with one set of values for the estimators at each iteration. The mean and standard deviation summary statistics can be calculated from this table using the ***coda*** R package (Plummer et al. 2011), or it is also possible to estimate quantiles. The use of quantiles has the advantage that it does not require any hypothesis on the output distribution; hence an asymmetric distribution is well accommodated. When the result of a MCMC analysis is used to estimate a function of these estimators (for example, the TRT), each individual set of parameters obtained during the MCMC search should be used to generate the posterior distribution of outputs and this distribution can be summarized using mean, standard deviation or quantiles. The standard deviation of the MCMC output is the standard error of the corresponding parameter.

We follow the ISO 80000-1:2009 standard indicating that numerical value and unit symbol are separated by a space, including the °C symbol (ISO/TC 12 Quantities and units & IEC/TC 25 Quantities and units 2009).

Results

Maximum likelihood estimates for grouped data

Logistic and *flexit* models were both fitted to the comprehensive dataset: 40 incubations with temperatures from 24 °C to 34 °C and 468 sexed embryos identified as males or females. Mixed-sex ratios were observed in embryos or hatchlings from 8 incubations (Table 1). Upon comparing both models (Fig. 2 and Table 2), the *flexit* model was selected based on AICc and Akaike weight as the latter suggested there was a 97% probability that the *flexit* model was the best. While the *logistic* model showed the typical symmetric shape, the *flexit* model showed a strong asymmetrical pattern: transition from all-male condition to the pivotal temperature is smooth, whereas the transition from the pivotal temperature to all-female production was abrupt (Fig. 2). As a consequence, the estimated pivotal and transitional range of temperatures obtained from both models were different (Table 1).

It is worthwhile noting that the standard error and therefore the coefficient of variation for the S, K_1 and K_2 parameters in the *flexit* model was large (S=-0.79 SE 0.3; K_1=-1.72 SE 1.59; K_2=200 SE 2485.61, see supplementary material). In other words, the *flexit* model did not precisely describe all parameters, even though it was the selected model.

The tests of the deviances indicated that the models fit the observations correctly: p=0.16 and p=0.56, respectively, for the *logistic* and *flexit* models on global data. The same is true for the fit at RMU or country scale except for Costa Rica data (p=0.01, Table 3). The distribution of 1000 estimates of deviance for both the *logistic* and the *flexit* models is shown in tables 2 and 3. All these tests gave a probability of more than 0.05, indicating that the models fit the observations relatively well. From these observations, we recommend against the use of $\square^2$ approximations to test the degree to which the models fit the data and, instead, use comparisons based on generated null distributions. Based on this methodology, the function tsd() of the ***phenology*** R package returns the probability that the observations fit the null model in the object p.Deviance.Null.model (Girondot 2019a).

Finally, because some parameters of the *flexit* model are nearly impossible to fit with these datasets (coefficient of variation for K_1=0.92, and for K_2=12.43), the *flexit* model will not be applied further in this paper (see supplementary 2 for a Bayesian MCMC with *flexit* model).

Comparison of the maximum likelihood estimates for RMU data

The characteristics of the fitted *logistic* model for the 3 RMUs and Mexico and Costa Rica within the East Pacific RMU are shown in Table 3. The fitted S values for East Pacific (Mexico) and Northeast India observations have substantial standard errors and confidence intervals. These two datasets have only one mixed-sex ratio, and the maximum likelihood approximation failed to provide a correct estimate of the S parameter.

The analysis shows that, within the East Pacific RMU, a single TSD pattern is sufficient to model the combined Mexico and Costa Rica data (w-value=0.87), whereas there is a 0.97 probability (*w-value*) that at a worldwide scale there are differences in TSD parameters for the different olive ridley populations (Table 4).

Bayesian estimates

Priors for the Bayesian process were chosen with a Gaussian distribution, with an average set as the fitted parameters, which were estimated from the global maximum likelihood analysis (Fig. 2, Table 2), and a standard deviation large enough to avoid imposing too high a constraint on the output. Minimum and maximum values for P were 25 and 35 °C, respectively, with S spanning from -2 to 2 and K_x from -500 to +500. The standard deviation for the new proposal was initially chosen to be 2 for P and 0.5 for S, K_1, and K_2. They were adjusted during the iterations to have acceptance rates closer to 0.234. A total of 100,000 iterations were performed.

Though both the *logistic* and *flexit* models were fitted using Bayesian MCMC, only the results for the *logistic* will be discussed here because some parameters of the *flexit* model are nearly impossible to fit with these datasets (coefficient of variation for K_2=12.43) (see supplementary 2 for a Bayesian MCMC with *flexit* model).

The distribution of priors and posteriors for the parameters derived from the East Pacific analyses is shown in Fig. 3A & B. The distinctness of the posterior and prior distributions verify that the fit was guided primarily by the observations. The posterior distributions of the P and S parameters were relatively independent (Fig. 3C). Distribution of other posteriors is shown in supplementary material 2.

TSD pattern fits using maximum likelihood and Bayesian MCMC (Figs. 4 and 5) were derived for the 3 RMUs, and separately for Mexico and Costa Rica (East Pacific RMU) together with the 0.025, 0.5 and 0.975 quantiles (95% of values are located between 0.025, and 0.975 quantiles) for pivotal temperature and transitional range of temperatures (Table 5).

The plotted posterior distributions for P vs. TRT from the 2 RMUs and Mexico and Costa Rica separately, is shown in Fig. 6. While posterior values for P suggest two separate groups (Northeast India on one side, and East Pacific and West Atlantic on the other), the spread of *TRT* values does not suggest any differences.

Discussion

After the discovery of temperature-dependent sex determination in reptiles (Charnier 1966; Pieau 1971; Pieau 1972), the description of the TSD pattern provided a basis for its understanding and for comparisons among species (Bull et al. 1982; Ewert et al. 2004; Ewert et al. 1994; Ewert et al. 2005; Ewert & Nelson 1991; Godfrey et al. 1999; Mrosovsky 1988; Yntema & Mrosovsky 1982). However, significant methodological advancements have been made since the original studies. For example, straight-line interpolations of the sex ratio values on each side of the 50 % level were proposed by N. Mrosovsky (pers. comm. in 1992) (see also Mrosovsky & Pieau 1991). Limpus et al. (1983) refined the approach using a graphical method (Litchfield & Wilcoxon 1949) using a curve from the intercept and the slope of a straight line in the log dose vs. probit effect scale. This method allowed statistical comparisons between samples but required at least two values in the 16% to 84% dose-effect range, confidence limit calculations, and re-testing between samples. Starting in 1999, new statistical methods were developed (Girondot 1999; Godfrey et al. 2003; Hulin et al. 2009) and the R package *embryogrowth* (Girondot 2019a) has included the tsd() function since version 2.0.0 in 2013.

More recently, an attempt to correlate TSD patterns with life-history parameters was successful (Carter et al. 2019; Hulin et al. 2009) but sometimes produced unexpected results. For example, when these were compared between populations in *Chrysemys picta, P* could not be explained by geography or local thermal conditions, but the *TRT* was wider at lower latitudes (Carter et al. 2019). An explanation for these difficulties could be that the models for the TSD pattern used did not correctly reflect the true TSD pattern.

The TSD pattern has also been used to estimate sex ratios from natural nests using the average nest temperature for the total incubation period, which can result in biased data because temperature determines sex only during the thermosensitive part of development. When the average of the temperature experienced during the absolute middle third of the incubation period is used, sex ratio is also potentially biased because the thermosensitive period for sex determination shifts depending on the temperature (Georges et al. 2005; Girondot et al. 2018b). Recently, improved models that take into account changes in the rate of embryonic development affected by variations in the incubation temperature have been developed (Massey et al. 2019; Monsinjon et al. 2019a; Monsinjon et al. 2019b). Promising results have been obtained, but further analyses of *in-situ* empirical data would benefit the field (Fuentes et al. 2017).

Nevertheless, the mathematical and statistical complications of currently available methods to analyze and study TSD patterns were a barrier for the full understanding and further analyses of sex ratios. The purpose of this article was to fully describe the procedures and the R code to familiarize readers with the analyses and the outputs.

The main conclusions of the example analysis are summarized here. A single model is sufficient to describe Mexican and Costa Rican rookeries within East Pacific RMU. This result is consistant with the phylogeography of this species (see figure 2 in Bowen et al. 1998). The pivotal temperature for the India rookery (Northeast Indian RMU) differs substantially from the values estimated for both Mexico and Costa Rica (East Pacific RMU), and also from the estimation for Brazil (West Atlantic RMU). However, no differences among the RMUs were observed from the estimated TRTs. The use of the Bayesian Metropolis-Hastings algorithm with Markov chains generated by a Monte-Carlo process provides a substantial improvement to the model fit as compared to the maximum likelihood fit, especially when few mixed-sex ratio results are available. The quality of the fit, as measured by a deviance test, was generally reliable, but in one case the generation of null distribution for deviance gave a result different from the χ^2 approximation. We suggest that null distributions of deviance is more reliable than χ^2 approximations to test deviance and should be chosen when possible.

We hope that this methodological paper will be useful and encourage researchers to explore new hypotheses to understand the ecological and evolutionary significance of temperature-dependent sex determination in reptiles. We also encourage more studies on TSD patterns for different populations and species, to help improve our understanding of this fascinating phenomenon.

References

Abreu-Grobois A, and Plotkin PT. 2008. *Lepidochelys olivacea*. The IUCN Red List of Threatened Species. Gland, Switzerland: IUCN SSC Marine Turtle Specialist Group.

Akaike H. 1974. A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19:716-723.

Bowen BW, Clark AM, Abreu-Grobois FA, Chaves A, Reichart HA, and Ferl RJ. 1998. Global phylogeography of the ridley sea turtles (*Lepidochelys spp.*) as inferred from mitochondrial DNA sequences. *Genetica* 101:179-189.

Bull JJ. 1983. *Evolution of sex determining mechanism*. Menlo Park, Ca: The Benjamin/Cummings Publishing Company, Inc.

Bull JJ, Vogt RC, and McCoy CJ. 1982. Sex determining temperatures in turtles: A geographic comparison. *Evolution* 36:326-332.

Burnham KP, and Anderson DR. 2002. *Model selection and multimodel inference: A practical information-theoretic approach*. New York: Springer-Verlag.

Carter AL, Bodensteiner BL, Iverson JB, Milne-Zelman CL, Mitchell TS, Refsnider JM, Warner DA, Janzen FJ, and White C. 2019. Breadth of the thermal response captures individual and geographic variation in temperature-dependent sex determination. *Functional Ecology*.

Castheloge VD, de D. dos Santos MR, de Castilhos JC, de J. Filho PR, de C. Gomes L, Clemente-Carvalho RBG, and Ferreira PD. 2018. Pivotal temperature and hatchling sex ratio of olive ridley sea turtles *Lepidochelys olivacea* from the South Atlantic coast of Brazil. *Herpetological Conservation and Biology* 13:488-496.

Charnier M. 1966. Action de la température sur la sex-ratio chez l'embryon d'*Agama agama* (*Agamidae*, Lacertilien). *Comptes Rendus des Séances de la Société de Biologie et de ses Filiales* 160:620-622.

Dimond MT. 1985. Some effects of temperature on turtle egg incubation. In: Goel SC, and Srivastava CBL, eds. *Recent Advances in Developmental Biology of Animals*. Poona, India: Indian Society of Developmental Biologists, 35-39.

Dutton PH, Whitmore CP, and Mrosovsky N. 1985. Masculinisation of leatherback turtle *Dermochelys coriacea* hatchlings from eggs incubated in styrofoam boxes. *Biological Conservation* 31:249-264.

Ewert MA, Etchberger CR, and Nelson CE. 2004. Turtle sex-determining modes and TSD patterns, and some TSD patterns correlates. In: Valenzuela N, and Bull JJ, eds. *Temperature-Dependent Sex Determination in Vertebrates*. Washington: Smithsonian Books, 21-32.

Ewert MA, Jackson DR, and Nelson CE. 1994. Patterns of temperature-dependent sex determination in turtles. *Journal of Experimental Zoology* 270:3-15.

Ewert MA, Lang JW, and Nelson CE. 2005. Geographic variation in the pattern of temperature-dependent sex determination in the American snapping turtle (*Chelydra serpentina*). *Journal of Zoology* 265:81-95.

Ewert MA, and Nelson CE. 1991. Sex determination in turtles: diverse patterns and some possible adaptative values. *Copeia* 1991:50-69.

Fuentes MMPB, Monsinjon J, Lopez M, Lara P, Santos A, dei Marcovaldi MAG, and Girondot M. 2017. Sex ratio estimates for species with temperature-dependent sex determination differ according to the proxy used. *Ecological Modelling* 365:55-67.

