



NOAA Technical Memorandum NMFS-SEFSC-777  
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# **PROCEEDINGS OF THE FORTIETH ANNUAL SYMPOSIUM ON SEA TURTLE BIOLOGY AND CONSERVATION**



**40<sup>th</sup> International  
Sea Turtle Symposium**

25 to 28 March, 2022

Perth, Australia (virtual)

Compiled by:

Paul A. Whittock, Anton D. Tucker, and Lisa Belskis

U.S. DEPARTMENT OF COMMERCE  
National Ocean and Atmospheric Administration  
National Marine Fisheries Service  
Southeast Fisheries Science Centre  
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Miami, Florida 33149

March 2024

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March 2024

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40<sup>th</sup> International  
Sea Turtle Symposium

## **PRESIDENT'S REPORT**

### **40<sup>th</sup> Annual Symposium on Sea Turtle Biology and Conservation**

25 – 28 March, 2022, Perth, Australia (held online)

Kellie L. Pendoley, President, ISTS

When the Board of the International Sea Turtle Society was forced to make the difficult decision to cancel the 40<sup>th</sup> International Sea Turtle Symposium in Cartagena, Colombia, in March 2020, the world was facing an unknown future as the reality of the COVID-19 pandemic started to emerge. It was a tough decision but in hindsight we feel it was the right one. The decision was also made to switch the location of the symposium to Perth, Western Australia and delay it until 2022.

The 40<sup>th</sup> International Sea Turtle Symposium (ISTS40) was therefore held between the 25<sup>th</sup> and 28<sup>th</sup> March 2022. The event was originally planned as a face-to-face symposium. However, with the ongoing global pandemic it was moved to an online event hosted on the Gather.Town platform and was managed by a team of people from Perth, Western Australia. Organising this event, the first ever online international sea turtle symposium, was incredibly challenging and would not have happened without the tireless work of our sea turtle biologist colleague, Dr Paul Whittock. In the two years leading up to the symposium, he single-handedly revamped the Society website, set up and managed the ISTS40 website ([ists40perth.com.au](https://ists40perth.com.au)), organised the online platform Gather.Town, managed the planning for regional meetings and workshops, worked out the program schedule, assisted the Session Chairs in session planning, drafted the Symposium Program, and responded to the hundreds of emails asking for help and advice. Paul's contributions to the Society and to the ISTS40 event were critical and without him it would not have been possible.

Over the four days of ISTS40, we attempted to recreate as many of the events found in a face-to-face Symposium as possible. On Friday 23<sup>rd</sup> March, we programmed and hosted 11 Workshops and seven Regional Meetings with up to 110 registered participants in some of them. On Saturday 24<sup>th</sup> March, we kicked off with a formal opening session which started with an Acknowledgement of the Traditional Custodians of the land upon which we met, for those of us in Perth the land of the Wadjuk Noongar, and a Welcome to Country by Traditional Custodian Nick Abrahams who welcomed the Symposium delegates to his Country. This was followed by keynote addresses by Abigail Ross (Principal Marine Environmental Advisor, Fortescue Future Industries), Dr Scott Whiting Principal Research Scientist and Coordinator of NWSFTCP, Department of Biodiversity, Conservation and Attractions (WA State Government), and Albert Wiggan, a Bardi, Nyul and Kija man, Indigenous Leader, Environmental Consultant and Social-Emotional Wellbeing Officer. This opening session started the three days of online presentations managed via the VirtualChair and ConfTool programs in the Gather.Town space. The space provided attendees the ability to view presentations in real time and for up to two weeks after the event, and gave people the opportunity to meet and chat via video streaming.

Statistics for this symposium:

- Held across 3 days and 43 hours of online content;
- Participants from 85 countries registered to attend (ranked #1 for all ISTS Symposia);
- 675 registered individuals (ranked #14 for all Symposia; ranked #4 of all ISTS Symposia outside USA after Loreto (1,016), San Jose (1,000), and Crete (700));
- 11 workshops were hosted;
- 7 regional meetings were hosted;
- 110 oral presentations delivered;
- 128 posters presented;
- Allowed students to attend for a cost of between US\$5 – \$20, making this the most accessible Symposium ever held by ISTS; and
- Despite the low registration costs, the event was one of the most profitable ISTS Symposia ever held.

As the first online Symposium held by the ISTS there was an enormous learning curve since none of us had ever done anything like this before.

I want to recognise the huge effort by Dr Paul Whittock and the whole team at the Pendoley Environmental office for giving up their weekend to help out and make this Symposium possible.

I also need to acknowledge all those people who embraced the online approach and went out their way to help make the event happen, specifically:

- Dr Nancy FitzSimmons for stepping up and taking on the Session Chair role, she and her committee were the heart of this Symposium, selecting and programming the 110 talks and 130 posters from 221 oral submissions. Nancy and the global committee accepted the challenge and embraced the new approach to accepting, reviewing, and selecting abstracts via a completely new abstract management program (ConfTool). This was all done while she was deep in our Southern Hemisphere field season, on a remote island with no internet and dodgy mobile phone reception that required a hike up a hill to communicate. In every way she went above and beyond.
- The oral and poster authors. When the call went out for abstracts, we were nervous that nobody would respond. But you did and in that typical last-minute flood of submissions you gave us a Symposium.
- Dr Manjula Tiwari for being the brains trust, holding all the thousands of pieces of information in her head that forms the corporate memory of the Society and always available to answer numerous questions. Her steady hand, wisdom and gentle diplomacy guided us through, and was all the more important in these COVID impacted years.
- Student Award judges, Awards Committee, Nomination Committee, Speed Chatting with Experts volunteers, Student Committee, Workshop and Regional Meeting organisers and assistants, and our future proceedings compilers.
- Our keynote speakers, for their passion, enthusiasm, and firm belief in the messages they delivered. They brought us new and challenging ideas to think about and introduced us to concepts and ideas beyond our own worlds and our own way of thinking.

While the online forum was not the first choice for those people who regularly enjoy attending the face-to-face Symposia, it did provide an opportunity for people who may not otherwise have been able to attend, the chance to join an ISTS event. There were many benefits that came out of this online meeting, including:

Substantially reduced carbon footprint.

- Using published criteria, the online Symposium produced ~10kg CO<sub>2</sub> per person compared to an in-person Symposium which produces ~1900kg per person. Travel, primarily long-distance flights accounts for 91–97% of total emissions.
- Blaine Friedlander of Cornell University in a Nature Communication piece concluded that moving a professional conference completely online reduces its carbon footprint by 94%, and shifting it to a hybrid model, with half of attendees online, reduces the footprint to 67%.
  - Consideration of our personal contributions to global warming, something that we as marine turtle biologists and conservationists should be conscious of since turtles are so hugely impacted by climate change.
  - Accessibility, reduced discrimination, and promotion of diversity and equal opportunities; the online event provided an opportunity for anyone who was interested to attend and allowed a greater inclusion of:
    - Non-European/non-North American researchers, students, and junior researchers and conservationists who otherwise would not have the opportunity to attend or present at the conferences due to travel, accommodation and registration costs.
- Parents and family caregivers who may not be able to leave home.
- People who do not have the luxury of personal leave time to travel.
- People who need to present at a Symposium for career advancement are not discriminated against if they cannot afford the time or costs of attending or travelling to a Symposium.
  - Pre-recorded presentations that were efficient, predictable, and had no time zone restrictions
  - The availability of presentations online for 2 weeks after the meeting gave everyone the chance to watch at their leisure

While the online event did have a few teething problems, overall the feedback was very positive, particularly from students and people who had neither the time or money to attend otherwise. For many I spoke to it was the first ISTS Symposium they had ever attended, and they loved it. I believe now might be the time for the ISTS membership and board to seriously consider different models for the annual ISTS Symposium, including; biennial international face to face meetings, online meetings, domestic satellite regional meetings, or a hybrid mix of all options.

So, thank you all for trusting my team and I to bring you an online Symposium. Thank you for stepping up, interacting, and trying out this new approach to an ISTS. We hope you enjoyed the virtual world we created for you and we look forward to catching up with you next time, either face to face in Cartagena, at another online meeting, or at some hybrid of these options. Who knows where the world will be in 12 months?



---

## EXECUTIVE COMMITTEE

Executive Committee Role	Member
President	Kellie Pendoley
Secretary	Manjula Tiwari
Treasurer	Nicholas Blume
President Elect	Diego Amorocho
Past President	Kenneth J. Lohmann

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## ORGANIZING COMMITTEE

Organizing Committee Role	Member
Registrar	Paul Whittock
Webmaster	Paul Whittock, Adam Mitchell
Virtual Space Designer	Rhonda Clark, Paul Whittock
Exhibitor/Vendor Chair	Paul Whittock
Speed Chatting Coordinators	Alexandra Fireman, Gabi Arango, Matthew Ramirez, Renato Saragoça Bruno
Nomination Committee Chair	Mustapha Aksissou
Nomination Committee Members	Ryan Welsh, Gabriela Vélez-Rubio, Nicholas Pilcher, Roldán Valverde
ISTS Awards Committee	Hector Alonso Barrios-Garrido, Ana Liria Loza, Mariluz Parga, Frank Paladino, Michael Salmon
Student Committee	Alexandra Fireman, Gabi Arango, Matthew Ramirez, Renato Saragoça Bruno
Student Awards Committee	Andrea Phillott, Matthew Godfrey
Grassroots Conservation Award Committee	Ingrid Yañez
Workshops Committee	Kellie Pendoley, Daniela Freggi, Sandra Hochscheid
Social Media	Paul Whittock, Andrea Phillott, Lauren Peel

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## PROGRAM COMMITTEE

Program Committee Role	Member
Program Chair	Nancy FitzSimmons
Program Committee	Nancy FitzSimmons, Kellie Pendoley, Paul Whittock
Session Chairs	Michael Paul Jensen, Jérôme Bourjea, Mayeul Dalleau, Kelly Stewart, Anton D. Tucker, Ana Rita Patricio, ALan Rees, George Shillinger, Jeanette Wyneken, Alexander Gaos, Alessandro Ponzio, Erin Seney, Brian Michael Shamblin, Kimberly Finlayson, Maximilian Polyak, Roldán Valverde, Erina J. Young, Matthew Godfrey, Peter Bradley Richardson, Andrea Phillott, Hector Alonso Barrios-Garrido, Seh Ling Long, Zoe A. Meletis, Nancy FitzSimmons, Tyffen Read, Mariana Fuentes, Simona A Ceriani
Proceedings Compilers	Paul Whittock, Anton D. Tucker, Lisa Belskis

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## REGIONAL MEETINGS

Regional Meeting	Member
Africa	Manjula Tiwari, Angela Formia, Andrews Aguekumhene
Caribbean (WIDECAST)	Karen Eckert, Kate Charles
IUCN Marine Turtle Specialist Group (MTSG)	Roderic B. Mast
Indian Ocean & Southeast Asia (IOSEA)	Lalith Ekanayake, Zahirul Islam
Latin America Meeting (RETOMALA)	Daniela Rojas, Jaime Restrepo, Daniel Gonzalez-Paredes, Carmen Mejías Balsalobre, Jimena Gutiérrez
Mediterranean	Sandra Hochscheid, Yakup Kaska, Aliko Panagopoulou
Oceania/Pacific	Irene Kelly

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## BOARD OF DIRECTORS AND THEIR END OF TERM

Marc Girondot (2022)  
 Felix Moncada (2022)  
 Richard Reina (2023)  
 Andres Estrades (2023)  
 Neca Marcovaldi (2024)  
 Marco García Cruz (2024)  
 Sandra Hochscheid (2025)  
 Daniela Freggi (2025)  
 Stephen Dunbar (2025)  
 Kenneth Lohmann (past President in 2021) (2022)

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## **SPONSORS AND CONTRIBUTORS**

The International Sea Turtle Society gratefully acknowledges the generous financial support from the following organizations and individuals:

### **Gold Sponsor**

Australian Government Department of Agriculture, Water and the Environment (DAWE)

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Australian Institute of Marine Science (AIMS)

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### **Vendors**

Lotek  
CLS-Argos  
A&F Wildlife Foundation  
Care for Hedland

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## **ISTS AWARDS**

Chair: Hector Alonso Barrios-Garrido

Members: Ana Liria Loza, Mariluz Parga, Frank Paladino, Michael Salmon

### **Lifetime Achievement Award**

Alan Bolten

### **Champions Award**

None

### **President's Award**

Anna Vitenbergs  
North West Shelf Flatback Turtle Conservation Program

### **Grassroots Conservation Award**

Tetepare Descendants Association & Community Rangers on Rendova Solomon Islands: Critical nest protection of leatherback turtles on two remote islands in Solomon Islands

### **The Ed Drane Award for Volunteerism**

Barry Gilmore

## STUDENT AWARDS

There were 38 oral presentations and 31 poster presentations entered by students for the Archie Carr Student Awards. Presentations were assessed under one of four award categories (nominated by the student presenter): Biology Poster, Conservation Poster, Biology Oral, Conservation Oral. Judges for each category selected a winner and a runner-up.

The Perth, Australia symposium was held virtually due to the ongoing COVID-19 global pandemic. The platform and organization for the virtual meeting was designed to allow for student presenters to be available synchronously for questions at the end of each oral session and designated times for each of three poster sessions. This greatly helped facilitate the opportunity for students to answer questions, which is incorporated in the judging. The greatest challenge reported by Student Award judges was coordinating their schedules (judges were based in several different time zones) with the talk and poster sessions, making it difficult to attend all student presentations and ask them questions, as well as to meet virtually with other judges during the ranking process at the end of the symposium.

Student presentations in Perth/Online were judged by Amanda Southward Williard, Ana Caldas Patricio, Connie Kot, Cynthia Lagueux, Erin Seney, Hector Barrios-Garrido, Larisa Avens, Joe Pfaller, Kostas Papafitsoros, Natalie Wilderman, Nicole Esteban, Wendy Dow Piniak, Yakup Kaska, and Zoe Meletis. A judge's meeting was not scheduled during the symposium due to the challenges of scheduling; instead, judges communicated using WhatsApp, email etc at a convenient time. Unfortunately, Carlos Carreros and Rupika Rajakaruna were unable to view presentations during the symposium due technical challenges.

Award amounts: Winners = US\$500 each, Runners-up = US\$250 each. Total for all awards = US\$3,000.

Student Awards for Poster and Oral Presentations at ISTS40, Perth, Australia (virtual):

Category	Prize	Student	Institution	Presentation Title
Biology Poster (n=18)	Winner	Alexandra G. Gulick	University of Florida, USA	A mechanism for compensatory growth in Caribbean seagrass meadows grazed by green turtles
	Runner up	Emily Turla	Florida Atlantic University, USA	Investigating the relationship between cheloniid hatchling sex and growth rate in captive conditions
Conservation Poster (n=13)	Winner	Gisela Marín-Capuz	University of Barcelona, Spain	Analysis of the scientific production on sea turtles in Cabo Verde
	Runner up	Mar Izquierdo	University of Valencia, Spain	Factors affecting marine debris ingestion by loggerhead turtles ( <i>Caretta Caretta</i> ) in the Western Mediterranean
Biology Oral (n=20)	Winner	Samantha Elizabeth Trail	Florida Atlantic University, USA	Differences in visual perception are correlated with variation in seafinding behavior between hatchling leatherback ( <i>Dermochelys coriacea</i> ) and loggerhead ( <i>Caretta caretta</i> ) marine turtles
	Runner up	Renato Saragoça Bruno	University of Florida, USA	Reproductive dynamics of female green turtles ( <i>Chelonia Mydas</i> ) on the Miskito coast, Nicaragua, an important Atlantic foraging ground
Conservation Oral (n=18)	Winner	Chia-Ling Fong	National Taiwan Normal University, Taiwan	Citizen scientists reveal the distribution, abundance, and main threats to the foraging sea turtles in Taiwan
	Runner up	Larissa Rosalie Young	University of Queensland, Australia	Increasing male hatchling production using seawater irrigation

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## **OPENING REMARKS, PLENARY, AND SPECIAL SESSIONS**

### **OPENING REMARKS**

Kellie L. Pendoley, President, International Sea Turtle Society

### **WELCOME TO COUNTRY**

Mervyn (Nick) Abraham

### **THE IMPORTANCE OF THE ENERGY TRANSITION FOR PROTECTING BIODIVERSITY AND MITIGATING THE EFFECTS OF CLIMATE CHANGE**

Guest Speaker: Abigail Ross

*Fortescue Future Industries*

### **TURTLE CONSERVATION – PLAYING THE SHORT AND LONG GAMES IN A CHANGING WORLD**

Guest Speaker: Scott Whiting

*Department of Biodiversity, Conservation and Attractions (WA State Government)*

### **PRESENTATION**

Guest Speaker: Albert Wiggan

*Indigenous Leader, Environmental Consultant and Social-Emotional Wellbeing Officer*

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## **SPECIAL FEATURES**

### **SPEED CHATTING WITH THE EXPERTS**

**Chairs:** Alexandra Fireman, Gabi Arango, Matthew Ramirez, Renato Saragoça Bruno

**Panel:** Tomo Eguchi, Irene Kelly, Nicholas Pilcher, Nancy Fitzsimmons, Takahiro Shimada, Kei Okamoto, David Booth, Michael Salmon, Simona Ceriani, Brian Shamblin, Katherine Comer Santos

## **WORKSHOPS**

### **UNDERSTANDING AND QUANTIFYING CUMULATIVE AND SYNERGETIC STRESSORS TO SEA TURTLES**

Organizer: Mariana Fuentes

### **COMBATting GLOBAL MARINE TORTOISESHELL TRADE**

Organizers: Brad Nahill, Christine Hof, Michael Jensen, Alex Robillard

### **SINGLE SPECIES ACTION PLAN FOR THE LOGGERHEAD TURTLE (CARETTA CARETTA) IN THE SOUTH PACIFIC OCEAN**

Organizers: Karen Arthur, Duane March

### **ASSESSMENT OF THE CONSERVATION STATUS OF HAWKSBILL TURTLES IN THE IOSEA REGION – LAUNCH OF PUBLICATION AND INTRODUCTION TO TURTLENET**

Organizers: Mark Hamann, Heidrun Frisch-Nwakanma

### **LIGHT POLLUTION SOLUTIONS FOR SEA TURTLES**

Organizers: Karen Arthur, Cesar San Miguel, Rachel Tighe, Kate Hofmeister

### **BRIDGING THE GAPS: THE ASIA-PACIFIC MARINE TURTLE GENETIC WORKING GROUP**

Organizer: Alessandro Ponzo

### **HOW TO QUANTITATIVELY DESCRIBE CORRELATIONS BETWEEN SEA TURTLE MOVEMENT AND OCEAN SURFACE CURRENT**

Organizer: Yaoting Tseng

### **SEA TURTLE MEDICINE, REHABILITATION AND RESCUE WORKSHOP**

Organizer: Daniela Freggi

### **NEW PARTNERSHIPS TOWARDS AN EFFICIENT CONSERVATION OF SEA TURTLES ALONG THE ATLANTIC AND MEDITERRANEAN COASTS OF AFRICA**

Organizer: Alexandre Girard

### **THE ICAPO NETWORK: THE EASTERN PACIFIC HAWKSBILL RESEARCH AND CONSERVATION: CURRENT STATUS**

Organizer: Ingrid Yañez

### **STUDENT COMMITTEE WORKSHOP: HOW TO SUCCEED IN THE TURTLE WORLD AND BEYOND**

Organizers: Alexandra Fireman, Gabi Arango, Matthew Ramirez, Renato Saragoça Bruno

## ANATOMY, PHYSIOLOGY AND HEALTH

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### PHARMACOKINETICS AND PHARMACODYNAMICS OF A SINGLE DOSE OF INTRAVENOUS ALFAXALONE IN JUVENILE GREEN SEA TURTLES (*CHELONIA MYDAS*)\*

**Julie A. Balko<sup>1</sup>, Kristen M. Messenger<sup>1</sup>, and Craig A. Harms<sup>1,2</sup>**

<sup>1</sup>*North Carolina State University, College of Veterinary Medicine, Raleigh, NC, USA*

<sup>2</sup>*Center for Marine Sciences and Technology, North Carolina State University, Morehead City, NC, USA*

Alfaxalone is frequently used for sedation or anesthetic induction of sea turtles, yet the pharmacokinetics in this cohort remain unknown. Study objectives were assessment of the pharmacokinetics and pharmacodynamics of alfaxalone in green sea turtles (*Chelonia mydas*). Six juvenile sea turtles (4.2-8.5 kg) were prospectively enrolled in a randomized crossover study with a 7-day washout. Intravenous alfaxalone 3 mg/kg or equal volume 0.9% saline was administered into the external jugular vein and time to initial effects, resumption of movement, and resumption of spontaneous ventilation, ability to intubate, and serial heart and respiratory rates and sedation scores were collected. Following alfaxalone administration, serial blood collection was performed using a sparse sampling technique at 14 time points over a 24-hour period. Plasma was analyzed via ultra-high performance liquid chromatography for quantification of alfaxalone plasma concentrations. A nonlinear mixed-effects model was fitted to the data. Results demonstrated a half-life ( $T_{1/2}$ ) of 1.40 hr, a volume of distribution of 0.56 L/kg, and a clearance of 0.91 L/kg/hr. Following alfaxalone, median (range) time to initial effects was 22 (20-34) seconds. While peak level of sedation varied among turtles, all reached this milestone at the 2-minute mark. All turtles demonstrated absent spontaneous movement with median (range) time to resumption of spontaneous movement of 10.6 (5.6-13.1) minutes. Only 3/6 turtles permitted intubation and time to resumption of spontaneous ventilation was 10.8 (0.1-37.8) minutes. Three turtles exhibited transient, relative bradycardia following alfaxalone administration. This is the first report of alfaxalone pharmacokinetics in a sea turtle species.

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### ULTRASOUND IMAGING OF OVARIES AND EGGS IN LOGGERHEAD TURTLES (*CARETTA CARETTA*) IN THE ADRIATIC SEA (ITALY)

**Stefano Ciccarelli<sup>1</sup>, Delia Franchini<sup>1</sup>, Serena Paci<sup>1</sup>, Carmela Valastro<sup>1</sup>, Daniela Freggi<sup>2</sup>, Pasquale Salvemini<sup>3</sup>, Davide Bellomo<sup>1</sup>, and Antonio Di Bello<sup>1</sup>**

<sup>1</sup>*University of Bari "Aldo Moro", Italy*

<sup>2</sup>*Lampedusa Turtle Rescue, Italy*

<sup>3</sup>*WWF Molfetta Rescue Center, Italy*

Reproductive anatomy of female loggerhead sea turtles (*Caretta caretta*) has been difficult to study. The main method for evaluation of the coelomic cavity in both adult and juvenile female sea turtles is celioscopy. In this preliminary study, ultrasound scans were performed in 60 subjects of *Caretta caretta*, admitted to the Sea Turtles Clinic (S.,TC) at the Department of Veterinary Medicine in Bari (Italy) for veterinary evaluation, with a curved carapace length (CCL) greater than 65 cm in the period between September 2020 and January 2021. All turtles examined were placed in dorsal recumbency on foam mattress. The ultrasound was performed through the right and left inguinal fossae and the cranial pole of

the kidney was identified as a landmark to identify the oviduct and the ovary. Preliminary results showed that subjects with a curved carapace length (CCL) less than 70 cm presented no follicular development and only the oviduct could be identified with values ranging from 2 to 5 mm. On the other hand, in sea turtles > 73 cm of CCL it was possible to identify large vitellogenic follicles (from 18 to 25 mm in diameter). Ultrasonography showed advantages because is non-invasive and did not require anesthesia. By evaluating ovarian morphology without laparoscopy approach, this non-invasive technique allows to monitor the reproductive season of these animals in order to accurately study their reproduction. Accurate measurements of follicles and eggs allow a turtle's reproductive status to be monitored at frequent intervals but only follicles larger than 3 mm in diameter could be measured. This would limit the use of ultrasonography in large immature female sea turtles where ovarian maturation is not complete. Moreover, the positioning of the sea turtle is essential to have a good visualization of the oviduct and other genital structures that may be hidden by the intestinal tract.

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### **SEVERE PNEUMOCELOMA ASSOCIATED WITH FISHING LINE CROSSING THE DIGESTIVE CANAL IN THREE LOGGERHEADS (CARETTA CARETTA)**

**Antonio Di Bello<sup>1</sup>, Stefano Ciccarelli<sup>1</sup>, Carmela Valastro<sup>1</sup>, Serena Paci<sup>1</sup>, Francesco Caprio<sup>1</sup>, Daniela Freggi<sup>2</sup>, and Delia Franchini<sup>1</sup>**

<sup>1</sup>*Department of Veterinary Medicine, University of Bari, Italy*

<sup>2</sup>*Lampedusa Turtle Rescue, Lampedusa (Ag), Italy*

Longline bycatch is one of the main causes of sea turtle deaths in the Mediterranean Sea. The most serious injuries are caused by the fishing line, which is nearly always eaten and causes damage to the digestive canal or entanglement of the flippers and/or neck if the fisherman cuts it too long and abandons the turtle in the sea. When the hook is placed into the esophagus and the fishing line goes along the digestive canal it can come out of the cloaca. In some circumstances, peristalsis causes a plication of the whole digestive tract, resulting in ischemia, intussusception, lacerations, necrosis, and the turtle's death. In last five years 107 loggerhead turtles were admitted to the Sea Turtle Clinic of the University of Bari's Department of Veterinary Medicine presenting severe digestive tract lesions caused by ingestion of fishing line. For three of them a very severe pneumoceloma was concurrently found. After a clinical evaluation, the leakage of a fishing line from the cloaca was evident and radiographic examinations revealed the presence of a hook embedded in the wall of the cervical esophagus. In Addition, a severe pneumoceloma determined the crushing of both lungs dorsally, against the inner surface of the carapace. The overdextension of the ligaments between the liver, the lungs, and the digestive canal was clearly highlighted by the radiographic contrast caused by the abundant gas in the coelomic cavity; additionally, the intestine appeared pulled in the center of the coelomic cavity due to the tension of the fishing line. An endoscopy of the coelomic cavity through the left inguinal fossa was performed during multiple surgeries to remove foreign bodies from the esophagus and intestine. This revealed in all three cases a rupture of the left lung in correspondence of the insertion point of the pneumocolic ligament, which was completely detached. The lesion was covered by extensive granulation tissue in two cases, which had closed the lung rupture; however, in one case, there was evidence of anesthetic mixture leaking during ventilation. An esophagogastric tube was implanted after the foreign body was removed to guarantee appropriate feeding during recuperation. Weekly radiological tests were undertaken in the postoperative term to confirm the condition of the pneumoceloma, pending full spontaneous healing of the pulmonary rupture. Between 20 and 50 days after surgery 2 or 3 centesis of the coelomic cavity for air aspiration was performed to accelerate pneumoceloma reduction. Within 4 to 6 months of surgery, all three turtles had recovered from their pneumoceloma and were released back into the wild. Endoscopic evidence and tests on carcasses simulating what can happen when a linear foreign



body is dragged across the intestine lead us to believe that if a fishing line is ingested and crosses the entire intestine, the lung can rupture due to detachment of the pneumocolic ligament and subsequent pneumoceloma, which is a rare but serious complication.

---

## IS DROWNING OR GAS EMBOLISM THE LEADING CAUSE OF DEATH OF TRAWLED TURTLES?

**Delia Franchini<sup>1</sup>, Stefano Ciccarelli<sup>1</sup>, Serena Paci<sup>1</sup>, Carmela Valastro<sup>1</sup>, Francesco Caprio<sup>1</sup>, Pasquale Salvemini<sup>2</sup>, and Antonio Di Bello<sup>1</sup>**

<sup>1</sup>*University of Bari, Italy*

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Incidental captures of sea turtles in fishing gear contribute significantly to the mortality of sea turtles. According to several studies, direct mortality due to trawling depends on tow duration and hence to the submergence time, with high mortality rates when apnoea is prolonged. Drowning can result when turtles have been held underwater after capture in fishing gear. More recently loggerhead turtles entrapped during fishing trawls or in gillnets have been found to develop gas embolism (GE) which may lead to severe organ injury and even mortality. Between January 2016 and December 2021, 1445 loggerhead sea turtles incidentally captured (bycaught) in trawls were admitted to the Sea Turtles Clinic (STC) at the Department of Veterinary Medicine in Bari (Italy) for veterinary evaluation after having been taken to the local Adriatic Sea turtle rescue centre (World Wildlife Found, WWF, Molfetta). The duration from surfacing (time of capture on fishing boat) until the animal arrived at the STC ranged from 5 to 7 h. Turtles were mainly incidentally captured (bycaught) in trawls in the winter months, with the highest incidence in December and January. We evaluated all the turtles bycaught both live and dead. Immediately upon admission, physical and radiographic examinations of each turtle were performed. All radiographs were assessed to evaluate signs of drowning, characterized by interstitial and peribronchial thickening, and the presence of GE within the cardiovascular system. In all turtles with radiographic signs of GE, we evaluated and listed the cardiovascular sites (CAS) where the gas was clearly detectable. The main objective of the study was to evaluate the percentage of animals that died from GE and/or drowning to understand the definitive cause of death in trawled turtles. Of 1445 trawled turtles, 540 (37.4%) showed radiographic signs of systemic GE of variable severity, of which 147 (27.2%) also showed radiographic signs of drowning. Of the 540 subjects with signs of GE, 102 (19%) died and of the 147 turtles with GE and signs of drowning, 67 (46%) died. By contrast, of the 905/1445 (62.6%) turtles without radiographic signs of GE, 30 (3.3%) showed radiographic signs of drowning, however, none died. We found 14 CAS where gas could be detected. In the univariate analysis, radiographic drowning signs of GE-affected turtles emerged as a statistically significant factor for increased risk of death. However, in the multivariable analysis, radiographic drowning signs were not a risk factor for death, while the number of the CAS (>12/14) and the presence of GE in the left atrium and sinus venosus/right atrium were the only risk factors. According to the data it can be deduced that trawled sea turtles rarely undergo drowning without also suffering from GE and that mortality is closely associated with the presence of drowning contextually to GE. Specifically, we can therefore hypothesise that the turtles probably first undergo severe GE involving the heart then subsequently drown. We therefore hypothesise that the leading cause of death of turtles caught in trawling gear is severe undiagnosed GE and not water aspiration associated with forced submergence.

## COMPARATIVE STUDY OF MITOCHONDRIAL DENSITY IN THE PECTORAL MUSCLE OF GREEN SEA TURTLES (*CHELONIA MYDAS*) AND DIAMOND-BACKED TERRAPINS (*MALACLEMYS TERRAPIN*).

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Sea turtles are fully aquatic turtles that migrate long distances between breeding and foraging grounds, and their capacity for these migrations are unique among reptiles. In comparison to estuarine or freshwater turtles, sea turtles may display differences in muscle morphology and capacity for sustained activity reflective of enhanced aerobic metabolism. Mitochondrial density within muscle tissues is a reliable indicator of the aerobic capacity. The goal of this study was to compare mitochondrial density in the swimming muscle of green sea turtles (*Chelonia mydas*) with diamond-backed terrapins (*Malaclemys terrapin*), a member of the Emydidae family. Skeletal muscle samples were collected from stranded, cold-stunned green sea turtles and diamond-backed terrapins that were incidentally captured in crab pots in North Carolina. Relative mitochondrial density was assessed by sectioning muscle samples, mounting them onto microscope slides, and staining for succinate dehydrogenase (SDH); SDH staining intensity was used as an index of mitochondrial density. I hypothesized that sea turtle muscle would exhibit higher mitochondrial density compared with diamond-backed terrapin muscle, which would be indicative of enhanced aerobic capacity beneficial for the long-distance migrations undertaken by sea turtles. The SDH staining intensity in green sea turtles ( $62.69 \pm 42.41$ , 110.6) and diamond-backed terrapins ( $73.70 \pm 40.67$ , 99.02), was not statistically significant when compared by Student's t-test ( $P = 0.32$ ,  $t = -0.46$ , d.f. = 10). Further studies to assess how seasonal variation in activity patterns and migratory status, as well as differences in the handling and processing of samples, affect measurements of mitochondrial density are warranted. Both sea turtles and diamond-backed terrapins are the focus of conservation efforts. Knowledge of their metabolic strategies under variable environmental conditions may provide insight into how they might respond behaviorally and physiologically to climate change.