Georges A. 1989. Female turtles from hot nests: Is it duration of incubation or proportion of development at high temperatures that matters? *Oecologia* 81:323-328.

Georges A, Beggs K, Young JE, and Doody JS. 2005. Modelling development of reptile embryos under fluctuating temperature regimes. *Physiological and Biochemical Zoology* 78:18-30.

Georges A, Limpus CJ, and Stoutjesdijk R. 1994. Hatchling sex in the marine turtle *Caretta caretta* is determined by proportion of development at a temperature, not daily duration of exposure. *Journal of Experimental Zoology* 270:432-444.

Girondot M. 1999. Statistical description of temperature-dependent sex determination using maximum likelihood. *Evolutionary Ecology Research* 1:479-486.

Girondot M. 2019a. embryogrowth: Tools to analyze the thermal reaction norm of embryo growth. 7.7 ed: The Comprehensive R Archive Network.

Girondot M. 2019b. HelpersMG: Tools for Environmental Analyses, Ecotoxicology and Various R Functions. 3.9 ed: The Comprehensive R Archive Network.

Girondot M, Godfrey MH, Guillon J-M, and Sifuentes-Romero I. 2018a. Understanding and integrating resolution, accuracy and sampling rates of temperature data loggers used in biological and ecological studies. *Engineering Technology Open Access Journal* 2:1-7.

Girondot M, and Guillon J-M. 2018. The w-value: An alternative to t- and X^2 tests. *Journal of Biostatistics & Biometrics* 1:1-4.

Girondot M, and Kaska Y. 2014. A model to predict the thermal reaction norm for the embryo growth rate from field data. *Journal of Thermal Biology* 45:96-102.

Girondot M, Monsinjon J, and Guillon J-M. 2018b. Delimitation of the embryonic thermosensitive period for sex determination using an embryo growth model reveals a potential bias for sex ratio prediction in turtles. *Journal of Thermal Biology* 73:32-40.

Godfrey MH, D'Amato AF, Marcovaldi MA, and Mrosovsky N. 1999. Pivotal temperature and predicted sex ratios for hatchling hawksbill turtles from Brazil. *Canadian Journal of Zoology-Revue Canadienne de Zoologie* 77:1465-1473.

Godfrey MH, Delmas V, and Girondot M. 2003. Assessment of patterns of temperature-dependent sex determination using maximum likelihood model selection. *Ecoscience* 10:265-272.

Godfrey MH, and Mrosovsky N. 2006. Pivotal temperature for green sea turtles, *Chelonia mydas*, nesting in suriname. *Herpetological Journal* 16:55-61.

Hastings WK. 1970. Monte Carlo sampling methods using Markov chains and their applications. *Biometrika* 57:97-109.

Hill AV. 1910. The possible effects of the aggregation of the molecules of hæmoglobin on its dissociation curves. *J Physiol* 40:IV-VII.

Hulin V, Delmas V, Girondot M, Godfrey MH, and Guillon J-M. 2009. Temperature-dependent sex determination and global change: Are some species at greater risk? *Oecologia* 160:493-506.

Hulin V, Girondot M, Godfrey MH, and Guillon J-M. 2008. Mixed and uniform brood sex ratio strategy in turtles: The facts, the theory and their consequences. In: Wyneken J, Bels V, and Godfrey MH, eds. *Biology of turtles*. Chigaco, IL: University Press of Chicago, 279-300.

Hurvich CM, and Tsai C-L. 1995. Model selection for extended quasi-likelihood models in small samples. *Biometrics* 51:1077-1084.

ISO/TC 12 Quantities and units, and IEC/TC 25 Quantities and units. 2009. ISO 80000-1:2009. Quantities and units. ISO.

Korpelainen H. 1990. Sex ratios and the conditions required for environmental sex determination in animals. *Biological Reviews* 65:147-184.

Lang JW, and Andrews HV. 1994. Temperature-dependent sex determination in crocodilians. *J Exp Zool* 270:28-44.

Lemoine NP. 2019. Moving beyond noninformative priors: why and how to choose weakly informative priors in Bayesian analyses. *Oikos* 128:912-928.

Lewontin R. 2000. *The Triple Helix: Gene, Organism, and Environment*. Cambridge, MA: Harvard University Press.

Limpus CJ, Reed P, and Miller JD. 1983. Islands and turtles: the influence of choice of nesting beach on sex ratio. In: Baker JJ, Carter RM, Saurmarco PW, and Stark KP, editors. Proceedings of the Inaugural Great Barrier Reef Conference: James Cook University Press of North Queensland. p 397-402.

Litchfield JT, and Wilcoxon F. 1949. A simplified method of evaluating dose-effect experiments. *Journal of Pharmacology and Experimental Therapeutics* 96:99-113.

López Correa JY. 2010. Diferenciación gonádica en crias de *Lepidochelys olivacea* (Eschscholtz, 1829) (Testudinata: Cheloniidae) PhD dissertation PhD dissertation. Instituto Politécnico Nacional, Centro Interdisciplinaria de Ciencias Marinas.

Massey MD, Holt SM, Brooks RJ, and Rollinson N. 2019. Measurement and modelling of primary sex ratios for species with temperature-dependent sex determination. *J Exp Biol* 222:1-9.

McCoy CJ, Vogt RC, and Censky EJ. 1983. Temperature-controlled sex determination in the sea turtle *Lepidochelys olivacea*. *J Herpetol* 17:404-406.

Merchant-Larios H, Ruiz-Ramirez S, Moreno-Mendoza N, and Marmolejo-Valencia A. 1997. Correlation among thermosensitive period, estradiol response, and gonad differentiation in the sea turtle *Lepidochelys olivacea*. *General and Comparative Endocrinology* 107:373-385.

Merchant-Larios H, Villalpando-Fierro I, and Centeno-Urruiza B. 1989. Gonadal morphogenesis under controlled temperature in the sea turtle *Lepidochelys olivacea*. *Herpetol Monographs* 3:43-61.

Metropolis N, Rosenbluth AW, Rosenbluth MN, Teller AH, and Teller E. 1953. Equations of state calculations by fast computing machines. *Journal of Chemical Physics* 21:1087-1092.

Miller JD. 1985. Embryology of marine turtles. In: Gans C, Billet F, and Maderson PF, eds. *Biology of the Reptilia*. New-York, US: Wiley-Liss, 270-328.

Mohanty-Hejmadi P, Behra M, and Dimond MT. 1985. Temperature dependent sex differentiation in the olive ridley *Lepidochelys olivacea* and its implications for conservation. Symposium on Endangered Marine Animals and Marine Parks. Cochin.: Marine Biological Association of India. p 1-5.

Monsinjon J, Lopez M, Lara P, Santos A, dei Marcovaldi MAG, Girondot M, and Fuentes MMPB. 2019a. Effects of temperature and demography on the phenology of loggerhead sea turtles in Brazil. *Marine Ecology Progress Series* 623:209-219.

Monsinjon JR, Wyneken J, Rusenko K, López-Mendilaharsu M, Lara P, Santos A, dei Marcovaldi MAG, Fuentes MMPB, Kaska Y, Tucek J, Nel R, Williams KL, LeBlanc A-M, Rostal D, Guillon J-M, and Girondot M. 2019b. The climatic debt of loggerhead sea turtle populations in a warming world. *Ecological Indicators* 107:1-13.

Morales Mérida A, Ruiz A, Navarro E, Sifuentes-Romero I, Abreu-Grobois FA, and Girondot M. 2015. Contrasting incubation data for *Lepidochelys olivacea* highlight the need for more experimentation and detailed reporting. *Marine Turtles Newsletter* 145:15-19.

Mrosovsky N. 1988. Pivotal temperatures for loggerhead turtles (*Caretta caretta*) from northern and southern nesting beaches. *Canadian Journal of Zoology-Revue Canadienne de Zoologie* 66:661-669.

Mrosovsky N, and Pieau C. 1991. Transitional range of temperature, pivotal temperatures and thermosensitive stages for sex determination in reptiles. *Amphibia-Reptilia* 12:169-179.

Navarro Sánchez EJ. 2015. Efecto de la temperatura de incubación y la diferenciación sexual sobre la morfología de crías de tortuga marina *Lepidochelys olivacea* Maestría Tesis de Maestría. Universidad Nacional Autónoma de México.

Pieau C. 1971. Sur la proportion sexuelle chez les embryons de deux Chéloniens (*Testudo graeca* L. et *Emys orbicularis* L.) issus d'oeufs incubés artificiellement. *Comptes Rendus de l'Académie des Sciences Paris Série D* 272:3071-3074.

Pieau C. 1972. Effets de la température sur le développement des glandes génitales chez les embryons de deux Chéloniens, *Emys orbicularis* L. et *Testudo graeca* L. *Comptes Rendus de l'Académie des Sciences Paris Série D* 274:719-722.

Pieau C, and Dorizzi M. 2004. Oestrogens and temperature-dependent sex determination in reptiles: all is in the gonads. *Journal of Endocrinology* 181:367-377.

Pieau C, Dorizzi M, Richard-Mercier N, and Desvages G. 1998. Sexual differentiation of gonads as a function of temperature in the turtle *Emys orbicularis*: endocrine function, intersexuality and growth. *Journal of Experimental Zoology* 281:400-408.

Plummer M, Best N, Cowles K, and Vines K. 2011. coda: Output analysis and diagnostics for Markov Chain Monte Carlo simulations. 0.14-6 ed: http://cran.r-project.org/web/packages/coda/index.html.

Raftery AE, and Lewis SM. 1992. One long run with diagnostics: Implementation strategies for Markov chain Monte Carlo. *Statistical Science* 7:493-497.

Roberts GO, and Rosenthal JS. 2001. Optimal scaling for various Metropolis-Hastings algorithms. *Statistical Science* 16:351-367.

Rosenthal JS. 2011. Optimal proposal distributions and adaptive MCMC. In: Brooks S, Gelman A, Jones G, and Meng X-L, eds. *MCMC Handbook*: Chapman and Hall/CRC, 93-112.

Sandoval S, Gómez-Muñoz VM, and Porta-Gándara MÁ. 2017. Expansion of the transitional range of temperature for sea turtle *Lepidochelys olivacea* from sex ratio data at controlled incubation temperatures. *Herpetology Notes* 10:63-65.

Tanabe K, and Sagae M. 1992. An exact cholesky decomposition and the generalized inverse of the variance–covariance matrix of the multinomial distribution, with applications. *Journal of the Royal Statistical Society: Series B (Methodological)* 54:211-219.

Tezak BM, Sifuentes-Romero I, and Wyneken J. 2018. A new approach for measuring temperature inside turtle eggs. *Journal of Experimental Biology*.

Valenzuela N. 2004. Evolution and maintenance of temperature-dependent sex determination. In: Valenzuela N, and Lance V, eds. *Temperature-Dependent Sex Determination in Vertebrates*. Whasington: Smithsonian Books, 131-147.

Verhulst PF. 1838. Notice sur la loi que la population suit dans son accroissement. *Correspondance Mathématique et Physique* 10:113-121.

Verhulst PF. 1846. Deuxième mémoire sur la loi d'accroissement de la population. *Mémoires de l'Académie Royale des Sciences, des Lettres et des Beaux-Arts de Belgique* 20:1-32.

Wallace BP, DiMatteo AD, Hurley BJ, Finkbeiner EM, Bolten AB, Chaloupka MY, Hutchinson BJ, Abreu-Grobois FA, Amorocho D, Bjorndal KA, Bourjea J, Bowen BW, Dueñas RB, Casale P, Choudhury BC, Costa A, Dutton PH, Fallabrino A, Girard A, Girondot M, Godfrey MH, Hamann M, López-Mendilaharsu M, Marcovaldi MA, Mortimer JA, Musick JA, Nel R, Seminoff JA, Troëng S, Witherington B, and Mast RB. 2010. Regional management units for marine turtles: a novel framework for prioritizing conservation and research across multiple scales. *PLoS One* 5:e15465.

Wibbels T, Rostal DC, and Byles R. 1998. High pivotal temperature in the sex determination of the olive ridley sea turtle, *Lepidochelys olivacea*, from Playa Nancite, Costa Rica. *Copeia* 1998:1086-1088.

Wilson EB. 1927. Probable inference, the law of succession, and statistical inference. *Journal of the American Statistical Association* 22:209-212.

Yntema CL, and Mrosovsky N. 1982. Critical periods and pivotal temperatures for sexual differentiation in loggerhead sea turtles. *Canadian Journal of Zoology-Revue Canadienne de Zoologie* 60:1012-1016.

Table 1: Constant temperature incubation data used in this study on TSD patterns in *Lepidochelys olivacea*. (*) maximum amplitude of temperatures within the middle-third of the incubation is shown. References: 1 - Castheloge et al. (2018), 2 - Dimond (1985), 3 - Mohanty-Hejmadi et al. (1985), 4 - McCoy et al. (1983), 5 - Wibbels et al. (1998), 6 - Merchant-Larios et al. (1997), 7 - Merchant-Larios et al. (1989), 8 - López Correa (2010), 9 – Navarro Sánchez (2015).

Area	Country	RMU	Incubation temperature °C	Temperature amplitude °C	Males	Females	Intersexes	Reference
Pirambu Beach	Brazil	West Atlantic	26.3	0.3	2	0	0	1
Pirambu Beach	Brazil	West Atlantic	28.5	0.2	3	0	0	
Pirambu Beach	Brazil	West Atlantic	29.2	0.2	8	1	0	
Pirambu Beach	Brazil	West Atlantic	29.9	0.5	7	0	0	
Pirambu Beach	Brazil	West Atlantic	30.4	0.2	5	2	0	
Pirambu Beach	Brazil	West Atlantic	31.2	1.9	0	1	0	
Pirambu Beach	Brazil	West Atlantic	31.8	1.2	0	3	0	
Pirambu Beach	Brazil	West Atlantic	32.1	0.4	0	6	0	
Pirambu Beach	Brazil	West Atlantic	32.2	0.4	0	2	0	
Pirambu Beach	Brazil	West Atlantic	32.4	1.9	0	1	0	
Pirambu Beach	Brazil	West Atlantic	32.9	0.6	0	5	0	
Pirambu Beach	Brazil	West Atlantic	33.1	0.3	0	4	0	
Orissa	India	Northeast Indian	26.5		3	0	0	2, 3
Orissa	India	Northeast Indian	28		8	0	0	

Orissa	India	Northeast Indian	29.5		2	3	0	
Orissa	India	Northeast Indian	30		0	4	0	
Orissa	India	Northeast Indian	31		0	2	0	
Orissa	India	Northeast Indian	31.5		0	9	0	
Nancite	Costa Rica	East Pacific	25		23	0	0	4
Nancite	Costa Rica	East Pacific	28		30	1	3	
Nancite	Costa Rica	East Pacific	30		12	8	5	
Nancite	Costa Rica	East Pacific	32		0	23	0	
Nancite	Costa Rica	East Pacific	27	0.5	15	0	0	5
Nancite	Costa Rica	East Pacific	29.4	0.5	23	1	0	
Nancite	Costa Rica	East Pacific	30.4	0.5	16	3	0	
Nancite	Costa Rica	East Pacific	32	0.5	0	19	0	
La Escobilla	Mexico	East Pacific	27		25	0	0	6
La Escobilla	Mexico	East Pacific	32		0	26	0	
La Escobilla	Mexico	East Pacific	27.5		15	0	0	7
La Escobilla	Mexico	East Pacific	32		0	9	0	
Playa La Destiladeras	Mexico	East Pacific	27.61	0.79 *	3	0	4	8
Playa La Destiladeras	Mexico	East Pacific	32.24	0.81 *	0	5	6	
Playa La Destiladeras	Mexico	East Pacific	28.62	0.86 *	2	0	0	

Playa La Destiladeras	Mexico	East Pacific	32.29	1.05 *	0	1	1	
El Verde Camacho	Mexico	East Pacific	24	0.1	16	0	0	9
El Verde Camacho	Mexico	East Pacific	26	0.1	24	0	0	
El Verde Camacho	Mexico	East Pacific	28	0.1	20	0	0	
El Verde Camacho	Mexico	East Pacific	30	0.1	15	4	0	
El Verde Camacho	Mexico	East Pacific	32	0.1	0	20	0	
El Verde Camacho	Mexico	East Pacific	34	0.1	0	5	0	

Table 2: Summary of *logistic* and *flexit* model fits for *Lepidochelys olivacea* incubation data (East Pacific, West Atlantic, and Northeast Indian). “p-random” column is to the probability that the observed deviance was obtained using a random sampling with the same characteristics (same number of temperatures and eggs per temperature and sex ratio probability obtained from the fitted model). Line with bold text indicates the best model.

Model	P (mean SE)	S (mean SE)	K_1 (mean SE)	K_2 (mean SE)	-LnL	AICc	ΔAICc	Akaike Weight	Deviance	df	p-value	p-random
Logistic	30.39 SE 0.09	-0.41 SE 0.05			32.6	69.52	6.92	0.03	45.68	38	0.183117	0.08
Flexit	**30.57 SE 0.11**	**-0.79 SE 0.3**	**-1.72 SE 1.59**	**200 SE 2477.58**	**26.73**	**62.6**	**0**	**0.97**	**33.95**	**36**	**0.566649**	**0.22**

Table 3: Characteristics of logistic models fitted for *Lepidochelys olivacea* incubation data (East Pacific, West Atlantic, and Northeast Indian). "p-random" column is to the probability that the observed deviance was obtained using a random sampling with the same characteristics (same number of temperatures and eggs per temperature and sex ratio probability obtained from the fitted model). Cells with large coefficient of variation are in bold and could produce a sign change for the parameter during resampling.

RMU	Country	P (mean SE)	S (mean SE)	-Ln L	Deviance	df	p-value	p-random
Northeast India	India	29.49 SE 0.33	**-0.03 SE 0.81**	1.06	0	4	1	0.4
West Atlantic	Brazil	30.63 SE 0.23	-0.36 SE 0.12	4.46	4.76	10	0.90	0.56
East Pacific	Costa Rica + Mexico	30.46 SE 0.1	-0.37 SE 0.05	16.32	19.43	20	0.49	0.28
"	Costa Rica	30.5 SE 0.13	-0.42 SE 0.07	13.5	16.83	6	0.01	0.26
"	Mexico	30.16 SE 1.9	**-0.12 SE 1.43**	1.52	0	12	1	0.41

Table 4: Comparison using BIC and BIC weight (or *w-value*) of the homogeneity of TSD pattern (A) within the East Pacific RMU and (B) at a global scale. Selected models are indicated in bold font.

A : Comparison within East Pacific RMU

Series	BIC	ΔBIC	BIC weight
All East Pacific grouped	**38.06**	**0.00**	**0.87**
Mexico and Costa Rica separated	41.82	3.76	0.13

B: Worldwide comparison

Series	BIC	ΔBIC	BIC weight
World	71.99	7.32	0.03
East Pacific, Northeast Indian, and West Atlantic separated	**64.67**	**0.00**	**0.97**

Table 5: Quantiles (0.025, 0.5, and 0.975) for pivotal temperature and transitional range of temperatures (5%) using maximum likelihood (MLE, upper line) and Bayesian MCMC (Bay. MCMC, lower line) with *logistic* and *flexit* model for TSD pattern.

		Pivotal temperature in °C			Transitional range of temperatures in °C		
Quantiles		0.025	0.5	0.975	0.025	0.5	0.975
RMU	*Logistic* model						
East Pacific	MLE	30.27	30.46	30.66	1.66	2.19	2.77
	Bay. MCMC	30.26	30.46	30.66	1.72	2.24	2.89
Northeast Indian	MLE	28.74	29.27	29.50	0.17	3.28	10.89
	Bay. MCMC	28.72	29.35	29.76	0.34	1.72	4.76
West Atlantic	MLE	30.18	30.63	31.06	0.79	2.14	3.51
	Bay. MCMC	30.23	30.65	31.15	1.29	2.44	4.53
RMU	*Flexit* model						
East Pacific	MLE	30.58	30.92	31.23	1.96	2.81	4.08
	Bay. MCMC	30.44	30.76	31.29	1.48	2.33	3.40
Northeast Indian	MLE	29.66	34.31	45.71	na	na	na
	Bay. MCMC	29.00	29.43	29.74	0.37	0.87	4.65
West Atlantic	MLE	30.12	30.36	30.56	na	na	na
	Bay. MCMC	30.34	30.82	31.52	1.02	2.37	28.13

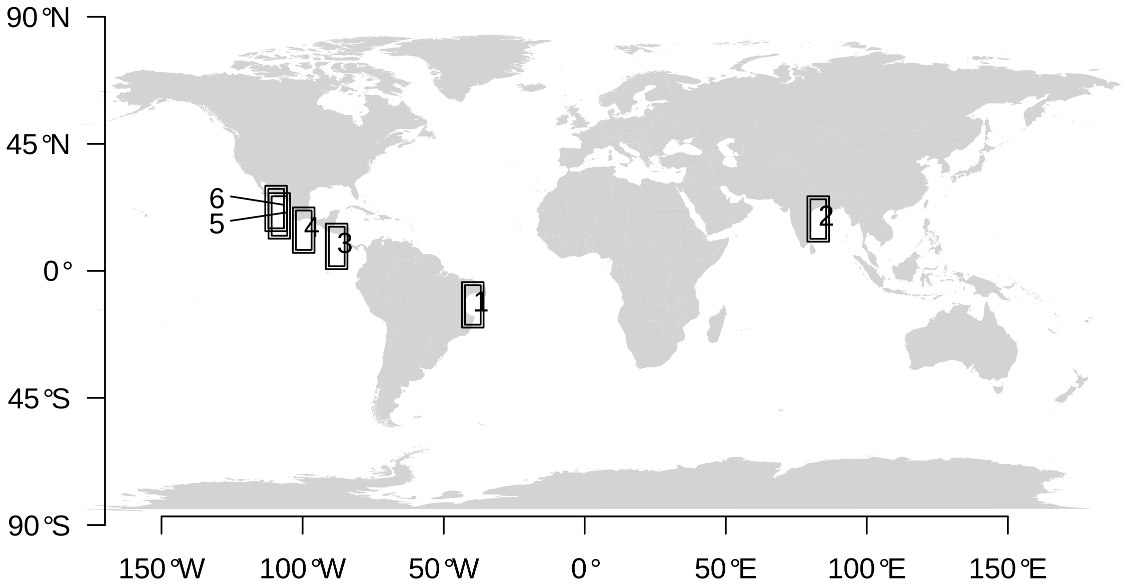


Fig. 1: Map showing locations of data collection. (1) Pirambu Beach, Sergipe State, Northeastern Brazil, (2) Odisha (formerly spelled as Orissa), India, (3) Nancite, Costa Rica, (4) La Escobilla, Mexico, (5) Playa La Destiladeras, Mexico, (6) El Verde Camacho, Sinaloa, Mexico.

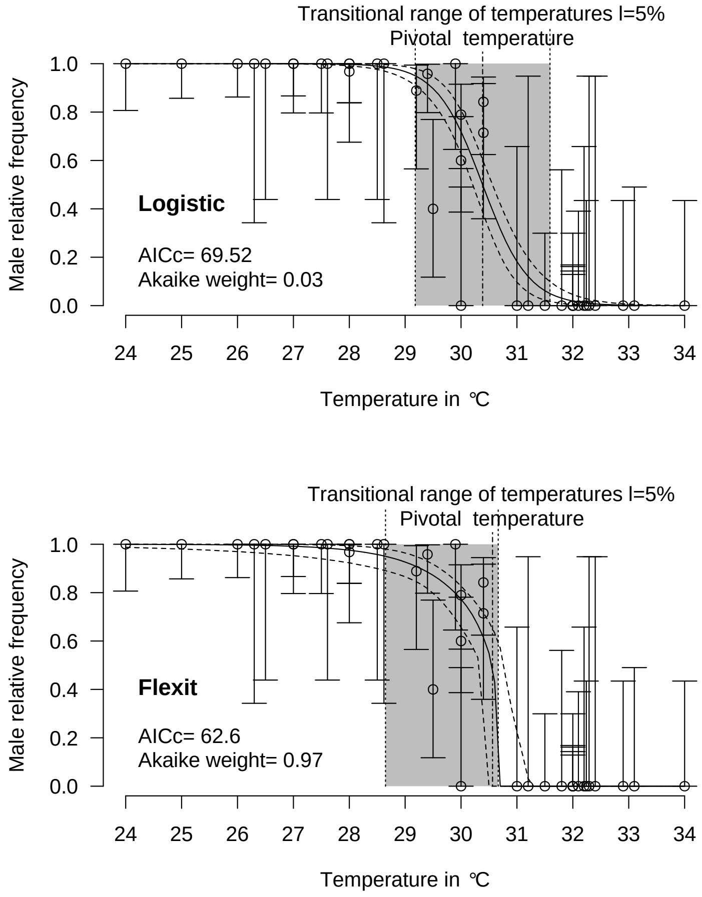


Fig. 2: *Logistic* and *flexit* models fitted with all olive ridley incubation data. Confidence intervals were estimated using 10,000 random numbers obtained from the Hessian matrix. The standard errors for the S, K_1, and K_2 parameters in the *flexit* model were high and during resampling some trials were removed as their signs had reversed (see text). As a consequence, the estimated confidence interval is biased towards a lower value. The dark grey zone is the TRT 5% and the light grey zone is the 95% confidence of the TRT. The points correspond to observations and the bars are their 95% confidence intervals for the sex ratios according to the Wilson method (1927). The plain curve shows the maximum likelihood model and its 95% confidence interval is shown as dashed lines. The pivotal temperature is indicated by the vertical dash-dotted line.