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## MYCOBACTERIUM TUBERCULOSIS INFECTION IN A FREE-RANGING GREEN TURTLE (*CHELONIA MYDAS*), BRAZIL

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Mycobacteriosis is a well-known disease of reptiles and have been reported in a variety of species. Of the few published reports of mycobacteriosis in sea turtles, most have been in captive-reared animals. An 11 kg juvenile green turtle (*Chelonia mydas*) (curved carapace length of 44.0 cm, and curved carapace width of 40.2 cm) was found stranded on March 16, 2020, in the city of Rio de Janeiro (lat: -22.91972; long: -

43.16995), Southwestern Brazil. The green turtle was rescued in Marina da Gloria Bay, one of the most urbanized and polluted areas in Rio de Janeiro. Physical examination revealed signs of weakness, anemia (PCV of 17%) and hypoproteinemia (Total protein: 0.60 g/dL). The turtle was in good body condition and exhibited multiple cutaneous fibropapillomas covering almost 60% of its body. Blood samples were collected from the dorsal cervical sinus for hematological examination and microbiological analysis. Colloid IV fluids (20 mL/kg) were administered to maintain a high osmotic pressure in the blood vessels. Then, an intravenous amino acid solution (Aminoven®) (13 mL/kg) was administered to correct hypoproteinemia. Two days after arrival, the turtle was placed in a 3000L tank one quarter-filled with fresh water, maintained at 27°C. The water level was gradually increased; however, the turtle was still lethargic. A variety of food items (e.g., fish, shrimp, squid) were offered, however the turtle did not eat on its own and, despite that, a slight clinical improvement was observed. Despite continued medical therapy, the animal succumbed to death 60 days after initial supportive care. Necropsy revealed multiple cutaneous fibropapillomas, granulomatous pneumonia, hydropericardium, small granulomas in the myocardium, liver, spleen, thyroid and brain and caseous material in the meninges of the brain. The histopathological analysis evidenced severe heterophilic meningitis, granulomatous splenitis with acid-alcohol-resistant bacteria (Ziehl Neelsen stain) and parasitic eggs, granulomatous pneumonia with hyphae and parasitic eggs, heterophilic hepatitis, kidney necrosis and tubular degeneration, granulomatous myocarditis and heterophilic adrenalitis. Post-mortem exams revealed systemic mycobacteriosis, which was further associated with *Mycobacterium tuberculosis* identified, at first, in blood culture and, after, by PCR amplification of DNA extracts from frozen tissue samples obtained from different organs, including liver, heart, lungs, kidneys, spleen, pancreas, brain, adrenals, thyroid, thymus and salt glands. The pathological findings were suggestive of septic shock as the main cause of death. The presence of intravascular trematode eggs in multiple organs is consistent with spirorchidiasis, which could have been a contributory cause for the turtle's death. Additionally, the animal had a concomitant fungal infection. To our knowledge, this is the first description of a disseminated infection caused by *Mycobacterium tuberculosis* in a free-ranging sea turtle. The source of this *Mycobacterium* infection remains unknown, but it could be due to waste dumping or leakage from landfill sites. Specific identification of *Mycobacterium* species associated with disease in wild sea turtles is important to understanding infections in free-ranging sea turtles and continued documentation of these cases will increase knowledge and understanding in caring for these chelonians. It is worth noting that tuberculosis is an important zoonotic disease.

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## THE 'POOPCORN' EXPERIMENT. AN APPROACH FOR DETECTION OF DIGESTIVE MOTILITY DISORDERS, ASSESSING GASTROINTESTINAL TRANSIT TIMES IN MARINE TURTLES\*

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The ingestion of anthropogenic marine debris can lead to injuries in the digestive system of marine turtles through blockages, lacerations and enteritis, as well as sub-lethal effects from bioaccumulation of adhered chemicals and toxic substances leached out into tissues and blood. The early detection of these impacts is central for the treatment and recovery of turtles in a rehabilitation setting. In this study, we provide baseline data on gastrointestinal transit times in healthy green turtles (*Chelonia mydas*) to enable non-intrusive detection of digestive motility disorders. We conducted two experiments with juvenile green turtles (N=14)

(curved carapace length range 33.7–47.0 cm) using inorganic and organic markers in order to estimate gastrointestinal transit times and assess the effectiveness of each marker type in recording them. The inorganic marker trial (IMT) was conducted over six green turtles (n=6) intentionally caught for scientific purposes from Uruguayan waters using scientific capture techniques. On the first day of the trial, each turtle was given five purple markers made from 7 mm diameter discs of polypropylene (Alfepa Ltd., Uruguay). The organic marker trial (OMT) was conducted over eight green turtles (n=8), 32-months post-hatching, originating from Heron Island (Queensland, Australia) and kept in captivity for scientific purposes in the Turtle Health Research Facility at James Cook University (Queensland, Australia). On the first day of the trial, each turtle was fed 15 pre-cooked corn kernels (Coles Group Ltd. Australia), as organic markers, in batches of five with other food. Turtles were monitored by veterinarians during the trial periods in order to detect any anomalies in activity and feeding behaviour. The monitoring tanks were checked several times each day for faeces collection. The presence and quantity of markers in each faeces were recorded. Gastrointestinal transit times were recorded as the time between the ingestion and expulsion of the markers. After completing the trials, all turtles were released to the marine environment. Gastrointestinal transit times for the IMT trial group ranged from  $14.6 \pm \text{SD } 3.6$  days for the first markers recovered to  $22.5 \pm \text{SD } 4.2$  days for the last markers recovered. The corresponding data for the OMT group ranged from  $6.63 \pm \text{SD } 1.6$  days to  $17.3 \pm \text{SD } 3.3$  days respectively. We obtained 96% recovery success of markers in the inorganic marker trial versus 72.5% in the organic marker trial. Thus, inorganic markers proved to be more efficient in reporting gastrointestinal transit times because they do not degrade or discolour as they pass along the digestive process, enabling higher recovery success. Opportunistically, veterinarians diagnosed an obstruction caused by plastic fragments, which had been swallowed in the wild prior to the trial, in one of the experimental animals after we failed to recover any markers. This incident is evidence that gastrointestinal transit time assessment is a useful approach for providing early warning of digestive system blockages. Furthermore, this knowledge on transit times could be of interest for toxicology studies regarding exposure to chemicals lixiviated from debris ingested, as an index of the time spent by these substances inside the organism.

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## **DEVELOPMENTAL STAGES AND CONGENITAL MALFORMATIONS OF DEAD EMBRYOS AND HATCHLINGS IN HATCHERY RELOCATED CLUTCHES OF GREEN TURTLES (CHELONIA MYDAS) IN SRI LANKA\***

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Many hatcheries are established in Sri Lanka as an ex-situ conservation tool, although their contribution towards conservation of sea turtles is debated. Hatchery managers purchase eggs from egg collectors and incubate the eggs in man-made nests at the hatchery and until the hatchlings emerge. The present study investigated embryonic stages and the types of malformations in the failed green turtle eggs. One of the oldest hatcheries on the southwestern coast in Bentota South was selected as the study site. The number of eggs in each clutch was recorded upon reburial and hatching and emergence success were calculated once the hatchlings emerged. Dead hatchlings and unhatched eggs were counted and preserved in 10% formalin. Data was gathered from 30 relocated nests and 1,768 eggs. An emergence success of 90.3% and hatching success of 90.0% were recorded. Preserved eggs (n=161) were opened and embryos categorized according to their developmental stages. Among them, 67.0% of the embryos had no visible signs of embryo development, 2.4% were developed to stages 17-20, 3.1% to stages 21 -25, 21.7% to stages 26 -29, and 5.6% were developed to stage 30 (pipped; within shell). We also examined 11 dead hatchlings (fully

developed; emerged from the eggshell). Congenital malformations were observed in embryos at stages 26 - 30 and dead hatchlings. Of the total 15 malformed cases, 73.3% exhibited scute malformations, 20.0% had leucism, and 6.6% demonstrated anophthalmia. The results indicate that embryonic mortality in hatchery relocated Green Turtle eggs are highest in early and then late development stages. The most common malformations, in scutes, could be the result of high temperature and low moisture availability during incubation in hatchery enclosures.

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## ASSESSING THE 30-YEAR TEMPORAL CHANGES IN HEAVY METALS IN THE NORTHWEST PACIFIC OCEAN BY ANALYZING SAMPLES FROM GREEN SEA TURTLES

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To measure heavy metal pollution, it is necessary to assess the spatiotemporal trends of heavy metals. Sea turtles are known to accumulate pollutants, including heavy metals, from their food and the surrounding environment. Thus, they are considered as potential bioindicators of regional heavy metal pollution. In addition, if historical data on heavy metal concentrations in turtle populations is available, current sea turtle samples can be used for better understanding of the temporal changes in heavy metal pollution. The Ogasawara islands are known to be an important rookery for green sea turtles (*Chelonia mydas*) in the Northwest Pacific population. In these islands, adult green sea turtles are traditionally captured for food during their mating period under the Fisheries Adjustment Rules of the Tokyo Metropolitan Government. The heavy metal concentrations of the adult turtles in this population collected in 1990 were previously reported. In the present study, we measured the heavy metal concentrations (Fe, Mn, Zn, Cu, Pb, Ni, Cd, and Co) of the tissues (liver, kidney, muscle, and yolk) from adult turtles collected in 2020 (male: n = 8; female: n = 8) to assess the 30-year temporal changes in heavy metals in the Northwest Pacific Ocean. A comparison between the heavy metal concentrations in the present study and those in 1990 revealed differences in the temporal changes of some elements between male and female turtles. This is assumed to be due to physiological and/or ecological sex differences such as metabolism, foraging habitat, and migration route. In addition, in samples from both sexes, the concentrations of Pb in the liver were notably increased from 1990 to 2020, whereas the concentrations of Cu in the kidney, and Cu and Mn in the muscle were notably decreased from 1990 to 2020. Therefore, results suggested that Pb levels increased from 1990 to 2020, whereas Mn and Cu levels decreased in the migration area of green sea turtles in the Ogasawara population. Pb is one of the heavy metals that is of particular toxicological concern. Thus, the toxicological risk to green sea turtles in the Northwest Pacific population has increased in the last 30 years, and it is necessary to investigate whether the current Pb level has detrimental effects on animals in this area. To better understand the spatiotemporal trends of heavy metal pollution, global and temporal collection of sea turtle samples is highly recommended. Such analysis could make an important contribution to the global assessment of marine pollution.



## POTENTIAL USE OF HOST BIOMARKERS FROM TUMOR BIOPSIES FOR PREDICTING THE OUTCOME OF SEA TURTLE PATIENTS WITH FIBROPAPILLOMATOSIS\*

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Sea turtle fibropapillomatosis (FP) is an epizootic tumor disease that has been found globally affecting all seven species of marine sea turtles. This disease presents itself with characteristic tumors that can be found both externally and internally and is often associated with chelonid alphaherpesvirus 5 infection. External tumors can be surgically removed, or in some cases, resolve on their own. However, the detection of internal tumors is fatal as there are currently no known treatments and patients are often humanely euthanized. Upon intake of these FP sea turtles, many require extensive rehabilitation which usually involves surgical removal and complicated clinical cases involving months of husbandry and medical attention which is costly. The goal of this study was to provide a secondary diagnostic tool (reverse transcription quantitative polymerase chain reaction, RT-qPCR) that could be utilized at patient intake to predict how well a patient would survive/recover from rehabilitation efforts resulting in either release or euthanasia. Such prognostic molecular biomarkers are routinely employed in human oncology to aid treatment decisions. We developed real-time TaqMan RT-qPCR gene expression assays to predict patient outcome based on 10 potential predictive host biomarkers. These biomarkers were chosen based on results of our previous study (Kelsey et al., *Comms Bio.*, 4, 2021) that consistently found these putative biomarkers to have high gene expression in released animals, and low gene expression in euthanized and died in care animals. To further test the potential use of these biomarkers as a diagnostic tool, an archive of external tumor samples representing 57 patients that were released or euthanized from 2015 to 2021 at the Whitney Sea Turtle Hospital in St. Augustine, Florida was used. Preliminary results showed that these novel RT-qPCR assays may be useful biomarkers to predict the outcome of FP patients, particularly the assay detecting proteasome subunit alpha type-6 (*PSMA6*) and cyclic GMP-AMP synthase (*cGAS*) expression. This study provides preliminary evidence that qPCR can be used as a prognostic tool to help direct rehabilitation efforts for FP patients. Such biomarkers may also hold diagnostic value as a secondary prognostic or screening tool to current diagnostic methods of internal tumor detection. qPCR is advantageous due to its quick turn-around time, minimal invasiveness, and low cost.

## **STOWAWAYS – MARINE LEECHES INFECTING OLIVE RIDLEY SEA TURTLES ENTANGLED IN GHOST NETS**

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Sea turtles are known to host a wide variety of organisms, including parasitic marine leeches of the family Ozobranchidae. Leeches are sanguivorous (blood feeders) and are subsequently found on soft skin areas of turtles, such as the cloaca and corners of eyes and mouth. Superinfection with this parasite can lead to severe damage to the host, for example sea turtle leech erosion disease (SLED). Turtles suffering from SLED can show severe disfigurement to erosion of skin, muscle and occasionally bone tissue (Bunkley-Williams et al. 2008). Ozobranchidae have been shown to be a potential candidate as a mechanical vector for the tumour inducing fibropapilloma-associated turtle herpesvirus (Greenblatt et al. 2004). Marine Ozobranchidae have been found in all major ocean basins infecting all hard-shelled sea turtle species. The primary focus of previous investigations has been in the Atlantic and Pacific Oceans. Records from the Indian Ocean are scarce but indicate a widespread distribution with specimens documented in Western Australia, Reunion, Seychelles and India (Baird 1869, Sanjeeva Raj 1959, Reme 1980, Göpper et al. 2018). In this study, we present the first cases of ozobranchid leeches found on sea turtles in the Maldives. The host turtles were entangled in abandoned, lost or discarded fishing nets, so called ghost nets, and treated at the Olive Ridley Project's Marine Turtle Rescue Centre in Baa Atoll in the Maldives. Both hosts were Olive Ridley turtles (*Lepidochelys olivacea*), which are typically not resident to the country, but are often observed entangled in ghost nets. The entangled turtles are thought to be carried over great distances when floating in various ocean currents. Ozobranchid leeches were identified to the species level with morphological and barcoding methods (Davies 1978, McGowin et al. 2011). The presence of parasitic leeches on entangled turtles may have epidemiological implications for the endemic populations of sea turtle species throughout the Indian Ocean.

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## **CALCIUM OXALATE, GREEN TURTLES, AND TURTLE GRASS: WHAT ARE THE INTERACTIONS?\***

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*Chelonia mydas* (green turtles) and *Thalassia testudinum* (turtle grass) have co-evolved for thousands of years, but the interrelationship is not well understood. *Thalassia testudinum* has responded to simulated and natural grazing by *C. mydas* with compensatory growth and increased nitrogen, phosphorus, and energy

content in leaf tissue. However, it is not known if *T. testudinum* develops defensive mechanisms to deter herbivores. Anti-herbivore responses can be physical or chemical and are well studied in terrestrial ecosystems. An increase of oxalates, whether insoluble calcium oxalate crystals or oxalic acid, is a known anti-herbivore response in plants. Calcium oxalate crystals have been documented in *T. testudinum*. However, oxalates have not been quantified in *T. testudinum* tissue nor evaluated in relation to *C. mydas* grazing. High intake of soluble oxalate is correlated with animal health issues such as renal oxalosis, or kidney stones. *C. mydas* recovered from Florida and Costa Rica have been diagnosed with renal oxalosis by histopathological examination, with varying levels of severity in crystal concentration. This study asks: Does *C. mydas* grazing induce the production of oxalates in *T. testudinum*, is there a seasonal effect on these oxalate concentrations, and what proportion of oxalates are degraded by the microbial fermentation in the hindgut of green turtles? We experimentally simulated *C. mydas* grazing by clipping *T. testudinum* blades for 16 months in The Bahamas. We quantify total oxalate concentrations in leaves from 10 clipped and 10 unclipped plots at months 0 and 11, as well as in leaves from unclipped plots from months with the highest and lowest water temperatures. We distinguish between soluble and insoluble oxalates to understand if oxalates in *T. testudinum* function primarily as a physical or chemical anti-herbivore response. We quantify oxalate concentrations in green turtle feces and hindguts and calculate apparent digestibility. We predict that (1) total oxalate concentrations will increase over time in simulated grazing plots and not increase in ungrazed plots, (2) there will not be a seasonal effect on oxalate concentration, (3) oxalates will mostly be insoluble, and (4) oxalates will be digestible by *C. mydas*. Studying responses of *T. testudinum* to *C. mydas* grazing will support more informed decisions when conserving an endangered species and its declining habitat. Understanding the interactions of oxalates in *T. testudinum* consumed by *C. mydas* will contribute to the knowledge of this intricate relationship.

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## MYSTERIES OF A MEDITERRANEAN LOGGERHEAD: A CASE STUDY ACCUSING HUMANS OF PLASTIC POLLUTION

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Plastic is recognized as a major pollutant of the marine environment, representing a serious threat to ocean wildlife. Plastic can often lead to several environmental problems and is especially dangerous for marine animals that can become entangled or accidentally ingest these items while feeding. Once ingested, plastic items can cause a variety of issues. Digestive tracts can become blocked, which can lead to starvation and death. Plastic blocking the gut which to an extent can plausibly be considered lethal, may appear relatively infrequent: 78.7% of our animals expel plastic during their convalescence, but only 5.4% of all autopsies performed showed plastic presence in the digestive tube responsible for their death. At the end of the 2019 summer, a young loggerhead was found around Lampedusa floating and struggling to dive. The body showed an old deep scar in the rear carapace and a big deformation in the rear plastron. Soon after it was brought to our rescue centre for rehabilitation, it was discovered that it was tagged by Spanish colleagues in 2014, when it was rescued for an old injury on the rear carapace. The evident debilitated condition was confirmed by a blood test, showing severe anaemia, low protein and albumin and high levels of white cells. The flotation, with an inclination of 40 degrees, highlighted a problem to the rear part of the body where the tail presented an intussusception, inhibiting the possibility to expel faeces. Radiograms couldn't show the lower part of the body, where a white spot was blocking details of the body. The animal showed low interest in the food. We decided to perform inguinal access to understand the situation of the digestive tract: the surgery allowed us to reach what we believed to be a tract of the intestine, from where we removed



more than 5 kg of faeces, full of an astonishing number of plastic debris. After the surgery, once back in the tank, the loggerhead showed a regular flotation and an increased interest in the food, but 5 days later the patient died. We performed a necropsy that highlighted an incredible situation: close to the cloaca, the body had built a new chamber to store faeces that couldn't be expelled, due to the injury that blocked the cloaca opening. The space reached by the surgeon, and considered intestine, in reality, was a pocket where more than 2 kg of faeces and plastic debris were stored! The formation of that chamber had deformed the plastron. We analysed the plastic debris, and we counted the stunning number of 505 pieces of plastic waste, ranging from rigid pieces of 5-8 cm to those soft and of few millimetres, showing once more how detrimental plastic waste is on wildlife health, and how high the price is in terms of the pain and lives that sea turtles have to pay for human ignorance and negligence.

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## CONCURRENT PAPILLOMAVIRUS-HERPESVIRUS INFECTION IN GREEN TURTLE FIBROPAPILLOMATOSIS TUMOURS\*

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Fibropapillomatosis (FP) is a pandemic debilitating disease of sea turtles, afflicting mostly juvenile green turtles. This cancerous disease was believed to be caused by Chelonid alphaherpesvirus 5 (ChHV5), however recently green turtle papillomavirus 1 (CmPV1) was also proposed to have a potential role in the development of this disease. Papillomaviruses cause benign to malignant cancers in humans and animals and the co-infection of papillomaviruses and herpesviruses in human and even marine animals such as dolphins has been reported before. In this study we have followed precision wildlife medicine. We have developed sensitive quantitative PCR (qPCR) and digital droplet PCR (ddPCR) detection and quantification techniques for CmPV1 and ChHV5 from sea turtle tumours, and optimised transcriptomic and genomic profiling of these tumours for both host and viral gene detection. We used the advances of Oxford nanopore sequencing to better characterise the viruses and their interaction with host such as possible CmPV1 integration sites. We identified CmPV1 in a high proportion of tumoured animals in Australia (51.7% out of 89 tumoured turtles), and at a slightly lower prevalence in the USA (33% out of 31 turtles tested so far). In both Australia and USA, CmPV1 was found along with ChHV5 in tumours and skin biopsies of FP-afflicted turtles. In USA, CmPV1 was found in tumours of green turtles, but also in marine leeches and tumours of two sea turtle species in which PV1 has not previously been reported: Kemp's ridley (*Lepidochelys kempii*) and Olive ridley (*Lepidochelys olivacea*). CmPV1 was also detected from green turtle internal tumor samples for the first time. CmPV1 was previously ruled out as a potentially aetiological agent of sea turtle FP, due to a lack of detection. However, the advent of ultra-sensitive qPCR and ddPCR assays reveals that CmPV1 is present within a high proportion of FP tumours. ChHV5 expression within tumours was predominantly latent, suggesting that cooperation between ChHV5, CmPV1 and other viruses may be responsible for continued FP tumour growth. Our ongoing research is investigating the contribution of CmPV1, ChHV5 and other viruses to tumour development, the interactions between these two potentially oncogenic viruses and the resulting effects on oncogenic signaling with hosts.

## **A NEW APPROACH TO AN OLD QUESTION: EVALUATION OF ECOLOGICAL BREEDING STRATEGIES IN RIDLEY SEA TURTLES\***

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Sea turtles have been traditionally classified as capital breeders, meaning they store energy (e.g., lipid) prior to the breeding season that they draw from to fuel reproductive effort through the end of nesting. However, the Ridley sea turtles (Genus *Lepidochelys*) have several life-history traits suggesting that they may be using an alternative, income breeding strategy in which feeding continues during nesting. We examined whether physiological approaches, including lipid and hormone measurements, can help elucidate nutritional state of olive ridleys nesting in the wild. Moreover, we evaluated the usefulness of these measurements to assess nutritive resource allocation in the critically endangered Kemp's ridley sea turtle. Reproductively-active turtles, including mating couples and mass-nesting females, were sampled in Ostional, Río Oro in Costa Rica. Females were recaptured during subsequent mass-nesting events to monitor blood and ultrasound profiles during the reproductive season. We measured the egg yolk-precursor vitellogenin (VTG) to measure reproductive lipid utilization over the course of the season. Additional sampling efforts were conducted along solitary nesting beaches further south, including Playa Hermosa and Río Oro when mass-nesting events were not occurring in Ostional. VTG concentrations remained elevated until final nesting, indicating a demand for lipid throughout the season. Subcutaneous fat width (SFW), determined by ultrasound, and blood beta-hydroxy-butyrate (BHB) are potentially valuable metrics for fat mobilization in sea turtles. SFW remained unchanged whereas BHB significantly increased over time in mass-nesting females. In contrast, SFW declined and BHB remained low in solitary-nesting females. Solitary females also showed a significantly higher body condition index and wider egg circumference than mass nesters. Preliminary measurements of the orexigenic hormone ghrelin have indicated it may also provide a helpful indicator of feeding status. These results suggest that an income breeding strategy may be used in mass-nesting *Lepidochelys olivacea*, and solitary females may use a different strategy. Further application of physiological metrics can help elucidate how Ridley sea turtles fuel reproductive effort.

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## **FIRST RECORD OF ARRHYTHMIA ASSOCIATED WITH OVIPOSITION REVEALED BY NON-INVASIVE HEART RATE MONITORING OF GRAVID LOGGERHEAD TURTLE\***

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Being adapted to marine life, nesting behaviours on land seems to be physically and physiologically demanding for sea turtles. Heart rate measurement is widely used to examine physiological condition of study animals. Using recently developed non-invasive heart rate measurement method, here we monitored

heart rate of a free-moving gravid loggerhead turtle throughout her nesting activities (ascent, body-pitting, digging, egg laying, covering, camouflaging, descent). A small accelerometer was also deployed to the turtle to measure activity. Heart rate ( $f_H$ ) significantly increased when the turtle landed (underwater,  $f_H = 13.1 \pm 5.8$  bpm, temp =  $27.9 \pm 0.1^\circ\text{C}$ ; on land,  $f_H = 29.1 \pm 5.2$  bpm, temp =  $26.7 \pm 0.1^\circ\text{C}$ ;  $p < 0.05$ ). While on land,  $f_H$  was positively related to dynamic body acceleration (DBA), the index of activity calculated from 2-axis acceleration (Spearman's rho = 0.79,  $p < 0.05$ ).  $f_H$  were constantly high during ascent, body-pitting and digging (30.7 – 32.7 bpm) but it suddenly dropped with the start of egg laying. While laying eggs,  $f_H$  remained low ( $21.0 \pm 5.9$  bpm) with increased R-R interval as well as increased R-R interval variability, indicating the occurrence of arrhythmia. It might be possible that decreased  $f_H$  during oviposition is a physiological reflex response controlled by parasympathetic nervous system as previously reported in spawning salmon.

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## PROGNOSTIC EVALUATION OF HEAD INJURIES IN 27 SEA TURTLES (CARETTA CARETTA)

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In sea turtle traumatic injuries can occur from a wide range of causes, mainly those caused by human activities. Skull trauma may result in reversible or irreversible neurological deficits, resulting in disorientation, debilitation, buoyancy disorder and difficulty in spontaneous feeding. Sea turtles may die under some circumstances due to direct impact of trauma, severe bleeding, or infection from exposed brain tissue. The majority of sea turtles with head trauma are found stranded or less frequently, accidentally trawled or rescued at sea in severe debilitation and poor physical condition. In the present study, 27 alive loggerhead sea turtles (*Caretta caretta*) out of 1755 admitted to the Sea Turtles Clinic (STC) at the Department of Veterinary Medicine in Bari (Italy) for veterinary evaluation, were assessed due to different degrees skull lesion. On admission, physical and neurological evaluation were performed to assess and grade the lesions and neurological deficits. According to the extent, depth, bone, and compromise of underlying soft tissues with brain exposure, a score from 1 (mild) to 3 (severe) was assigned for skull lesions. Neurological and sensory deficits were recorded, and a score was given from 1 (alert) to 3 (lethargic/comatose). Based on the severity of head injuries, the scores of the 27 sea turtles were: (1) mild in 4 turtles, (2) moderate in 6, and (3) severe in 17. Neurological examination performed on all 27 sea turtles showed that the mentation state was: (1) alert in 14 sea turtles, (2) depressed in 7 and (3) lethargic/comatose in 6. Surgical curettage of the skull wounds and application of Primary Wound Dressing® was performed in all 27 sea turtles and an esophagostomy tube was started in 14 out of 27 sea turtles to support them in feeding. In addition to daily primary dressing, a specific antibiotic therapy was formulated based on culture and susceptibility tests performed on wound lesions. Six out of 27 turtles died during the hospitalization, exhibited severely altered neurological status and brain tissue involvement. The data presented in this study consider a wide range (1755) of sea turtles evaluated over seven years and allowing to quantify the percentage of sea turtles (1.5%, 27/1755) affected by head trauma in Adriatic Sea. Moreover, the study compares the mentation state and the degree of head injuries in all 27 loggerhead sea turtle, highlighting how there is not always correlation between the severity of the head injury and the development of serious neurological deficits or alteration of the sensory state. In the present study, 26% (7/27) of the turtles showed head damage related to severe neurological deficits. These findings suggest that turtles with severe head trauma run a serious risk of death or permanent brain damage, and the importance of early diagnosis and appropriate treatment to improve the mortality rates associated with intracranial injury and the prognosis.

## INTRAVENOUS LIPID EMULSION REDUCES SYMPTOMS OF BREVETOXICOSIS IN SEA TURTLES\*

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The southwest coast of Florida experiences annual red tides, a type of harmful algal bloom (HAB) that results from high concentrations of *Karenia brevis*. These dinoflagellates release potent, lipophilic neurotoxins known as brevetoxins. *K. brevis* is a naturally occurring organism; however, during favorable environmental conditions, these organisms may multiply rapidly and release enough toxins to result in mass strandings of marine birds, fishes, mammals, and turtles. A recent red tide bloom persisted from Nov 2017–Jan 2019 and was described as one of the worst HABs that the state has ever seen, with nearly 600 sea turtles stranding as a result. Another red tide bloom of lesser magnitude occurred from Sep–Dec 2019. Red tides appear to be increasing in frequency and duration; therefore, our objective was to develop and test a rapid treatment protocol known as intravenous lipid emulsion (ILE) in sea turtles experiencing brevetoxicosis. Sea turtles exhibiting neurological symptoms related to brevetoxicosis were brought to the Clinic for the Rehabilitation of Wildlife in Sanibel Island, Florida for treatment. Upon arrival, blood samples were collected followed by administration of 25 mg ILE/kg body mass given slowly intravenously. Additional blood samples were collected at numerous intervals (1h, 2h, 6h, 24h, 48h, 72h, 168h) post-ILE delivery. Whole blood was spun down and the resulting plasma was harvested and frozen at –80°C and subsequently analyzed for brevetoxins using enzyme-linked immunosorbent assays. In total, 9 loggerheads (*Caretta caretta*), 5 Kemp’s ridleys (*Lepidochelys kempii*), and 4 green turtles (*Chelonia mydas*) were included in this study. We found that plasma brevetoxins declined faster using ILE compared to turtles from separate studies that received standard and supportive care (SSC). Survival rate of patients receiving ILE (94%; 17/18) was significantly higher than previous studies that used SSC and/or dehydration therapy (47%; 46/99). Additionally, nearly all symptoms were eliminated within 24–48 hours, whereas symptom elimination using SSC could take up to seven days or more. The dosage given here (25 mg/kg) was sufficient for turtles in this study, but the use of a higher dosage (50–100 mg/kg) for those animals experiencing severe symptoms may be considered. These types of fast-acting treatment plans are necessary for rehabilitation facilities that are already resource-limited. ILE therapy has the potential to reduce rehabilitation time, save resources, and increase survival of sea turtles and other marine animals experiencing brevetoxicosis.

## POST-MORTEM FINDINGS OF A PULMONARY BULLAE IN BUOYANT OLIVE RIDLEY TURTLES

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Olive ridley turtles commonly suffer from buoyancy syndrome, generally directly attributable to being entangled in ghost gear in Maldivian waters. However, a small proportion are found floating in open waters, or washed ashore with no obvious signs of external injury. All buoyant turtle patients are sent to the Olive Project's Marine Turtle Rescue Centre and undergo triage before beginning a tailored treatment plan. Basic haematology, cytology, radiographs, and ultrasound (when indicated) allow for a better understanding and diagnosis of the underlying aetiology and any potential co-morbidities. Usually the inciting cause of the buoyancy syndrome is not evident from radiographs, with lung tears being the most likely cause. However, in two cases in 2021, pulmonary bullae of varying severities were found to be the cause of their buoyancy syndrome, and were visible radiographically. The first case was an adult female Olive Ridley who was found floating, with no signs of entanglement but multiple full thickness cracks to the carapace. On radiographs, there was a moderate pneumocoelom, an ingested oesophageal long line tuna fish hook and a very large thick walled bullae occupying the whole area of the left lung. On post mortem, there was no normal lung tissue visible grossly, and on dissection the bulla was filled with extensive white fungal plaques. Her carapace trauma appeared incidental, as it was not exposing the coelom and was not in the area of the lungs. The second patient was also found floating, with no signs of entanglement but soft tissue was significantly distended grossly around the femoral fossa and at the margin of the marginal and inframarginal scutes, indicative of severe pneumocoelom. On radiographs, she had a severe pneumocoeolom and a significant air-filled bulla was visible occupying the majority of the area of the right lung. On post mortem it was evident that it had started to leak, thus leading to a pneumocoelom that would explain the inability to dive despite no outward signs of trauma. These cases were the first of their kind at the rescue centre and unfortunately neither patients responded to treatment.

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## EVALUATION OF THE PRESENCE OF MICRONUCLEI IN ERYTHROCYTES OF GREEN TURTLES (*CHELONIA MYDAS* LINNEAUS, 1758) FROM THE URUGUAY COAST\*

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Sea turtles spend their life migrating to different places, which aim at reproduction, nesting, feeding and growing. *Chelonia mydas* (Green turtle) (Cheloniidae, Testudines) is one of five species which use coastal



water from Uruguay, being the coast of Rocha State the one that records the largest number. This area is used for juvenile to feed and grow. There are investigations which indicate that the marine vertebrates are good bioindicators, therefore they can be implemented to assess environmental quality. A test of micronuclei in erythrocytes is used to assess the health status of the animal. The micronuclei are chromosomal alterations that appear in answer to the presence of genotoxics substance. The aim of this research is to analyse the health situation of green turtles in one sector of the Rocha department that includes a protected area as well as disturbed areas due to agricultural runoff. The working hypothesis is that there could be differences in micronucleus frequency (MNF) presented in the different areas studied. Samples of blood were collected from 20 individual captured by ONG during the summer of 2017. The samples were stained and observed under microscope by an immersion objective. It has been found that in all specimens studied micronuclei are present in their erythrocytes. The mean value of MNF was  $13,62 \pm 8,68$  micronuclei every 1000 erythrocytes with a high coefficient of variation 2,61 to 35,97. No significant relationship was found between the frequency of micronucleus (FMN) and the size of the individuals analyzed ( $p \geq 0.05$ ), which would indicate that there is no associated preponderance within the age group studied. Although a decrease in FMN was observed in relation to the potentially most polluted site (mouth of the Andreoni Channel), this relationship was not statistically significant, probably associated with the low number of repetitions. The results may also be associated with the form of habitat occupation and the great spatial mobility of the turtles. From the data obtained, it can be indicated that the technique can be used to evaluate micronuclei in green turtles; that its presence is highly frequent and that the FMN would not be associated with either size or distance to the source of contamination.

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## **PLASMA HORMONE LEVELS IN THE GREEN TURTLE *CHELONIA MYDAS* REARED UNDER CAPTIVITY AS A TOOL TO PREDICT MATING AND OVIPOSITION\***

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Circulating estradiol (E2), Testosterone (T) and progesterone (P4) levels were monitored in green turtles *Chelonia mydas* during 3 annual continuous seasons from 2017 to 2020 in the Israel sea turtle rescue center. The breeding center is located near Michmoret as part of the NPA turtle conservation program. The breeding stock is composed of 28 sea turtles. The facility has two outdoor holding tanks (450m<sup>3</sup>) with an open-water system and a sandy nesting area (200-400 m<sup>2</sup>). The water temperature of the tank ranges between 20°C and 22°C across a 12-month period. The turtles are fed every day with green lettuce in quantities equivalent to 1.5-3% of their body weight and with a diet that includes fish in quantities equivalent to 0.15% of their body weight once a week. Female turtles presented consistent E2 profiles from year to year, a rise from January to June with a peak towards the end of April, females that were able to present oviposition had higher levels. P4 levels in females were high in April (mating) and higher towards the start of June due to oviposition, and females that presented with levels above 1ng/ml had successful oviposition (1 female in 2018, 5 in 2019 and 2 in 2020). T profiles were high at the onset of vitellogenesis towards November and again, high during April (maybe due to mating). Males T levels followed a pattern of elevated levels from June to November, and a decrease from January to April. P4 levels were high in males during April probably due to mating and higher towards November probably due to the interaction with the female in the tank (i.e. level of pheromones). The E2 levels were high toward April probably due to mating and again in November. We were able to predict females that will present for oviposition with a profile of P4 in males and females combined that showed elevation from February toward June. This will allow us in the future to selectively couple mating pairs we desire to ensure that the Mediterranean

population genetic variability is maintained. Numbers of clutches per female was between 2 to 6, and the total number of clutches was 25. On average the females laid  $91 \pm 7$  eggs (range 39-139 eggs) per nest with an average emergence rate of ~10% (0–58%). Overall, we saw the same trend in circulating steroid levels over the years in females and males, but only when a certain threshold was achieved oviposition occurred. Our assumption is that the similarity of the environment at the facility to that in the wild contributed to the breeding success. Further studies are required to extend our knowledge of the reproductive biology and ecology of sea turtles and improve the emergence and survival rates. Success of the Israeli breeding group should lead the way for implementation of more captive breeding programs to assist the recovery of these endangered species, in addition to the existing conservation programs.

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## HEMATOLOGY, BLOOD GASES AND BIOCHEMISTRY PROFILES OF WILD-NESTING SEA TURTLES IN TERENGGANU, MALAYSIA\*

Syamsyahidah Samsol

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*Chelonia mydas* is an endangered marine species globally, which its reference blood parameters intervals have been published for some group populations, but baseline health status values are lacking from Malaysia. This study aims to better understand the hematology, biochemical, and blood gas of a wild-nesting green turtle population in the South China Sea. The health status of nesting turtles at the Chagar Hutang Turtle Sanctuary, Pulau Redang, were performed on different individuals (26 females laid one nest and 2 females laid two nests). A portable handheld blood analyzer (iSTAT) used in the field to obtain immediate results of pH, lactate, pO<sub>2</sub>, pCO<sub>2</sub>, HCO<sub>3</sub><sup>-</sup>, Hct, Hb, Na, K, iCa, and glucose levels in blood samples, while standard laboratory hematology techniques were conducted at Universiti Malaysia Terengganu (UMT) for red and white blood cell counts. The results for all blood analytes (except Na, iCa, and hematology values) were not within the healthy index range for wild turtles, compared to previous studies from different geographic populations. Therefore, the results of this current study help to develop a baseline profile of healthy wild-nesting turtles as a global reference for detecting abnormalities in blood chemistry and the health status of turtle populations worldwide.

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## GONADAL DYNAMICS OF FEMALE GREEN TURTLES (*Chelonia mydas*) NESTING AT TORTUGUERO NATIONAL PARK, CARIBBEAN COAST OF COSTA RICA, THE MOST IMPORTANT NESTING BEACH IN THE ATLANTIC BASIN

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In addition to migrating long distances to breed, female sea turtles produce enough yolk to nurture several hundred embryos. Yolk deposition starts 8 to 12 months prior to the nesting season and may be complete when female sea turtles arrive at nesting beaches. The number of egg clutches laid by a sea turtle population annually is often used to determine population status, but this metric can be confounded by the variation in the number of clutches each female lays in a season (clutch frequency). To further understand timing of completion of yolk deposition into ovarian follicles and to refine estimates of clutch frequency, we collected the gonads of 20 female green turtles (*Chelonia mydas*) preyed upon by jaguars (*Panthera onca*) while

nesting at Tortuguero National Park, on the Caribbean coast of Costa Rica, in 2021. We measured and counted all vitellogenic ovarian follicles, recently formed ovarian scars, and oviducal eggs in the samples. Our study yielded four conclusions. First, vitellogenic follicles of nesting green turtles increase in diameter until ovulation, which differs from the patterns observed via ultrasonography in leatherback (*Dermochelys coriacea*) and Kemp's ridley (*Lepidochelys kempii*) turtles. This means that yolk deposition may still be happening during the nesting season of Tortuguero green turtles. Second, mean clutch frequency of Tortuguero green turtles based on the visual inspection of gonads is double the clutch frequency estimated based on mark and recapture data. Thus, visual inspection of gonads provides a tool for refining estimates of the number of females reproducing at Tortuguero every year. Third, ovarian follicles recruited to form egg clutches come from both the right and left ovaries. Moreover, although contribution of each ovary varies per egg clutch, both ovaries contribute a similar number of follicles throughout the nesting season. Fourth, the distribution of follicles and scars is uniform across the ovary of female green turtles nesting at Tortuguero, which makes it possible to subsample ovaries to facilitate data collection.

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## REPRODUCTIVE DYNAMICS OF FEMALE GREEN TURTLES (*CHELONIA MYDAS*) ON THE MISKITO COAST, NICARAGUA, AN IMPORTANT ATLANTIC FORAGING GROUND\*

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The green turtle (*Chelonia mydas*) population in the greater Caribbean has decreased by more than 90% since pre-Columbian times due mostly to direct exploitation for human consumption. Tortuguero, on the Caribbean coast of Costa Rica, is the most important nesting ground for green turtles from this aggregation. The majority of female green turtles nesting at Tortuguero forage off the Miskito coast, Nicaragua, where a legal green turtle harvest exacts an average toll of over 6,000 individuals per year. Understanding the reproductive dynamics of green turtles at nesting and foraging grounds will allow us to better predict population responses to accumulating threats in a rapidly changing planet and to devise conservation strategies accordingly. We studied green turtle reproductive dynamics by measuring and counting ovarian follicles and scars and measuring oviduct length of 201 females legally harvested by local fishers from foraging grounds off the Miskito coast between 1993 and 1995 (samples collected by CJL) and in 2020 (samples collected by RB). We 1) defined threshold sizes and characteristics of gonadal structures that are indicative of maturity as well as recently past and impending reproductive events; 2) described the probabilities of turtles being mature or having already bred based on gonadal characteristics and body size; 3) quantified the ratio of immature to mature turtles harvested in 2020; and 4) determined the proportion of mature females that became reproductively active per year. The threshold sizes of gonadal structures we found in this study were similar to those used for studying green turtle reproductive dynamics in Australia. Additionally, models using gonadal measurements fit the data better compared to those using body size to predict probabilities of female green turtles being mature and having bred in the past. Nevertheless, with this study we provide a tool for more accurately assessing maturity status and breeding history based on Caribbean green turtle body size, which is easier to obtain than gonadal measurements. Finally, over a quarter of mature females in this green turtle aggregation may become reproductively active in a year. However, this proportion did not correlate with the number of green turtle nests recorded at Tortuguero during the 2020 nesting season.



## HOW STRONG ARE SEA TURTLE SHELLS?

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The hard, boney shells of turtles act as the organism's first line of defense against a variety of natural and, increasingly, anthropogenic threats. The shell has long been perceived as a form of armor because it consists of strong bone encasing the spinal cord, limb girdles, and viscera. The carapace arises from the expansion of ossified ribs and neural arches joined via collagenous sutures. Green turtle (*Chelonia mydas*) shells obtained through rehabilitation facilities and fresh strandings were tested to create force-deformation curves in order to quantify how resilient shells can be and how they fail under compressive loads. Our data suggests that sea turtle shells are much less stiff than those of their freshwater and semiterrestrial counterparts. Marine turtle shells thus have the capacity to deform substantially under relatively low stresses. This trait likely reflects the behavior of marine turtles that experience oscillations from low to high pressures as they dive and surface to breathe. Additionally, juvenile shells were significantly more compliant than those of adult turtles. This flexibility may reflect the effect of ossification throughout ontogeny whereby increases in cortical bone density and the trabecular core increase the stiffness of the shell's sandwich-like composition. Understanding shell stiffness and resilience under biologically relevant loading regimes are the first steps in understanding how juvenile and adult sea turtles have been successful for millions of years even in the presence of large predators and how damage from boats can occur.

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## MUTATIONAL DRIVERS OF FIBROPAPILLOMATOSIS TUMORS IN GREEN SEA TURTLES\*

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Fibropapillomatosis (FP) is a panzootic disease outbreak spreading in sea turtles, particularly green turtles (*C. mydas*), that expresses itself as both external and internal tumors. Each afflicted individual can have tens to hundreds of tumors simultaneously, which can hamper vision, feeding, and swimming and can be fatal. Chelonid alphaherpesvirus 5 (ChHV5) is likely the major causative agent but is not the only contributing factor. Turtles showing clinical FP are often immunosuppressed, but not in all cases, and causality is undetermined. The full scope of causes--viral, host, and environmental--is currently uncertain. The aims of this study were to identify possible underlying mechanisms, molecular drivers, and therapeutic targets for FP, and the relationship between different tumors in the same individual. Whole genome sequencing was conducted on rehabilitation patient paired tumor and non-tumor tissue to identify tumor-specific mutations. From this data, single nucleotide variants, copy number variants, and structural variants were called using Somatypus, cn.MOPs, and Manta respectively. Tumor-specific mutational signatures were compared to known human cancer signatures arising from specific carcinogens. Tumors displayed a large amount of heterogeneity in tumor-specific mutational variants, however there were significant

commonalities, including recurrent mutations in genes related to oncogenic and immune functions. Genes with unique variations in individual tumors also displayed significant enrichments in immune-related pathways. This study reveals for the first time that FP tumors can harbor genetic mutations, with many of these occurring in known cancer-associated genes. Improved understanding of the mutational landscape of sea turtle FP will help identify the environmental and viral triggers of this devastating disease, and reveal the host molecular mechanisms driving tumor growth.

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## SEA TURTLE HEALTH BASELINES: SUPPORTING CONSERVATION SCIENCE IN WESTERN AUSTRALIA\*

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The current state of sea turtle health in the Indian Ocean, especially for the endemic flatback turtle (*Natator depressus*) is largely unknown. Anecdotally, the causes of illness, injury, and death in Western Australian turtles are comparable to those in other parts of Australia and the world (e.g., spirorchidiasis, fibropapillomatosis, and marine debris interaction) but scientific studies to validate these reports are particularly limited in this region. To address these knowledge gaps, causes of both live and dead turtle strandings (n=75) in Western Australia (WA) were investigated through an array of veterinary diagnostic techniques including necropsy, clinical pathology, diagnostic imaging, histopathology, parasitology, microbiology, toxicology, and molecular analyses. Health assessments were conducted on live animals (n=220) to determine baseline levels of health and disease for specific populations, predominately nesting and foraging flatback turtles. Through these health and disease investigations, baselines were developed, along with the discovery of new diseases including a novel haemoparasite, *Haemocystidium* spp.; a potentially emerging zoonotic bacterium, *Streptococcus iniae* associated with a multi-species mass mortality event involving post-hatchling flatbacks; as well as spirorchidiasis in flatback turtles previously unreported in this species. In this study, natural disease-related causes of mortality (69.3%) occurred more frequently than direct anthropogenic causes (12.0%). Spirorchidiasis was the most common cause of mortality in this study (32.0%), with a prevalence of 93.2% in turtles susceptible to the disease (i.e., excluding the post-hatchling cohort). The next most common cause of mortality was unknown (18.7%), followed by trauma (13.3%), and endoparasitosis (10.7%). We developed the first flatback turtle reference intervals (RIs) following American Society of Veterinary Clinical Pathology (ASVCP) guidelines. We found flatback turtle RIs were generally similar to other published sea turtle RIs but detected significant differences in our study for the various boundary conditions including life stage (nesting or foraging), as well as for measurement methodology (field or laboratory tests), justifying the establishment of separate RIs. This study is the first health and disease investigation in WA and the eastern Indian Ocean and will offer broader insights into sea turtle health and disease status on a regional scale, and, will provide a framework to integrate health into future conservation management decisions.

## CONSERVATION, MANAGEMENT AND POLICY

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### LIGHT POLLUTION GUIDELINES FOR WILDLIFE INCLUDING MARINE TURTLES, SEABIRDS AND MIGRATORY SHOREBIRDS\*

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Light pollution is increasing globally by ~2% per year and is a common problem near turtle nesting beaches. Artificial light can disrupt critical behaviours in sea turtles. Nesting female turtles may avoid artificially lit beaches and hatchling turtles may not be able to find the ocean when coastal areas are artificially lit at night, making them more vulnerable to predation. Recent studies have demonstrated that hatchlings in the water are attracted to light, which may delay their dispersal to the open ocean or trap them in light pools increasing their risk of predation at sea. To address this conservation challenge, the Australian Government developed Light Pollution Guidelines for Wildlife including Marine Turtles, Seabird and Migratory Shorebirds (Light Pollution Guidelines). The Light Pollution Guidelines aim to raise awareness of the potential impacts of artificial light on marine turtles and provide a framework for assessing and managing these impacts near important nesting beaches. The Guidelines provide foundational knowledge on the potential biological impacts of artificial light, as well as consistent, standardised and transparent processes and expectations for assessing, measuring, auditing and managing artificial light around wildlife. Although the Light Pollution Guidelines were developed within the Australian context, the pervasive nature of light pollution means that the broad principles, process, and technical information provided in the Light Pollution Guidelines can be applied in other countries experiencing similar challenges. On this basis, the Australian Light Pollution Guidelines were presented to 13th Conference of the Parties to the Convention on the Conservation of Migratory Species of Wild Animals (CMS) in Gandhinagar, India in February 2020. The Guidelines were endorsed, and the Secretariat requested to promote the Light Pollution Guidelines amongst subsidiary agreements to the CMS, such as the Indian Ocean South East Asian Marine Turtle Memorandum of Understanding (IOESA).

## **MARINE TURTLE CONSERVATION IN CUBA: ACHIEVEMENTS AND CHALLENGES FACING PERSISTENT AND EMERGING THREATS**

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Specialists declare that many, marine turtle populations world-wide have become seriously depleted by the impact of numerous factors. Cuban turtle conservation efforts have led to important achievements but it is still not enough, given that illegal capture continues and the black market trade in marine turtle products is increasing, and new threats are being identified. For this reason, our objectives are to identify and assess main threats and challenges to sea turtle conservation in Cuba; to identify main conservation strategies and discuss the tools that have been implemented to address these threats; and to summarize main achievements, drawbacks and current challenges in marine conservation in Cuba. Fourteen threats were identified as affecting nesting populations of marine turtles in Cuba with illegal fishing and poaching in nesting areas being the most critical. Management and enforcement in protected areas is uneven and as a result, some nesting areas are seriously threatened while others have the capacity to ensure marine turtle conservation. In the first case, the protected area of managed resources in the south of the Isla de la Juventud stands out as being the one with the highest number of threats identified and Cayo Largo for having the least management capacity despite being the largest nesting population in Cuba. These results should provide guidance for managers to set conservation priorities and management improvement in areas identified as vulnerable to threats.

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## **SEA TURTLE CONSERVATION AND PARTICIPATORY MANAGEMENT AT THE BIJAGÓS ARCHIPELAGO, GUINEA-BISSAU: BENEFITS FOR LOCAL COMMUNITIES AND FOR THE SEA TURTLE CONSERVATION PROCESS**

**Castro Barbosa, Justino Biai, Emanuel Dias, António Jesus Pires, Aissa Regalla, and Quintino Tchantchalan**

*Instituto da Biodiversidade e das Áreas Protegidas (IBAP), Dr Alfredo Simão da Silva*

Offshore Guinea-Bissau, West Africa, lies the Bijagós Archipelago, a sanctuary for iconic marine megafauna, such as manatees, seabirds and marine turtles. Its biodiversity has led to the designations of the Bolama-Bijagós Biosphere reserve and Ramsar site, and to the creation of three Marine Protected Areas (MPAs). The archipelago is mainly inhabited by the Bijagó ethnic group, a farmer-gatherer people with a strong dependence on natural resources, both for food and cultural purposes. Four species of sea turtles nest in the archipelago's sandy beaches: green turtles *Chelonia mydas*, hawksbills *Eretmochelys imbricata*,

leatherbacks *Dermochelys coriacea*, and olive ridleys *Lepidochelys olivacea*, while its coastal waters sustain foraging grounds for juvenile and adult green and hawksbill turtles. The region is of global importance for the green turtle, with ca. 25,000 postures laid annually at Poilão, a sacred island according to Bijagó tradition. Among the Bijagó people, the sea turtles represent a high source of protein, but also a sacred symbol, their meat and carapace being used in religious ceremonies, and their eggs in traditional medicine. Traditionally, harvesting of sea turtles followed strict rules and taboos, which promoted a sustainable use. More recently, however, there has been a breaking-up of traditional practices along with a rise in the exploitation of marine resources, both by locals as by migrant fishers, jeopardizing the sustainability of sea turtle populations. To address these threats, the Institute of Biodiversity and Protected Ares of Guinea-Bissau (IBAP), responsible for the country's biodiversity conservation, adopted a participatory management approach, engaging the local stakeholders in conservation actions and MPA decision processes. This has contributed to a) reduce conflict between communities and authorities; b) increase income sources of local communities (e.g. through conservation jobs, support of alternative sustainable economic activities, and appointment of tourism fees in community events); c) raise awareness and capacity among locals; and d) protect sea turtles in their nesting and feeding habitats. Annually, 55 youngsters from the Bijagós participate in sea turtle monitoring and research activities, 31 locals are currently full-time employed by the IBAP – as park wardens, sailors, logistics – some of which were former poachers, and 18 Bijagós villages have representatives in MPA Management Boards. Additionally, the fishing communities recognize that the surveillance of sea turtle marine habitat and the implementation of fisheries regulations (e.g. fishing gear of low impact, temporary fishing bans), negotiated in Parks' Management Board meetings, greatly benefits them, giving communities priority access to resources by limiting the access of illegal foreign fishers. Throughout 17 years of this approach, respecting the local traditions and beliefs, the IBAP has gain the trust of the Bijagós community, and has gathered stories of success, as well as new challenges, both worth to share. One major achievement of the participatory conservation process is the full protection and growing trend of the main African green turtle nesting population at Poilão Island.

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## TWO DECADES OF WORK CONSERVING SEA TURTLES ON THE PACIFIC COAST

**Blanca Alicia Bojórquez Martínez**

*University of Guadalajara, Mexico*

In 1995 during a vacation in Puerto Vallarta, I had my first experience with nesting sea turtles at Boca de Tomates beach and I was left grateful and impressed. Later, in 2001, we made agreements with Selva Negra Ecological Foundation of rock band MANA, to mutual support in our projects; theirs being the turtle conservation camps in Jalisco and Nayarit; ours being a development project for Wixárrica communities. And so, with little information on it, in March 2001, we made our first incursion in Chalacatepec camp, on Tomatlán Municipality, Jalisco and in Platanitos camp on Compostela municipality, Nayarit. From there we began collaborating with Majahuas camp, Cruz de loreto camp and Mayto camp, all in Jalisco, while maintaining a permanent collaboration with Palatanitos camp, Nayarit. In 2006 I was fortunate to be added at the works of a new camp beach near Platanitos camp called Chila Beach, over which we were made responsible on December 7<sup>th</sup> 2007; a night made unforgettable to me, in addition, by the sighting of the largest sea turtle in the world: a leatherback (*Dermochelys Coriacea*). It was then that our hardest efforts began towards the recouperation of this important and endangered reptile, a task that would take us to know other turtle camps all over the Pacific, from Baja California to Chiapas as well as meet with incredible collaborators from students with whom I am deeply thankful, particularly with Leonardo Alvarado, former student turned assistant for all endeavors over the last 13 years, as well as many professors. So it was that

the Selva Negra/UdeG Program for Protection and Conservation of the Sea Turtle came to life; its main objective being to protect populations, nests and individual specimens of sea turtle as well as their immediate environment by constant patrolling along the beaches and close environmental education work with the coastal communities adjacent to these beaches. With these actions we aim to favor the recovery of the various species of sea turtle that nest on our protected beaches by preventing the sacking of their nests and the slaughtering of the females that otherwise occur, by involving the communities in the upkeep of natural spaces and creating awareness and sympathy for the need of preserving these species, allowing them to freely reproduce and aiding in the preservation of local ecosystems. This latter is done by visiting educational campuses on these communities to make environmental education workshops for both children and their parents. The most relevant results for these twenty years of work are the following: Capacitation of students for Preservation and Protection: 15,700 students, amongst whom we've had foreign exchange students from countries such as Chile, Argentina, Colombia, Germany, France, Spain and the USA. Involvement in 12 preservation camps, reduced to only Platanitos camp, Nayarit, since 2013. Up until December 31<sup>st</sup> 2021, 2'242,570 nests collected and 1'838,907 hatchlings released of all four species which arrive at those beaches: *Lepidochelys olivacea* mainly, followed by *Chelonia mydas*, *Eretmochelys imbricata* third and *Dermochelys coriacea* last.

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## TOWARDS TRANSBOUNDARY MONITORING AND CONSERVATION OF MARINE TURTLE IN THE MEDITERRANEAN WITHIN THE MEDPAN NETWORK

**Susan Gallon<sup>1</sup>, Reda Neveu<sup>1</sup>, Dune Ganot<sup>1</sup>, Laurent Sourbes<sup>2</sup>, Marie Romani<sup>1</sup>, Pierre Vignes<sup>1</sup>, and Purificacio Canals<sup>1</sup>**

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The Mediterranean Sea is recognised as one of the world's 25 top biodiversity hotspots. Although it represents only 0.7 % of the global ocean surface, it comprises 4 to 18 % of the world known marine species, depending on the taxonomic group considered. The Mediterranean Sea marine life, however, undergoes multiple soaring pressures, mostly due to human activities, such as professional and recreational fishing, maritime traffic, water pollution, coastal development, introduction of non-indigenous species, and offshore oil and gas prospection and exploitation. In this context, Marine Protected Areas (MPAs) are key tools to mitigate human impacts in coastal environment and promoting sustainable activities to conserve Biodiversity. Mobile and migratory species depend on critical habitats throughout their seasonal movements, including breeding and foraging sites as well as the pathways between them. Loggerhead sea turtles, for example, can be found nesting on a beach in Greece, but forage along the coast of Tunisia, France or Sardinia. During their migrations, marine turtle experience varying levels of protection and face many threats as they travel through multiple countries' jurisdictions and across ocean basins. Some populations are under threat and an efficient network of MPAs can contribute to their long-term conservation by facilitating the implementation of management and conservation measures in key habitats of their life cycle. Since 2018, the network of Marine Protected Areas managers in the Mediterranean (MedPAN) is developing and supporting activities, bringing together MPA managers and involving NGOs and researchers working on marine turtle, to implement an integrated management strategy for these species. This network, that counts to date 130 strongly committed organisations from 21 Mediterranean countries, represents more than 90% of the Mediterranean MPAs that are managed. Within MedPAN, a monitoring guide dedicated to managers has been developed. In addition, a recurrent training programme for the management of marine turtles and a Cooperation Framework for monitoring marine turtles, which includes a Data Sharing Charter are under way. This work is coordinated by the Mediterranean MPAs



Marine Turtle Working Group (MPATWG). Indeed, marine Protected Areas (MPAs) managers can adopt measures that help decrease pressures on nesting beaches and sometimes in waters around and by collaborating, they can be increasingly effective at curbing pressures and impacts in mating, migrating, foraging and wintering areas.

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## FROM LITTLE THINGS BIG THINGS GROW: LOCALISED SMALL-DRONE NESTING BEACH SURVEYS CAN SUPPORT A REGIONAL DATASET

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Globally, the conservation status of sea turtles ranges from critically endangered to data deficient. Many populations utilise nesting habitats which are remote and difficult or expensive to access for the purposes of scientific investigation and population monitoring. As a result, they are understudied which hinders conservation and management decisions. We provide a case-study from north Western Australia (WA) that shows how localised monitoring programs can use small drones to contribute to a larger dataset. Six of the world's seven species of sea turtles occur in Australian waters and are protected under the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999*. The north-west marine region of WA provides important nesting habitat for several species along a vast and remote coastline under various pressures from developments. The WA *Environmental Protection Act 1986* and WA *Biodiversity Conservation Act 2016* both aim to help achieve environmentally sustainable development while protecting environmental values, including biodiversity, across the State. The latter is implemented by the State Department of Biodiversity, Conservation and Attractions (DBCA) who are tasked with monitoring and managing threatened species and communities. The purpose of our study was to support an environmental impact assessment, by identifying the presence of nesting turtle species and determining the regional significance of the beaches at our site as nesting habitat. We used a DJI Phantom 4 remotely piloted aerial system (RPAS), or 'drone', which is relatively small, affordable, and easy to acquire. The use of RPAS in marine wildlife monitoring is rapidly growing, with benefits including reduced costs and health and safety risks due to less field time required and increased transparency and reproducibility due to permanent data storage for future verification and autonomous operations along pre-defined transects. As the use of RPAS in sea turtle studies remains novel, we consulted with DBCA scientists during the early stage of survey design. The outcome was to, where possible, mirror photographic data capture, processing and analysis methods used by a regional-scale study conducted by an occupied aircraft with mounted cameras. RPAS flights were planned and flown along transects using the freely available DJI GS Pro application. The pilot moved by small vessel or four-wheel drive vehicle to maintain visual line of sight with the RPAS. While a larger RPAS would allow data to be gathered from large stretches of inaccessible coastlines and offshore islands beyond visual line of sight, associated licensing and permitting times can be prohibitive. A trained and experienced observer identified flatback (*Natator depressus*) and green (*Chelonia mydas*) turtle track presence, distribution, density, and nesting outcome. We were able to directly compare our local data with results obtained for the region. By focussing surveys to a localised area, we were able to repeatedly survey beaches within a survey period and the turtle nesting season which increased capture probability. The methods proved reliable with the potential to be standardised for uptake by various groups including grass-roots NGOs, indigenous rangers, and environmental consultants to contribute to data for informed sea turtle population-level conservation and management.

## USING SATELLITE TRACKING TO INFORM CONSERVATION ACROSS OCEAN BASINS AS WELL AS AT LOCAL LEVELS\*

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Satellite tracking is widely used to assess the movements of sea turtles, often with purported goal of improving conservation management. However, often the links between tracking data and conservation policy are unclear. Using case studies around the world as well as our own work in the Indian Ocean, we highlight where tracking data have been used to directly to drive management actions (<https://doi.org/10.1016/j.tree.2019.01.009>). In this way we highlight best practices that allow the translation of tracking data into conservation policy and management. We consider how satellite tracking data can help support the creation and continuation of both massive marine protected areas that are many 1000s of km<sup>2</sup> (e.g. <https://doi.org/10.1111/cobi.12325>) as well as important localised areas, such as heavily used foraging sites (<https://doi.org/10.1002/eap.2418>). We consider the value of data sharing to help maximise the conservation benefits from satellite tracking studies (e.g. <https://doi.org/10.1038/s41586-019-1444-4>). Early engagement between the data collectors and the stakeholders involved in policy development was often extremely important to help translate tracking data into conservation outcomes. Early engagement can help ensure that tracking programs provide the data and products (maps, analyses, etc.) needed for management. Furthermore, direct communication with managers and policy makers is important because academic publications are often inaccessible to policy makers. Tracking data alone may be insufficient to estimate the threats that species face in different areas, so synergistic use of tracking along with threat and resource maps might help drive the use of tracking data to gain conservation benefits. Tracking data can sometimes make a contribution to decisions via what is termed the ‘web of influence’. Through effective science communication, the results of conservation-related tracking studies are being integrated into the consciousness of stakeholders, whose opinions can influence policy. The value of the sea turtle tracking community coming together to collectively highlight further examples of where tracking data has driven conservation policy, will be discussed.

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## LIVING IN COEXISTING: A NOVEL PREDATOR EXCLUSION CAGE DESIGN

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Coyotes (*Canis latrans*) have become the main source of loggerhead sea turtle (*Caretta caretta*) nest depredation on Bald Head Island, North Carolina despite the use of predator exclusion cages (PEC). While some mammalian predators may be legally trapped and euthanized in North Carolina, this management strategy is not always politically supported. We engineered a new PEC design using a PVC frame with MasterNet fencing and evaluated its efficacy against mammalian depredation, cost, and time to construct



compared to three PEC designs used by sea turtle monitoring programs throughout the southeastern United States. We assessed each design's efficacy and mammalian predator behavior during two 14-day trials conducted with artificial baited nests in November and December of 2020. The two most effective designs were then installed over live sea turtle nests during the 2021 nesting season to assess each design's efficacy. In November and December, coyotes and red fox (*Vulpes vulpes*) were observed attempting depredation on the artificial nests in each trial. The MasterNet and welded wire cages were 100% effective against depredation attempts, therefore they were then installed over live sea turtle nests during the 2021 nesting season. There was little variation between designs as the MasterNet cage had a 97.2% efficacy rate, while the welded wire cage produced a 94.9% efficacy rate during the 2021 sea turtle nesting season. Our findings show that the MasterNet cage is a suitable option for sea turtle nest protection and will aid sea turtle program managers in their depredation mitigation strategies while continuing the effort in closing the knowledge gaps described in Kays' 2018 IUCN assessment of coyotes.

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## **COMMUNITY MONITORING, CONSERVATION & SECURING THE FUTURE OF FLATBACK TURTLES (NATATOR DEPRESSUS) IN PORT HEDLAND, WESTERN AUSTRALIA**

**Kelly Ann Howlett**

*Care For Hedland Environmental Association Inc, Australia*

The Flatback Turtle (*Natator depressus*) is the only species of marine turtle endemic to Australia. Port Hedland is 1,800km north of Perth, in the resource rich Pilbara region of Western Australia. Local Aboriginal rock carvings depicting turtles have been dated to approximately 8,000 years of age. Much has changed in the Port Hedland area, with the lifting of the iron ore export embargo in the late 1960s, the subsequent dredging and transformation of the mangrove harbour has today, lead to the Port Hedland harbour now being the largest bulk tonnage export port in Australia. Today with a population of 15,000 people, Port Hedland and its associated beach areas are a far cry from what they once were. Each of Port Hedland's three nesting beaches represents a unique monitoring and management opportunity. All beaches are predisposed to a number of threatening factors that could detrimentally affect the turtles nesting in the area: high level of disturbance by people (direct contact, off road vehicles, tourism, disturbance of nests and Indigenous take, harbour dredging), residential lighting and feral animal predation. The monitoring methods used are track identification, mark/recapture and nest excavation/success. These methods, used in a community monitoring program setting, have enabled the documentation of seasonality, numbers, inter-nesting rates, recruitment rates, spatial distribution, familial relations, successful nests, hatchling emergence, hatchling orientation and any apparent disturbance such as nest predation by the European Red fox or human disturbance such as off-road vehicles and inappropriate people-turtle interactions. The monitoring data has been integral in guiding local light management, residential and street side development, a nearby hotel development and a large-scale marina development. This year will be the program's nineteenth season and already it has been able to deliver quantifiable data that has been able to be input into local management decisions. Assisting greatly to secure the future of Flatback Turtles (*Natator depressus*) in Port Hedland, for future generations.