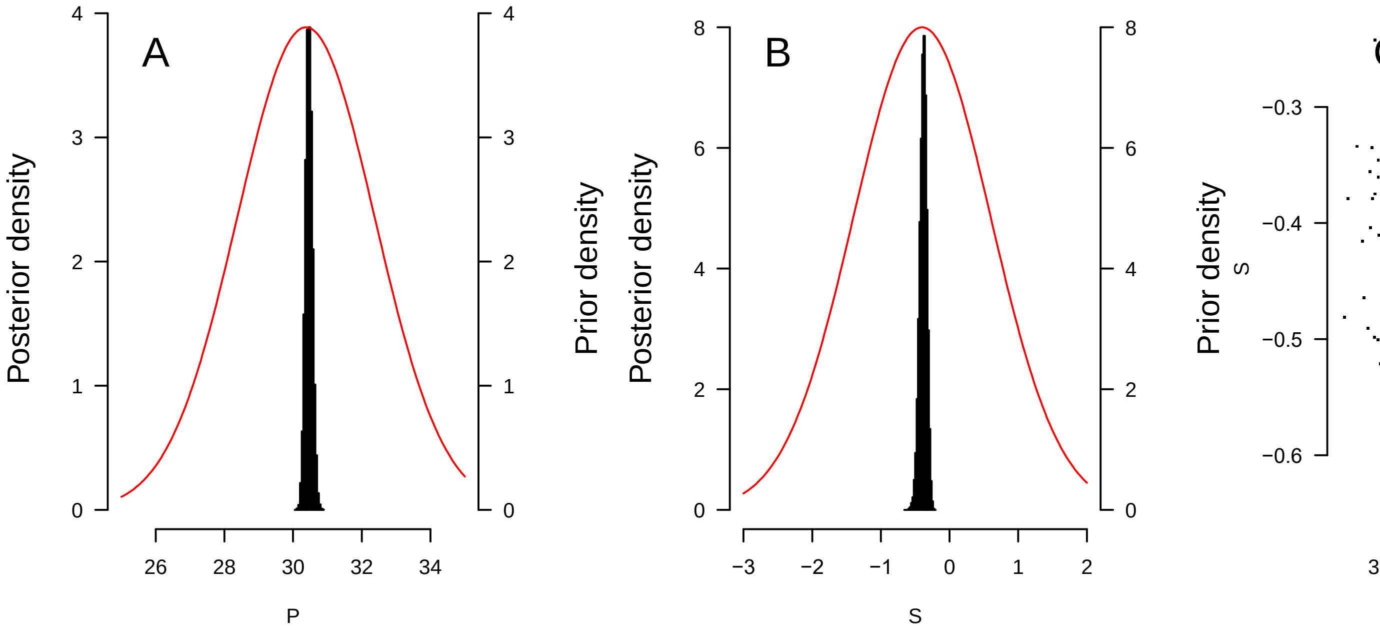


Fig. 3: A, B: Distribution of priors (plain line) and posteriors (histograms) for *P* (A) and *S* (B) for East Pacific RMU. The covariation of posteriors for *P* and *S* is shown in C.

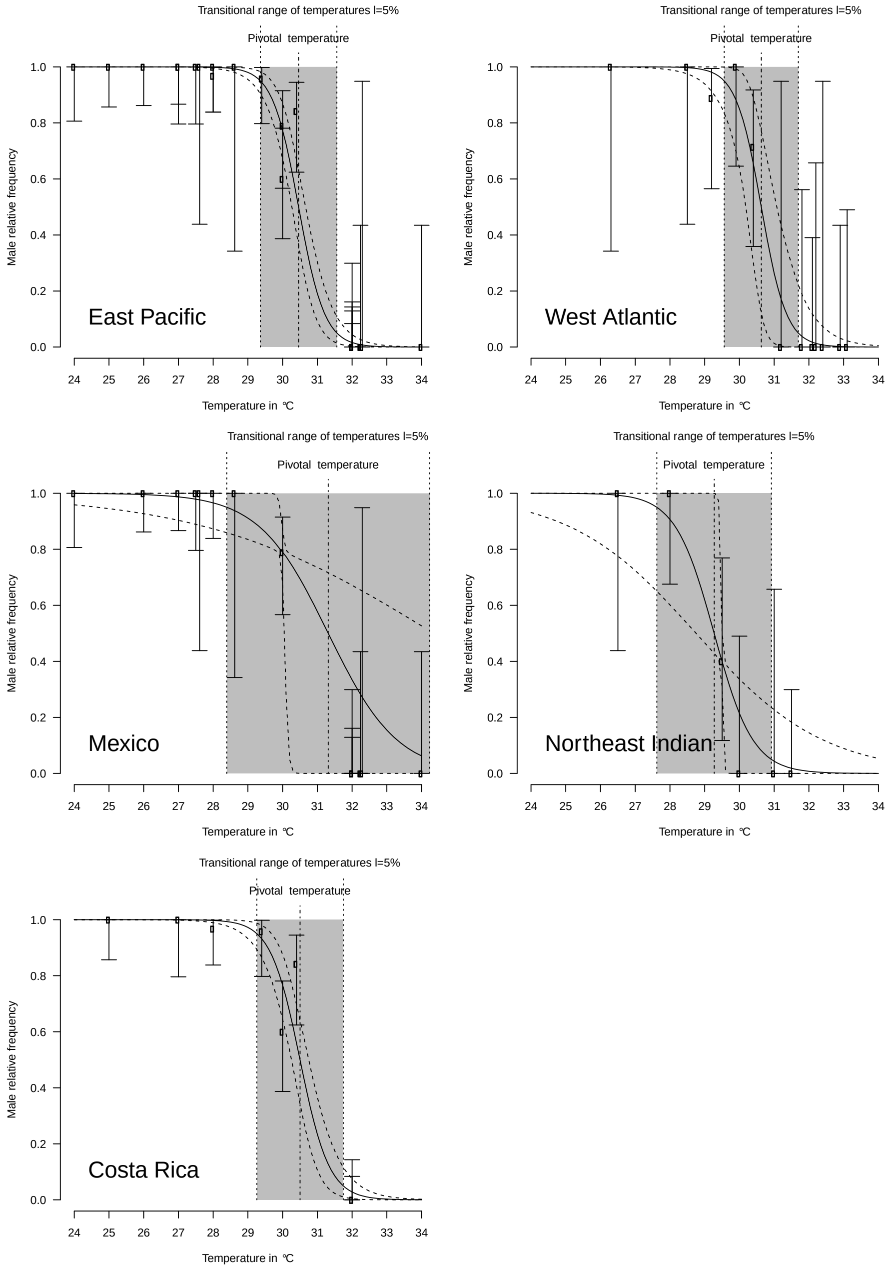


Fig. 4: TSD patterns as modeled by a *logistic* function and fitted using maximum likelihood. The dark grey zone is the TRT 5% and the light grey zone is the 95% confidence of the TRT. The points correspond to observations and the bars are their 95% confidence intervals. The plain curve shows the maximum likelihood model and its 95% confidence interval is shown as dashed lines. The pivotal temperature is indicated by the vertical dash-dotted line. Note that Mexico, East Pacific and Northeast Indian datasets each have only one temperature with mixed sex ratio.

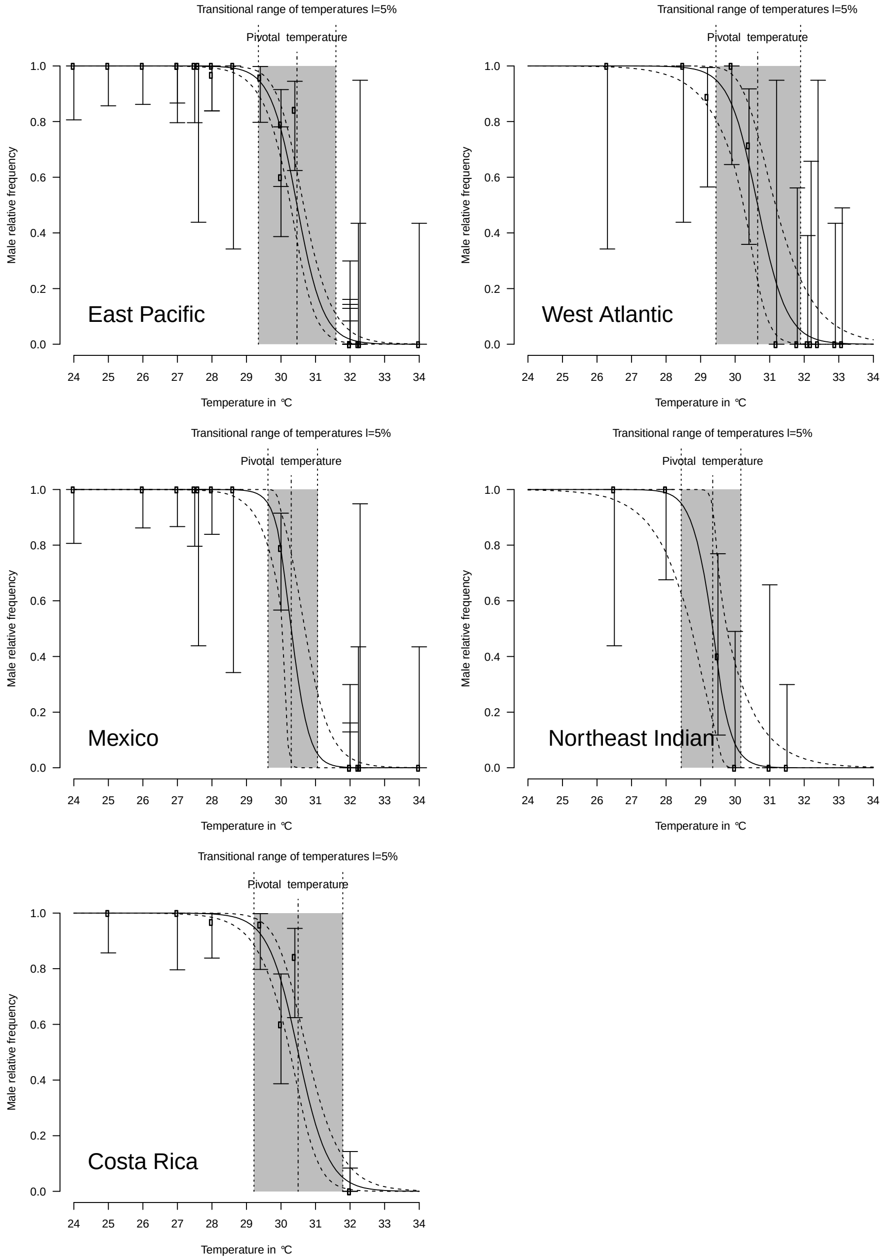


Fig. 5: TSD patterns modeled as a *logistic* function fitted using Bayesian MCMC. The dark grey zone is the TRT 5% and the light grey zone is the 95% confidence of the TRT. The points correspond to observations and the bars are their 95% confidence intervals. The plain curve shows the maximum likelihood model and its 95% confidence interval is shown as dashed lines. The pivotal temperature is indicated by the vertical dash-dotted line.

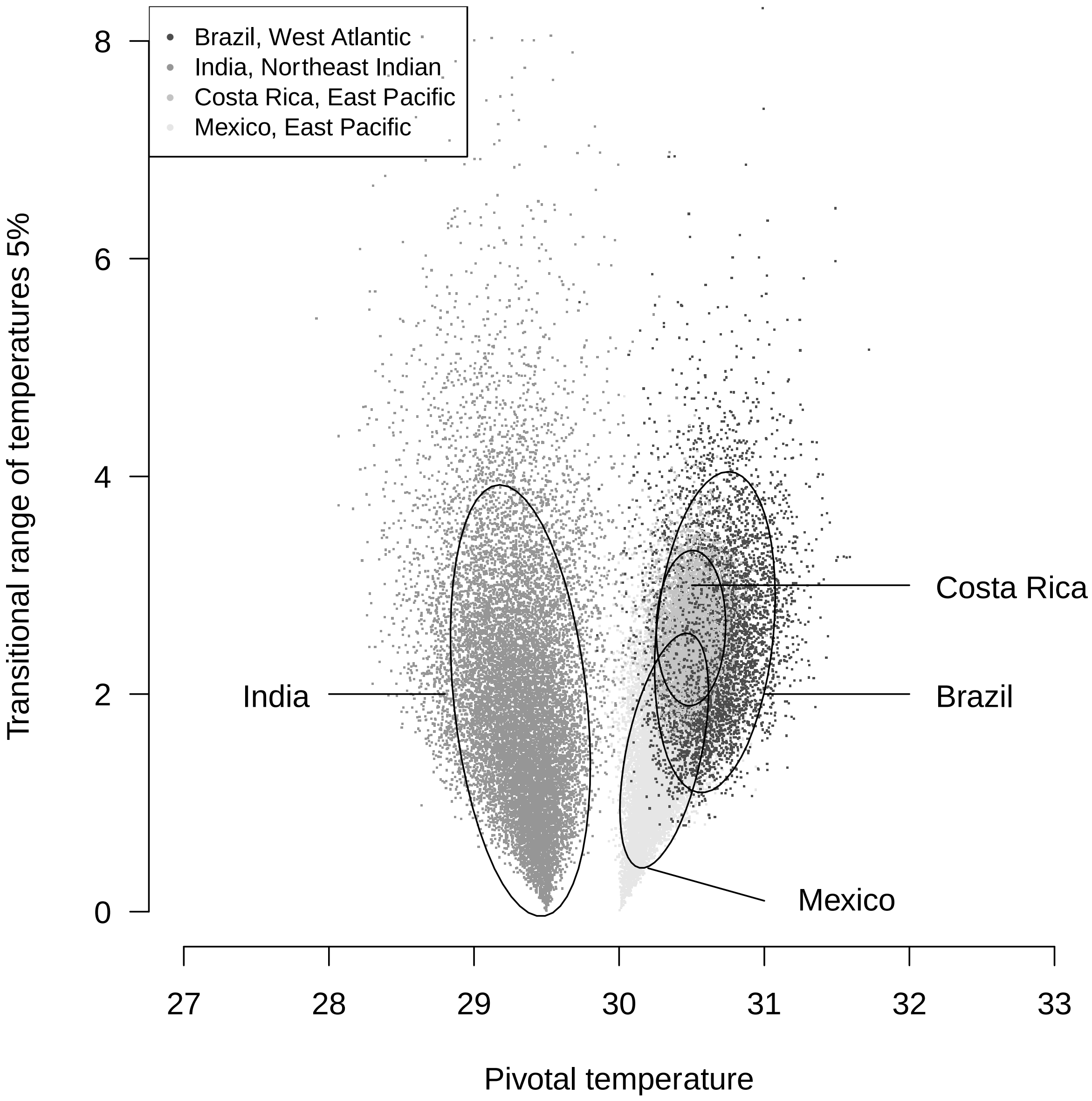


Fig. 6: Distribution of 100,000 posteriors for pivotal temperatures and transitional range of temperatures for the *logistic* model fitted using Bayesian MCMC. A 20,000 random subsample of posteriors is shown. Ellipses including 75% of the points are drawn.

CHAPTER 3. Management of *Lepidochelys olivacea* nests on the eastern coast of the Pacific of Guatemala

ARTICLE 6. Recommendations for the management of nests of the marine turtle *Lepidochelys olivacea* on the Pacific coast of Guatemala

Introduction

Marine turtles are long-lived species that have inhabited the planet for millions of years (Lawson & Rollinson, 2019). They represent an essential component of biological diversity and play important roles in the food chain, fundamental for the health and structure of critical marine-coastal ecosystems (Lovich et al., 2018). In addition, they are deeply rooted in the social and economic aspects of various cultures (Eckert & Abreu Grobois, 2001).

Marine turtles are widely distributed in the oceans of the planet. Despite global efforts to conserve these species, some populations are considered to be among the most endangered marine fauna (Darmawan et al., 2018). In addition, their life history means they are dependent on sandy beaches that are conducive to successful egg incubation, which requires further conservation measures to protect and conserve their nests in various places around the world (Hudgins et al., 2017). Thus, a deep understanding of the biology of reproduction and egg incubation is essential for the recovery and management of marine turtles (Richardson, 2000).

Three species have been reported nesting on beaches in Pacific Guatemala: *Lepidochelys olivacea*, *Dermochelys coriacea*, and *Eretmochelys imbricata* (Montes Osorio, 2004). Of the three, only *L. olivacea* commonly nests along the coast, according to the head of the Hydrobiological Resources Section of the National Council of Areas Protected (CONAP), the entity responsible for protecting biological diversity in Guatemala, according to Decree 4-89, Law on Protected Areas (López-Roulet, February 13th, 2018).

The reported leading causes of the historical decline of *L. olivacea* populations include the human exploitation of their eggs, meat, and shell for subsistence and trade, bycatch in longline and shrimp fisheries, pollution, and destruction of their habitats (CONAP, 2009; Panamá, 2017a). In Guatemala, marine turtles have been protected by law since the 1970s, including conservation programs that rely heavily on artificial enclosures for incubating eggs, called hatcheries, located along the country's coasts (CONAP, 2015). Freshly laid eggs are relocated from the nesting beaches to these hatcheries, and at the end of incubation, when hatchlings emerge, they are released to the sea (CONAP, 2009).

Hatcheries have been used throughout the world as a conservation measure for sea turtle populations. However, the impact of this practice on the recruitment and quality of hatchlings produced has rarely been evaluated, apart from impacts in sex ratio (Maulany et al., 2012b; Morreale et al., 1982); (Godfrey et al., 1996); (Mrosovsky, 2008). All hatcheries are supported by private entities, non-governmental or academic institutions, and governed by CONAP standards (CONAP, 2016). Despite its obvious importance, Guatemala’s hatcheries lack adequate technical management, and only a few collect data and handle eggs in a standardized way (CONAP, 2015). In this system, the commercialization of *L. olivacea* eggs is legal, and egg collectors are allowed to sell them as long as they hand over a "conservation quota" of 20% of each nest to an officially registered hatchery. The remaining 80% of the eggs of each nest are available for either local consumption, commercial sale in the marketplaces of big cities, or can be sold to the hatcheries for incuabation. Some hatcheries use volunteers to conduct daily patrols to find nests, the eggs of which are all collected for incubation in the hatcheries.

In general terms, the success of the conservation of marine turtles in Guatemala depends mainly on the proper management and functionality of these hatcheries. Considering that apparently all eggs are collected, either for commercialization or artificial incubation in hatcheries, there are no nests being left to incubate in natural conditions. This can be problematic in the long term because it limits the exposure of the nest to natural and selective pressures, which would promote natural selection to act (Maurer et al., 2021).

Nevertheless, it is generally accepted that an understanding of the nesting habitats used by marine turtles is essential for the recovery and management of their populations in a particular region or country, as well as for the design of the conservation program based on information provided by scientific research (Eckert et al., 2000). Generally, one should avoid generalizing data from a different country or region to make conservation proposals or scientific analyses of the species due to site-specific differences in behavior, physiology, and habitats (Morales-Mérida et al., 2015).

In various locations, the management of marine turtle hatcheries has been evaluated, taking into account various aspects such as sex ratio, hatching success, emergence success, among others, and placing in context the impact hatcheries can have on conservation at various scales (Phillott et al., 2018);(Mutalib & Fadzly, 2015);(Sari & Yakup, 2017);(Hudgins et al., 2017). Considering these assessments, the following recommendations are proposed, based on the information obtained as a result of this doctoral thesis, including tangible data on the production of hatchlings of *L. olivacea* under relocated conditions and the factors that likely influence the survival of hatchlings.

Management of *Lepidochelys olivacea* nests in Guatemala: what to expect and how to proceed with the information we currently have

As has been widely discussed, in Guatemala, the conservation of marine turtles has relied on hatcheries as an exclusive conservation tool since 1971. Since this decade, the permanence and promotion of the establishment of these sites by the authorities have been increasing (CONAP, 2015). Given this situation and the recent data on hatchlings produced in hatcheries conditions, the following recommendations are proposed as a possible solution. We propose a hybrid system involving the long-term goal of creating conditions such that some or most sea turtle nests laid on beaches in Pacific Guatemala can incubate naturally (*in situ*).

A recognized goal of *in situ* conservation is the protection of larger populations through the conservation of organisms and their habitat so that the species can continue their usual life cycles and selective processes without human manipulation (Fotedar, 2018);(Patrício et al., 2019). The *in situ* strategy allows natural selection and local adaptation to act, especially in response to diverse climate change episodes that, as it has been stated, can vary among locations, whereas some populations might be more affected by climatic events than others (Rivas et al., 2019);(Maurer et al., 2021).

While international recommendations for the conservation of marine turtles promote approaches that allow turtles and their eggs to remain in their natural environment (*in situ*) (Chacón et al., 2007);(Eckert et al., 2000), in Guatemala, there has been nearly 50 years of manipulation of egg incubation through the use of hatcheries. Therefore, an alternative approach is needed through which a new stage in the conservation of marine turtles in the country can begin. This approach must consider the biological needs of marine turtles and the human needs of communities that depend on the income from the sale of the eggs in the nests of *L. olivacea* found along the Pacific coast of Guatemala.

As established in the 2030 Sustainable Development Agenda (ONU, 2015);(Palmer & Flanagan, 2016), each country faces specific challenges in its search for sustainable development. As a first step, it is recommended the establishment of community workshops to explore alternative income sources to replace the sale of olive ridley eggs. These workshops could be accompanied by an economic valuation study of the eggs throughout the nesting season. In Guatemala's case, it would be expected that plans represent a new synthesis of economic development and environmental protection, and the empowering of vulnerable people. However, it remains a challenge to be fulfilled.

In this context, the need to achieve economic growth in coastal communities should include ensuring economic growth that also allows the protection of marine turtles and the environment in the long term (González Guevara, 2000). Given that the hatchlings' release has become an economic income to the current "hatchery industry" (with a few exceptions), it is controversial to state the possibility of transforming hatcheries into complete working community projects. Nevertheless, there must be a clear separation between conservation, sustainable development, and the commercialization of releasing hatchlings from hatcheries and the sale of eggs. It is also important to remember that any conservation action must invariably prioritize natural processes when establishing or updating conservation strategies and programs (Chacón et al., 2007).

In the second instance, ensuring adequate education opportunities and more straightforward communication with community members is essential when executing any participation plan for the management and conservation of marine turtles, especially when concrete negotiations with the various stakeholders must be had (Chacón, 2000). For example, conservation workers in Honduras have recognized that it is impossible to help the turtles without helping the local communities that depend on these species (Nuila Coto, 2010). In this case, they argued that people could have a better life by maintaining the turtle population, avoiding egg commercialization, and killing turtles. Without a doubt, it is something that the community members in Guatemala should be very aware of. This is the only way that local communities, researchers, and turtles can all benefit (Nuila Coto, 2010).

Following the workshops and the subsequent establishment of alternative economic incomes, improved community education, and clear communication, the next step would be to establish an “environmental window” on egg collection, establishing two days per week when all freshly laid eggs remain in place. During the remaining days of the week, egg collection and relocation to hatcheries would continue, and they have always been. This initial partial ban on egg collection should remain in place for at least one or two seasons and will include regular workshops to discuss and assess the outcomes of this system. Based on the results, the egg collection ban may be altered, perhaps to include more days per week, based on scientific assessments and updates of the National Strategy.

It is essential to take into account that the previous planning and analysis of the factors described above may provide benefits at the levels where the plan is applied, from the local efforts made in the nesting beaches to cooperative international initiatives that are essential for the management of populations that are spread across several countries. It is necessary to recognize that the operation and success of conservation programs depend heavily on communities. Therefore it is essential to maintain an objective and respectful attitude that allows listening to and learning from local people and supporting the value of the local traditional knowledge during the development of conservation planning for marine turtles (Eckert, 1999).

Finally, a biological component should be mandated in the National Strategy, where population data is being taken to understand and predict the population trend. Such data can be the track record on an annual basis. Also, because each hatchery has specific and different conditions, the data obtained and analyzed from the hatchlings incubated during the execution of this thesis cannot address the specific measurement of depth and amount of eggs to all artificial nests being relocated in Guatemala's hatcheries. These conditions may vary from one hatchery to another. Therefore, the recommendation would be more research on this aspect, using different depths, and adjusting the conditions to each hatchery's specific conditions, using the hatching success value as the measurement for the nest-specific conditions in each hatchery.

References

Chacón, D. (2000). *Memorias del IV Taller Regional para la Conservación de las Tortugas Marinas en Centroamérica*. Belice.

Chacón, D., Sánchez, J., Calvo, J., & Ash, J. (2007). *Manual para el manejo y la conservación de las tortugas marinas en Costa Rica, con énfasis en la operación de proyectos en playa y tortugarios*. San José, Costa Rica: : Ministerio de Ambiente y Energía (MINAE).

CONAP. (2009). *Manual para la conservación de las tortugas marinas en Guatemala, con énfasis en el manejo de tortugarios*. Guatemala: Asociación Rescate y Conservación de Vida Silvestre (ARCAS) y el Consejo Nacional de Áreas Protegidas (CONAP).