## SEA TURTLE CONSERVATION IN CAMBODIA: PROGRESS AND CHALLENGES\*

**Sour Kim and Henry Duffy**

*Fauna & Flora International, United Kingdom*

In Cambodia's coastal and marine waters, five species of sea turtles have historically been reported, namely, 1) green (*Chelonia mydas*), 2) hawksbill (*Eretmochelys imbricata*), 3) olive ridley (*Lepidochelys olivacea*), 4) loggerhead (*Caretta caretta*), and 5) leatherback (*Dermochelys coriacea*). However, Cambodia's turtle populations have undergone significant declines, with only green and hawksbills observed in recent years, and the remaining populations are severely threatened by fisheries bycatch, loss of nesting beach and foraging habitat, marine plastic pollution and some ongoing local consumption. Sea turtle conservation efforts have been undertaken by Fauna & Flora International (FFI) since 2010, in close collaboration with the Fisheries Administration of the Cambodian government. Recent work has focused on understanding fisheries impacts on turtles, with bycatch surveys conducted in 2018 through interviewing local fishers about the frequency and location of turtle interactions with fishing gear. Another survey is planned in 2022 to assess temporal and spatial changes in bycatch, and to examine the outputs and outcomes of conservation initiatives. Recording, tagging and releasing of sea turtles from bycatch events have been ongoing since 2010, and to date 174 sea turtle bycatch releases have been recorded; of which 26% were tagged. Efforts are also ongoing to locate any remaining nesting beaches, and in 2021, volunteers walked about 160 km of beaches in five priority islands in search of nesting sites, although none were recorded. Awareness events are also very important for local fishing communities to understand and be aware of the legislation which protects these marine endangered species. Between October 2019 and July 2020, three awareness events were conducted in nine coastal villages, focusing on conservation of marine endangered species including sea turtle and seahorse. Even in the mid of the Covid-19 pandemic, conservation efforts continue, most recently through installation of signboards displaying sea turtle conservation and protection messages, which were erected in coastal fishing communities and fish landing sites. These conservation activities are planned to further intensify in the coming years, as nesting site survey effort will be increased through camera trap and drone monitoring, with the purpose of identifying priority nesting beaches for future protection. Capacity building will also continue for both Fisheries Administration staff and local fisheries committees, focusing on sea turtle bycatch management, data recording and tagging. More awareness raising events will also be carried out with fishing communities. Provision of technical advice on policies relevant to sea turtle conservation is also important to ensure implementation of the sea turtle National Plan of Action in Cambodia. A final key priority is an initiative to pilot Turtle Excluder Devices (TEDs) on trawlers, with the aim of securing fishery sector buy-in to reducing sea turtle bycatch in trawl nets.

## USING BAMBOO NEST COVERS TO PREVENT PREDATION ON SEA TURTLE EGGS

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<sup>3</sup>*COTORCO*

Predation on sea turtle nests is a widespread issue that contributes to the decline of sea turtle populations worldwide. While a certain degree of predation on sea turtle eggs is caused by native species, domestic and stray dogs have substantially increased predation rates and altered local food chains at many beaches. A range of methodologies have been trialed to reduce predation, including the use of nest covers to restrict predators' access to turtle eggs. Previous research has described nest cover designs that result in significant reductions in predation; however, setbacks in cost, practicality, and reliance on synthetic materials such as plastic can render such designs unsustainable. Additionally, many of these nest covers require removal prior to the expected hatching date, thus exposing the nest to predation at a vulnerable time. In contrast, the present study used bamboo nest covers that were designed to be inexpensive, constructed of natural and locally grown materials, and enable hatchlings to emerge without prior removal of the nest cover. The bamboo nest covers were constructed by weaving or tying bamboo strips into a grid formation and were then placed on a portion of newly laid nests on Playa Carate, Costa Rica. Daily census surveys were conducted for one year from May 1st, 2017 to April 30th, 2018 to identify predation patterns and assess the effectiveness of bamboo nest covers as a predation prevention tool. It was found that nests with bamboo covers were 2.49 (95% CI: 1.50 - 4.15) times less likely to be predated upon than bare nests, with predation rates of 6.6% and 17.6% respectively. Dogs, crabs, birds of prey and raccoons were all identified as predators, with dogs and birds of prey causing the greatest overall damage. Predation occurred year-round but was greatest in peak nesting season (June to December). Nests were most likely to be predated on the first night laid or near the time of expected hatchling emergence, emphasizing the importance of using covers that do not require removal prior to nest hatching. Bamboo nest covers were inexpensive and easy to use but labor-intensive to construct. Extreme weather conditions in the study region occasionally displaced nest covers, emphasizing the importance of using natural materials to reduce harmful ocean litter. Overall, bamboo nest covers are an effective method of preventing predation on sea turtle eggs and provide an inexpensive and more environmentally friendly alternative to synthetic covers. Depending on the resources available, nesting beaches in other regions around the world could use bamboo nest covers as a sustainable method of reducing predation on turtle eggs.

## **IMPROVISATION OF HATCHERY MANAGEMENT PRACTICE TO MITIGATE THE IMPACT OF THE SHIFT IN THE NESTING SEASON OF OLIVE RIDLEY TURTLES ON THE WEST COAST OF INDIA\***

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Increased sea temperature has a profound effect on sea turtle breeding and nesting. On the west coast of India, the olive ridley peak nesting season has shifted drastically from winter to summer season. It resulted in a total failure in the emergence of hatchlings due to the hardening of the nest and increased incidence of predatory ant attack inside the hatchery. This was in addition to the usual impact of high summer temperature viz increased mortality of developing embryos and highly skewed female-biased sex ratio. A protocol to rescue the trapped hatchlings inside the hardened nest was developed and implemented. An Indigenously developed temperature datalogger unit having an advanced GPRS system for data transfer were installed at seven major rookery sites to monitor the incubation temperature of the nest from 2019 - 2021. The temperature data showed variation in incubation temperature among the nesting sites when there was an insignificant variation in the ambient air temperature. The shade net installed above the hatchery was found to be ineffective in restricting the incubation temperature below the threshold temperature of 33°C. Increased incubation temperature of the nest can be lowered by the sprinkling of water or keeping a wet jute bag over an inverted cane basket above the nest after looking into the temperature data which resulted in an increased emergence rate. The most important outcome of the study was observed with temperature data the advantage of erecting turtle hatchery inside the sparse plantation of *Casuarina equisetifolia* on one of the nesting beaches. The incubation temperature on this site was below 33°C throughout the summer incubation period resulting in natural emergence of hatchlings, non-hardening of nest sand, the well-balanced sex ratio of the clutch as estimated by indirect incubation temperature in the second trimester and increased emergence rate. It was observed that no extra efforts like the use of shade or sprinkling of water were required to reduce or restrict the incubation temperature below a threshold. The red ants *Dorylus orientalis* predating on turtle eggs and hatchlings inside the nest was reported for the first time in India. The increased incidence of this ant attacking the turtle nest was due to a shift in the nesting season. This predatory ant native to the Indian subcontinent has a natural presence on the nesting sites and are active at high temperature and moisture. Management of this ant can be done outside the hatchery site by using *Azadirachta indica* seed residue rich in alkaloids and mixed with kerosene to prevent their invasion. Direct application of residue without kerosene on the infested nest has proved to be effective against ants. The variation in incubation temperature among the nesting sites suggests a tailor-made localized approach than a current generalized approach of hatchery management practice for mitigation of high temperature.

## ANALYSIS OF THE SCIENTIFIC PRODUCTION ON SEA TURTLES IN CABO VERDE

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Sea turtles constitute a threatened group of fauna, thus to know gaps in their knowledge it is crucial to lead research efforts. The present study aims to analyse the scientific literature published on marine turtles in Cabo Verde by means of bibliometric analysis. This objective will be achieved by using the main database of scientific literature (Web of Science, Scopus, Plos One, PubMed, BioOne, and Google Scholar). We analysed the temporal and geographical variation in the publications, the number of authors and nationalities involved, and the extent of study of different research topics by species. We found a total of 168 publications as a result of the bibliographic search and screening in all different databases. The number of publications grew through the analysed time period (1979-2020), as well as the number of authors. The countries with the highest number of authors were Spain followed by Cabo Verde. Research areas of greater investigation effort were "Breeding and reproductive success", followed by "Conservation and management" and "Population dynamics". However, there were differences between species and islands. Most of the studies were conducted at Boa Vista, followed by Sal and Maio Islands. The most studied species was *Caretta caretta*, being the only one addressed in all research topics. This study contributes to understanding trends and gaps in sea turtle knowledge that could help guide future research lines and conservation efforts in areas of particular concern.

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## BENEFITS OF A LEATHERBACK CONSERVATION PROJECT TO LOCAL COMMUNITIES IN AN EXTREMELY POOR REGION IN PAPUA, INDONESIA\*

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Improving the capacity and livelihood of local communities is essential for promoting lasting conservation of natural resources. Approximately 75% of leatherback nesting activity in the western Pacific occurs at the Bird's Head region of West Papua every year at Jamursba Medi and Wermon Beaches. The Bird's Head leatherback population has declined more than 60% since the 1980s due to large-scale turtle egg harvesting between the 1970s and 1990s. Although local communities no longer rely on egg harvesting for income, they are one of the poorest. West Papua is one of the poorest provinces in Indonesia, and Tambrau Province, where these communities are located, is one of the poorest regencies in West Papua. Twenty-two percent of its population lives in extreme poverty, defined as living on less than 1.90 USD per day; Tambrau residents live on 1 USD per day. Tambrau also has a low Human Development Index (HDI) of 53.45 (West Papua HDI: 65.09 and Indonesia HDI: 71.94). Although located on the coast, most of Tambrau's coastal community members are farmers, not fishers. Coconut is the primary commodity in the region, and most farmers sell copra. However, the copra trade is not growing because the villages are remote, and community members do not have reliable and affordable transportation. The State University

of Papua (UNIPA) Leatherback Project has been leading leatherback research and conservation in the region. One key component of UNIPA's holistic conservation approach is the community empowerment program, which helps build the capacity of community members living in the villages near Jamursba Medi and Wermon through mentorship. The program focuses on improving the reading, writing, and arithmetic skills of local students through an after-school program called House of Learning and livelihood through employment at the nesting beaches and the processing of coconut, the region's primary commodity, into coconut oil that meets market standards. Here, we summarize data on educational and financial benefits that local community members received in 2019 and 2020. We summarized data on educational and economic benefits that local community members received in 2019-2021. Ninety-seven local community members received a total of 47,086 USD per year through UNIPA's nesting beach and community empowerment programs, providing them with an additional 1.33 USD to spend per day. More than 100 local students attended our House of Learning, and at least 53% of the participating students showed marked improvement in reading, writing, and arithmetic. Over 200 people (24% of the total population living near Jamursba Medi and Wermon) have benefitted from UNIPA's leatherback conservation project every year. UNIPA's leatherback conservation project has contributed to local capacity and livelihood. In the coming years, we hope to improve the livelihood of more community members by teaching them to process coconuts into different products and empower them to supply a bigger market.

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## PELAGIC AREAS WITH INCREASED RISK OF EXPOSURE TO MARITIME TRAFFIC FOR LOGGERHEAD TURTLES IN THE TYRRHENIAN (MEDITERRANEAN) SEA\*

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Concern about the impacts of vessel collisions with marine animals is increasing worldwide. To date, vessel collision is a recognized significant threat for large whales, and there is evidence that other marine species are also affected, including sea turtles. All species of sea turtles are vulnerable to vessel collisions as they surface to breathe or stay close to the surface to bask, forage or re-oxygenate and rewarm after deep dives in the cold waters. Unfortunately, our current knowledge of vessel collision consequences on sea turtles is mainly based on animals stranded along the coastline. In contrast, the degree of the impact in offshore pelagic habitats, where turtles are likely to spend more time near the surface and where fatal collisions go likely unnoticed due to the sinking of the carcasses, is largely unknown. Interestingly, during systematic monitoring of marine megafauna using passenger ferries as observation platforms, sea turtles were often observed vanishing under the ship's hull, without re-emerging behind, indicating a likelihood for collision events. The current study reports on the results from a spatially explicit risk assessment identifying pelagic areas of higher risk of exposure to maritime traffic in the Mediterranean Sea, one of the world's busiest waterways. The assessment is based on satellite tracking data of loggerhead turtles moving in the pelagic habitat of the Tyrrhenian Sea, off Italy's western coast, yielding estimates of animal utilization distributions (UDs) through the Brownian bridge approach of the kernel method for auto-correlated relocations. Maritime traffic was estimated using density maps provided by The European Marine Observation and Data Network (EMODnet), summarized and sorted by ship category. Also, a Generalized Additive Model (GAM) was fitted to data to see the effect of explanatory variables (shipping densities and temporal patterns) on turtles' distribution. The results show that loggerheads reside regularly in the Eastern



Tyrrhenian, especially in the autumn-winter period, and that 64% of their average core home range (85% contour) overlaps with relatively high-density marine traffic. The risk approach allows us to identify that the exposure for loggerhead turtles in this pelagic area is primarily associated with cargo and passenger vessels due to the leading global routes passing through the Messina Strait or connecting Sicily to the mainland. Temporal areas of relatively high risk are identified in the middle of the eastern Tyrrhenian and off the Salerno Gulf according to the season. Our results indicate that the risk approach can intuitively and effectively identify high-exposure areas to which research and conservation efforts should be directed. To our knowledge, this study is the first spatial risk assessment of exposure to maritime traffic for loggerhead turtles in pelagic habitats. Further research is needed to measure the risk of vessel collision and inform optimal mitigation strategies. In this perspective, other animal-related factors, such as surface time and the behavioral responses to vessel approaches, should be considered in future analysis. The study also outlined the power of satellite telemetry in the collection of direct observations of movement patterns of highly migratory species to identify potential risks for their conservation.

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## **AN ASSESSMENT OF SEA TURTLE LIGHTING COMPLIANCE, ORDINANCE STRENGTH, AND DISORIENTATION IN PALM BEACH COUNTY, FLORIDA**

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Florida beaches provide vital nesting grounds for three species of sea turtles; the loggerhead (*Caretta caretta*), the green turtle (*Chelonia mydas*) and the leatherback (*Dermochelys coriacea*) (FWC 2022). Palm Beach County's (PBC) 46 miles of shoreline consistently have the most densely nested beaches (nests per mile) in the state reporting a total of 36,155 nests in 2020 which equates to approximately 786 nests per mile (FWC 2021). Artificial lighting on nesting beaches may cause sea turtle hatchlings to disorient, leaving them at high risk of dehydration, predation, and death (Witherington, 2003). PBC's Unified Land Development Code Article 14 Chapter A (ULDC 14.A), Sea Turtle Protection and Sand Preservation, establishes lighting standards for coastal properties within an established Sea Turtle Protection Zone. The adoption of the State of Florida's Model Lighting Ordinance for Sea Turtle Protection, Chapter 62B-55, in 2021 has provided guidance for local governments in updating sea turtle lighting to the most current standards. The first purpose of this study is to provide a draft of proposed updates to PBC's lighting ordinance that reflects the current standards outlined in the State of Florida's Model lighting Ordinance for Sea Turtle Protection. In PBC, municipalities can "opt-in" to PBC's regulation and enforcement of ULDC 14.A, or "opt-out", and independently enforce an equally or more stringent coastal lighting ordinance than the County's. Overall, 11 municipalities opt-into PBC's regulation of ULDC 14.A and 8 municipalities opt-out. The secondary purpose of this study is to investigate how lighting compliance, hatchling disorientation, and lighting ordinances differ across PBC's "opt-in" versus "opt-out" municipalities. Lighting data from PBC Environmental Resources Management (ERM) nighttime lighting surveys, and disorientation data from Florida Fish and Wildlife Conservation Commission (FWC) was used for analysis. Surveyors recorded noncompliant lighting throughout the entirety of PBC's beaches during the 2021 sea turtle nesting season. Each property was scored on a scale from 0-5; 0 representing total compliance with PBC's ULDC 14.A and 5 representing highest levels of non-compliant lighting. Hatchling disorientation data was reported in ranges of number of hatchlings disoriented, so data entries were re-assigned a value based on their lower-most bound. Two hot spot analyses were conducted using lighting data and hatchling disorientation data for the year of 2021. Generally, municipalities that have "opted-out" of Palm Beach County's ULDC 14.A are at high priority for sea turtle lighting improvements. Data analysis suggests that opt-out municipalities

display more lighting noncompliance hot spots than opt-in municipalities and hot spots for hatchling disorientation were identified in Riviera Beach, Palm Beach Shores, Palm Beach, and Ocean Ridge. Overall, this study found that more robust sampling of both coastal lighting and sea turtle nesting will help create a more complete understanding of the state of lighting compliance and hatchling disorientation in Palm Beach County. The results of this study shall be used to provide suggestions for improvements to PBC's sea turtle lighting ordinance, as well as for PBC ERM's coordination with municipalities to optimize sea turtle-safe lighting practices along their coasts.

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## **SEE SHELL: A DEEP LEARNING MODEL FOR DETECTING HAWKSBILL DERIVED PRODUCTS\***

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Despite current regulations prohibiting the sale and distribution of hawksbill sea turtle-derived products, the tortoiseshell trade continues internationally, in both physical and online marketplaces. Here we present the application of a convolutional neural network (CNN), which can be widely deployed by law enforcement, to help curb the sale of illegal tortoiseshell. We describe a curated dataset (n = 4,428 digital images), which was used to develop a deep learning model we are calling "SEE Shell." When deployed, SEE Shell identifies real and faux hawksbill-derived products from digital images. Developed on a MobileNetV2 architecture using the TensorFlow library, SEE Shell was tested against a validation (n = 665) and test (n = 649) set where it achieved an accuracy between 82.6-92.2% depending on the certainty threshold implemented. We expect SEE Shell will give potential tortoiseshell consumers more agency in their purchasing decision, and will enable retailers to rapidly filter their online marketplaces to exclude illegal products.



## ILLEGAL TAKE OF NESTING SEA TURTLES IN TORTUGUERO ROOKERY, COSTA RICA: CONSERVATION, TRADE, OR TRADITION?

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Tortuguero, Costa Rica is considered the second largest rookery for green turtles (*Chelonia mydas*) in the world. In 1950, Tortuguero was one of the sites with more take of green turtles in the Caribbean Sea. Nowadays, this rookery is a global example for sea turtle conservation based on ecotourism; however, there is still take of sea turtles happening in the area. Therefore, we aim to describe the drivers and dynamics of this illegal activity at Tortuguero rookery. Sea Turtle Conservancy (STC) has been working together with local authorities to protect sea turtles for over 60 years. Using the data collected during STC's daily monitoring activities on the northmost 8 km of the beach and weekly track surveys on the entire 29.6 km conducted between 2005-2021, we expanded on the estimates reported by Pheasey et al. (2021) to assess the minimum number of sea turtles taken during the study period. Additionally, we conducted 12 semi-structured in-depth interviews with local key informants to get a better understanding of the drivers and techniques behind sea turtle take at Tortuguero. We documented 733 ( $43.1 \pm 18.5$  year) nesting turtles illegally taken, being the green turtle the most taken species (96.3%), a finding which was also supported by the interviewees (100%). According to some of the responders (33.3%), take techniques have improved over time; however, dragging the turtles from the beach into the vegetation to be butchered is still the most common method implemented for this end (83.3%). Interviewees also asseverate that the two hot spots for sea turtles take are: the Dr. Archie Carr Wildlife Refuge (91.7%) and Tortuguero National Park (75.0%). Furthermore, evidence has shown that take techniques implemented on each one of these sites can be completely different according to the accessibility conditions, portraying the adaptation capacity of the people involved in the illegal take. Tortuguero hosts the oldest conservation project in the world, yet, sea turtle take is still occurring every nesting season. Traditions regarding sea turtle meat use are still present in the community of Tortuguero, even when consumption of sea turtle products is considered to be shameful in the village. Take techniques and their evolution to pass undetected represent the biggest challenge to estimate sea turtle take rates in the rookery. Still, our findings represent the minimum take in the last 16 years. Despite the constant conservation efforts carried out in Tortuguero by environmental authorities, NGO's and some sectors of the community; further changes in the management plans of Tortuguero National Park are needed as well as the incorporation of more vigilant personnel, this could be a necessary tool to reduce the take impacts on these sea turtle populations in the near future.

## THE SEA TURTLE EXPLOITATION'S PARADOX: A ONE HEALTH PLEA

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Whether their exploitation is legal or illegal at the national level, sea turtles seem unsustainably fished, and their specimens widely consumed and traded in several States. Sea turtle fishing, defined as the fishing activity that consists of willfully extracting sea turtles from the sea in order to consume them and their parts, and related activities, may indeed be considered traditional practices and part of the cultural heritage of some indigenous tribes. Indigenous peoples' rights are at the core of international law and include, in particular, the fundamental right of tribal people to subsistence and family perseverance. This forms the legal basis for the persistence of sea turtle exploitation in many countries. At this time of the 6th mass extinction crisis, when climatic changes threaten a million species with extinction and our own lives at unprecedented levels, the recent developments of international environmental law have emphasized indigenous peoples' right to a healthy environment, to be appropriately informed on environmental matters, and to participate in decision-making processes and environmental justice. This presentation focuses on the Wider-Caribbean region, and demonstrates that indigenous people are the primordial pillars for the enhancement of sea turtle conservation. We will discuss the fact that maintaining the lawful exploitation of sea turtles contradicts tribal communities' inherent right to a healthy environment, and damages their perspectives for economic progression. This presentation also offers recommendations on the way forward for efficient implementation of environmentally and economically sustainable activities for communities involved in sea turtle fishing and sea turtle specimen exploitation.

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## TRENDS AND NESTING PATTERNS OF CHELONIA MYDAS ON THREE BEACHES OF THE PASO DEL ISTMO BIOLOGICAL CORRIDOR, RIVAS, NICARAGUA: A REVIEW OF PASO PACÍFICO'S CONSERVATION PROGRAM OVER THE PAST DECADE

Osmar Benito Sandino, Sarah Marie Otterstrom, Jairo Coronado, Julio Collado, Liessi Calero Jiménez, Darling Delgado Jiménez, Karen Lacayo Santana, Elena Yajaira Vargas Martinez, and Yorlin Vargas Collado

*Paso Pacifico, Nicaragua*

The Eastern Pacific green turtle (*Chelonia mydas agassizi*) is listed as globally endangered on the IUCN Global Red List (International Union for Conservation of Nature 2017). The turtle conservation program promoted by Paso Pacifico since 2009, is an initiative that promotes the conservation, reproduction and research of the reproductive biology of the green turtle in three solitary nesting beaches of the Eastern Pacific (EP) in southwestern Nicaragua (Brasilón, Guacalito, Florcita beaches). Through our study, we have recorded nesting females that likewise nest in the neighboring Costa Rican Guanacaste Conservation Area, thus we believe that this bi-national nesting subpopulation of nesting females is one likely unit. The individuals that frequent these beaches, according to the data collected in the last 10 years, present nesting

peaks in the last quarter of the year, reaching an average of 250 nests per season together on these beaches. With a reproductive success of between 63% - 70% (> 2500 turtles/ nesting season) when the conditions and influence of ENSO and other environmental variables are favorable. This success rate has also been by the activities of poaching of nests that take place in these beaches, which are located next to the olive ridley arribada beach in the *La Flor Wildlife Refuge*. Over the decade that our program has functioned in the area, poaching pressure on green sea turtle nests has declined, but it has persisted toward olive ridley nests, a species that is more abundant and which has historically has received much more pressure due to its demand in local markets. The progress in sea turtle protection is thanks to our community ranger program in which rangers are full-time, professionalized employees. Paso Pacifico has emphasized the importance of having a program of more than 10 beach guards that have a presence on these coasts 24 hours a day throughout the year where nests are protected in-situ with the exception of on neighboring beaches where nests turned in by poachers are placed in a hatchery. This conservation initiative is one of the few that are active in the country and its objective is to guarantee the immediate protection of the nests at the time of spawning. In the 2021 season, the trend and increase in arrivals has allowed the collection of more behavioral data and to document fluctuations in nesting. This will allow for improved conservation strategies and increase the reproductive success of naturally-placed nests. In Nicaragua, the lack of scientific documentation on the reproductive biology and monitoring over time of this species is evident. Therefore, sustaining this program and closely coordination with conservation efforts in Northern Costa Rica will make it possible to better protect the species and to enhance our knowledge of this Eastern Pacific green turtle in the Salinas Bay area.

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#### **EFFECTIVENESS AND DESIGN OF MARINE PROTECTED AREAS FOR MIGRATORY SPECIES OF CONSERVATION CONCERN: A CASE STUDY OF POST-NESTING HAWKSBILL TURTLES IN BRAZIL**

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Marine protected areas (MPAs) are among the most widely used strategy to protect marine ecosystems and are typically designed to protect specific habitats rather than a single and/or multiple species. There remains the need to assess whether existing and proposed MPA designs provide protection to species of conservation concern. For this, information on species spatial distribution and exposure to threats is necessary. However, this information is often lacking, particularly for mobile migratory species, such as marine turtles. To highlight the importance of this information when designing MPAs and for assessments of their effectiveness, we identified high use areas of post-nesting hawksbill turtles (*Eretmochelys imbricata*) in Brazil as a case study and assessed the effectiveness of Brazilian MPAs to protect important habitat for this group based on exposure to anthropogenic threats. MPAs were categorized based on management intent, according to the International Union for Conservation of Nature (IUCN) criteria; no-take, where only non-

extractive activities such as educational and scientific activities are allowed, extractive reserves (RESEX), where sustainable use by traditional communities are allowed, and multiple use, where sustainable use by several groups is promoted. The anthropogenic threats considered included industrial and artisanal fisheries, marine traffic, mining, ports and oil and gas production fields across our study region. The majority (88%) of high use areas of post-nesting hawksbill turtles were exposed to human threats, with artisanal fishery being the most prevalent threat in high use areas (78%), followed by marine traffic (76.7%), industrial fisheries (20.7%), ports (1.8%) and mining (0.4%). Most (88.1%) of high use areas by post-nesting hawksbill turtles are not protected by any type of MPA, with only 4.8% of high use areas protected by no-take MPAs and 7% of high use areas are protected by multiple use MPAs. Among the areas protected 86% are exposed to threats. This incongruity is driven by a lack of explicit conservation goals and targets for turtles in MPA management plans, limited spatial information on species' distribution and threats, and a mismatch in the scale of conservation initiatives. To inform future assessments and design of MPAs for species of conservation concern we suggest that managers: clearly state and make their goals and targets tangible, consider ecological scales instead of political boundaries, and use adaptive management as new information become available.

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## **TURTLE LOVE: CONSERVING SEA TURTLES ON THE CARIBBEAN COAST OF COSTA RICA THROUGH COMMUNITY INVOLVEMENT, APPLIED RESEARCH, AND LIAISON WITH ENVIRONMENTAL AGENCIES**

**Renato Saragoça Bruno, Gustavo Adolfo Ortiz Lopez, Gilberto Rafael Borges Guzmán, Camille Fleury, Guilherme Lessa Ferreira, Melissa Msuo, Melissa Serrano, Braulio Piedra Leiton, Andrés Salas Chaverri, Daniele Macedo, Devon Valverde, and Nerine Constant**

*Turtle Love, Barra de Parismina, Limón, Costa Rica*

Turtle Love is a nonprofit conservation organization with the mission to conserve sea turtles on the Caribbean coast of Costa Rica through community involvement, applied research, and liaison with environmental agencies. Turtle Love runs a community-based conservation project working to protect sea turtles nesting at Playa Tres, the 5-km stretch of beach immediately south of Tortuguero National Park (TNP). High levels of illegal harvest still threaten sea turtles and their eggs in areas adjacent to TNP, and Turtle Love extends protection to 5 km of important nesting beach for green (*Chelonia mydas*), leatherback (*Dermochelys coriacea*), and hawksbill (*Eretmochelys imbricata*) turtles. In addition to monitoring the spatiotemporal distribution of nesting activity and illegal harvest at Playa Tres, Turtle Love engages residents of local communities through outreach events and fosters development based on sustainable use of sea turtles through ecotourism. By running a monitoring project, we discourage illegal harvest in the short term, and by involving local community members in the conservation effort, we foster long-term change by providing a sustainable income source that does not rely on harvesting eggs and adult sea turtles. Additionally, Turtle Love's monitoring provides the Costa Rican Ministry of Environment and Energy (MINAE) with data to guide management of this nesting beach and helps ensure the continued survival of sea turtles nesting in the project area. From June 2019 to December 2021, we collected data on 392 individual green turtles, 59 leatherback turtles, and 6 hawksbill turtles during 375 night surveys (4,500 km walked). In this period, there were 1,575 green turtle nests, 170 leatherback nests, and 31 hawksbill turtle nests laid at Playa Tres. Average hatching success was 83.7% for green turtle nests, 54.5% for leatherback turtle nests, and 86.7% for hawksbill turtle nests. We found 83% of green turtle nests ( $n = 1320$ ) and 70% of leatherback turtle nests ( $n = 120$ ) were laid in the northernmost half of Playa Tres. Nesting activity was lower near the river mouths that delimit our project area to the north and south. Based on preliminary data collected in 2018, we estimated over 90% of sea turtle nests laid at Playa Tres were harvested illegally.

Illegal harvest during our first season affected 18% (n = 131) of all nests laid at Playa Tres in 2019, 13.5% (n = 104) of nests in 2020, and 8% (n = 23) of nests in 2021. Illegal harvest was more intense in the dates preceding local festivals and at the middle of the beach, where local landowners abet this activity. Turtle Love reports the distribution of nesting and illegal harvest at Playa Tres to MINAE, which helps local authorities target management actions and protect the second most important nesting beach for green turtles in Costa Rica more effectively. Turtle Love aims to make a long-term contribution to the wellbeing of both local communities and sea turtle populations, and we will resume monitoring and outreach activities for the leatherback turtle season in February 2022.

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## A NEW METHOD TO PREVENT SEA TURTLE NEST PREDATION BY WILDLIFE AND DOGS

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Sea turtles are threatened species with many populations in declining numbers. One of their threats affecting nests and hatchlings is predation by wildlife and feral dogs. On the Osa Peninsula, Costa Rica, predation by raccoons, coatis, and vultures is a major cause of a highly reduced hatching success in nests in situ. In this study, we aimed i) to weekly record the number of nests laid by Olive ridley sea turtles (*Lepidochelys olivacea*) on two different beaches (Pejeperro and Piro) along the coast of the Osa Peninsula, and ii) to implement a novel anti-predator system for *L. olivacea*'s nests made of recyclable plastic mesh and string, which protected the nests from the top to the clutch sides. From April to December 2021, 178 nests were found at Pejeperro beach (4.5km), of which 48 were predated within the first 12 hours of being laid. At Piro beach (2km), 1042 nests were recorded, of which 171 were predated in the first 12 hours. For the anti-predator system, two different approaches (in situ and ex situ) were followed. At Pejeperro beach, 21 anti-predator systems were installed around in situ nests that were laid the night before and marked for weekly monitoring of their status (predated, poached, eroded, washed over, camouflaged or destroyed by other turtles). After 40 days the top part was removed to let the hatchlings emerge once the eggs hatched, and after 10-20 days, we performed the nest excavation to assess hatching success. The anti-predator system had an 81% of success in preventing any form of predation, and showed a hatching success of 65%. On Piro beach, 337 nests were relocated in a hatchery (ex situ approach). The first 58 nests were protected with the typical basket buried close to the sand surface, aiming to control hatchling emergence and prevent fly predation. This had a 69% of success in preventing nest's predation. The remaining 279 nests were protected with the same anti-predator system made of recyclable plastic mesh and string used on Pejeperro beach. In this case, the anti-predator system had a 90% of success in preventing predators from reaching the eggs of the relocated nests, with a hatching success of 73.4%. Our results from in situ and ex situ approaches indicate the potentiality of using these anti-predator systems to protect sea turtles' nests from predation, and can be considered an effective turtle conservation tool for some beaches.

## THE IMPORTANCE OF RAINFALL IN MODULATING HATCHLING SEX-RATIOS AND DETERMINING BREEDING SEX-RATIOS OF SEA TURTLE POPULATIONS IN A WARMING CLIMATE\*

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The ecological impacts of atmospheric warming have been the focus of investigations over the past few decades. However, variations in rainfall patterns are elements of climate change that are often overlooked in most theoretical models that predict the impact of climate change on threatened species and ecosystems. For many tropical regions around the world, heavy rainfall events are predicted to increase in frequency with rising air temperatures. Based on previous findings (Staines et al. 2020), we theorise that the subsequent cooling effect of heavy rainfall may counteract the general warming trend. The feminisation of sea turtle populations may cause lower genetic variation and lower fertilisation of clutches due to the lack of males, and in extreme cases, potentially drive populations to extinction. It remains unknown how widespread the feminisation of sea turtle populations is throughout the Asia-Pacific, a biologically significant region for both green (*Chelonia mydas*) and hawksbill turtles (*Eretmochelys imbricata*). Additionally, there is no estimate of what operational sex-ratio (the sex-ratio at mating grounds) is optimal for a sea turtle population. As part of the WWF-Australia *Turtle Cooling Project*, we aimed to; (1) determine the importance of heavy rainfall events at key hawksbill and green turtle nesting beaches for male hatchling production, specifically in the Asia-Pacific region; (2) assess the relative threat from feminisation for these populations based on models of the past and future hatchling sex-ratios using sand and air temperature data; and (3) identify the functional operational sex-ratio (fOSR) at a regionally significant green sea turtle mating ground in the southern Great Barrier Reef (GBR) of Australia using unmanned aerial vehicle (UAVs) surveys. Our results from aerial surveys of Heron Island Reef indicated a greater proportion of females in the foraging population than what was expected for this region in the southern GBR. However, during the courtship period, there was an influx of both males and females that resulted in a relatively balanced fOSR. This is a promising outcome as most sea turtle rookeries with available sand temperature data globally have a female-biased primary sex-ratio. Hence, our results assist with our understanding of how different sea turtle populations are responding to rising temperatures. It is hoped that outcomes from the ‘Turtle Cooling Project’ will provide new insights to better inform conservation managers on sex-ratio trends of key sea turtles populations and their relative risk to feminisation. This is particularly critical for the Asia-Pacific region where protection or interventions (e.g. shading and nest irrigation) may be required to maintain cool temperatures in a drying and warming climate, thus avoiding critical population feminisation.



**UNOCCUPIED AIRCRAFT SYSTEM SURVEYS TARGET LEATHERBACK  
(DERMOCHELYS CORIACEA) CONSERVATION IN THE RIO DE LA PLATA ESTUARY,  
ARGENTINA**

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Research and conservation efforts for sea turtles have long benefited from technological advances that expand our understanding of their population trends and threats. Better information on habitat use, population densities, and demographic distribution, as well as interaction with anthropogenic threats is essential for the conservation of these species. Consequently, a need exists for better and more efficient ways to study them in areas like the Rio de la Plata Estuary (RLPE) in Southwestern Atlantic Ocean (SWAO). This is an annual migratory foraging destination for adults and large juveniles leatherback turtles (*Dermochelys coriacea*). Several studies on sea turtles in SWAO have given an essential, yet partial description of habitat use and population abundance, highlighting the importance of the RLPE as a key foraging ground for sea turtles in the SWAO, and the importance of considering the region as focus for conservation efforts. Furthermore, this area is a highly important conjoint fishing ground for Argentina and Uruguay, representing a high susceptibility risk as bycatch. During the last decade Unoccupied Aircraft Systems (UAS, also known as drones) have been identified as highly valuable tools in wildlife ecology, showing to be highly valuable, especially to reach restricted or difficult-access habitats and to study endangered species. In addition, the use of UAS allows the scientific community to reduce their cost of research and carbon footprint. Although UAS use is starting to become more common in sea turtle research and shows promising advantages for population studies, this technology has not been fully applied in different sea turtle habitats such as in RLPE. This study represented the first to evaluate the applicability of UAS technology for sea turtle monitoring in the region of RLPE and for developing pilot standardized protocols for UAS future implementation worldwide. At the same time, our aim was to test different strategies for using this tool by evaluating the presence of leatherback turtles in RLPE. We conducted surveys over the water with a UAS in the Santa Teresita (Argentina), in March 2020 evaluating environmental variables and different flight patterns. We tested three surveys transect techniques for detecting individuals over the water from the coast and from a boat. The survey testing allowed us to standardize a methodology for UAS surveys in the area and to show the potential use of this technology in poor water clarity is feasible for sea turtle monitoring. We recorded 72 leatherback turtles and 10 Franciscana dolphins (*Pontoporia blainvillei*) in the study area using a zigzag flight pattern around a boat. Consequently, this study has the potential for not only revolutionizing traditional surveying methods by applying the newest technologies in sub-optimal conditions in SWAO, but also for providing comprehensive, highly valuable, and needed information on habitat use, distribution and abundance of the leatherback turtle in one of the main feeding grounds in SWAO, providing the explanatory power needed for managers to design regional plans for fisheries management and to establish effective conservation efforts in the area.



## SEA TURTLE CONSERVATION AND SOCIAL OUTCOMES THROUGH INDIGENOUS INITIATIVES AND COLLABORATIONS IN AUSTRALIA\*

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Successful long-term conservation programs often depend on locally engaged stakeholders where nature assets have inherent conservation, cultural or monetary values. This is especially true for Indigenous Australians who have intricate cultural links to sea turtles through lore, food, ceremony, songs, stories, dance, art, health and conservation. Over the last 30 years in Australia, changing attitudes and government policies and recognition of Indigenous Knowledge globally have led to many initiatives, public policy changes and different operational behaviours that have increased support for local on-country management including through two-way knowledge systems and jointly managed protected areas. In Australia, this progress is the result of many factors including the Native Title Act 1993 which preserves native title rights and interests of Indigenous people and does not prohibit activities such as hunting, fishing, gathering or cultural or spiritual activities (including the take of turtles). To support this, many groups have developed community management plans to ensure, that in these modern times, cultural and community needs are met in a sustainable manner. In addition, the expansion of Australia's protected area network over the past two decades has occurred across different government jurisdictions with many areas jointly managed with Indigenous groups (e.g. <https://www.dpaw.wa.gov.au/parks/aboriginal-involvement/91-joint-management>). Added to this are 78 Indigenous Protected Areas (IPAs) established since 1999 which are areas of land and sea managed by Indigenous groups to protect biodiversity and culture. Many have healthy country plans (e.g. <https://wunambalgaambera.org.au/healthy-country/healthy-country-plan/>) and employ rangers through various government and non-government sources. The perspective of western scientists has also changed over decades with many recognising the value of two-way knowledge systems and Indigenous collaborations for joint outcomes which is acknowledged in Australia's marine turtle recovery plan (<https://www.awe.gov.au/sites/default/files/documents/recovery-plan-marine-turtles-2017.pdf>). This includes recognising that many Indigenous groups lead their own effective monitoring programs (e.g. li-Anthawirriyarr Rangers Gulf of Carpentaria, Torres Strait, Mapoon <https://apps.des.qld.gov.au/land-sea-rangers/?ranger=mapoon>) and work collaboratively with diverse partners on research and monitoring initiatives. A recent example is the North West Shelf Flatback Turtle Conservation Program ([www.flatbacks.wa.gov.au](http://www.flatbacks.wa.gov.au)) which has collaborated with over 13 Indigenous groups to deliver shared outcomes including employment and education opportunities. The Western Australia Marine Science Institute (<https://wamsi.org.au/research/programs/kimberley-marine-research-program/>) also had successful collaborations across the Kimberley with legacy outcomes including a formalised process for research engagement. Following this the Indigenous Saltwater Advisory Group (ISWAG) formed which is

a non-binding consortium of 9 Indigenous groups to develop solutions to conservation problems with a unique turtle and dugong conservation initiative developed in 2021 (<http://www.mosaicenvironmental.com.au/iswag.html>). The benefits to turtle conservation from these collaborative initiatives include project continuity, better planning, onground implementation, more complete knowledge and community support. Added to this are the equally valuable social outcomes in remote parts of Australia related to education, school attendance, local job prospects and opportunities, health, community cohesion and community finances which could be enhanced further through broader cross-discipline and cross-sector collaborations, and could in part, contribute to Australia's "Closing the Gap" (<https://www.closingthegap.gov.au/>) targets for Indigenous Australians.

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## **THE MARRIAGE BETWEEN BASIC AND APPLIED SCIENCE ENHANCES SEA TURTLE CONSERVATION**

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There are fundamental links between basic science and sea turtle management/conservation. Scientists who use marine turtles for basic science studies must often make those links apparent. Fundamentally, such an approach is consistent with the basics of the scientific method, (observe, hypothesize, test the hypothesis, use the data, and refine the hypothesis). The outcome is what we refer to as "foundational science." The application of basic science provides the building blocks or tools for understanding how conservation and management may work most effectively and helps prevent conservation missteps. Where possible, emphasis on factors that are common to all marine turtle species sets up a basic framework for understanding the species. Comparisons gained through basic science approaches highlight contrasting aspects because species, as well as the several life stages, differ in their ecological specializations, providing contrasts that point to conservation options. Examples of applications of basic science approaches that became conservation and management tools include studies of sea turtle vision leading to the management of artificial lighting at nesting beaches, as well as the use of specific wavelengths of lights on fishing lines and nets to reduce sea turtle bycatch while not affecting the capture of target species. Similarly, studies of early embryonic development led to understanding of movement-induced egg mortality, solutions used for safe egg translocation, and focus on middle development points to the drivers of hatchling sex determination that can lead to shifts in population sex ratios. Collectively, these studies span the basic to applied science continuum and illustrate how foundational knowledge provides tools for more informed and effective conservation and management alternatives, even when those links initially are unanticipated.

## INCREASING MALE HATCHLING PRODUCTION USING SEAWATER IRRIGATION\*

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The northern Great Barrier Reef green turtle, one of the world's largest populations, nests almost exclusively on Raine Island in the northern Great Barrier Reef, Australia. Recent research suggests that Raine Island has been producing almost exclusively female hatchlings for the last two or three decades, feminising to approximately 99% over this time. The *Turtle Cooling Project*, a cooperation between WWF Australia, the University of Queensland and the Conflict Islands Conservation Initiative, has been exploring ways to cool nests to increase male hatching production since 2018. In the project reported here, over two separate nesting seasons we trialled seawater irrigation to cool green turtle nests at Heron Island, southern Great Barrier Reef, Australia. In both trials we had control (no irrigation), freshwater irrigation, and seawater irrigation treatments. Nests were irrigated once immediately before (2019/20 season) or in the middle (2020/21 season) of the sex determining period, by application of the equivalent of either 100 mm or 200 mm of rainfall. The simulated rainfall was applied with a watering can in a circular 80cm diameter area directly above the eggs. There was no difference in hatching success in either year across the treatments, with hatching successes pooled across years: control 70% (n = 8), freshwater 82% (n = 6), and seawater 74% (n = 12). Freshwater and seawater had a similar cooling effect on nest temperature, causing nest temperature to decrease by between 1.0 °C and 1.5 °C immediately on water application, and nest temperatures remained below control nest temperatures for at least 5 days, a sufficient temperature drop to increase male hatchling production. In the 2020 - 2021 trial, mean nest temperatures during the five-day period immediately after irrigation were: control 28.3°C (n = 4), 100 mm freshwater 28.0°C (n = 2), 100 mm seawater 28.0°C (n=6), and 200mm seawater 27.7°C (n=3), and during the sex determining period were control 29.0°C, 100 mm freshwater 28.8°C, 100 mm seawater 28.9°C, and 200 mm seawater 28.5°C. In the 2020 - 2021 trial, 10 hatchlings were sampled on nest emergence and their sex determined by histological examination of their gonads. The proportion of male hatchlings was: control 12.5%, 100 mm freshwater 71.8%, 100 mm seawater 27.1%, and 200 mm seawater 63.3%. If once-off seawater irrigation is used as a management tool to increase male hatchling production, we recommend the application of the equivalent of 200 mm rainfall as this results in the largest fall in nest temperature, and consequently the largest increase in male hatchling production which was a 5-fold increase in our trial, without compromising hatching success. We recommend this intervention is trialled on Raine Island as a nesting cooling strategy as a next step to the *Turtle Cooling Project*.

## **STRENGTHENING THE ROLES OF BEACH OWNERS IN MANAGING NESTING BEACHES AT THE JEEN WOMOM COASTAL PARK, PAPUA, INDONESIA**

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The Jeen WOMOM Coastal Park is a Marine Protected Area (MPA) in the Bird's Head region of Papua, established in 2017 to protect Jeen Yessa (formerly Jamursba Medi) and Jeen Syuab (formerly Wermon) beaches, the most important nesting beaches for Pacific leatherback turtles. The local Abun community legally own the beaches and have the rights to control access to the beaches and determine types of activities allowed. One instrument the community uses to manage natural resources is called "Sasi," a set of customary rules for extractive activities. When Sasi is "closed," certain extractive activities are prohibited. They can be resumed after the Sasi is "opened." To control the number of native animals taken by non-local hunters, Jeen Yessa Beach owners closed Sasi in September 2019, and marine turtles, wallabies, tree kangaroos, cendrawasih, cassowaries, parrots, echidnas, deer, etc. could not be hunted. Only take of pigs and monitor lizards was allowed because they depredate marine turtle nests. We predicted that when Sasi is closed, turtle nest predation by pigs would increase because local people would not want to place pig snares near the beach out of fear of capturing deer. We compared turtle nest predation data from a section of Jeen Yessa, before and after Sasi was closed (April-August 2019 versus April-August 2020), and surveyed local community members to learn how closed Sasi influence their hunting behavior. As predicted, locals' hunting activity near the beach was reduced during closed Sasi, which led to increased pig activity. Nest predation increased from 0% during opened Sasi to 23% during closed Sasi. In 2021, the beach owners accepted our recommendation and opened Sasi on 11 June 2021. When comparing nest predation data between closed Sasi (1 April- 11 June 2021) and opened Sasi (12 June-September 2021), turtle nest predation was reduced by 85% during opened Sasi compared to closed Sasi. We have learned that a partnership with beach owners through their customary instrument can bring immediate positive results to the marine turtles of Jeen WOMOM Coastal Park. We are committed to strengthening the roles of beach owners in beach management by providing recommendations based on scientific data and information.

## EDUCATION, OUTREACH AND ADVOCACY

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### LAMPEDUSA, A PLATFORM IN THE MIDDLE OF THE MEDITERRANEAN: A CASE STUDY ON THE SOCIAL AND ECOLOGICAL IMPACTS ON A SEA TURTLE CONSERVATION PROJECT

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The island of Lampedusa is located in the middle of the Mediterranean Sea, a strategic point where sea turtles and fishery activity often meet. Over the last 30 years, Lampedusa Turtle Rescue's focus has been on sea turtle rehabilitation and conservation, especially in consequence of fishery interactions. The centre has hosted over 5000 turtles and performed about 2500 surgeries, implementing the latest surgical techniques, and improving medical care throughout. Alongside other Mediterranean Groups, Lampedusa Turtle Rescue has organised several veterinarian seminars to create an International Rehabilitation Network with standardised medical management for emergencies. Since 2010, Lampedusa Turtle Rescue has also coordinated the Medical and Health workshops for the International Sea Turtle Society (ISTS) Symposia. A change for experts to share their knowledge and experiences while promoting awareness-raising and research actions to other rehabilitation centres, and to inspire future sea turtle experts. Volunteering is the main reason for the success of Lampedusa Turtle Rescue's projects, as over 700 people have offered their time to support sea turtle conservation efforts at the centre. The change in volunteer activity over the years against the change in the interest of the local community and economy was analysed and compared. From this study, the demographic group most likely to volunteer is young women in higher education, mainly motivated by an interest in sea turtles. Only a small number of them have had former wildlife volunteering experience. In terms of feedback, the most common theme highlighted was the knowledge acquired on sea turtle biology and ecology during their time at the centre. Particularly the majority emphasised how the most rewarding part was realising the impact of their work and its importance in conservation. During summertime, volunteers speak with about 30 thousand visitors every year, the centre's largest awareness-raising project. The vast number of people interested in sea turtles provides an opportunity to transmit the importance of these rehabilitation and conservation projects. Consequently, impacting the mindset of visitors but also economically benefiting the local community resultant from the increased tourism to the island. Lampedusa Turtle Rescue hosts people from all over the world, from all age groups, eager to be a part of awareness-raising projects and contribute to sea turtle conservation. An opportunity that enables the development of communication skills and knowledge expansion through first-hand observation of the daily threats sea turtles face. Experiences like this open minds and create advocates, motivating young people to change the future of sea turtles. A unique place where you live and work with a team of people from different backgrounds, with a range of expertise and views, learning from sea turtles that there are no borders on Earth.

## **SEATURTLESIGHTINGS.ORG – VESSEL OPERATOR OUTREACH/EDUCATION/ OCCURRENCE DATA**

**Karen Moore Dourdeville and Robert L. Prescott**

*Mass Audubon Wellfleet Bay Wildlife Sanctuary, United States of America*

Mass Audubon Wellfleet Bay Wildlife Sanctuary (WBWS) in Wellfleet, Massachusetts, US, is known for its work rescuing cold-stunned Kemp's ridley (*Lepidochelys kempii*), loggerhead (*Caretta caretta*) and green (*Chelonia mydas*) sea turtles during the late fall/early winter along Cape Cod Bay beaches. Since 2002, the sanctuary has also operated a hotline and website, [seaturtlesightings.org](http://seaturtlesightings.org), aimed at communicating with marine vessel operators in coastal waters off New England. The goals of the hotline/website are: 1) to gather data points showing where and when sea turtles are seen in these NW Atlantic foraging grounds, and 2) to reduce vessel strikes by educating vessel operators that sea turtles are present in local waters and by conveying information about how to spot them. The resulting database of sightings contains over 2,400 credible reports, mostly of leatherbacks (*Dermochelys coriacea*) and loggerheads. Although these opportunistic sightings are not collected systematically and are not a count of turtles, they have become useful in showing trends of occurrence, especially of leatherbacks. The sightings also are a minimum count of close vessel/sea turtle interactions in highly trafficked waters. Outreach to vessel operators includes: describing species by text, photos and videos on the website; posting fliers at marinas, yacht clubs, bait and tackle stores and harbormaster offices; and giving talks to boating and fishing groups. Recreational fishermen are often important sea turtle reporters. WBWS partners with the Martha's Vineyard Striped Bass and Bluefish Derby, the oldest fishing derby in the US, which registers over 3,500 participants annually. This five-week recreational fishing derby coincides in time and space with leatherback occurrence in the fall. Several applications for sea turtle conservation have ensued from the WBWS hotline/website. Vessel operators reporting entangled sea turtles, many of which are alive, are instructed how to immediately call the Center for Coastal Studies (CCS) in Provincetown, MA. CCS are the designated sea turtle disentanglers, and timely reports from vessel operators allow CCS staff to begin travel to the entanglement as soon as possible. The US Coast Guard has issued "Notices to Mariners", aimed at reducing vessel strikes, based on sightings of feeding leatherbacks reported consistently in an area, or "hotspot". "Unresolved floaters", i.e. dead sea turtles reported and photographed at sea but never reported on shore, have been documented every year. Many of these floaters show clear evidence of vessel strike. Vessel strike may be a significantly under-reported cause of leatherback and loggerhead mortality in the coastal NW Atlantic.

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## **WHAT DO TOURISTS LIKE? KEY ELEMENTS FOR A SATISFACTORY TURTLE TOUR IN TORTUGUERO, COSTA RICA\***

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In the community of Tortuguero, Costa Rica, tourism is the main source of income for its inhabitants since the 90s and plays a very important role in sea turtle conservation, because it raises awareness about the protection of the various species, provides funding for conservation projects, and creates alternative livelihoods and revenue for people who otherwise would be engaged in direct consumption or sale of sea



turtle products. In 2017, a total of 46,480 tourists from different parts of the world visited Tortuguero to participate in the green turtle (*Chelonia mydas*) observation tour. For this reason, guiding and informing tourists is essential for the success and long-term viability of the local economy and the conservation of this endangered species. Different studies analyzing the relationship between tourists and the turtle tours in Tortuguero have been conducted in the past, and considering pertinent to keep the information up to date to be able to identify possible improvements to the experience, a new survey was developed based on the formats and methodology from the previous ones. Our goal was to identify the key factors that determine an optimal experience for the tourists that take the turtle tour. More than 700 tourists were surveyed after conducting the turtle tour from August to October 2018 and from July to October 2019. These surveys focused on crucial elements that influence tourist satisfaction, such as the educational content of the tour, as well as the aesthetic, cultural and experiential elements related to nature. Preliminary results indicate that more than 90% of tourists rated the tour as "good or excellent". Among the factors that negatively influenced the experience are the high concentration of tourists and the perception of disturbing the turtles during the tour. On the other hand, the factors that influence positively the experience are the observation of some stage of the nesting process, a good preparation of the guide, and the perception of positively influencing conservation activities. The information obtained in this study will serve to improve the service offered during turtle tours, in addition to promote a quality tourism experience based on the opinion of visitors. This work contributes to the conservation and management of sea turtles in the community of Tortuguero, adding information to existing assessment and monitoring tools that allow decision makers to continue with the conservation success that this community represents.

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## **THE LAST TWO NESTING GREEN SEA TURTLES (CHELONIA MYDAS): AN INCENTIVE TO MOBILIZE THE PRESERVATION AND ADVOCACY OF NESTING BEACHES IN REUNION ISLAND\***

**Anne-Emmanuelle Landes<sup>1</sup>, Léo Pairain<sup>1</sup>, Claire Jean<sup>2</sup>, and Stéphane Ciccione<sup>1,2</sup>**

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Reunion Island was an important nesting site for green sea turtles *Chelonia mydas* before human colonization. Nesting activity has since then almost disappeared following massive historical hunting and rapid coastal development. Although sightings of foraging juvenile turtles have significantly increased in coastal waters over the past three decades, only three sea turtle tracks were recorded between 1990 and 2002 on the 39 km of sand beaches in Reunion Island. Rehabilitating nesting beaches appeared as an urgent conservation action, in an attempt to attract nesting turtles. A pilot beach restoration program was launched in 1999 on a site located where no sign of turtle nesting activity had been reported for the past 50 years. The program restored the upper vegetation, potentially used by females as clues for their nesting site selection. A female laid its eggs four times on the rehabilitated beach in 2004, returned in 2007, but has unfortunately not been sighted again thereafter. A second identified female successfully nested on that site in 2011, and then in 2018. On another unrehabilitated beach, a nesting activity from a third identified female was also recorded in 2007. This female successfully nested on the same location every three years until 2019. These promising events have led to a larger beach rehabilitation program conducted by the Centre d'Etude et Découverte des Tortues Marines (CEDTM) between 2017 and 2021, targeting four beaches for a total linear of 3 km. A total of 222 field operations have been conducted, combining awareness-raising talks and specific onsite actions: alien species' control and removal, debris collection, native species planting and light pollution reduction. About 8,000 people have participated in those actions, of which more than 50% were school children. Besides, monitoring of eventual nesting activities was carried out by



volunteers and beachside residents. A total of 50 different local companies working in the study or management of the coastal ecosystem components, including NGOs, public communities, scientists, seaside tourism actors, were informed about the program. As a result, multiple partnerships with the CEDTM have been established, leading up to four complementary funds to achieve the common objectives of restoring and preserving the coastline of Reunion Island. Actions with similar protocols have been deployed by partners on five other beaches, expanding the restored coastal line by 4.5 km. Since the beginning of the restoration program, the two last aforementioned breeding females were reported nesting on five occasions each. Indeed, the relict breeding population of Reunion Island, although small and threatened, mobilizes human and financial resources for the protection of its nesting habitat, acting as an umbrella species. The involvement of children and the local community has generated a virtuous dynamic that will contribute to sustain the necessary conservation efforts over time. Restored nesting beaches will hopefully welcome an increasing number of reproductive turtles and their offspring, which will in turn benefit the coastal ecosystems of Reunion Island.

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### **THE AGA SEA TURTLE EXHIBITION: AN ADAPTABLE EDUCATION TOOL TO BUILD ECOLOGICAL AWARENESS AND FOSTER RESPONSIBLE BEHAVIOR IN TOURISTS' HOME COUNTRIES AND ON SITE**

**Miriam S. Mueller, Birgit Braun, and Stephan Kieninger**

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Environmental education and awareness building activities can contribute to marine turtle conservation and restoration efforts. Besides local residents, tourists are a key user group to be reached by environmental education programs since many turtle nesting sites around the world are also important tourist destinations. In order to address both groups, the non-governmental organization Aktionsgemeinschaft Artenschutz (AGA) e.V. has developed an adaptable and easy-to-use sea turtle exhibition with an accompanying environmental education program that centers not only on the biology of marine turtles, their habitat and threats to sea turtles, but also puts emphasis on activities and behavioral decisions that help to protect the marine environment. On the one hand, the exhibition is used in schools, during action days and on trade fairs in tourists' home countries in order to raise awareness and foster the idea of environmental responsible behavior at home as well as at tourist locations. On the other hand, the exhibition is presented in different turtle nesting areas in order to address local stakeholders and visitors alike. For those reasons, the exhibition has been translated and adjusted to different target groups and locations. It is currently available in German, English, Turkish, Spanish, Portuguese and Singhalese and has been adapted to the local conditions in eight countries so far. Teachers and multipliers can lend the exhibition and use the accompanying game material free of charge to design their own education event while sea turtle conservation projects can adjust the content to their local circumstances. In this way, the exhibition helps to create knowledge about sea turtles for visitors as well as local residents and thereby supports conservation activities. Here, we would like to present the exhibition, build synergies with other educators and present the existing possibilities to extent the exhibition's reach.

**IMPLEMENTING A LIGHT POLLUTION MITIGATING SYSTEM; A COMMUNITY BASED INITIATIVE BY SEA TURTLE CONSERVANCY'S JUNIOR ASSISTANTS AT TORTUGUERO, COSTA RICA**

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Marine turtle populations represent a main and singular component among worldwide biodiversity, but over the last centuries the number of individuals registered has decreased drastically. As a group, marine turtles are considered endangered, exposed to different pressures throughout their biological cycle, such as a bycatch, illegal hunting, egg and meat harvesting, increasing of ocean contamination and coastal development. One of the main objectives of marine turtle conservation organizations is to reduce the effect of human action on nesting sites, which are critical places for the welfare of future generations of sea turtles. Of the threats that affect nesting sites, the presence of artificial lights directly affects both adult female and hatchling's behavior. Previous studies have shown that there is a negative correlation between light registered on nesting sites and the number of females that nested successfully. Furthermore, hatchling emergence and orientation are also affected negatively. Light pollution from surrounding urbanized areas affects turtle's vision, and thereby their orientation along the beach. Tortuguero, Costa Rica, is the second most important nesting beach for the green turtle (*Chelonia mydas*). Here, since 1959 the Sea Turtle Conservancy has conducted many studies regarding nest distribution for this species. We have analyzed the influence of light pollution conducting light surveys on the beach since 2013. Based on these, we have worked on mitigation strategies against light pollution with different projects in collaboration with other organizations. Although changes have been positive, human population and development in Tortuguero is increasing every year and thereby public, houses and hotel lights are as well, leading to increased pressure on sea turtle populations. Given the situation, a new strategy was proposed that includes the collaboration with the community and contemplates the limitations of the resources available. Along with the group of Junior Research Assistants, teenagers from Tortuguero's community, a project was initiated to decrease the influence of light contamination on the most vulnerable areas for sea turtles. As a first step, surveys were conducted to sensitize and evaluate the knowledge of the residents about how light pollution affects sea turtles and which could be viable methods to improve light conditions on the beach. Using survey results, deflecting devices were created to cover the lights affecting the beach. These devices were made by the Junior Research Assistants from plastic containers donated by a local establishment. During a trial test, brightness was measured on three lights that had been covered with the new devices, recording the brightness before and after installing the cover device. Results obtained showed that light covers are a viable solution to decrease the impact of light brightness that reaches the beach. Therefore, with the help from the Junior Research Assistants, new devices were installed in the most critical points in terms of light pollution along the beach and coastal areas of Tortuguero. The presence of new light covers reduced the visibility of the lights from the beach and thereby resulted in better conditions both for nesting females and hatchlings to return safely to the ocean.

## TEACHING EVIDENCE-BASED PROTECTION OF SEA TURTLE EGGS

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It is important for emerging conservationists and wildlife managers to gain experience in the use of evidence-based conservation, by way of critical analysis and understanding of the context and application of conservation actions. We developed a teaching case and activity for undergraduate and graduate courses in conservation biology, wildlife management etc, although it could also be adopted for upper-level high school classes. The case is based on a recent paper by Phillott et al. (2021) on hatchery practices in India. Hatcheries are commonly established to protect sea turtle eggs and hatchlings from abiotic (e.g., tidal inundation, light pollution) and biotic (e.g., depredation, illegal take) threats. Eggs are collected from the nesting beach, transported to a protected area (the hatchery) located on or close to the beach, and buried in artificial nests for incubation. When hatchlings emerge from the nest, they are released to the sea to complete the sea turtle life cycle. Guidelines for sea turtle hatcheries have been widely followed by sea turtle conservationists for decades, and their use has potentially contributed to successful conservation of some sea turtle populations worldwide. However, the conservation value of hatcheries has long been debated. Unless best practices in the collection, transport, and incubation of eggs, and holding and release of hatchlings are followed (reviewed by Phillott & Shanker 2018), relocation of clutches to hatcheries may result in lower hatchling production, reduced hatchling fitness, and/or skewed sex ratios. The teaching activity builds conservation science literacy as students identify studies describing methods to assess risks to *in situ* clutches and mitigate threats including tidal inundation, depredation, and illegal take. A practical exercise asks students to assess threats at multiple nesting beaches on an island and propose which protection strategy (protect *in situ*, relocate to safer individual location on the beach, or relocate to a hatchery) would be most appropriate at different locations.

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## CAREY'S SALE IN BOGOTÁ

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The Tortugas Foundation is a non-profit organization, based in Bogotá, that works for the protection and conservation of sea turtles from environmental education and the dissemination of knowledge of the species. In 2018, thanks to the valuable collaboration and disposition of the Tortugas del Mar Foundation, our organization became a strategic ally of the country's initiative against the hawksbill turtle "I don't buy hawksbill turtles", framed in the international campaign "Too much weird to use it. " When we began to spread the campaign, we thought that the possibilities of informing about hawksbill turtle objects in Bogotá would be zero and we would focus on promoting responsible tourism with turtles, so as not to buy souvenirs made of hawksbill turtles. Unfortunately, in October 2018, we made a fortuitous discovery of the street sale of handicrafts made with Carey in Bogotá (approximately 100 pieces); in a sector recognized for the sale of handicrafts and supplies for them. After this episode, we are more attentive to the craft and jewelry stores of the city, focusing on 2 sectors: Chapinero, place where the first fortuitous discovery was made and the

Historic Center, a tourist reference of Bogotá. First, Chapinero, a selection was made of stores that had potential characteristics for the sale of hawksbill turtles and a survey was designed to apply them. However, in this place, people were reluctant to apply the survey and take photographs; the reasons given were the copying or plagiarism of their designs and not being the business owners, so they did not have permission to respond. We found 18 places, including galleries and street stalls (the latter on the rise) that could become a stage for selling hawksbill; as it happened in the finding of 2018. In 2019, in the historic center, a visit was also made to characterize the main stores that sell jewelry and supplies closest to the tourist sites. In these places, it was shown that mainly gold, silver and precious stones are sold. However, at the end of the tour we found a craft fair in the street, with approximately 40 tents, where we recorded the sale of items made with hawksbill, by an exhibitor. In this same neighborhood, earlier this year, he was "denounced" on social networks, another traveling fair where they sold products made of hawksbill turtles and sea turtle shells. From the participation scenarios in which we have an impact in some way, we continue working to disseminate the initiative "I do not buy hawksbill turtle", to the community in general, so that citizens know the origin of hawksbill, identify and denounce its sale. It is also important to promote this initiative, in the sectors of the district administration, responsible for organizing sporadic craft fairs; taking into account that the scenarios where the sale of hawksbill have been found are traveling scenarios, and although they have been reported to the authorities, it is difficult to effectively monitor them by not staying long in the same place.

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## GLOBAL DISTRIBUTION OF PUBLIC HEALTH RISKS LINKED TO SEA TURTLES' SPECIMEN CONSUMPTION

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Sea turtles are vectors of numerous health hazards known to potentially induce severe adverse health effects on people, sea turtles, and other animals. Probably the most known affliction linked to sea turtle specimen consumption is a toxin-induced syndrome called chelonitoxism. Often lethal, this syndrome is thought to be caused by an unknown marine toxin, able to pass into breast milk, reported as systematically fatal to breastfed infants, and for which no specific treatment is available. Lethal chelonitoxism human and animal cases have been regularly reported and could be increasing due to climate change and rise of sea temperature. Numerous zoonotic and anti-microbial resistant microbes are known to infect sea turtles: several species of *Vibrio*, *V. parahaemolyticus*, *V. alginolyticus* and the pandemic strains of *Vibrio cholerae*, have been isolated in green turtles in the Gulf of California, and show intermediate or complete antibiotic resistance to at least one antibiotic. *Vibrio mimicus* in Costa Rica, *Vibrio cholerae* in China, and *Salmonella chester* in Australia, have been reported to be associated with human disease following consumption of sea turtle meat or eggs. The zoonotic potential of *Leptospira interrogans*, bacteria that may circulate in *Eretmochelys imbricata* and *Chelonia mydas* in Baja California, Mexico, is unknown. Zoonotic parasites have been isolated from sea turtles and their eggs such as *Cryptosporidium* spp. which has been found in green turtles in Hawaii. The presence of zoonotic spirorchid trematodes in sea turtles is also regularly documented, for instance in Panama, Puerto Rico, Bermuda, Australia and the southeastern USA. The contamination of Olive Ridley eggs by *Salmonella typhimurium* in the Pacific has been described, as has the contamination of leatherbacks eggs by *Salmonella enterica* in Grenada, representing a risk of "food-

borne” disease for sea turtle specimen consumers. More generally *Caretta caretta* has been suggested as a prime reservoir of antimicrobial resistant bacteria due to the species unique ecological and physiological characteristics, including a diverse omnivorous diet, long lifespan, and high site fidelity to coastal nesting habitats. High levels of antibiotic resistance were described in *Citrobacter* spp., *Pseudomonas aeruginosa*, *Morganella morganii*, and *Proteus vulgaris* isolated from loggerheads, namely to penicillin and tetracycline. *Shewanella putrefaciens*, *Morganella morganii*, and *Vibrio alginolyticus* were isolated from samples of loggerheads in Cabo Verde, showed multi-antimicrobial resistance and presented complex virulence profiles. Many more health hazards may not be known today due to the costs of the laboratory analysis required to be performed at a scale large enough to bring statistically significant results. This scientific poster aims to inform indigenous and non-indigenous people of the inherent public health risks from consuming sea turtle specimens. Vulnerable communities are more susceptible to adverse health effects due to environmental harm, including malnutrition. Moreover, exposure to pollution and other environmental harm in childhood can have lifelong consequences, including diminished mental capacity and an increased likelihood of cancer and other diseases. These public health hazards are visually presented to efficiently bring awareness to communities at risk.