CONAP. (2015). *Estrategia Nacional de Manejo y Conservacion de Tortugas Marinas de Guatemala*. (Documento técnico No. 02-2015). Guatemala, Guatemala.

CONAP. (2016). *Listado de tortugarios de Guatemala: actualización 2016, según el libro de registros*. Consejo Nacional de Áreas Protegidas (CONAP). Guatemala

Darmawan, A., Saputra, D. K., Wiadnya, D. G. R., & Gusmida, A. M. (2018). *Prediction of supratidal Zones as turtle nesting sites using remote sensing and geographic information system, a case study in Pacitan, Southern Java Sea.* Paper presented at the IOP Conference Series: Earth and Environmental Science, Batu, East Java, Indonesia.

Eckert, K. L. (1999). Designing a conservation program. In K. L. Eckert, K. A. Bjorndal, F. A. Abreu-Grobois, & M. Donnelly (Eds.), *Research and Management Techniques for the Conservation of Sea Turtles* (pp. 45-55). Washington, DC: IUCN/SSC Marine Turtle Specialist Group Publication No. 4.

Eckert, K. L., & Abreu Grobois, F. A. (2001). *Conservación de tortugas marinas en la Región del Gran Caribe–Un diálogo para el manejo regional efectivo.* (Vol. 13). St. Croix, U.S. Virgin Islands: WIDECAST, UICN/CSE Grupo Especialista en Tortugas Marinas (MTSG), WWF y el Programa Ambiental del Caribe del PNUMA.

Eckert, K. L., Bjorndal, K. A., Abreu-Grobois, F. A., & Donnelly, M. (2000). *Técnicas de Investigación y Manejo para la Conservación de las Tortugas Marinas*. Washington, DC: Grupo Especialista en Tortugas Marinas UICN/CSE.

Fotedar, A. (2018). Difference between In Situ and Ex Situ Conservation. Retrieved from http://www.differencebetween.net/science/nature%20/difference-between-in-situ-and-ex-situ-conservation/

Frazier, J. (2003). Prehistoric and ancient historic interactions between humans and marine turtles. In P. L. Lutz, J. A. Musick, & J. Wineken (Eds.), The Biology of Sea Turtles (Vol. 2, pp. 1-38). Boca Raton: CRC Marine Biology Series

Godfrey, M. H., Barreto, R., & Mrosovsky, N. (1996). Estimating past and present sex ratios of sea turtles in Suriname. Canadian Journal of Zoology-Revue Canadienne de Zoologie, 74, 267-277.

González Guevara, L. F. (2000). *PROPUESTA DEL PLAN DE MANEJO PARA LAS ZONAS DE RESERVA Y SITIOS DE REFUGIO DE TORTUGA MARINA: EL PLAYON DE MISMALOYA EN JALISCO; MEXICO, COMO ESTUDIO DE CASO.* (Master), Universidad de Colima, Colima, Mexico.

Hudgins, J., Mancini, A., & Ali, K. (2017). *Marine turtles of the Maldives: A field identification guide*. Gland, Switzerland: IUCN, Gland, Switzerland and the Government of Maldives in collaboration with USAID.

Lawson, L., & Rollinson, N. (2019). Artificial incubation in species with temperature-dependent sex determination. How hot is too hot? *BioRxiv*. doi: http://dx.doi.org/10.1101/791202

López Roulet, A. A. (2018, February 13th). Personal Communication.

Lovich, J. E., Ennen, J. R., Agha, M., & Gibbons, J. W. (2018). Where Have All the Turtles Gone, and Why Does It Matter? *Bioscience, , 68*, 771-781.

Maulany, R. I., Booth, D. T., & Baxter, G. S. (2012a). The effect of incubation temperature on hatchling quality in the olive ridley turtle, *Lepidochelys olivacea*, from Alas Purwo National Park, East Java, Indonesia: implications for hatchery management. *Marine Biology, 159*(12), 2651-2661. doi: 10.1007/s00227-012-2022-6

Maulany, R. I., Booth, D. T., & Baxter, G. S. (2012b). Emergence success and sex ratio of natural and relocated nests of olive ridley turtles from Alas Purwo National Park, East Java, Indonesia. *Copeia, 2012*(4), 738-747. doi: 10.1643/ch-12-088

Montes Osorio, N. L. (2004). *Estimación de la abundancia relativa de tortugas marinas que anidan en las costas de Guatemala.* (Bachelor), Universidad de San Carlos de Guatemala, Guatemala, Guatemala.

Morales Mérida, A., Ruiz, A., Navarro, E., Sifuentes-Romero, I., Abreu-Grobois, F. A., & Girondot, M. (2015). Contrasting incubation data for *Lepidochelys olivacea* highlight the need for more experimentation and detailed reporting. *Marine Turtles Newsletter, 145*, 15-19.

Morreale, S. J., Ruiz, G. J., Spotila, J. R., & Standora, E. A. (1982). Temperature-dependent sex determination: Current practices threaten conservation of sea turtles. Science, 216, 1245-1247. https://doi.org/10.1126/science.7079758

Mrosovsky, N. (2008). Against oversimplifying the issues on relocating turtle eggs. Environmental Management, 41(465-467).

Muccio, C., & Izquierdo, S. (2019). First confirmed hawksbill nesting on the Pacific coast of Guatemala. Marine Turtle Newsletter 158: 12-13.

Mutalib, A. H. A., & Fadzly, N. (2015). Assessing hatchery management as a conservation tool for sea turtles: A case study in Setiu, Terengganu. *Ocean & Coastal Management, 113*, 47-53. doi: http://dx.doi.org/10.1016/j.ocecoaman.2015.05.010

Nuila Coto, R. W. (2010, October 3rd.). Erika, la de Punta Ratón, primera tortuga golfina hondureña con monitoreo satelital. *La Tribuna. .* pp. 2B-3B. Retrieved from http://www.turtleprotector.org/SatTrack/PDF/ERICA%20primera%20tortuga%20golfina_Complete.pdf

ONU. (2015). *Transformar nuestro mundo: Agenda 2030 para el Desarrollo Sostenible*. A/70/L.1. ONU, Asamblea General

Palmer, T. B., & Flanagan, D. J. (2016). The sustainable company: looking at goals for people, planet and profits. Journal of business strategy.

Panamá, M. d. A. d. (2017). *Diagnóstico de la Situación de las Tortugas Marinas y Plan de Acción Nacional para su Conservación*. Panamá: Gaceta Oficial Digital.+ Fotedar, A. (2018, August 14). Difference between In Situ and Ex Situ Conservation. Difference Between. http://www.differencebetween.net/science/nature%20/difference-between-in-situ-and-ex-situ-conservation/

Patrício, A. R., Varela, M. R., Barbosa, C., Broderick, A. C., Catry, P., Hawkes, L. A., Regalla, A., & Godley, B. J. (2019). Climate change resilience of a globally important sea turtle nesting population. Global Change Biology, 25(2), 522-535.

Phillott, A. D., Firdous, F., & Shahid, U. (2018). Sea turtle hatchery practices and hatchling production in Karachi, Pakistan, from 1979-1997. Indian Ocean Turtle Newsletter, 27, 7.

Richardson, J. I. (2000). Prioridades para los Estudios sobre la Biología de la Reproducción y de la Anidación. In K. L. Eckert, K. A. Bjorndal, F. A. Abreu-Grobois, & M. Donnelly (Eds.), *Técnicas de Investigación y Manejo para la Conservación de las Tortugas Marinas* (pp. 9-12). Washington, DC: Grupo Especialista en Tortugas Marinas UICN/CSE.

Rivas, M. L., Esteban, N., & Marco, A. (2019). Potential male leatherback hatchlings exhibit higher fitness which might balance sea turtle sex ratios in the face of climate change. Climatic Change. doi: 10.1007/s10584-019-02462-1

Sari, F., & Yakup, K. (2017). Assessment of hatchery management for the loggerhead turtle (Caretta caretta) nests on Goksu Delta, Turkey. *Ocean & Coastal Management 146*, 89-98.

Global Discussion

Turtles are among the most primitive groups of vertebrates, appearing in the fossil record over 200 million years ago, giving rise to different lineages along the way, including sea turtles whose oldest fossils are over 100 million years old (Lockley et al., 2019). Marine turtle survival can be attributed to the development of adaptations to the environment in which they live, including a hydrodynamic body, glands that excrete excess salt, limbs that facilitate swimming, specialized diving systems, olfactory receptors, etc. (Hudgins et al., 2017). They are oviparous animals without parental care, where the females use sandy beaches for nesting and then return to the sea, leaving their eggs to incubate and after the hatchlings emerge from the nest and head towards the sea where they will spend the rest of their lives (Hudgins et al., 2017).

Their complex life cycles are affected, in all phases, by temperatures (Gane et al., 2020; Morales-Merida et al., 2018). Sea turtles are ectothermic and poikilothermic animals, such that their caloric exchange with the environment occurs via convection, radiation, and conduction (Vitt & Caldwell, 2014), and they depend on environmental resources to obtain body heat, which arises from cellular or mitochondrial metabolism (Merchant-Larios, 2000; Schmidt-Nielsen, 1976). However, they can regulate their temperature in relatively short ranges, using the sun and surrounding environmental surfaces to obtain heat, and lose it through the shadow, the water, and the cold surfaces (Vitt & Caldwell, 2014).

To make adjustments in the management policies of this species is essential to understand their physiology and thermal behavior, including Temperature-Dependent Sex Determination (TSD) (Taylor et al., 2020). This mechanism determines sex according to the accumulation of temperatures to which they are exposed during a certain period of their embryonic development during egg incubation (Girondot et al., 2018). In addition, temperature affects embryonic development directly, both in rates of growth and metabolism and also in hatching and emergence success (Maulany et al., 2012a).

Marine turtles nesting beaches are spread across vast regions of the world, and individual females return to the same region where they were born to lay eggs, a phenomenon called philopatry (Moncada et al., 2019). Each of the beaches where these species lay their eggs constitutes a nesting habitat, with specific characteristics that vary from one region to another. For this reason, it has been suggested that it is necessary to consider the attributes and factors that are likely specific to each nesting population (Morales-Mérida et al., 2015). Understanding the nesting habitat is also crucial for the development of proposals that allow the recovery and management of marine turtles (Richardson, 2000). The general recommendations of marine turtle conservation experts include documentation of when and where nests occur, determination of hatching success, characterization of genetic variability, evaluation of population parameters including trends, and investigation of issues relevant to conservation, such as nesting behavior and factors affecting the survival of embryos and hatchlings (Rees et al., 2016).

Characterizing nesting beaches facilitates a deeper understanding of the various components important for selecting this habitat by marine turtles, including geophysical factors such as the slope and width of the beach, the presence of interspecific competition, artificial lighting, and human activities (Narayani et al., 2018). When this detailed information is available in a specific country, more responsive and meaningful conservation planning can occur. In the case of Guatemala, there should be an effort to prioritize research and the collection of data on nesting females to assess population trends while also investigating the negative consequences of long-term egg collection and relocation to hatcheries. More work is needed to understand the beneficial impacts of allowing nests to incubate *in situ* on nesting beaches, including genetic and evolutionary adaptations to current conditions.

In Guatemala, *Lepidochelys olivacea* and *Dermochelys coriacea* nest along the 255 kilometers of the Pacific coast, and *Caretta Caretta, Chelonia mydas,* and *Eretmochelys imbricata* on the 148 kilometers of the Caribbean coast (CONAP, 2015). It is presumed that these species have been in danger of extinction for several decades, and starting in the 1970s, an informal system of conservation efforts was enacted through the establishment of hatcheries (CONAP, 2015). Within this established system, the commercialization of *L. olivacea* eggs is legal as long as the egg collectors turn over 20% of the eggs collected from each nest to an officially registered hatchery at the National Council of Protected Areas, a governmental entity in charge of the country’s biodiversity management (CONAP, 2018).

Hatcheries have been used throughout the world as a tool to increase the populations of marine turtles. However, the impacts of this practice on recruitment and quality of hatchlings have rarely been evaluated (Maulany et al., 2012a). In Guatemala, hatcheries have been the only conservation strategy, while no nests are left in place to incubate naturally and undisturbed on nesting beaches. While the number of operational hatcheries has fluctuated over time, there has been a tendency for their number to increase. As of 2017, 23 hatcheries were officially registered, 22 in the Pacific and one on the Caribbean coast (CONAP, 2016).

Hatcheries in Guatemala rely on the support of private, non-governmental, and academic entities and are promoted and governed by governmental norms (CONAP, 2009, 2016). Despite their essential role in local sea turtle conservation, hatcheries in Guatemala lack adequate technical management, and few of them gather data and handle eggs in a scientific and standardized way (CONAP, 2015 p.14). In addition, in Guatemala, hatchlings are used as a tourist attraction. Through a sponsorship system, tourists can pay a fee that allows them to carry a hatchling down the beach and release it to the sea (Morales-Mérida, 2013). This enables the hatcheries to raise money and obtain income, to buy more eggs from collectors, and to conduct other activities (Morales-Mérida, 2013). This system has remained and increased, despite the legal code that states that manipulation of the hatchlings, apart from hatchery personel, is officially prohibited (CONAP, 2018).

It was found with this thesis that the current management conditions have an impact on the offspring's behavior and fitness, making it clear that successful conservation of marine turtles depends on the good management and functionality of the hatcheries in Guatemala, especially as it appears that no nests are allowed to incubate naturally on beaches. We found that factors such as the nest depth and the amount of eggs in the nest have a significant influence over the self righting performance, where a higher number of eggs increases the time of self righting, and deeper nests produce hatchlings with a faster flipping response. With the information found about the impact of specific management conditions, it can be stated that the management at a hatchery is affecting the hatchling performance and thus fitness, which should be explored with more data in the future. Other studies have found differences in performance data measured in the same groups of turtles. For example, Sim et al. (2015), with *C. caretta*, stated the importance of crawling performance to make assumptions about survival. While, Gatto and Reina (2020), using *L. olivacea*, found a positive relationship between crawling performance and self righting performance.

Another important aspect is that surveys revealed that some beaches in Guatemala have dark sand color and/or high wall formations, which have implications for natural egg incubation. The understanding and knowledge of the nesting habitats of marine turtles are essential for the recovery and management of their populations and the design of the conservation program based on information provided by scientific research (Eckert et al., 2000; Miller Martin et al., 2019). Previously, little information about local nesting habitats was available for Guatemala, which hampered the development of effective conservation planning, given that there can be biological differences among marine turtle populations (Morales-Mérida et al., 2015). The work presented in this thesis is specific to Guatemala and should help promote more effective conservation planning.

The use of hatcheries has been recommended when the protection of marine turtle eggs *in situ* is impossible, for example, in the face of illegal egg collection (Mutalib & Fadzly, 2015). According to the most recent assessment for the IUCN Red List, *L. olivacea* is categorized as Vulnerable, with a decreasing trend population globally (Abreu-Grobois & Plotkin, 2008). Even if the Red List assessment says there has been a 69% decline in olive ridley nests in Guatemala, this number is based on too many assumptions and extrapolations. Better data are needed for a proper assessment, and therefore it can be stated that there is no evidence of the populations decreasing in Guatemala, where the trend is rather stable with some peaks. The cultural and local context is somewhat related to the increase in the number of eggs incubated in each hatchery. Each hatchery has independent means of purchasing eggs in each nesting season, depending on their funding, which could explain why many hatcheries are buying the rest of the nests from collectors or incubating the whole nest. However, this can affect turtles and their habitats if not correctly performed (Katselidis et al., 2013). Thus, the exponential increase in the number of incubated eggs cannot be used as evidence for an increase in the number of nests deposited on the Pacific coast of Guatemala. The evaluation of the population status should focus on the nesting phenology and the total number of nests deposited on the beach as opposed to the number of incubated eggs.

Aside from the population trend found, it is important to allow some sea turtle nests to remain in place for incubation on the beaches to establish a baseline against which can be used to compare the operations and results of the hatcheries. This would adhere to the recommendation that hatchery management should be based on results from scientific research on reproductive physiology, morphology, and other aspects of marine turtle species, *in situ* and *ex situ*, on the nesting beaches of each country (Morales-Mérida et al., 2015).

When created, the conservation strategy of sea turtles in Guatemala adopted the hatchery system without adaptation to local conditions or without any adjustments, mainly because there is little to no local information about nests and nesting habitats in the country. This thesis constitutes a first step towards improving our knowledge of nesting and nesting habitats in Guatemala so that adjustments to a national strategy can be implemented to benefit sea turtle conservation, including highlighting future research needs. We suggest a mixed system where both natural nests and hatcheries remain. Nevertheless, we suggest that hatcheries should be mainly managed by community members.

The idea of community members managing the hatcheries is to promote community sustainability and provide alternative incomes to collectors, offering the opportunity to grow and develop in another area related to sea turtle management. This could be a way of ensuring and providing a profitable and fulfilling way of life and that economic and social progress occurs in harmony with nature, such as presented in the 2030 Agenda for Sustainable Development (Lee et al., 2016).

Global Conclusions

Based on the results and analyses presented in this thesis, it has been established that contrary to what has been previously stated, the olive ridley population in Guatemala does not appear to be drastically decreasing, and the nesting activity reveals a trend with several peaks that tend to stabilize and show a normal behavior, throughout the years.

Incubation and management conditions such as nest depth and amount of eggs in each artificial nest at the hatcheries influence the locomotor performance of hatchlings, which is an indirect measurement of survival. Further research on the conditions of each hatchery must be undertaken to evaluate the impacts and make recommendations for best practices for each one.

Temperatures have shifted in the past decade, which can affect populations of species such as sea turtles with TSD. Therefore it is necessary to incorporate this information into national and regional conservation strategies for more effective outcomes.

In Guatemala, hatcheries are managed mainly by non-profit organizations or private entities. At the same time, coastal communities play a small part in the management and conservation of the *L. olivacea* population. With all that has been done and considering the 2030 Agenda for Sustainable Development, it is necessary to promote community involvement in the conservation and preservation of this species to ensure that the policies are being implemented.

Recommendations

The gathering of nesting data is vital to better understand trends in nesting density. Therefore, it is recommended that as part of a national plan, standardized counts of nesting tracks that are georeferenced are needed from all parts of the nesting season and all parts of the coast. With these data collected over the long term, trends of nesting populations can be made.

Current recommendations of hatchery management typically state that nest conditions in these artificial enclosures must mimic as much as possible the natural conditions. This would mean that for each hatchery, there should be dedicated personnel collecting data and information from nearby natural nesting beaches. In Guatemala, this is not currently done, mainly because hatcheries obtain their eggs from local collectors who may be taking eggs from different beaches (although some hatcheries may collect a portion of their eggs from nearby beaches). Therefore another recommendation is that each hatchery should evaluate the conditions of nearby nesting beaches and adjust the hatchery conditions to mimic natural conditions as much as possible to minimize potential impacts to incubating eggs and hatchlings. For example, incubation conditions in the hatcheries can alter the performance of hatchlings, which in turn may affect their long-term fitness.

More research and data are needed on incubation conditions of nests left on nesting beaches to assess the possibility of starting at a long-term mixed strategy approach (incubation of eggs in hatcheries and *in situ*). In addition, it is necessary to investigate the incubation conditions of each hatchery, which would facilitate the establishment of standardized protocols and management of all hatcheries in Guatemala.

Finally, in terms of community development, whenever possible and appropriate, local traditions and practices should be included in conservation programs, plans, strategies, and actions, integrating them into environmental education planning and programs

(Frazier, 2000). The capture of this information contributes to the improved knowledge of the populations, and the involvement of the multisectoral stakeholders is fundamental for the program's success (Eckert, 1999). This will facilitate and support social and political organizations within the community, leading to further development for leaders and political structures. The active participation of the local stakeholders is necessary to develop conservation actions that address local needs and expectations while minimizing damage to the environment or society (Frazier, 2000). Therefore, it is recommended that as long as hatcheries are used in Guatemala, the benefit to local coastal communities should be prioritized, and therefore these communities should play an active role in the administration of nearby hatcheries, as well as being active stakeholders in managing the conservations of these protected species.

References

Abreu-Grobois, A., & Plotkin, P. (2008). Lepidochelys olivacea. The IUCN Red List of Threatened Species 2008: e. T11534A3292503. In.

Bladow, R. A., & Milton, S. L. (2019). Embryonic mortality in green (Chelonia mydas) and loggerhead (Caretta caretta) sea turtle nests increases with cumulative exposure to elevated temperatures. *Journal of Experimental Marine Biology and Ecology*, *518*, 151180.

Blechschmidt, J., Wittmann, M. J., & Blüml, C. (2020). Climate Change and Green Sea Turtle Sex Ratio -Preventing Possible Extinction. *Genes*, *11*(5), 588.

Chacón, D. (2000). Memorias del IV Taller Regional para la Conservación de las Tortugas Marinas en Centroamérica. In F. A. PROARCA/CAPAS (Ed.), (pp. 165). Belice.

Chacón, D., Sánchez, J., Calvo, J., & Ash, J. (2007). *Manual para el manejo y la conservación de las tortugas marinas en Costa Rica, con énfasis en la operación de proyectos en playa y tortugarios*. San José, Costa Rica: : Ministerio de Ambiente y Energía (MINAE)

CIT. (2011). *Cooperando para la Conservación de las Tortugas Marinas* (B. Dick, M. Donnelly, & V. C. Ch., Eds.). Secretaría Pro Tempore Convención Interamericana para la Protección y Conservación de las Tortugas Marinas (CIT)

http://www.iacseaturtle.org/docs/publicaciones/17-Revista10yearsESPANOLLR.pdf

CONAP. (2009). *Manual para la conservación de las tortugas marinas en Guatemala, con énfasis en el manejo de tortugarios*. Guatemala: Asociación Rescate y Conservación de Vida Silvestre (ARCAS) y el Consejo Nacional de Áreas Protegidas (CONAP)

CONAP. (2015). *Estrategia Nacional de Manejo y Conservaciσn de Tortugas Marinas de Guatemala*. (Documento técnico No. 02-2015). Guatemala, Guatemala

CONAP. (2016). *Listado de tortugarios de Guatemala: actualización 2016, según el libro de registros*. Consejo Nacional de Áreas Protegidas (CONAP).

CONAP. (2018). *Normativo para el Manejo y Conservación de Tortugas Marinas Documento Técnico No. 36-2017*. (Documento Técnico No. 36-2017). Guatemala

Darmawan, A., Saputra, D. K., Wiadnya, D. G. R., & Gusmida, A. M. (2018). *Prediction of supratidal Zones as turtle nesting sites using remote sensing and geographic information system, a case study in Pacitan, Southern Java Sea.* IOP Conference Series: Earth and Environmental Science, Batu, East Java, Indonesia.

Eckert, K. L. (1999). Designing a conservation program. In K. L. Eckert, K. A. Bjorndal, F. A. Abreu-Grobois, & M. Donnelly (Eds.), *Research and Management Techniques for the Conservation of Sea Turtles* (pp. 45-55). IUCN/SSC Marine Turtle Specialist Group Publication No. 4.

Eckert, K. L., & Abreu Grobois, F. A. (2001). *Conservación de tortugas marinas en la Región del Gran Caribe–Un diálogo para el manejo regional efectivo.* (Vol. 13). WIDECAST, UICN/CSE Grupo Especialista en Tortugas Marinas (MTSG), WWF y el Programa Ambiental del Caribe del PNUMA.

Eckert, K. L., Bjorndal, K. A., Abreu-Grobois, F. A., & Donnelly, M. (2000). *Técnicas de Investigación y Manejo para la Conservación de las Tortugas Marinas*. Grupo Especialista en Tortugas Marinas UICN/CSE.

Fotedar, A. (2018, August 14). Difference between In Situ and Ex Situ Conservation. *Difference Between*. http://www.differencebetween.net/science/nature%20/difference-between-in-situ-and-ex-situ-conservation/

Frazier, J. G. (2000). Conservación Basada en la Comunidad. In K. L. Eckert, K. A. Bjorndal, F. A. Abreu-Grobois, & M. Donnelly (Eds.), *Técnicas de Investigación y Manejo para la Conservación de las Tortugas Marinas* (pp. 16-22). Grupo Especialista en Tortugas Marinas UICN/CSE.

Gane, J., Downs, C., Olivier, I., & Brown, M. (2020). Nesting ecology and hatching success of the hawksbill turtle (2004–2014) on Cousine Island, Seychelles. *African Journal of Marine Science*, *42*, 53-65. https://doi.org/10.2989/1814232X.2020.1727952

Gatto, C. R., & Reina, R. D. (2020). Sea turtle hatchling locomotor performance: incubation moisture effects, ontogeny and species-specific patterns. *Journal of Comparative Physiology B*, *190*(6), 779-793.

Girondot, M., Monsinjon, J., & Guillon, J.-M. (2018). Delimitation of the embryonic thermosensitive period for sex determination using an embryo growth model reveals a potential bias for sex ratio prediction in turtles. *Journal of Thermal Biology*, *73*, 32-40.

Godfrey, M. H., Barreto, R., & Mrosovsky, N. (1996). Estimating past and present sex ratios of sea turtles in Suriname. *Canadian Journal of Zoology-Revue Canadienne de Zoologie*, *74*, 267-277.

González Guevara, L. F. (2000). *PROPUESTA DEL PLAN DE MANEJO PARA LAS ZONAS DE RESERVA Y SITIOS DE REFUGIO DE TORTUGA MARINA: EL PLAYON DE MISMALOYA EN JALISCO; MEXICO, COMO ESTUDIO DE CASO* Universidad de Colima]. Colima, Mexico.