## FISHERIES AND THREATS

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### FORAGING GROUNDS OF YOUNG CHELONIA MYDAS AND THE INTERACTION WITH MULLET (MUGIL SPP.) FISHING IN THE RIGHT WHALE ENVIRONMENTAL PROTECTION AREA (“ÁREA DE PROTEÇÃO AMBIENTAL DA BALEIA FRANCA”), SOUTHERN COAST OF BRAZIL

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The foraging grounds of the green sea turtle (*Chelonia mydas*) are located in tropical and subtropical habitats of all major oceanic basins. In the Southwest Atlantic, a foraging ground for young *C. mydas* was monitored from 2010 to 2014 at the Itapirubá inlet (28°18'S, 48°41'W), within the APABF (“Área de Proteção Ambiental da Baleia Franca” – Right Whale Environmental Protection Area), Southern Brazil, and this monitoring was resumed in March 2021. At Itapirubá and other inlets of the marine protected area, the foraging grounds overlap with local small-scale and artisanal fishing sites, the incidental catch of green sea turtles being common. Small-scale fishing of mullet (*Mugil spp.*) in the region is carried out in a traditional fashion by artisanal fishers with pelagic seine nets and small boats during that species' reproductive migration period. Mullet schools are spotted from higher points in hills by a lookout who guides the boat's maneuvers. When the school approaches the coast, the fishers surround the fish and haul the net to the beach. This method is called “beach-seining” and is very lucrative and culturally important. The rules and standards for mullet fishing in Brazil are defined by federal law and fishing season for artisanal fishers begins on May 1<sup>st</sup> and continues for as long as there are schools in the region. In 2021, fishing season lasted from May to June and there were 110 incidental catches of *C. mydas* and 38 recapture events in nine beach-seining activities in the inlet. Researchers were actively following up on the fishing season and all green sea turtles were recorded alive and conscious, having been temporarily held for external body assessment and biometry and tagging. Seventy-two young specimens were identified with average curved carapace length (CCL) of 43.8 cm (34.9-61.5 cm; SD = 6.1 cm; N = 72). The handling and release procedures for all turtles were followed by fishers, family members and local inhabitants. Monitoring of the 2021 mullet fishing season showed an increase in the number of incidental catches of green sea turtles in relation to previous monitoring seasons, which indicates the need to continue monitoring this activity for the next years. Artisanal mullet fishing presents a low risk for green sea turtles because the nets remain submerged only for the time needed to surround the schools and drag them to the beach (less than one hour), and this avoids the risk of catch and death by asphyxiation for the turtles. However, a follow-up of fishing activity and a prior understanding by fishers as to the importance of monitoring and maintaining foraging areas for the conservation of green sea turtles are essential, since fishing is one of the major human activities threatening the species worldwide. Feeding areas are also resting sites during this relatively sedentary phase of the life of the green sea turtles, and the Itapirubá inlet is one of the important grounds for *C. mydas* that have been identified in the Southern coast of Brazil, Uruguay, and Argentina.

## **ANALYSIS OF DIGESTIVE TRACT CONTENTS FROM LOGGERHEAD SEA TURTLES CARETTA CARETTA (LINNAEUS, 1758) STRANDED ALONG THE NORTHWEST COAST OF MOROCCO**

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Understanding the foraging habitat preferences of marine megafauna is important to avoid the impact of fishery bycatch and develop appropriate management measures. Here, digestive tract contents of 20 dead stranded loggerhead turtles (*Caretta caretta*) in northwest Morocco were examined between 2002 and 2007. The weight occurrence of preys demonstrated a very diverse diet: 41 taxa were identified belonging to 8 Phyla, but were dominated by fish (*Engraulis*), crustaceans (*Polybius henslowii*) and mollusks. Anthropogenic debris, such as plastics, were also found among the gut contents. Adult turtles had fed largely on benthic preys (94%) whereas juveniles had consumed pelagic preys in large quantities (79%). The size class between 50 cm and 70 cm carapace length (sub-adults) consumed 63% benthic preys and 37% pelagic preys. The diversity in prey species consumed increased with turtle size as would be expected from the carnivorous nature of loggerhead turtles.

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## **IDENTIFICATION OF RISK AREAS OF SEA TURTLE POACHING ON THE ISLAND OF BOA VISTA, CAPE VERDE, USING A STATISTICAL AND GIS-BASED APPROACH**

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Poaching of nesting females for meat remains a great anthropogenic threat to the endangered loggerhead sea turtle population on Boa Vista, Cabo Verde. Due to the recent introduction of a law that can send poachers to jail for their crimes, the nightly activities of a special Sea Turtle Surveillance Task Force (STSTF) employing conservation dogs and night vision drones, and increased opportunities for community members to work in sea turtle conservation, poaching has significantly decreased. However, illegal taking of sea turtles from the beaches is still a problem that must be addressed by appropriate conservation actions. This project aims to identify areas on Boa Vista that have a high incidence of sea turtle poaching and to determine the geographic features that contribute to the selection of these areas by evaluating poaching locations in their geographical context. To do so, the GPS points that represent past poaching events were analyzed to determine whether the distribution is clustered, random, or dispersed. The output of this analysis was a Kernel Density map, where clear hotspots were visible in the northern and eastern coasts of the island. G and K functions with Monte Carlo simulations were then performed to determine the degree of significance of clustering. The data displayed statistically significant clustering, meaning that there must be a cause or causes for the locations seen as hotspots on the Kernel Density map, as the dispersal could not occur randomly. The following geographic features were selected due to informed belief that they influence



locations where poaching occurs: roads, hotels, research camps, and towns. The distances from each poaching site to the nearest of each geographic feature were calculated. An equal number of pseudo-absence points were created in the interior of the island, and the distances from each of these points to the nearest geographic feature were also calculated. Logistic regression analysis was then performed on all the distances to determine the strength of the influence that each geographic feature has on the likelihood of poaching, and to rank the features. Geographic elements that more strongly influence where poachers work were given more weight to reflect the reality of the situation. Interviews with ex-poachers were also used as expert consultation to further inform the weighting of elements. Finally, areas of high risk for poaching were determined by overlaying the weighted geographic features and identifying areas with the highest scores. Known hotspots should receive higher scores, and other areas that score high indicate areas that require increased surveillance, as it is predicted that poaching may occur more frequently in those areas than has been reported. This analysis therefore not only identifies areas of risk, but also predicts risk areas that have been missed by beach surveys, possibly due to a lack of patrols in those areas. The results of this project will be used for mission planning of the STSTF and to help all conservation organizations on Boa Vista to concentrate efforts for efficient beach patrolling to end sea turtle poaching on the island Boa Vista.

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## **MONITORING OF MARINE DEBRIS INGESTION IN ALIVE LOGGERHEAD SEA TURTLES FROM TUNISIAN WATERS (CENTRAL MEDITERRANEAN)**

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High interaction between sea turtles and marine debris is being increasingly reported worldwide through ingestion and entanglement. The threatened loggerhead sea turtle, *Caretta caretta*, has been considered as a bioindicator species to monitor marine debris in the Mediterranean and European marine areas. The ingestion of marine litter by 32 alive loggerheads, in rehabilitation at the INSTM Sea Turtle Rescue Centre, was investigated from faeces according to the SPA-RAC/INDICIT protocol. During 2020 and 2021 faeces were rinsed after collection in a 1 mm sieve and debris were collected manually by visual observation directly from the sieve. Debris was detected in 28.125% of the large juvenile turtles (mean CCL= 59.5 cm). Plastics (1 cm -12 cm) was the main debris defecated by turtles with a mean mass of 0.297 g by turtle. It was composed mainly of Sheet category (71.428%). Ingestion of plastics by turtles was discussed taking into consideration the external body condition of the turtles and their response during the rehabilitation period.

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## **SEA TURTLE INTERACTIONS WITH THE ARTISANAL FISHERIES IN BELYOUNECH (STRAIT OF GIBRALTAR)**

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Bycatch is one of the most serious threats to sea turtles all around the world, including the Mediterranean. In this paper we focused on assessing interactions between sea turtles and the artisanal fisheries in Belyounech, situated in northern Morocco on the Strait of Gibraltar. We conducted an interview with 20 fishermen who were on average  $41.5 \pm 12.8$  years old with an average fishing experience of  $18.4 \pm 11.9$

years. All fishermen claimed to have seen a turtle at least once in their life. Loggerheads and leatherbacks were the most frequently observed in the region throughout the year, however, results showed a low interaction between artisanal fisheries and sea turtles. This is probably due to the use of selective gears (hook lines and bottom longlines), which had no impact on sea turtles. Only four cases of bycatch were identified, which were due to the use of trammel nets; two turtles (Loggerhead) were released and two were already dead (Loggerhead). Despite the enthusiasm and moral commitment expressed by fishermen for the protection and preservation of sea turtles, it remains essential to plan meetings and workshops with the fishermen to discuss turtle protection, the laws and conventions that protect them, and incentives for collaboration to reduce sea turtle bycatch.

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## INCIDENTAL CAPTURE OF SEA TURTLES IN THE NORTH-EAST ATLANTIC PORTUGUESE PELAGIC LONGLINE FISHERY\*

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Incidental catches or bycatch of sea turtles by pelagic longline fisheries is a major concern worldwide. Fisheries bycatch has been linked to population declines and combined with high fishing effort, it may compromise population recovery. The Northeast Atlantic hosts foraging and developmental key areas for oceanic juvenile loggerhead sea turtles originated mainly from the south eastern USA and Cape Verde. Worryingly, it may be one of the most heavily fished areas by pelagic longline for which no recent assessments of fisheries interactions exist. We analyzed fishery observer data collected between 2015 and 2020 to assess sea turtle bycatch by Portuguese commercial pelagic longliners targeting swordfish (*Xiphias gladius*) and blue shark (*Prionace glauca*) in the Northeast Atlantic (latitude: 20-46°N, longitude: 10-42°W). The spatial and temporal distribution of fishing effort, and gear characteristics are presented. A total of 174 sea turtles interacted with the gear during the 896 fishing sets (887,641 hooks) monitored off the coast of mainland Portugal, the Azores and the Canary Islands. Loggerheads (*Caretta caretta*; n=139) ranging between 32 and 78 cm of curved carapace length (CCL) were caught at a rate of  $0.152 \pm 0.711$ (SD) turtles per thousand hooks and leatherbacks (*Dermochelys coriacea*; n=35) at a rate of  $0.043 \pm 0.215$ (SD) turtles per thousand hooks. Temporal and spatial patterns of sea turtles bycatch distributions within the fishery patterns were examined. Results indicate that sea turtle bycatch distribution change seasonally, with loggerhead bycatch higher during summer and autumn months and clustered in space mainly off Canary Islands and in international waters between Portugal mainland and the Azores, while leatherback bycatch occurred from spring to autumn scattered across the studied area, mostly within the Portugal mainland 200NM Economic Exclusive Zone (EEZ) and in international waters. Mortality rates of oceanic stage juvenile loggerhead turtles from the Portuguese pelagic longline fishery in the Northeast Atlantic were estimated at 30%. Leatherbacks physical condition at release was inferred to be strong for all individuals since they were not boarded due to their large size. This study presents information that can be useful to delineate effective management strategies for sea turtle conservation in the Northeast Atlantic.

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## **PERSISTENT ORGANIC POLLUTANT IN THE EGGS OF LEPIDOCHELYS OLIVACEA IN BRAZIL**

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Persistent Organic Pollutants (POPs) are among the threats that affect sea turtles. These synthetic contaminants were widely disseminated as pesticides, electrical insulation, flame retardants and industrial by-products. POPs, such as polychlorinated biphenyls (PCBs), organochlorinated pesticides (OCPs) and polybrominated diphenyl ethers (PBDEs), have high stability, toxicity, low degradability, and lipophilicity that favor the persistency in the environment and bioaccumulation in organisms. Studies showed that these pollutants can interfere in the immune system of sea turtles, as well as in their embryonic development. These contaminants are accumulated in the females mainly through food ingestion and consequently transferred to the eggs. Thus, the analysis of POPs in eggs is a way to assess the maternal transfer. In addition, studies that comprise eggs are scarce, mostly related to the north hemisphere. Therefore, the present study aimed to evaluate, for the first time, POPs in the unhatched eggs of olive ridley (*Lepidochelys olivacea*). To achieve this goal, eggs from 13 females were collected in the north coast of the state of Bahia, in Brazil. The content of the eggs of each nest that was in good conservation condition, as well as classified until the middle stage of development was used to constitute a *pool*. POPs were extracted in Soxhlet apparatus and quantitatively analysed in a gas chromatograph coupled to a triple quadrupole mass spectrometer for fifty-one congeners of PCBs, fifteen OCPs and seven PBDEs congeners. All results below were given in wet weight (ww). PCBs were predominant, ranging from 0.487 to 10.75 ng g<sup>-1</sup>. The hexa and heptachlorinated congeners were predominant, and PCB 153 and PCB 138 presented the highest concentrations. The DDTs (dichlorodiphenyltrichloroethane), composed almost entirely by *p,p'*-DDE were the most prevalent pesticide, with values from 0.168 to 1.23 ng g<sup>-1</sup>. The HCH (hexachlorocyclohexane) values occurred from 0.064 to 0.383 ng g<sup>-1</sup> and were constituted mainly by  $\gamma$ -HCH, followed by  $\beta$ -HCH. HCB (hexachlorobenzene) and Mirex occurred in all samples, with values between 0.025 to 0.116 ng g<sup>-1</sup>, and 0.008 to 0.100 ng g<sup>-1</sup>, respectively. Chlordane (0.007 to 0.060 ng g<sup>-1</sup>) were also detected and the oxychlordane was the most frequent compound, followed by *cis*-chlordane and *trans*-chlordane. The PBDEs were detected in 85% of the eggs but showed the lowest concentrations, from below the limit of quantification (<LOQ) to 0.078 ng g<sup>-1</sup>, compared to the other contaminants. Five PBDE congeners were detected: PBDE 47, 99, 100, 153 and 154. In agreement with previous studies, the pattern of POPs presented higher concentrations of PCBs, followed by DDT. Moreover, the results showed that *L. olivacea* that nest in Brazil are exposed to POP contaminations and corroborate with the maternal transfer to the eggs.

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## **COMPARATIVE STUDY OF A BOTTOM-SET GILLNET DESIGNED TO REDUCE SEA TURTLE BYCATCH IN THE U.S. MID-ATLANTIC MONKFISH GILLNET FISHERY\***

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Monkfish (*Lophius americanus*) is a commercially valued species that supports a lucrative fishery in the Mid-Atlantic and Northeast regions of the United States. Primarily targeted through sink gillnets and trawls, the fishery is known to incidentally catch sea turtles and Atlantic sturgeon, both of which are protected

under the Endangered Species Act. Although several years of research have demonstrated that an experimental, low-profile gillnet reduces the bycatch of Atlantic sturgeon while maintaining acceptable landings of target catch, there is a lack of information on its effects on sea turtle bycatch. In this study, we compare the difference in turtle catch between a low-profile (experimental) gillnet and a standard (control) gillnet that is typically used in the monkfish fishery. This study was conducted off Cape Hatteras, North Carolina from mid-February to mid-March 2021. This location is known to have high densities of loggerhead turtles in late winter/early spring, which allowed us to collect enough turtles within reasonable time and budget constraints. Over 14 days, 68 paired hauls with an average soak time of 42 minutes were completed. The control net caught 19 loggerhead turtles and the experimental net caught 6. Following a repeated measures design, we used generalized linear mixed models with a Poisson distribution to model the dependent variable of turtle catch numbers. Fixed effects included gear type and the environmental variables of surface temperature/bottom temperature differential, time of day, depth, and wind speed. A trip identifier was included in the model as a random effect and soak duration was included as an offset variable. The corrected Akaike Information Criterion (cAIC) was used to find the best fit model from a model suite. The model with the single fixed effect of gear type had the lowest cAIC. The estimate of gear type indicated that the experimental net reduced turtle catch by approximately 68% compared to the control net. In addition to the model results, underwater video of loggerhead turtles interacting with the experimental net was captured.

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## **BYCATCH OF SEA TURTLE**

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Interactions between marine turtles and fisheries represent one of the most serious threats to marine turtle populations. Our study aims, on the one hand, to highlight the extent of the incidental catches of marine turtles, and on the other hand to describe and analyze the interactions of fisheries with marine turtles. To achieve these objectives, we surveyed the fishermen in the port of Jebha using a questionnaire. The majority of the fishermen (77.08%) stated that they had accidentally caught marine turtles, of which the loggerhead turtle (89.19%) was the species most captured by the different fishing gear operating in the port of Jebha. Moreover, the purse seine is the most threatening fishing gear for the marine turtles according to the fishermen (54.05%). Moreover, subadult loggerhead turtles (40.54%) and juveniles (32.43%) are the most caught. Indeed, bottom-set nets and purse seiners (15.15%) and longlines (12.12%) are the fishing gears that cause more mortality among loggerhead turtles. In addition, all the fishermen surveyed (100%) stated that the sea turtle population is declining. This data is the most worrying since marine turtles are threatened with extinction. In this sense, all the fishermen saw that the protection and conservation of marine turtles is necessary, and 89.6% of them were interested in the application of tools for the release of accidentally captured marine turtles. Improving fishing techniques, raising fishermen's awareness, as well as the implementation of conservation measures for marine turtles remain the best solutions to avoid their bycatch.

## **FACTORS AFFECTING MARINE DEBRIS INGESTION BY LOGGERHEAD TURTLES (CARETTA CARETTA) IN THE WESTERN MEDITERRANEAN**

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Marine pollution is one of the main threats affecting marine biodiversity conservation. Here we analyzed debris ingested by 63 loggerhead sea turtles (*Caretta caretta*), live (n= 24) and dead (n= 39), stranded or accidentally caught at the Valencian Community (East Spain) between 2017 and 2019. The aim of this study was to elucidate which factors could be determining debris ingestion by this species. Totally, 843 debris items (dry weight: 230 g; wet volume: 346,3 ml) were found, being white-transparent plastic items from types “sheet” (51%) and “fragment” (32%) the most frequent type of debris ingested. Anthropogenic debris’ frequency of occurrence for dead turtles was 66,7%, while all samples from live turtles analyzed contained marine debris items. Anthropogenic debris ingestion was conditioned by the Curved Carapace Length (CCL) and seemed to be related with the season of the year and the area. Diet analysis carried out for necropsied dead turtles showed that ingested more debris at the bento neritic zone. Information about interaction between loggerhead turtles and anthropogenic debris is provided, which reaffirms the species’ role as an indicator of marine pollution at the study area.

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## **IDENTIFICATION OF ARGENTINE FISHING FLEETS OPERATING IN AREAS USED BY SEA TURTLES IN THE SOUTHWESTERN ATLANTIC OCEAN**

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Bycatch of non-target species in fisheries is considered a serious problem worldwide for the conservation of marine megafauna, including sea turtles. It is estimated that at least 85,000 turtles are caught by fisheries worldwide each year, and the frequency depends on the temporal and spatial overlap of critical species habitat and fishing activities. Argentina is home to the southernmost foraging and development areas for three species of sea turtles (*Caretta caretta*, *Chelonia mydas*, and *Dermochelys coriacea*), which seasonally overlap with areas where intensive fishing occurs. Although the number of bycatch and the impact of fishing activity on these reptiles are unknown, there are sporadic reports caused by different fishing gears. The objective of this study is to characterize the fleets that operated in 2020 in the area between 34°- 41°S and 52°- 63°W, previously identified in the bibliography as an area of high-susceptibility to bycatch, and to assess their fishing effort. Information was obtained from landing declarations provided by the National Undersecretariat for Fisheries. The following data were collected: Number of vessels, length, type of operation, fleet category, and tons landed for each of the rectangles (latitude/longitude) with low, medium, and high susceptibility to bycatch in each of the seasons (summer, autumn, winter, and spring). It was found

that in 2020 there were 188 vessels operating, with length ranges between 4 and 30 m, of which 182 belonged to fresh vessels (166 coastal fleet and 16 fresh high-seas vessels) and 6 belonged to freezer vessels. The reported catches in the study area were higher for the fresh vessels (31,030 tons), representing about 14% of the total landings in the ports of Buenos Aires province for the same type of operation. The results show that a large number of small- and large-scale vessels operate in areas of great importance for sea turtles. As well as that, they show the need to carry out systematic monitoring programs to quantify possible interactions with fishing fleets in order to evaluate possible mitigation measures that will benefit the conservation of these animals in the long term.

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## **FISHERIES INTERACTIONS WITH CRITICALLY ENDANGERED LEATHERBACK SEA TURTLES IN THE CALIFORNIA CURRENT LARGE MARINE ECOSYSTEM: WHAT IS THE IMPACT AND HOW IS IT MEASURED?**

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The threats to western Pacific leatherback sea turtles are truly global, yet actions at the state and national level are critical for conservation. Fisheries is one example of a threat under direct control of states and the federal government. With various state and federal protections for leatherback sea turtles, including listing on the California Endangered Species Act and designation of critical habitat off the U.S. West Coast pursuant to the federal Endangered Species Act, the question remains whether a patchwork of management measures can meaningfully reduce fisheries' bycatch of leatherback sea turtles. This poster reviews the methods for estimating and monitoring the take of endangered leatherback sea turtles in state (California) and federal fisheries' impact analyses. Piecemeal approval of different fisheries' incidental take has long been an obstacle in assessing the aggregate impact of fisheries on sea turtle populations. This issue of cumulative impacts from multiple sources of entanglements becomes more insidious as bycatch in unknown fishing gear and fisheries without observers (like pot and trap gear) increases. Estimating take from entanglement records provides only a minimum estimate of actual interactions. It is unclear whether the National Marine Fisheries Service has adequate methods to evaluate fisheries' impacts on leatherback sea turtles or whether the population can sustain any additional fisheries mortality. In late 2019 a federal district court ruled that the National Marine Fisheries Service failed to adequately analyze threats to critically endangered leatherback sea turtles when it allowed longline fishing off California. Two years earlier, a federal appeals court ruled the National Marine Fisheries Service failed to properly analyze the Hawaii-based swordfish longline fishery's impacts on the endangered loggerhead sea turtles before permitting an expansion of that fishery in 2012. Consistent oversight of permitting decisions and best practices of leatherback sea turtle avoidance by fisheries can greatly enhance leatherback sea turtle conservation. California's application for an incidental take permit in the Dungeness crab fishery, which would permit the take of one leatherback sea turtle every ten years, is an example of a precautionary permitting scheme that would help conserve these seafaring giants. It requires the closure of fishing in areas where tagged sea turtles are present. Environmental groups would like California to go one step further and transition to "ropeless" fishing gear, which is the only way to eliminate entanglement risk entirely. In the meantime, methods for estimating ongoing incidental take in fisheries is needed especially where observer coverage is nonexistent or very low. Science-based assessments of fisheries' impact to vulnerable populations is critical in fighting the extinction crisis.



## **ANALYSIS OF DIFFERENCES IN THE CHARACTERISTICS OF WATERCRAFT INJURIES AMONG THREE SPECIES OF SEA TURTLES**

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Interaction with watercraft is a common, but understudied, anthropogenic threat to sea turtles worldwide. Some populations suffer mortality rates of 25% or more from vessel strikes, which are the most common cause of traumatic or blunt-force injury in stranded sea turtles. Vessel strikes are also predicted to increase over time, making this a growing area of concern. Very few studies have focused on species and life stage differences with regard to boat strikes. Understanding potential differences in how sea turtles react to oncoming boats, as well as how boat strikes affect different populations of sea turtles, may help inform management as to areas of focus for sea turtle protection. Mote Marine Laboratory's Stranding Investigations Program (SIP) responds to stranded sea turtles in southwest Florida and has records of stranded sea turtles dating back to 1987. Historical stranding data collected from 1987-2020 were reviewed, and sea turtles with documented wounds consistent with boat strikes were evaluated. Wounds were classified by anatomical zones that corresponded with underlying organ systems. Analyses examined injury location by species, sex, age class, cause of death (if determined), and the likelihood that the boat strike contributed to the cause of stranding. In addition, we compared the general anatomical region of injury (i.e., *cranial*, *medial* and *caudal*) to determine if there were any potential differences in strikes among species attributable to turtle orientation in the water column or directionality of collisions. There were significant differences in boat strike locations by sex, cause of death, and likelihood of contributing to the cause of stranding. We found no statistically significant differences among species. Our findings characterize in detail vessel strikes and their effects on sea turtles with potential implications for management and mitigation strategies.

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## **REPORTING SEVERE DEGRADATION OF NESTING BEACH DUE TO EXTENSIVE SAND MINING THREATENING THE NESTING OF OLIVE RIDLEY AND GREEN SEA TURTLE IN GUJARAT, THE WEST COAST OF INDIA**

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The 120 km Saurashtra coast of Gujarat in West India from Dwarka in the north to Mangrol in the south has olive ridley (*Lepidochelys olivacea*) and the green sea turtle (*Chelonia mydas*) sporadic nesting sites. The green sea turtle nesting (70%) dominates the olive ridley nesting (30%) in this area. The olive ridley nesting sites of Gujarat is unique as compared to nesting sites in other states on the west coast of India. The peak nesting season for olive ridley is July in Gujarat whereas in other states it is February. In India, the endangered green sea turtle has nesting sites only in Gujarat and Lakshadweep. In Gujarat, the peak nesting for green sea turtles is in November and the second peak is in February. Two permanent hatcheries exist for relocating the nest of green sea turtle and occasionally olive ridley turtle. To study the unusual nesting period of olive ridley turtles a survey was initiated on the Saurashtra coast in 2019. The status of sea turtles



in previous surveys viz 2006 and 2012 has given the sizeable number of the nest in the peak season (> 80 nests) in approx. 35 km stretch. In our study period, 60km of the beach was surveyed from June till August. Sites having nesting possibilities were repeatedly surveyed. Four olive ridley nests with 0% emergence were found in the 3km stretch of the surveyed sites. The nesting sites of olive ridley turtle reported in earlier studies was found to be heavily mined for fine sand. Active mining was observed during the survey. At the site where the beach was intact (unmined sites), the foredune was steep with a height > 15 feet. Green turtle nests were found at the high tide mark below the foredune with chances of inundation by seawater. The beaches of Saurashtra are reflective, wide (at least 100mts) and static. Most of the places have fine sand on the foredune area and hard rock clay at the back of foredune till back dune vegetation. Nesting sites like Karunga- Okhamadhi (6km), Ratadi – Katela (3km), Atroli – Divasa (4km) and Mangrol bara- lohej (3km) were protected by the forest department. These sites (approx. 16km out of 60 km surveyed) have conducive conditions for the nesting of green sea turtles and olive ridley. The rest of the nesting sites are lost to heavy sand mining. The survey has uncovered a destructive and environmentally degrading activity of sand mining (probably illegal) going on a large scale at a landscape level. It is very well observed that olive ridleys nesting will not be seen from this region in the coming years. Immediate remedial actions are required with help from the international body to implement an effective strategy for the conservation of the green sea turtle nesting sites in Gujarat.

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#### A GLOBAL TRAIT-BASED CLIMATE VULNERABILITY ASSESSMENT FOR SEA TURTLE\*

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Climate-driven changes in environmental conditions have significant implications for the distribution, abundance, and phenology of sea turtles. Nest incubation temperatures, which are affected by beach temperatures, influence sea turtle embryo survival and sex ratios. Changes in ocean temperature, pH, and salinity may affect the distribution, health, and behavior of sea turtles as well as the abundance, distribution, and quality of sea turtle forage. Sea turtle nesting and foraging habitats are already affected by sea level rise, shifting currents, and extreme storm events. Non-climatic stressors (e.g., bycatch, direct take, pollution) may be exacerbated or become more impactful with shifts in sea turtle distribution, abundance, and phenology. The conservation and management of sea turtles in a changing climate requires an understanding of how vulnerable populations are to the projected future conditions. To better understand the impacts of climate change on regional sea turtle populations, we applied a climate vulnerability assessment framework developed by NOAA Fisheries that uses available population-specific information and expert elicitation to score each population's (i) exposure to climate change and (ii) combined sensitivity and capacity to adapt to climate change. An international group of 18 sea turtle experts evaluated the projected impact upon 51 Regional Management Units and Distinct Population Segments of sea turtles from changes in climate and environmental variables (e.g., sea surface temperature, ocean pH, sea level rise) out to the year 2055. The experts evaluated climate sensitivity and adaptive capacity using life history traits as proxies, then used the exposure component scores, and combined sensitivity and adaptive capacity component scores, to calculate relative vulnerability to climate change for each population. Results indicate that all populations are expected to be impacted by climate change. Most populations (92%) have very high exposure to climate change, with the remaining 8% having high exposure, meaning all populations are expected to experience a meaningful shift ( $>1.5$  standard deviation) in at least two environmental variables by 2055 relative to 1956–2005. Forty-one percent of populations scored very high sensitivity to climate change, while 49% scored high sensitivity, 8% moderate sensitivity, and 2% low sensitivity. The resulting climate vulnerability score was very high for 86% of populations, high for 10%, moderate for 2%, and low for 2%. Exposure scores were generally driven by similar climate variables for populations within each region. Sensitivity scores were generally driven by similar attributes for populations within each species. In addition to quantifying the vulnerability, exposure, and sensitivity of regional sea turtle populations to climate change, this assessment identified population-specific data gaps (e.g., reproductive rates) that, once filled, could improve our understanding of climate change threats to sea turtles. These results will help inform the development and implementation of activities that conserve, manage, and recover sea turtle populations. This study highlights populations and drivers that may require urgent attention, such as focused conservation and research as the climate changes, and provides baseline comparators for similar future efforts to assess sea turtle populations at global, regional, and local scales.

## NEW THREAT ON SEA TURTLES: MASS STRANDING OF TURTLES SUFFERING FROM SHOCK WAVE TRAUMA\*

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During the months of December 2018 to March 2019, 250 sea turtles were stranded along Israel's Mediterranean coastline. In January 2019 alone, 96 turtles were stranded. 69 loggerheads (*Caretta caretta*), 16 green sea turtles (*Chelonia mydas*) and 11 unidentified sea turtles were located. Only 30% (29) of the turtles were still alive when located, all suffering from serious injuries, and were brought to the Israel Nature & Park Authority (INPA) Sea Turtle Rescue Center for medical treatment. This represents an alarming ten-fold increase in the number of stranded turtles located yearly in the month of January. CT scans of live injured sea turtles revealed that 83% (19 of 23 examined) showed symptoms of soft tissue trauma: pulmonary hemorrhage and accumulation of fluids in the middle ear. Such symptoms are consistent with shock-wave trauma, suggesting a fatal exposure to a yet undetermined strong impulsive sound source, such as underwater explosions, on a significant level. Similar stranding events, with identical clinical symptoms (Aizenberg et al., 2013), were previously documented by the INPA on a smaller scale in 2012 and 2015. Both in 2012 and during the current event (but not in 2015), seismic surveys for gas exploration were carried out in the territorial waters off the Israeli coast (2012) or within Israel's Exclusive Economic Zone (EEZ, 2019). The temporal overlap between the current stranding events and seismic surveys has raised alarm by the INPA and prompted a joint investigation headed by the Ministry of Energy together with environmental experts from the Ministry of Environment, INPA, Israel Oceanographic and Limnological Research Institute and leading academic professionals. The team of experts investigated the turtle distribution in the region as well as the physical properties of offshore air-gun seismic survey technology. The team found no scientific evidence that the seismic air guns used in the survey have sufficient power to induce the unique shockwave trauma observed. Oceanographic models were not able to connect the stranding sites with the seismic survey sites, further discrediting the seismic survey as the cause. Other sources of underwater strong impulsive sounds such as: explosives (military or fisheries) lightning storms and active sonar were also examined. The results were inconclusive. Hence, while discrediting the seismic survey, it was not possible at this time to connect the turtle mortality event to any specific cause. Such a large-scale mortality event of sea turtles may negatively impact endangered populations of the two turtle species within the entire eastern Mediterranean, both which are already threatened by human activities. Thus, there is an urgency to ascertain the possible cause (natural or anthropogenic) leading to this incident and determine where in the Mediterranean it had occurred in order to establish the necessary national and regional guidelines (Popper et al., 2014), so as to mitigate future negative effects of various levels of man-made underwater acoustic activity on sea turtles.

## EFFECTS OF GEAR MODIFICATIONS ON SEA TURTLE BY-CATCH CARETTA CARETTA (LINNAEUS, 1758): A MULTI-YEAR EXPERIMENT IN THE SURFACE LONGLINE FISHERIES OF THE AZORES

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Sea turtles are among the groups of animals most affected by incidental catch in pelagic longline fishery. Juveniles loggerhead sea turtles (*Caretta caretta*) use Azorean waters during the oceanic stage and are commonly caught accidentally by the pelagic longline fishery, which targets mainly swordfish (*Xiphias gladius*) and blue shark (*Prionace glauca*). To characterize the incidental catches of juveniles sea turtles, mainly the loggerhead *Caretta*, and investigate the effects of gear modifications on the by-catch fauna (using eight different types of hook), a multi-year fishing experiment was conducted in the Azorean archipelago from 2000 to 2004. At the end of the experiment, 27,214 individuals of 19 Osteichthyes species, 11 of Chondrichthyes, and three species of sea turtles were captured. The most abundant species was the blue shark (81.43%), followed by swordfish (11.85%). Three species of turtles were recorded: *Caretta caretta* (401 individuals), *Dermochelys coriacea* (15), and *Chelonia mydas* (1). Of the 491 loggerhead sea turtles recorded in 335 fishing sets, 292 were hooked in the mouth, 141 in the throat, and 58 were hooked externally. Six turtles were dead and all the others were released. The mean number of loggerhead turtles caught per 1000 hooks (CPUE) was 1.01 individuals, varying between a minimum of 0.03 in December 2004 and a maximum of 2.6 in August 2000. A GLM showed that hook type, year, and month influenced turtle catchability. There was also a higher rate of capture in September, decreasing progressively in October, July, November, and December. Some previous studies concluded that the turtle capture rate is highest between July and November during the swordfish fishing season (May to December), with peaks at the beginning and end of summer. These peaks may be associated with the passage of a thermal front through the Archipelago. It was also verified that the RT hook is significantly associated with a higher capture rate, followed by the CM16, JM9, CM16O, and CM18 hooks. On the other hand, a lower catch rate was associated with the CLP18O, CLP18, and JM9O hooks. Although the circle hooks seem to be more selective for sea turtles, it is difficult to affirm which of the hooks studied is the best to minimize by-catches, as the different options can have different consequences for the variables under study. In addition, to propose the use of a particular hook over another it is also necessary to take into account the effect of hook types on other animals, both target and accessory species. For example, previous studies have indicated that the catchability of blue sharks increased for certain types of circle hooks as compared to J- hooks. In the Azores, the use of circular hooks in pelagic longline fishery is already implemented as a management strategy. Therefore, global analyzes of the effects of hook type on the catchability of target and accessory species will allow evaluating the effectiveness of these management measures, as well as proposing alternative strategies to minimize the impact of this fishing activity on the marine fauna.

## TRENDS IN SEA TURTLE STRANDING ALONG THE SOUTH WESTERN ITALIAN COASTS, 2007-2021\*

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We present a detailed analysis of spatio-temporal patterns of sea turtle strandings (N=1167) recorded over a 15-year period (2007-2021) along the south western Italian coasts. The word stranding here refers to all injured, sick, debilitated or dead sea turtles that washed ashore, were found at sea or were accidentally caught during fishing operations and reported to the local Authority or directly to the sea turtle stranding network. Loggerhead turtles comprised 99.6% of the strandings with only 4 green and 1 kemp ridley turtles recorded over the study period. The majority of turtles was found dead (N=842), stranded on the beach (86%) or floating at sea (12.5%). Only 0.3% of the dead turtles were reported directly by fishermen as bycatch, in stark contrast with the 52% for the alive subgroup (N=319). Overall, for approximately 70% of the stranded turtles it was not possible to identify any macroscopic evidence of acute interaction with human activities as possible cause of the stranding (87.5% and 23.6 % in dead and alive turtles, respectively). Monthly stranding time series were decomposed in the seasonal, trend and irregular components using an additive model. Stranding exhibited relevant inter annual fluctuations both in the alive and dead subgroups but without a clear trend over the last 15 years. The seasonal component was stronger in the monthly strandings of dead turtles that showed a peak during spring, in May and June (seasonal factors of 4.1 and 8 respectively) and the lowest values in autumn, from October to December (seasonal factors of -2.8, -2.7 and -2.9 respectively). Seasonality in the stranding of alive turtles was less evident, with the largest seasonal factor detected in February (1.4) and the lowest in October (-1.2). Although strandings were recorded throughout the study area, spatial analysis identified two zones of higher concentration corresponding to the coastal sectors located in the northern and southern portion of the Campanian coasts with the widest continental shelf where trawling activity is most intense. To sum up, the long-term series of stranding data suggest a strong correlation with coastal fishing activity, particularly trawling, and also indicate critical times of the year and areas where mitigation of fisheries interaction would be most effective. There's still much that needs to be done to turn the tide of turtle strandings, and most of all engagement and collaboration with the fishermen need to be constantly reinforced to promote their direct involvement in the conservation of sea turtles.

## **HISTORICAL TRENDS IN NEW YORK STATE COLD STUNNED SEA TURTLE STRANDING-TO-RELEASE: 1998-2019**

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Long Island Sound and the Great Peconic Bay (New York, USA) contain southern barrier lagoons and eastern bays and are known habitats for foraging juvenile populations of sea turtles during summer months. Every year, sea turtles strand throughout these areas due to climate-related cold snaps that typically occur in the late fall and lead to cold stunning, a physiological temperature shock similar to hypothermia which renders turtles unable to swim and prone to washing up onto beaches. Cold stunning events in this area tend to last longer than a few weeks and typically affect juvenile Kemp's ridley (*Lepidochelys kempii*), juvenile green (*Chelonia mydas*) and sub-adult loggerhead (*Caretta caretta*) sea turtles. The New York Marine Rescue Center (NYMRC), formally known as the Riverhead Foundation for Marine Research and Preservation (RFMRP), is the sole rehabilitation facility for cold-stunned sea turtles in New York and responds to the second largest number of cold stuns in the Greater Atlantic Region; which encompasses marine ecosystems from Maine to North Carolina. Since 1998, a total of 510 sea turtles were recovered from New York state waters or beaches, between the months of October and February. Of these 510 cases, five individuals re-stranded under similar conditions a second time following rehabilitation and release, resulting in 505 distinct sea turtles stranding due to cold stunning. These 505 cold-stunned sea turtles were comprised of three different species; 281 *L. kempii* (56.0%), 174 *C. mydas* (3.1.3%), 48 *C. caretta* (9.5%), and two hybrids (0.4%). Over the course of 22 years, stranding frequency varied from 3–85 turtles per season, with an average of 23. However, a large increase in stranding numbers began in 2007; average stranding numbers from 1998–2006 were seven per season, increasing to 34 per season from 2007–2019. Multiple factors are likely contributing to the increase in stranding/rescue frequency such as the gradual warming of northern waters (which may entice turtles further north and prevent their timely southern migration), development of a free public outreach program targeted at educating patrons about local sea turtle populations, and implementation of an effective beach patrolling system. More efficient management of patrolling efforts has contributed to the quick response time and resulting increase in live turtle rescues. In addition, modification and enhancement of in-house treatment protocols have contributed to the upward trend of successfully rehabilitated cold-stunned turtles. Understanding historical cold stun trends will allow local and national organizations to identify needs and allocate funding for conservation initiatives of endangered Atlantic sea turtle populations.



## **PLASTIC DEBRIS INGESTED BY SEA TURTLES FROM THE KOREAN WATERS: QUANTITY, SHAPE, ORIGINS, AND POLYMER COMPOSITION\***

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Plastics are most bountiful debris in the marine environment affecting wildlife. All seven species of sea turtles and about 52% of global sea turtles were estimated to have ingested plastic debris. However, the reports on plastic ingestion by sea turtles were concentrated in America and Europe, while the information on the East Asia are scarce even though more than half of mishandled plastics is emitted to the ocean from this region. Notably, no such study has been conducted in Korea. This study examined the sea turtles found stranded, floating, or by-captured between 2012 and 2018 in Korean waters. The quantity, shape, color, size, origin, and polymer type of plastic debris ingested by sea turtles were analyzed after being sorted from gastrointestinal (GI) tract of 34 turtles: 21 loggerheads, 9 green turtles, 2 leatherbacks, and 2 olive ridley turtles. The plastic ingestion frequency of green turtles, loggerheads, leatherbacks, and olive ridleys were 100%, 81%, 50%, and 50%, respectively. The overall amount of plastics were in the range of 0 – 1.313 g/kg turtle (0 – 229 pieces/turtle). There was a negative correlation between body size (CCL) and mass of plastics per kg of turtle. The GI tract contents were sieved over a 1 mm steel mesh, and plastic items were classified to macro (>25 mm), meso (5 – 25 mm), and large-micro (1 – 5 mm) sized groups. The number-based composition of macro, meso and micro plastics were 63%, 31% and 6%, respectively. Film and fiber were found as dominant shapes in all species, accounting for 81% of total plastics. Light color (such as white and transparent) of plastics constituted the majority of the debris. Some plastic items labeled with certain languages (9 items for Korean and 10 items for Chinese) were found, mostly in loggerheads. Single-use or fishery-related plastics such as filmed packaging, plastic bags, twine, net, and rope predominated in origin distinguishable plastics. A total of 17 different polymer types were identified, among which the polymers lighter than seawater (specific density = 1.02) such as polyethylene, polypropylene, polypropylene [poly(ethylene:propylene)], and expanded polystyrene comprised a large portion (> 90%). Green turtles ingested the largest amount of plastics (mean value: 0.26 g/kg), followed by loggerheads (0.073 g/kg), leatherbacks (0.0007 g/kg), and olive ridleys (0.0004 g/kg). Green turtles ingested mostly fiber type plastics such as rope, string, fishing line, and net, while loggerheads ingested largely film type plastics such as plastic bag, filmed packaging, and tape. The difference in ingested plastics between greens and loggerheads may be related to feeding habit and geographical range of movement of each species. The overall result of this study demonstrates that sea turtles inhabiting around the Korean waters are seriously affected by marine plastic debris, especially single-use and fishery-related plastics and recommends green and loggerhead turtles as bioindicator of marine plastic debris in this region.



## ANTIBIOTIC-RESISTANCE OF PSEUDOMONADACEAE AND ENTEROBACTERIACEAE ISOLATED FROM UNHATCHED EGGS OF LOGGERHEAD SEA TURTLES IN THE WESTERN MEDITERRANEAN\*

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Antibiotic-resistance is deeply concerning from the ecological, economical and medical perspective. The phenomenon is influenced by anthropogenic pressure, resulting from the overuse of antibiotics in animal productions, human and veterinary medicine. Nowadays, antibiotic-resistant bacteria are almost ubiquitous, and even wildlife could acquire antibiotic-resistant strains without previous antibiotic treatment. Loggerhead sea turtles are important sentinels for the Mediterranean Sea, and antibiotic-resistant strains have been isolated from various samples collected from them, including eggs. Indeed, the nest environment is ideal for bacterial colonization, where antibiotic-resistant strains might arrive from maternal or environmental sources. This study evaluated the antibiotic-resistance of bacteria isolated from unhatched eggs of loggerhead sea turtles, focusing on *Pseudomonadaceae* and *Enterobacteriaceae*, frequently detected in sea turtles and commonly exhibiting antibiotic-resistance phenotypes. During summers 2015-2020, we examined 40 nests from 14 locations along Campania and Lazio (south-western Italy). After nest final excavation, surface and fluid swabs were collected from 279 unhatched eggs, and processed to isolate *Enterobacteriaceae* and *Pseudomonadaceae*, according to laboratory internal standard procedures. Colonies were identified through biochemical tests, and submitted to antibiotic-susceptibility testing, using the disk-diffusion method. We selected antibiotics among the most common in human and animal medicine, with available standardized breakpoints, and the results were classified as susceptible, intermediate or resistant, according to the Clinical and Laboratory Standards Institute documents. All strains were tested with amikacin, ceftazidime, ciprofloxacin, enrofloxacin, gentamicin. *Pseudomonadaceae* were additionally tested with colistin sulphate, whereas *Enterobacteriaceae* were additionally tested with ampicillin, chloramphenicol, doxycycline, nalidixic acid, streptomycin, tetracycline, trimethoprim-sulfamethoxazole, and the combination disk test to assess the production of Extended Spectrum Beta-Lactamase (ESBL). *Pseudomonadaceae* were isolated from 84.84% of samples, whereas *Enterobacteriaceae* from 70.36%. Among 885 strains, 48.59% exhibited non-susceptibility to one or more antibiotics, differing between *Pseudomonadaceae* (15.64%) and *Enterobacteriaceae* (86.41%). *Pseudomonadaceae* exhibited non-susceptibility towards enrofloxacin (24.10%), ceftazidime (20.51%), amikacin (5.07%) and gentamicin (0.85%), whereas all strains were susceptible to ciprofloxacin and colistin-sulphate. *Enterobacteriaceae* exhibited non-susceptibility towards ampicillin (92.48%), tetracycline (39.81%), streptomycin (28.88%), doxycycline (25.73%), ceftazidime (19.42%), amikacin (8.74%), nalidixic acid (3.88), chloramphenicol (2.43%), gentamicin (1.94%), and enrofloxacin (0.73%). No resistance was detected towards ciprofloxacin and trimethoprim-sulfamethoxazole, and no strain produced ESBL. Only 8.93% of strains (0.85% of *Pseudomonadaceae* and 18.20% of *Enterobacteriaceae*) exhibited multidrug resistance, frequently towards penicillins, tetracyclines, cephalosporins and/or aminoglycosides. No strain showed non-susceptibility to more than four antibiotics. Our results highlight the role of sea turtles as reservoir of resistant strains and, more importantly, the anthropogenic impact endured by their habitats. Antibiotic-resistance in commensal or environmental strains might be transferred to pathogenic ones, with serious repercussions for animal and human health, also considering that these strains might enter the food web and disseminate across species, through predators consuming sea turtle eggs. Moreover, antibiotic-resistant strains might interfere with

embryo development or with the establishment and maturation of a healthy microbiota. Therefore, this phenomenon could threaten sea turtle conservation, especially during this delicate first stage of life, crucial to provide individuals fit to sustain populations through time.

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## **TWENTY YEARS OF SEA TURTLE STRANDINGS IN NEW CALEDONIA\***

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In this study, we investigated cause-specific temporal and spatial trends in sea turtle strandings in New Caledonia. Five species of sea turtles were recorded in the 406 strandings between January 1999 and March 2021. Green turtles represented the majority of the species found stranded (68%) which is reflecting the important resident green turtle population in New Caledonian waters. Nearly half of the individuals stranded were juveniles (48%). The larger majority of strandings were recorded in the South Province, the most populous province of New Caledonia (73%). A large proportion of the recorded strandings were of unknown cause (50%) followed by poaching (17%), by-catch (15%), collision (10%), natural (8%), plastic ingestion (0.5%) and other (0.5%). This study is the first official record of the presence and relative importance of fibropapilloma in New Caledonia but it could not be determined if it was the cause of death for the individuals stranded. Two individuals, after necropsies, were found to have died of plastic ingestion (one in 2011 and the other in 2020). This is the first record of death by plastic ingestion for sea turtles in New Caledonia. Significant trends over the study were also discovered: an increase in the number of individuals reported in the study since 2004, was a seasonal effect with most strandings occurring in summer (November to January) and stranding hotspots (Noumea, Bourail and Boulouparis). Rehabilitation allows for 35% of individuals found alive to be released back in the wild. This study suggests that mitigation strategies such as go-slow zones should be put in place in New Caledonia as well as a functioning stranding network.

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## **DIRECT BEHAVIORAL MEASUREMENTS OF SEA TURTLE INTERACTIONS WITH FISHING GEAR: A PEEK INTO THE BYCATCH BLACK BOX\***

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Numerous anthropogenic hazards in the ocean, especially those associated with fisheries, cause sublethal effects and mortality to sea turtles. Bycatch, or the incidental capture of non-target species in fisheries, is a major threat to sea turtle populations worldwide. The purpose of this research was to develop and test solutions to reduce sea turtle bycatch in gillnet fisheries. Here, we measured sea turtle behavior in relation to gillnet fishing gear. Under controlled conditions, we recorded the interactions of wild-caught juvenile green and loggerhead turtles with modified gillnets in a 13.9 x 2.3 x 1.5 m tank at the St. Lucie Nuclear Power Plant in Jensen Beach, Florida. Our binary choice trials consisted of a gillnet vs. no gillnet set up

during the day and at night. Thus, each turtle was given the choice between a pathway with a gillnet fully blocking it or an open pathway. We identified two novel evasive maneuvers that both green and loggerhead turtles exhibit in the presence of gillnets. A “reversal” behavior was characterized by contact with the gillnet followed by backward movement and a forward orientation, whereas a “U-turn” behavior was a quick 180-degree pivot before making contact with the gillnet or other barrier. Following an entanglement event, and escape involving one of these two behaviors, green turtles were more likely than loggerheads to swim into the gillnet again. These findings support the hypothesis that sea turtles detect gillnets either visually or tactilely but may not perceive them as impassable barriers, at least initially. The “reversal” and “U-turn” behaviors are evidence of sea turtles’ ability to potentially avoid entanglement. Our experimental approach has the potential to contribute to “fine tuning” of bycatch reduction techniques. By having a detailed understanding of sea turtle behavior, including attraction, avoidance, and detection, in relation to fishery hazards along with potential deterrents, we will be able to inform the development of improved deterrents that effectively reduce sea turtle bycatch.

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## **A TWENTY-YEAR ANALYSIS OF MARINE LITTER INGESTED BY LIVE LOGGERHEAD SEA TURTLE, *CARETTA CARETTA*, FROM THE MEDITERRANEAN SEA**

**Chiara Roncari, Antonino Pace, Andrea Affuso, Mariapia Ciampa, Fulvio Maffucci, Gianluca Treglia, and Sandra Hochscheid**

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The Marine Strategy Framework Directive (2008/56/EC) is an EU’s legal instrument developed to protect the marine environment and biodiversity with the ambitious aim to achieve the Good Environmental Status (GES) through the guidance of eleven qualitative descriptors. The tenth descriptor concerns the evaluation of the proprieties and quantities of marine litter (ML); one of the indicators used to assess ML and its relative harm is the “*Trend in the amount and composition of the ML ingested by marine animals*”. For the Mediterranean Sea, *Caretta caretta* (Linnaeus, 1758) was chosen as the bioindicator in light of its biological features. The analysis of gut contents from dead stranded marine turtles is the official tool used to assess ML ingestion and estimate GES values, whereas the effectiveness of monitoring ML from faecal samples obtained in rescue centres is still under evaluation. The present study aims at assessing the litter presence in live loggerhead and the validity of data collected by faecal analysis, using an adequate experimental design and precautions to avoid contamination or loss of material. During the period from 2000 to 2020 (excluding 2017), a total of 417 specimens of loggerhead sea turtles were rescued along the Italian coast, from three of the four sub-regions of the Mediterranean Sea (*sensu* MSFD). Each specimen was examined by a vet to assess its health status and record additional parameters which may be correlated to litter ingestion, such as principal injuries and biometric measures. Turtles ranged from 10.4 to 88 cm CCL (n=407), allowing us to acquire data on animals of different life stages. Post-hatchlings with CCL<10 cm (n=6) have not been taken into account due to the difficulties to sample faeces of such small sizes. For the remaining 411 animals, faecal samples were collected during the first period of rehabilitation, to analyse ML contents (>5mm) and record dry mass and number for each litter category. The percentage of animals positive for litter ingestion over the total of examined animals (Frequency of Occurrence, FO%) is 68.9%. ML was detected with an average of  $1.15 \pm 0.12$  grams and  $10.19 \pm 1.16$  items per specimen. Fragments were the most represented plastic shape category for dry mass (39%), although sheetlike items were the most abundant in terms of number (65%). No clear distribution pattern emerged along two decades of data monitoring, with FO% ranging from 27% (2020) to 92% (2005). The present work is the first to provide a long time series of data collected from live loggerheads, that could be used to develop GES evaluations by employing *in vivo* specimens. In rescue centres the samples are readily available and, although this

methodology needs standardization, it can provide additional information on the phenomenon of litter pollution with low-cost analysis. Furthermore, the possibility to monitor live animals poses an opportunity to evaluate the potential impacts on animal behaviour due to the ingestion of litter (e.g. buoyancy disorder).

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## **GHOST GEAR ENTANGLEMENT ON LOGGERHEAD SEA TURTLES IN THE MEDITERRANEAN SEA**

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Abandoned, lost, or otherwise discarded fishing gear (ALDFG) is one of the deadliest forms of anthropogenic debris in the marine environment. In this study, we examined the reasons for the stranding of sea turtles in the Balearic Sea between 1993-2020. Despite entanglement causing the lowest mortality rate in comparison to the other causes, an increasing trend of their incidence was detected. We categorized the types of marine litter causing entanglement (n=110) into 7 groups. The most prevalent forms of *ghost* gear were a form of illegal drifting fish aggregating devices (dFADs) (n=47). A backtracking approach was utilized to identify the potential origin of the ghost dFADs found in the Balearic Sea and general ALDFG in the Western Mediterranean Basin. Visual marks and backtracking suggest there was a large proportion of ghost dFADs originating in Algerian Exclusive Economic Zones (EEZ). Nevertheless, ALDFG is present across all the Western Mediterranean, and their sources potentially involve every coastal country. Overall, the study highlights the need to address the increasing rate of ghost dFADs in conservation plans at a regional level.

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## **PREVALENCE OF FISHING HOOK TYPE IN INCIDENTAL CAPTURE OF KEMP'S RIDLEY (LEPIDOCHEYL KEMPII) SEA TURTLES\***

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Kemp's ridley sea turtles (*Lepidochelys kempii*) are the most critically endangered species of sea turtle and are prone to incidental capture by recreational fishermen in Mississippi<sup>1-2</sup>. These anthropogenic interactions lead to the need for medical intervention of these turtles, where the hooks are often removed. In this study, the type of hook and location of each type of hook was evaluated for rehabilitated turtles from the Northern Gulf of Mexico in the state of Mississippi. Hook types and hook locations were also compared to patient outcome. The prevalence of hook types and locations were compared to a previous study of a similar population of Kemp's ridley sea turtles, Heaton 2016. Radiographs or endoscopy were used to determine hook locations. This information can be utilized to determine what types of hooks pose a stronger threat to sea turtle health based on prevalence and common locations where they get embedded. From 2016 to 2020,

89 Kemp's ridley sea turtles were admitted to a stranding network in Mississippi. A total of 72 hooks were removed or passed through the gastrointestinal tract from these turtles. Of these, 7 were Kahle hooks, 20 were circle hooks, 52 were J hooks, and 9 of the hooks were of unknown type. A total of 14 hooks were removed prior to intake, which accounts for the 9 unknown hook types. The locations that were evaluated for hook location were the oral cavity, tongue, cranial esophagus, mid-esophagus, caudal esophagus, unspecified esophagus, coelom, and externally. Of the 20 hooks found in the oral cavity, 45% were J hooks, 30% were circle hooks, 10% were Kahle hooks, and 15% were hooks of unknown type. Of the 17 hooks found in the cranial esophagus, 59% were J hooks, 29% were circle hooks, and 12% were Kahle hooks. Of the 30 hooks found in the mid-esophagus, 70% were J hooks, 23% were circle hooks, and 7% were Kahle hooks. Of the 8 hooks found in the caudal esophagus, 87.5% were J hooks and 12.5% were circle hooks. Of the 5 hooks that were found in an unspecified region of the esophagus, 60% were J hooks, 20% were circle hooks, and 20% were Kahle hooks. The one coelomic hook was identified as either circle or Kahle and was not removed prior to release. Of the 8 external hooks removed, 50% were J hooks, 37.5% were hooks of unknown type, and 12.5% were Kahle hooks. Nearly a third of all hooks were in the mid-esophagus over the 2016–2020-time frame, and J hooks predominated for hook type. Of the 89 turtles that presented for incidental hook capture, only 2 died while in hospital. One mortality was an individual that had a J hook in the esophagus, and the other was an individual that had a circle hook in the oral cavity. These mortalities could not be used to relate type of hook or hook location to case outcome. The remaining 87 turtles were successfully rehabilitated and released.

## IN-WATER BIOLOGY

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### USING FORENSIC ANALYSIS OF BITE-RELATED INJURIES ON SEA TURTLES TO DETERMINE SPECIES AND SIZE OF SHARK PREDATORS

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Predators play an important role in ecosystems because they influence the distribution, behavior, and abundance of their prey. Sharks are the primary predators of immature and mature sea turtles, particularly tiger (*Galeocerdo cuvier*), white (*Carcharodon carcharias*), and bull (*Carcharhinus leucas*) sharks, and injuries inflicted by sharks are sometimes encountered in stranded and nesting sea turtles of all species. Sharks are also facultative scavengers that feed on sea turtle carcasses, further confounding efforts to accurately assess their role in sea turtle mortality. The shark species that inflict such injuries are rarely identified because attacks are infrequently observed. When combined with observations of shark feeding behavior, geographic distribution, and habitat preference, forensic analysis of bite wounds can be used to obtain a species- and size-specific assessment of the predation or scavenging event. The goal of this study was to conduct a forensic analysis of bite wound patterns on free-ranging sea turtles in the southeastern United States to better understand their predators. We compiled images for 13 cases from Florida and Alabama during 2010–2019 that documented predation or scavenging attempts on loggerhead (*Caretta caretta*), green (*Chelonia mydas*), Kemp's ridley (*Lepidochelys kempii*), and leatherback (*Dermochelys coriacea*) sea turtles of differing life-stage classes. For each image with discernable tooth marks, we measured the distance between tooth marks (interdental distance) and bite circumference using ImageJ software. Using pre-established, species-specific regressions of these values against the shark's total length, we were able to identify predator species for six cases, including two mature loggerhead turtles and one immature green turtle bitten by great white sharks, one mature loggerhead and one immature green turtle bitten by tiger sharks, and one immature green turtle bitten by a bull shark. In five cases, including one case each of white, tiger, bull, sandbar (*Carcharhinus plumbeus*), and dusky (*Carcharhinus obscurus*) sharks, two possible shark species were identified based on their interdental distance and bite circumference. We were then able to use bite wound characteristics (i.e., sawing versus tearing) to identify one probable species. A single predator species could not be determined for two cases due to similar interdental distance and bite circumference measurements among the candidates. Based on gross observations including tissue characteristics of the bite wounds, 11 cases were considered to be antemortem predation events, while two cases were identified as postmortem scavenging events. This forensic analysis approach enables us to better understand the behavioral dynamics between sharks and sea turtles, including attack motivation and bite patterns that are rarely observed.



**ACOUSTIC TELEMETRY ENABLES ANALYSIS OF POST-NESTING MOVEMENT PATTERNS OF NORTHWEST ATLANTIC LEATHERBACK SEA TURTLES (*DERMOCHELYS CORIACEA*)**

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Acoustic telemetry is a type of biotelemetry that gathers data on animal presence when animal-borne transmitters pass within the detection range of an acoustic receiver. While acoustic telemetry provides relatively precise locational data, as receivers are typically stationary and fixed to the ocean floor, it does not generate fine-scale data on par with satellite telemetry. It can, however, provide a higher number of detections within acoustic receiver arrays, improved resolution, and fewer positional errors than satellite tags. Additionally, acoustic telemetry is useful for providing arrival times, which has implications for fisheries management to key habitats, and can be used to better understand human impacts on marine organisms. In leatherback sea turtles (*Dermochelys coriacea*), acoustic telemetry within active tracking studies has only been previously used to study hatchling dispersal, partly because the migration routes of adult leatherbacks cover areas that lack acoustic receivers. The aggregation of leatherbacks that nest in southeastern Florida, USA presents a unique opportunity for using acoustic telemetry, as they tend to migrate near the continental shelf and could therefore interact with the extensive arrays of acoustic receivers situated along the eastern seaboard (Florida Atlantic Coast Telemetry [FACT] and Ocean Tracking Networks [OTN]). During June 2021, we attached Vemco V16 acoustic transmitters to five nesting leatherbacks on Juno Beach, Florida in the first known US-based acoustic tracking study involving adult leatherbacks. Each tag was attached to the caudal peduncle of a selected turtle through a single hole drilled with a disinfected, 7/32- (0.2) inch drill bit and aseptic technique. Transmitters are typically attached by the two hole attachment method, where two holes are used to secure the tag. In an effort to minimize the animal's stress and pain, we used the one-hole attachment method and turtle's exhibited little to no reaction to the drilling process. Preliminary results show that four of the five turtle-borne acoustic transmitters were detected by 13 acoustic receivers within five separate acoustic arrays managed by FACT Network members between Tequesta, Florida (26.7847°N, -80.0273°W) and Cape Lookout National Seashore, North Carolina (34.6187°N, -76.5628°W). On average, there were  $16 \pm 6$  receiver detections per individual turtle. The fifth transmitter has not been detected, possibly due to the turtle migrating further offshore and out of range of acoustic receivers. As we continue to receive tag detection data and tag more turtles, we hope to be able to use acoustic transmitters to confidently identify the turtles' foraging ground location(s) and/or detect shifts between current and historical foraging areas. If successful, acoustic telemetry tracking represents an alternative or complimentary method to the more costly satellite telemetry, especially in areas where acoustic receiver arrays are present and actively managed. Leatherback movement data generated by acoustic tags can also be overlaid with maps of fishery and boating activities to identify potential areas of conflict, and help inform management and conservation decisions.



## **DIVING BEHAVIOR AND ENERGETIC STRATEGY OF LEATHERBACK SEA TURTLES DURING INTERNESTING INTERVALS ON ST. CROIX, U.S. VIRGIN ISLANDS\***

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Female leatherback sea turtles (*Dermochelys coriacea*; hereafter referred to as leatherbacks) nesting on St. Croix in the U.S. Virgin Islands in the spring lay 5-6 clutches, with an ~10-day internesting interval at sea between clutches. Although leatherbacks feed on gelatinous zooplankton during the winter, foraging and prey preference during the internesting interval has remained speculative. The goal of this study was to use miniature, animal-borne video and data recorders and satellite telemetry to monitor the diving behavior and movements of leatherbacks during the internesting interval on St. Croix. Our hypothesis was that foraging is rare and opportunistic during the internesting interval. Our results show that leatherbacks make two types of dives: Shallow Transit (ST) and Deep Transit (DT). ST dives were short (1.14 min), shallow (3.0 m) and associated with slow ( $0.6 \text{ m sec}^{-1}$ ), subsurface swimming to reduce drag with no periods of gliding descent. DT dives were longer (13.42 min), deeper (84.0 m), and covered a 10-fold greater distance (446 m) than ST dives but at the same slow speed. The deepest and longest DT dive was 531 m in depth and 28.9 min in duration, but only 4% of DT dives were deeper than 200 m. Leatherbacks spent 94% of the time at sea making ST and DT dives with short resting periods at the surface. Six species of gelatinous prey were identified from recorded video, but feeding was rare and opportunistic. Reduced foraging effort indicated fasting during the nesting season. The estimated energy expended while at sea for five internesting trips over six weeks was equivalent to the catabolism of 25.4 kg of fat. An additional 12 kg of fat was needed for egg production, resulting in a total energy requirement equivalent to ~36 kg of fat (~11% of body mass). Our results indicate that the purpose of the internesting interval is not foraging and that leatherbacks are capital breeders, which fast during the nesting season after acquiring energy reserves (i.e., fat) during the non-breeding season at higher latitudes where foraging effort may be more efficient.

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## **PHTHALATE ESTERS (PAES) CONCENTRATION PATTERN REFLECTS DIETARY HABITATS ( $\Delta^{13}\text{C}$ ) IN BLOOD OF MEDITERRANEAN LOGGERHEAD TURTLES (*CARETTA CARETTA*)**

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Phthalic acid esters (PAEs) are classified as endocrine disruptors, but it remains unclear if they can enter the marine food-web and result in severe health effects for organisms. Loggerhead turtles (*Caretta caretta*) can be chronically exposed to PAEs by ingesting plastic debris, but no information is available about PAEs levels in blood, and how these concentrations are related to diet during different life stages. This paper investigated, for the first time, six PAEs in blood of 18 wild-caught Mediterranean loggerhead turtles throughout solid-phase extraction coupled with gas chromatography-ion trap/mass spectrometry. Stable isotope analyses of carbon and nitrogen were also performed to assess the resource use pattern of loggerhead turtles. DEHP (12-63 ng mL<sup>-1</sup>) and DBP (6-57 ng mL<sup>-1</sup>) were the most frequently represented PAEs, followed by DiBP, DMP, DEP and DOP. The total PAEs concentration was highest in three turtles (124-260 ng mL<sup>-1</sup>) whereas three turtles had concentrations below the detection limit. PAEs were clustered in three groups according to concentration in all samples: DEHP in the first group, DBP, DEP, and DiBP in the second group, and DOP and DMP in the third group. The total phthalates concentration did not differ between large-sized (96.3 ± 86.0 ng mL<sup>-1</sup>) and small-sized (67.1 ± 34.2 ng mL<sup>-1</sup>) turtles (p < 0.001). However, DMP and DEP were found only in large-sized turtles and DiBP and DBP had higher concentrations in large-sized turtles. On the other hand, DEHP and DOP were found in both small- and large-sized turtles with similar concentrations, i.e. ~ 21.0/32.0 ng mL<sup>-1</sup> and ~ 7.1/9.9 ng mL<sup>-1</sup>, respectively. Winsored robust models indicated that  $\delta^{13}\text{C}$  is a good predictor for DBP and DiBP concentrations (significant AIC<sub>wt</sub>). Our results indicate that blood is a good matrix to evaluate acute exposure to PAEs in marine turtles. Moreover, this approach is revealed as a useful tool to explain the internal dose of PAEs in term of dietary habits ( $\delta^{13}\text{C}$ ), suggesting that all marine species at high trophic levels may be particularly exposed to PAEs, despite their different dietary habitats and levels of exposure.