Guillon, J.-M., Guéry, L., Hulin, V., & Girondot, M. (2012). A large phylogeny of turtles (Testudines) using molecular data. *Contributions to Zoology*, *81*(3), 147-158.

Hudgins, J., Mancini, A., & Ali, K. (2017). *Marine turtles of the Maldives: A field identification guide*. IUCN, Gland, Switzerland and the Government of Maldives in collaboration with USAID.

Katselidis, K. A., Schofield, G., Stamou, G., Dimopoulos, P., & Pantis, J. D. (2013). Evidence-based management to regulate the impact of tourism at a key marine turtle rookery on Zakynthos Island, Greece. *Oryx*, *47*, 584-594.

Lawson, L., & Rollinson, N. (2019). Artificial incubation in species with temperature-dependent sex determination. How hot is too hot? *BioRxiv*. https://doi.org/http://dx.doi.org/10.1101/791202

Lee, B. X., Kjaerulf, F., Turner, S., Cohen, L., Donnelly, P. D., Muggah, R., Davis, R., Realini, A., Kieselbach, B., & MacGregor, L. S. (2016). Transforming our world: implementing the 2030 agenda through sustainable development goal indicators. *Journal of Public Health Policy*, *37*(1), 13-31.

Lockley, M. G., Cawthra, H. C., Vynck, J. C. D., Helm, C. W., McCrea, R. T., & Nel, R. (2019). New fossil sea turtle trackway morphotypes from the Pleistocene of South Africa highlight role of ichnology in turtle paleobiology. *Quaternary Research*, *92*(9), 626-640. https://doi.org/10.1017/qua.2019.40

López-Roulet, A. A. (February 13th, 2018). In.

Lovich, J. E., Ennen, J. R., Agha, M., & Gibbons, J. W. (2018). Where Have All the Turtles Gone, and Why Does It Matter? *Bioscience,* , *68*, 771-781.

Marco, A., Abella, E., Martins, S., López, O., & Patino-Martinez, J. (2017). Female nesting behaviour affects hatchling survival and sex ratio in the loggerhead sea turtle: implications for conservation programmes.

Ethology Ecology & Evolution, *30*, 141-155. https://doi.org/https://doi.org/10.1080/03949370.2017.1330291

Martín-del-Campo, R., & García-Gasca, A. (2019). Sea turtles: ancient creatures, mysteries, adaptations, and threatens. *Research in Marine Sciences*, *4*(3), 556 - 562.

Martínez Mencos, J. E., & Calderón Pérez, V. (2011). Determinación de la frecuencia de anidación de la tortuga marina Parlama (Lepidochelys olivacea) por medio de marcaje, en la playa El Paredón del Parque Nacional Sipacate Naranjo, La Gomera, Escuintla. In P. U. d. I. e. C. B. (PUICB) & U. d. S. C. d. Guatemala (Eds.), (pp. 25). Guatemala: Programa Universitario de Investigación en Ciencias Básicas (PUICB).

Maulany, R. I., Booth, D. T., & Baxter, G. S. (2012a). The effect of incubation temperature on hatchling quality in the olive ridley turtle, *Lepidochelys olivacea*, from Alas Purwo National Park, East Java, Indonesia: implications for hatchery management. *Marine Biology*, *159*(12), 2651-2661. https://doi.org/10.1007/s00227-012-2022-6

Maulany, R. I., Booth, D. T., & Baxter, G. S. (2012b). Emergence success and sex ratio of natural and relocated nests of olive ridley turtles from Alas Purwo National Park, East Java, Indonesia. *Copeia*, *2012*(4), 738-747. https://doi.org/10.1643/ch-12-088

Maurer, A. S., Seminoff, J. A., Layman, C. A., Stapleton, S. P., Godfrey, M. H., & Reiskind, M. O. B. (2021). Population Viability of Sea Turtles in the Context of Global Warming. *Bioscience*.

Merchant-Larios, H. (2000). Determinación del Sexo en Crías (R. Briseño-Dueñas, F. A. Abreu-Grobois, L. Sarti Martínez, A. Barragán Rocha, J. C. Cantú, M. d. C. Jiménez, & J. Peña, Trans.). In K. L. Eckert, K. A. Bjorndal, F. A. Abreu-Grobois, & M. Donnelly (Eds.), *Técnicas de Investigación y Manejo para la Conservación de las Tortugas Marinas* (pp. 150-155). Grupo Especialista en Tortugas Marinas UICN/CSE.

Miller, J. D. (2000). Determinación del Tamaño de la Nidada y el Éxito de la Eclosión. In K. L. Eckert, K. A. Bjorndal, F. A. Abreu-Grobois, & M. Donnelly (Eds.), *Técnicas de Investigación y Manejo para la Conservación de las Tortugas Marinas* (pp. 143-149). Grupo Especialista en Tortugas Marinas UICN/CSE.

Miller Martin, J., Jambeck, J. R., Ondich, B. L., & Norton, T. M. (2019). Comparing quantity of marine debris to loggerhead sea turtle (Caretta

caretta) nesting and non-nesting emergence activity on Jekyll Island, Georgia, USA. *Marine Pollution Bulletin*, *139*, 1-5.

Moncada, F., Coppenrath, C. M., Hirsch, S., Nodarse, G., Page-Karjian, A., Reeves, A. M., & Perrault, J. R. (2019). Nesting Green Turtle Tagged in Cuba, Recaptured in Florida. *Marine Turtle Newsletter*, *156*, 1-2.

Montes Osorio, N. L. (2004). *Estimación de la abundancia relativa de tortugas marinas que anidan en las costas de Guatemala* Universidad de San Carlos de Guatemala]. Guatemala, Guatemala.

Morales-Mérida, A., Ruiz, A., Navarro, E., Sifuentes-Romero, I., Abreu-Grobois, F. A., & Girondot, M. (2015). Contrasting incubation data for *Lepidochelys olivacea* highlight the need for more experimentation and detailed reporting. *Marine Turtles Newsletter*, *145*, 15-19.

Morales-Merida, B. A., Bustamante, D. M., Monsinjon, J., & Girondot, M. (2018). Reaction norm of embryo growth rate dependent on incubation temperature in the Olive Ridley sea turtle, *Lepidochelys olivacea*, from Pacific Central America. *Journal of Embryology*, *1*(1), 12-24.

Morales Mérida, A. (2013). *Relationship between incubation length and the sex ratio of the sea turtles Lepidochelys olivacea in the Natural Reserve of Multiple Uses, in the Pacific Coast of Guatemala* [Bachelor in Biology, Universidad de San Carlos de Guatemala]. Guatemala City, Guatemala.

Morreale, S. J., Ruiz, G. J., Spotila, J. R., & Standora, E. A. (1982). Temperature-dependent sex determination: Current practices threaten conservation of sea turtles. *Science*, *216*, 1245-1247. https://doi.org/10.1126/science.7079758

Mrosovsky, N. (2008). Against oversimplifying the issues on relocating turtle eggs. *Environmental Management*, *41*(465-467).

Mueller, U. G., Eickwort, G. C., & Aquadro, C. F. (1994). DNA fingerprinting analysis of parent-offspring conflict in a bee. *Proceedings of the National Academy of Sciences of the United States of America*, *91*(11), 5143-5147.

Mutalib, A. H. A., & Fadzly, N. (2015). Assessing hatchery management as a conservation tool for sea turtles: A case study in Setiu, Terengganu. *Ocean & Coastal Management*, *113*, 47-53. https://doi.org/http://dx.doi.org/10.1016/j.ocecoaman.2015.05.010

Narayani, S., Venu, S., & D'Silva, A. J. (2018). Comparison of beach profiles conducive for turtle nesting in Andaman. *Journal of*

Threatened Taxa, *10*, 12337–12343. https://doi.org/ https://doi.org/10.11609/jott.3373.10.10.12337-12343

Nuila Coto, R. W. (2010, October 3rd.). Erika, la de Punta Ratón, primera tortuga golfina hondureña con monitoreo satelital. *La Tribuna. .* 2B-3B. http://www.turtleprotector.org/SatTrack/PDF/ERICA%20primera%20tortuga%20golfina_Complete.pdf

ONU. (2015). Transformar nuestro mundo: Agenda 2030 para el Desarrollo Sostenible. In O. d. N. U. (ONU) (Ed.), *A/70/L.1* (pp. 40). Asamblea General ONU.

Palmer, T. B., & Flanagan, D. J. (2016). The sustainable company: looking at goals for people, planet and profits. *Journal of business strategy*.

Panamá, M. d. A. d. (2017a). *Diagnóstico de la Situación de las Tortugas Marinas y Plan de Acción Nacional para su Conservación*. Panamá: Gaceta Oficial Digital

Panamá, M. d. A. d. (2017b). *Plan de Acción para la Conservación de Tortugas Marinas en Panamá 2017-2021*. Panamá

Patrício, A. R., Varela, M. R., Barbosa, C., Broderick, A. C., Catry, P., Hawkes, L. A., Regalla, A., & Godley, B. J. (2019). Climate change resilience of a globally important sea turtle nesting population. *Global Change Biology*, *25*(2), 522-535.

Phillott, A. D., Firdous, F., & Shahid, U. (2018). Sea turtle hatchery practices and hatchling production in Karachi, Pakistan, from 1979-1997. *Indian Ocean Turtle Newsletter*, *27*, 7.

Rees, A. F., Alfaro-Shigueto, J., Barata, P. C. R., Bjorndal, K. A., Bolten, A. B., Bourjea, J., Broderick, A. C., Campbell, L. M., Cardona, L., Carreras, C., Casale, P., Ceriani, S. A., Dutton, P. H., Eguchi, T., Formia, A., Fuentes, M. M. P. B., Fuller, W. J., Girondot, M., Godfrey, M. H., Hamann, M., Hart, K. M., Hays, G. C., Hochscheid, S., Kaska, Y., Jensen, M. P., Mangel, J. C., Mortimer, J. A., Naro-Maciel, E., Ng, C. K. Y., Nichols, W. J., Phillott, A. D., Reina, R. D., Revuelta, O., Schofield, G., Seminoff, J. A., Shanker, K., Tomás, J., Merwe, J. P. v. d., Houtan, K. S. V., Zanden, H. B. V., Wallace, B. P., Wedemeyer-Strombel, K. R., Work, T. M., & Godley, B. (2016). Review: Are we working towards global research priorities for management and conservation of sea turtles? *Endangered Species Research*, *31*, 337-382.

Richardson, J. I. (2000). Prioridades para los Estudios sobre la Biología de la Reproducción y de la Anidación. In K. L. Eckert, K. A. Bjorndal, F.

A. Abreu-Grobois, & M. Donnelly (Eds.), *Técnicas de Investigación y Manejo para la Conservación de las Tortugas Marinas* (pp. 9-12). Grupo Especialista en Tortugas Marinas UICN/CSE.

Rivas Chacón, A. B. (2002). *Evaluación del efecto de la profundidad sobre el éxito de la eclosión de los huevos de Tortuga Parlama (Lepidochelys Olivacea, Eschscholtz, 1289: Chelonidae) bajo condiciones controladas, en la Reserva Natural de Usos Múltiples Monterrico, Taxisco , Santa Rosa, Guatemala* Universidad de San Carlos de Guatemala]. Guatemala, Guatemala.

Rivas, M. L., Esteban, N., & Marco, A. (2019). Potential male leatherback hatchlings exhibit higher fitness which might balance sea turtle sex ratios in the face of climate change. *Climatic Change*. https://doi.org/10.1007/s10584-019-02462-1

Sari, F., & Yakup, K. (2017). Assessment of hatchery management for the loggerhead turtle (Caretta caretta) nests on Goksu Delta, Turkey. *Ocean & Coastal Management 146*, 89-98.

Schmidt-Nielsen, K. (1976). *Fisiología animal: adaptación y medio ambiente* (2nd ed.). Omega.

Sim, E. L., Booth, D. T., & Limpus, C. J. (2015). Incubation temperature, morphology and performance in loggerhead (*Caretta caretta*) turtle hatchlings from Mon Repos, Queensland, Australia. *Biology Open*, *4*(6), 685-692. https://doi.org/10.1242/bio.20148995

Taylor, E. N., Diele-Viegas, L. M., Gangloff, E. J., Hall, J. M., Halpern, B., Massey, M. D., Rödder, D., Rollinson, N., Spears, S., Sun, B. j., & Telemeco, R. S. (2020). The thermal ecology and physiology of reptiles and amphibians: A user's guide. *J Exp Zool*, 1-32. https://doi.org/10.1002/jez.2396

Vitt, L. J., & Caldwell, J. P. (2014). *Herpetology: an introductory biology of amphibians and reptiles* (4th ed.). Academic press.