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## **POLYMER COMPOSITION ANALYSIS OF PLASTIC DEBRIS INGESTED BY LOGGERHEAD TURTLES (*CARETTA CARETTA*) IN SOUTHERN TYRRHENIAN SEA THROUGH ATR-FTIR SPECTROSCOPY**

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The ingestion of anthropogenic plastic debris by marine wildlife is widespread in the Mediterranean Sea and loggerhead turtle (*Caretta caretta*) is one of the most vulnerable species. In this study, macro-/meso-plastics (6-170mm) collected from faeces of twelve loggerhead turtles rescued (live) in the Aeolian Archipelago (Southern Tyrrhenian Sea) were analysed by size, weight, shape, color and polymer type through ATR-FTIR Spectroscopy. The defecation rate during hospitalization (7-14 days) varied among turtles (from 0.08-0.58). The mean number of plastic expulsions (2.7 ± 1.8) was higher during the 5th day

of hospitalization (Kruskal-Wallis test,  $P = 0.01$ ). However, the mean number of plastic-like items defecated during the common days of hospitalization did not vary among turtles (Kruskal-Wallis test,  $P > 0.05$ ). All individuals were found to have ingested marine debris, composed of plastic items (113/114), mainly white-transparent (64.9%) and light (19.3%) plastic fragments (52.6%), sheet (38.6%), nylon, net-fragments, elastic plastic, foamed plastic and industrial granules (8.8%). Meso-plastics (5-25 mm) represented 72% of the total number of debris and were found more frequently in small juvenile (CCL = 30-60 cm,  $n = 5$ ) than in large juvenile/adult (CCL = 60-71 cm,  $n = 7$ ) turtles. Plastic items were composed mainly of polyethylene (48.3%) and polypropylene (34.2%). Polypropylene ( $R^2 = 0.95$ ,  $P < 0.001$ ) and polyisoprene ( $R^2 = 0.45$ ,  $P = 0.017$ ) were more common in meso-plastics while polyethylene ( $R^2 = 0.44$ ,  $P < 0.01$ ) in macro-plastics. Finally, high-density polyethylene, polyvinyl chloride, polyamide and polyurethane were also found in single turtles. This study reveals high spreads of plastic contamination in faeces of both juvenile and adult loggerhead turtles, particularly vulnerable to the increasing quantity of floating plastic into their foraging sites highlighting the need of further research to associate debris ingestion with turtle diet during different life stages.

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## **ECOLOGICAL TRAP OR FAVORABLE HABITAT? FIRST EVIDENCE THAT IMMATURE SEA TURTLES MAY SURVIVE AT THEIR RANGE-LIMITS IN THE NORTH-EAST ATLANTIC\***

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Unusual environmental events can push marine animals outside their physiological tolerances through changes in trophic and/or thermal conditions. Such events typically increase the risk of stranding. Rescue Centers offer a unique opportunity to report animals in distress and satellite track rehabilitated individuals to identify potential new habitats and support an effective conservation of these endangered species. By combining sightings (1988–2020) and tracking data (2008–2020) collected along the French Atlantic and English Channel coasts, our study assessed if the Bay of Biscay is an ecological trap or a favorable habitat for immature sea turtles. The largest tracked individuals migrated westward to pelagic waters, likely toward their natal beaches, while smaller individuals remained within the Bay of Biscay (BoB) and crossed colder (mean: 17.8 3.0C) but more productive waters. The turtles' directions differed from the ones of ocean currents, excluding a passive advection to these unexpected habitats. Although the BoB might be thermally unsuitable in winter, the higher micronekton biomass predicted in this region could offer a productive foraging habitat for immature turtles. However, the majority of the sightings referred to individuals stranded alive (75%), suggesting this area could also act as an ecological trap for the smallest individuals that are mostly reported in winter suffering cold-stunning. Assumed to be outside the species range, our results reveal a potential foraging ground in the North-East Atlantic for these young turtles, confirming the crucial role of the rehabilitation centers and the need to continue prioritizing conservation of these endangered species, particularly vulnerable at this stage and at such temperate latitudes.

## DYNAMICS AND AGING OF GREEN TURTLE GRAZING PLOTS IN SEAGRASS MEADOWS\*

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Green turtles (*Chelonia mydas*) are specialized grazers that maintain grazed areas by repeatedly cropping areas of seagrass. Naturally grazed areas in the Caribbean are mosaics of ungrazed seagrass and grazing plots. As population recovery continues in response to long-term conservation efforts, green turtles will play an increasingly important role in seagrass ecosystems. Given global seagrass declines, concerns over the sustainability of green turtle grazing have become a central topic in discussions of seagrass protection. Understanding grazing plot dynamics and aging is critical to evaluating the sustainability of grazing and understanding the role green turtles play in structuring seagrass ecosystems. We investigated grazing plot dynamics and aging at two naturally grazed seagrass meadows – one experiencing increasing grazing and one experiencing decreasing grazing – on the east coast of Eleuthera, The Bahamas. Both meadows are shallow (<4 m in depth), dominated by the seagrass *Thalassia testudinum*, and grazed by juvenile green turtles. To map the grazing mosaics at both sites, we captured aerial imagery of grazing plots using an unmanned aerial vehicle (UAV or drone) and conducted in-water characterization of primary producer communities to ground-truth the imagery. We processed imagery using Pix4Dmapper and imported the resulting spatially referenced GeoTIFF files into ArcMap 10.6. We tracked grazing plot fate (maintained, expanded, merged, reduced, regrowing, abandoned) over 13 months and quantified the morphology (area, edge length, edge-to-area ratio) of grazing plots. To update estimates of how long green turtles maintain grazing plots, we used archived satellite imagery of these meadows to calculate the minimum age of each grazing plot based on the earliest appearance of the plot in the time series of satellite and UAV imagery. By integrating in-water characterization of grazing plots with aerial imagery of naturally grazed meadows, we show that green turtle grazing plots are dynamic, with establishment of new plots, expansion and merging of existing plots, and abandonment and regrowth of plots occurring within 3- and 13-month periods. We also document that green turtles contribute to habitat complexity by increasing edge habitat in seagrass meadows and that they can maintain grazing plots for more than 9 years. By July 2019, green turtles had been maintaining grazing plots for at least 6.65 years at the meadow where grazed area was decreasing and for at least 9.22 years at the meadow where grazed area was increasing. Our results offer new insight into the life cycle of grazing plots and show green turtle grazing is sustainable over longer periods than previously documented. Future studies investigating grazing plot dynamics and evaluating the sustainability of green turtle grazing over longer periods, across seasons, and in different regions as well as evaluating cumulative effects of anthropogenic stressors will be key to effective co-management of seagrasses and green turtles.

## **SPECIES IDENTIFICATIONS AND ANCIENT FORAGING HABITAT USE FROM FRAGMENTED MID-HOLOCENE MEDITERRANEAN SEA TURTLE REMAINS**

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Two species of sea turtle breed and nest in the Mediterranean, these are the Green turtle (*Chelonia mydas*) and the Loggerhead turtle (*Caretta caretta*). In order to better understand past human-turtle interactions in the Mediterranean, and the biological impact thereof, we studied skeletal remains of turtles from three mid-Holocene archaeological sites located on the Northern Levantine coast. To identify the heavily fragmented bone specimens, we used comparative osteomorphology and Zooarchaeology by Mass Spectrometry (ZooMS), to distinguish between different species. Together with species identifications, we investigate sea turtles' foraging ecology using  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  stable isotope analysis with the aim of uncovering baseline information about ancient Levantine sea turtle species distributions and geographic habitat use. Liquid chromatography tandem mass spectrometry (LC-MS/MS) collagen sequencing (98% of amino acid positions covered in the Loggerhead sequence) confirmed seven previously undescribed peptide markers capable of distinguishing between the two species. We anticipate these markers will facilitate further investigation of sea turtle palaeoecology. Plotted stable isotope data showed two distinct clusters which resolved to the two species. Ancient Loggerhead turtle samples have a much larger variation in stable isotopes whilst Green turtle samples clustered more tightly. This was expected given the more varied diet of Loggerheads versus Green turtles. An important finding was evidence that suggests that foraging sites in the eastern Mediterranean basin have likely been in use for thousands of years and efforts should be made to protect these crucially important sites in a similar manner to nesting sites.

## **SEVEN YEARS OF HAWKSBILL TURTLE MONITORING IN THE EASTERN PACIFIC'S MOST IMPORTANT FORAGING SITE\***

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The Coiba Hawksbill Project was launched in 2014 through the larger Eastern Pacific Hawksbill Initiative (ICAPO: <http://www.hawksbill.org>), when visiting researchers observed high densities of this species in the waters of Coiba National Park, Pacific Panama, which were previously unreported to science. Since then, an international research team has been monitoring the population within the Park via 5-day field expeditions undertaken twice annually. Field efforts consist of in-water captures to weigh, measure, flipper tag, and collect biological samples for genetic and isotope analysis. Our overall goal was to determine the health, status, and size of the hawksbill population at Coiba National Park, and the information collected has been critical to scientists' understanding of the species across the southeastern Pacific region. To date over 600 individual hawksbills have been captured and tagged. Current proportions of new vs. recaptured turtles encountered during each visit remains ca. 1:1, showing that our tagging efforts have not yet achieved population saturation; in part, due to high annual recruitment rates to the local foraging population. In addition, we have deployed 12 satellite transmitters to understand hawksbill movement behavior and potentially identify migratory routes and nesting beaches, which remain poorly understood. Thanks to this ongoing research, it has been possible to ascertain that Coiba National Park hosts the largest hawksbill foraging aggregation in the entire Eastern Pacific and represents a key conservation area. However, protection within the National Park is not enough to ensure the future well-being of this species, considering that nesting does not occur at the site. Consequently, identifying the source nesting beaches for this foraging population is of paramount importance, and connections have been recently established via flipper tag returns between foraging grounds in Coiba National Park and nesting beaches in the Osa Peninsula in Costa Rica and the Azuero Peninsula in Panama. Research efforts will benefit from ongoing collaborative synergies with local conservation initiatives and businesses across the Pacific coast of Panama and beyond.



## IDENTIFYING THE ISOTOPIC NICHE OF HAWKSBILL SEA TURTLES IN ANTIGUA, WEST INDIES\*

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As adults, hawksbill sea turtles (*Eretmochelys imbricata*) are considered primarily spongivores. Because of their diet, hawksbills play an ecologically important role in coral reef habitats, and they are considered a keystone species. However, their resource use strategies (e.g., generalism vs. specialism) in these foraging grounds are poorly understood. Stable isotope analysis of carbon and nitrogen ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values) can provide information on the trophic niche and foraging location of the assessed organism. Evaluating stable isotope composition through layers of continuously accreting hard tissue, such as keratin, can reveal information on the long-term trophic niche of individuals. Here, we identify resource use strategies for 100 females within a nesting aggregation of hawksbills in Antigua, West Indies. From June through November each year, the Jumby Bay Hawksbill Project monitors hawksbills that nest on Long Island, Antigua to identify virtually every individual nester (i.e., saturation tagging). In 2017, 2018, 2019 and 2021, we collected keratin samples from the carapaces of nesting individuals and assessed  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values along the keratin growth axis. We compared among-individual and within-individual isotopic variance to evaluate resource use through time. Using MANOVA of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values together, we tested a null hypothesis of a specialist population (low variation) versus the alternative hypothesis of a generalist population (high variation). ANOVA tests were also used on  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values individually to assess element-specific variation and inter-individual variation. For individual hawksbills, standard ellipse area (SEA) of the isotopic niche can be used as a proxy for the ecological niche of individuals over time. We compared SEA values with reproductive age (the number of years between first observation and year of keratin sampling) and body size using linear regression models. Population-level analysis of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of keratin demonstrated significant differences among individuals (MANOVA,  $df = 52$ ,  $F = 61.35$ ,  $P = 0.0001$ ). ANOVA tests indicated significant variance in both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values individually, with among-individual variance ( $\delta^{13}\text{C}$  sum of squares = 1813.3,  $\delta^{15}\text{N}$  sum of squares = 2001.3) accounting for more variance than within-individual variance ( $\delta^{13}\text{C}$  sum of squares = 38.5,  $\delta^{15}\text{N}$  sum of squares = 35.2). Reproductive age of individuals was significantly negatively related to isotopic niche area (estimate = -0.014,  $R^2 = 0.16$ ,  $df = 48$ ,  $P = 0.003$ ), indicating niche width contraction as hawksbills age. These isotopic changes support previous evidence of an ontogenetic shift from a juvenile diet incorporating primary producers to a sponge-dominated diet in adults. These findings provide evidence of high variability in hawksbill diet and differences in ecological niche use across different ages. Alongside evidence that hawksbills use foraging grounds in at least 14 different countries around the Caribbean, these findings emphasize the necessity of protection of multiple marine habitats to aid in conservation of this critically endangered species.



## USING SATELLITE TELEMETRY TO IDENTIFY FORAGING GROUNDS OF LOGGERHEAD SEA TURTLES (*CARETTA CARETTA*) FROM SANIBEL ISLAND FL, USA

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One of the most significant loggerhead sea turtle (*Caretta caretta*) nesting aggregations is located in Florida, USA. Nesting is primarily concentrated on Florida's eastern coastline but is also dispersed across its western coast along the Gulf of Mexico. Due to their nesting distribution and relative abundance, satellite telemetry studies have been previously used as a tool to learn more about their migrations and other in-water behaviors. In 2020, for the first time, nesting loggerhead sea turtles were tracked from Sanibel Island, FL, USA, a 19 km barrier island located off of Florida's southwestern coast. This study used satellite telemetry to track nesting females and identify the location of their foraging grounds. Satellite telemetry studies have been previously conducted on Florida's gulf coast to the north and south of Sanibel Island but never directly on this nesting beach. Individuals were identified from Inconel flipper tags and passive integrated transponder (PIT) tags applied in previous nesting seasons on Sanibel, and only individuals encountered nesting on Sanibel in previous seasons were targeted. One turtle had been encountered in 2018, and another had been recorded nesting in 2016 and 2018. The third had been encountered in 2016, 2018, and 2019. The Argos platform transmitter terminals (PTT) transmitted across a range of 215 to over 565 days (as of January 2022, two individuals are continuing to transmit location data). Our data suggest that two of the turtles' core use areas are located in the Florida Keys, a chain of islands off of Florida's southernmost tip, and the third went to Bahamian waters, potentially establishing residency approximately 45 km off the north coast of Cuba. To determine their core use areas, location data from each turtle was used for kernel density estimation (KDE) analysis. 50% KDEs were used to represent core areas of activity, and their centroids were calculated to further identify potential foraging or residence areas. Only one of the individuals was encountered nesting post attachment of PTT, while the others migrated away from the nesting beach following PTT application. The turtle encountered nesting with its PTT had an 11-day internesting period, traveling to a site approximately 25 km off Sanibel's southern coast between the two nesting events. Due to undetermined causes, there is inconsistent data regarding possible migratory pathways for two individuals. The PTTs transmitted intermittently or did not provide location data for extended periods until the turtles had reached their potential foraging ground. Consistent data were collected for one individual, and locations were transmitted from nearshore waters as the individual made the over 180 km migration from nesting beach to potential foraging ground. Continuation of loggerhead satellite telemetry on Sanibel Island should be conducted to increase the sample size in order to provide a better representation of the nesting population.

## A MECHANISM FOR COMPENSATORY GROWTH IN CARIBBEAN SEAGRASS MEADOWS GRAZED BY GREEN TURTLES

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Recovery of green turtle populations, mega-herbivores that consume seagrasses, is resulting in dramatic ecosystem-wide shifts as meadows are returned to a natural grazed state. The green turtle grazing strategy, with long-term cultivation of meadows and high foraging site fidelity, is distinct from other terrestrial and aquatic mega-herbivores and may affect seagrass compensatory growth responses. Identifying mechanisms of compensatory growth responses to grazing is essential to understanding the functioning of plant systems under natural grazing regimes. In a Caribbean seagrass ecosystem at Buck Island Reef National Monument (St. Croix, USVI), we identify a mechanism for compensatory growth responses to grazing by evaluating the dynamics between *Thalassia testudinum* morphology and growth, the canopy light environment, and *in situ* grazing intensity in grazed and ungrazed areas. Cultivation grazing by green turtles reduces aboveground biomass of *T. testudinum*, which increases light availability at canopy height relative to ungrazed areas, stimulating leaf growth and turnover (i.e. compensatory growth). Shoot density increases with grazing intensity, maintaining the light harvesting potential of the canopy despite the removal of leaf biomass. This shift in growth allocation allows leaf area index to be maintained within the range of grazing intensity values of our study (10-70% removal of leaf biomass), thereby maximizing the potential for leaf photosynthetic activity and providing the plant with the capacity to sustain mass growth (g dry mass (DM) m<sup>-2</sup> d<sup>-1</sup>) and support a compensatory response to grazing. In addition, mass growth in grazed areas exhibited an increasing trend with shoot density and was maintained above a threshold of 2.5 g DM m<sup>-2</sup> aboveground biomass. This study presents novel insight for assessing the underlying mechanisms of seagrass compensatory growth responses to green turtle grazing and proposes potential thresholds that may be used to evaluate the sustainability of *in situ* grazing pressure by recovering green turtle populations.

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## DIURNAL HOME-RANGE PATTERNS IN LOGGERHEAD TURTLES\*

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Human use of marine habitats is increasing in many areas of the world, and increasingly, this use overlaps habitats of threatened species. To balance this, spatial-based or temporal-based management is used to minimise interactions. Understanding how habitats are used by wildlife species, such as marine turtles is therefore vital in providing improved understanding of the potential overlap and assessing management effectiveness. Advancements in satellite telemetry have provided many opportunities to advance

understandings of the behaviour of marine turtles while in their foraging habitats. In this project we use data from FastLoc GPS transmitter tags to improve our understanding of the temporal behaviours of loggerhead and flatback turtles foraging in the Great Barrier Reef. Overall, the research on loggerheads identifies that activity levels were higher during the day than at night and distances travelled was higher during day light hours. Finally, distances travelled by while foraging were larger during the day. The patterns are less clear in flatback turtles, which show considerable inter and intra-individual variation in patterns. Further studies are required to determine how turtles' behaviour influences marine and coastal ecosystems and therefore provide greater understanding for effective management and conservation measures to efficiently enhance the role sea turtles play within their habitats.

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## **COMPARISON OF ACOUSTIC AND SATELLITE TELEMETRY AS METHODS FOR QUANTIFYING SPACE USE OF MARINE TURTLES WITHIN FORAGING GROUNDS\***

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Knowledge of species habitat use and spatial distribution is important for establishing targeted conservation efforts. Telemetry methods such as satellite and passive acoustic telemetry have emerged as commonly used methods for tracking marine species. While these methods are inherently different and have important advantages as well as specific limitations, they are often used for similar purposes of delineating habitat use or identifying home ranges of individuals or species. However, data-driven comparisons of these telemetry methods, including their effects on our interpretation of species' spatial distribution, are lacking. To systematically compare telemetry methods, we simultaneously tracked nine juvenile green turtles within a foraging area in Bimini, Bahamas with both satellite and passive acoustic telemetry. We compared the temporal durations, spatial resolution, and spatial extent of each tracking technology. Dynamic Brownian Bridge Movement Models (dBBMM) were applied to both acoustic and satellite data separately to estimate and compare the areas of 50% and 95% utilization distributions (UDs) derived from each data source. Additionally, we assessed the costs associated with each tracking technology under 4 scenarios of varying sample sizes and temporal durations. Tracking durations were similar between telemetry methods, with a mean tracking duration of 90 days (range: 10 to 240) for acoustic and 119 days (range: 20 to 234) for satellite telemetry. Data resolution was lower for satellite telemetry, with only 7% of raw locations estimated as having an accuracy similar to that of the acoustic receiver detection probabilities (~185 - 350 m). On average, areas of satellite 50% and 95% UD were larger than acoustic UD, although not significantly so ( $p$ -value = 0.12 (50% UD) and 0.27 (95% UD)). The UD estimated from both technologies typically overlapped, with acoustic 95% UD corresponding well with satellite 50% UD in both location and extent. The costs associated with each technology varied based on the scenario. At small sample sizes, acoustic telemetry was up to 8 times more expensive than satellite telemetry, while at larger sample sizes, acoustic telemetry was less expensive. Based on the results of this comparison, selection of a telemetry method should be based on variety of factors, including the specific research question at hand, the study site, and the resources available. Our hope is that this study can serve as a resource for managers and researchers in identifying appropriate tracking methods for their project and financial constraints.

**FIRST SATELLITE TRACKING STUDY OF POST-NESTING HAWKSBILL TURTLES ALONG PACIFIC MEXICO; INSIGHTS FROM A SMALL, GENETICALLY ISOLATED AND HIGHLY VULNERABLE ROOKERY\***

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The hawksbill sea turtle (*Eretmochelys imbricata*) is rare and highly threatened throughout the eastern Pacific Ocean. Previous satellite telemetry research has helped shed light on the species' movement ecology in some parts of the eastern Pacific, particularly along Central and South America. Nonetheless, satellite telemetry research has never been conducted on post-nesting females along the Pacific coast of Mexico, where hawksbills represent a genetically distinct nesting population. The lack of research is primarily due to extremely low levels of nesting (average of <50 nests documented annually) along Pacific Mexico. Here we present preliminary results of nine post-nesting female hawksbills equipped with satellite tags at two nesting sites along the Mexican Pacific, including seven females at Punta de Mita, Nayarit during 2018 (n=2), 2019 (n=2) and 2021 (n=3), and two females at Costa Careyes, Jalisco in 2019. We augment our findings with telemetry data from three additional adult-sized females captured by fishers in Jalisco in 2016 and 2019 and Nayarit in 2018, as well as two adult males, one captured in Jalisco and one in Islas Marias, Nayarit. Turtles were tagged with Argos transmitters K2G 376 (Lotek, New Zealand). To date (December 2021) tracking duration varies from 586 to 1174 days and three turtles continue to transmit. Two of the post-nesting females, one from each rookery, travelled to Islas Marias where they have remained in the vicinity of Isla San Juanito and Isla Magdalena respectively. Three post-nesting females travelled to southern Sinaloa, where they established home ranges near the coastal city of Mazatlán. Three post-nesting turtles moved from Punta de Mita, Nayarit to Chacala, Nayarit a relatively short distance between their nesting and foraging site. One post-nesting turtle tagged in June 2021 travelled away from the coast presenting a behaviour not before registered for East Pacific hawksbills, finally arriving at the Revillagigedos islands where transmissions ceased. Post-nesting displacements ranged from 55 to 509 km, with turtles from Costa Careyes undertaking longer post-nesting migrations (mean displacement: 386km) than those from Punta de Mita (mean displacement: 143km). The two adult males had short transmitting durations 118 and 145 days respectively and remained in the vicinity of the area where they were captured. Movements were highly neritic in most cases, except for the turtle that travelled to Revillagigedos and turtles migrating from nesting beaches to the Islas Marias, which transited over open coast before settling at insular habitats. All turtles studied used areas that are developed as tourist destinations for at least a portion of their tracked movements. The Islas Marias appear to be of particular importance to foraging hawksbills in the Mexican Pacific and is rumoured to also host nesting by the species. Efforts to develop the area for tourism, coupled with increasing illegal fishing pressure represent important threats to this key habitat.

## **DIVING ACTIVITY OF OCEANIC-STAGE LOGGERHEAD SEA TURTLES USING TRI-AXIAL ACCELEROMETER DATA LOGGERS**

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Behavioral ecology of sea turtles is mainly focused on the neritic-stage of their life cycle, hatchlings or mature adults. Oceanic-stage of juvenile sea turtles remain enigmatic in many aspects, especially on their swimming behavior. The aim of this novel study is to characterize fine scale activity of juvenile oceanic-stage loggerhead sea turtles (*Caretta caretta*) in the Azores Archipelago, and to describe and classify diving and surface behavior. Here we present the first results of this ongoing study. During 2020 and 2021 we deployed 5 tri-axial multi-sensor recorders (accelerometer, magnetometer, gyroscope and time-depth recorder) in juvenile *Caretta caretta* ( $n=5$ ) ranging from 44 to 63 cm carapace length. A total of 76 hours of activity were recorded and analyzed, and five different types of dives were identified based on accelerometry and time-depth data. We will discuss these first result as well as lessons learned and future prospects of this project.

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## **MACHINE LEARNING AND MULTI-SENSOR TAGS BREATHE NEW LIFE INTO THE SPATIAL ECOLOGY OF SEA TURTLES\***

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Effective management and mitigation of in-water threats affecting sea turtles (e.g., gear entanglement, habitat modification) require a spatially explicit understanding of sea turtle activity, since the impact of threats is most likely context dependent. However, data on the spatial ecology of sea turtles generally only allows for minor inference regarding how a given location is used. Contemporary multi-sensor biologging tools allow us to quantify behaviour in highly resolved detail, via high-resolution movement sensors (e.g., accelerometers, magnetometers), which can be linked to distinct behaviours (e.g., resting, foraging, mating) when ground-truthed by direct observation, such as via animal-borne video cameras. Here, we demonstrate how such data, combined with automatic machine learning and GPS telemetry can generate spatially explicit maps of sea turtle activity, which may prove valuable for spatial management. From 2018 – 2021 we equipped adult flatback turtles (*Natator depressus*) ( $n = 51$ ; CCL 72.5- 98.9 cm) with biologgers (CATS-Diary,  $n = 22$  and CATS-Cam,  $n = 29$ ) at Roebuck Bay, Western Australia for up to 1 week each. We collected > 102 days total movement data, with ~ 5 % (115 h) of the data associated with concurrently recorded video. Behavioural activity-budgets were estimated by first isolating dives ( $n = 7074$ ) from the depth records and describing their respective features (phase durations, depth, activity, body posture, track characteristics). Dives with associated video footage were allocated with presence or absence of foraging and resting behaviours and a supervised machine learning algorithm was employed to assign behaviours to the remaining dives without available video footage, based on the dive features. Boosted regression tree



(BRT) models were trained and validated using the descriptive dive features. Validated BRTs for each behaviour were then used to predict presence or absence of foraging and resting during each dive over the entire dataset (including data where no video observation was available) and integrated with tags' GPS location data to create activity specific maps of turtle behaviour. Both validated BRT models performed exceptionally well (AUC; foraging = 0.939, resting = 0.926). Foraging dives were characteristic of shorter duration bottom phases with increased depth fluctuation and greater tortuosity than dives where no foraging was evident. Whereas resting dives were characterised by lower locomotory activity during the bottom phase of the dive and longer duration bottom and ascent phases. Predicted event rates for each behaviour overall were 43.23 % (foraging) and 33.29 % (resting). For 24.17 % dives, both behaviours occurred during the dive, reinforcing that sea turtle dives are often multi-purpose in nature. The location of foraging behaviour displayed a high degree of overlap with dives that did not include foraging, however foraging behaviour occurred over a sub-section of the total area occupied. Our automated machine learning approach is applicable to a broad range of other species; therefore, our method could significantly contribute to spatial management, for instance by regulation of vessel traffic and improving marine park zoning.

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## **PREDICTING THE NEARSHORE DISPERSAL OF SEA TURTLE HATCHLINGS: A MULTIDISCIPLINARY APPROACH TO ASSESSING DISPERSAL BY COMBINING FIELD DATA WITH SIMULATED TURTLE 'PARTICLES' VIA HYDRODYNAMIC MODELLING\***

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The first hours of a sea turtle hatchling's life are critical to its survival. After the hatchling emerges from the nest and makes its way to the sea or ocean, the hatchling must swim rapidly, avoiding predators until it reaches deeper, safer waters. However, this nearshore dispersal is challenging to study. The small size of the animal does not support active tracking such as GPS or Satellite tags and being so small they are more susceptible to environment conditions such as wind, waves and currents controlling their dispersal. In addition, tides dominate the coastline of many turtle rookeries, further affecting dispersal under various tidal movements and limiting sufficient sample size collection for accurate determination of movement patterns. Orientation cues presented in the scientific literature provide insight into potential swimming behaviour of dispersing hatchling such as orientation into wave fronts, with ocean currents, and in response to light horizons. However, when studied in the field, the range of environmental variables confound the study of these orientation cues. The direct tracking of sea turtle hatchlings in large numbers via acoustic telemetry or vessel-based tracking is costly, yet the need for understanding of movement patterns is essential for population modelling and species conservation. Here we present a multidisciplinary study approach using hatchling data collected at a rookery situated in North-West Australia in conjunction with a numerical approach that considers coupled hydrodynamic and wave modelling with particle motility to simulate specific hatchlings behaviour as they enter the marine environment. Over 350 Flatback sea turtle (*Natator depressus*) hatchling tracks were collected over a nine-year period, 2010 to 2019, using conventional vessel-based tracking. Track duration ranged from approximately 1 minute to 350 minutes and up to 7.5 kilometres in length under a range of environmental conditions. Travel speed and swimming speed were calculated, where possible, using available wind, wave, and current data. Swimming behaviours, such as diving and floating/drifted were recorded. The hydrodynamic model TUFLOW FV coupled to the SWAN spectral wave model was then developed and validated to simulate water levels, currents and wave

properties and subsequently adopted as the driver for the particle tracking model. A range of motility functions were developed within TUFLOW FV's particle tracking model to simulate animal movement in response to different environmental cues hypothesised as the main influencing factors controlling hatchling dispersal patterns. Particle behaviour included influences from hatchling swim speed, response to nearshore currents, incoming wave direction and light sources. Turtle nesting information from a long-term marine turtle monitoring program was used to estimate reproductive output and allocate proportional dispersal from sections of coastline. From this initial information, a range of scenarios were constructed assuming different hypotheses for hatchling behaviour. Each of the scenarios were qualitatively and quantitatively compared with the hatchling tracks measured in the field to infer likely cues for specific hatchling behaviour. It is proposed that the outputs of this multidisciplinary approach can be used to assist population viability analysis of the local turtle population and assign conservation measures, if required.

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## CONNECT 6: A GLOBAL NETWORK ANALYSIS OF MOVEMENTS TRACKED FOR SIX SEA TURTLE SPECIES\*

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A critical element in conserving vulnerable sea turtle populations is an understanding of their migratory connectivity, or how their extensive movements link areas used throughout their life cycle. Identifying the important areas for each life-stage and the way these areas are connected can highlight the need for coordinated management, especially when areas connect more than one jurisdiction. Many animals also depend on areas beyond national jurisdiction, which can increase the complexity of determining best management practices for conservation. Conservation measures within a network of habitats and corridors need to take into consideration the available scientific research describing connectivity among highly used areas. Various disciplines have used spatial networks (graphs connected by vertices and edges) to understand connectivity on a global and local scale, but this has yet to be fully utilized for highly migratory species in marine environments. We present global spatial networks that have been developed from synthesizing satellite tag data collected from a large number of tracked loggerhead *Caretta caretta*, green *Chelonia mydas*, leatherback *Dermochelys coriacea*, hawksbill *Eretmochelys imbricata*, Kemp's ridley *Lepidochelys kempii*, and olive ridley *Lepidochelys olivacea* sea turtles. The analysis and visualization of movements builds upon our understanding of species ecology, promotes scientific knowledge exchange on connectivity within the network structure, and provides a tool to prioritize conservation of areas and connections necessary to maintain spatial network function.



## **SNAPPERGPS: DEPLOYMENT OF A LOW-COST SNAPSHOT GNSS RECEIVER TO TRACK LOGGERHEAD SEA TURTLES\***

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Location tracking is a valuable tool for ecologists. Typically, this is done using global navigation satellite systems (GNSS), like the GPS. However, GNSS signals do not travel underwater. This makes tracking marine animals like sea turtles challenging because they only surface for brief intervals, sometimes as short as one second. Traditional GNSS receivers need to regularly be at the surface for at least 30 consecutive seconds to decode satellite orbit data from satellite signals. Assisted GNSS can reduce this time, but typically still requires several seconds to decode transmission timestamps. In contrast, snapshot GNSS is an approach that does not decode any satellite data and only needs to receive a few milliseconds of the signal to resolve a position fix. However, the few existing commercial snapshot GNSS systems for wildlife tracking are expensive. Furthermore, they are not always as accurate or reliable as traditional GNSS receivers. Here we present the first deployment of SnapperGPS, a small, low-cost, low-power snapshot GNSS receiver. Jonas Beuchert, Amanda Matthes, and Alex Rogers developed SnapperGPS at the University of Oxford. The device captures low-resolution twelve-millisecond satellite signals snapshots whenever the turtle surfaces. The tag stores the snapshots until it is recovered from the turtle. Finally, the data is uploaded to the cloud via our public web application (<https://snapper-gps.herokuapp.com>) where we employ novel probabilistic signal processing algorithms to calculate the track of the turtle. All signal processing algorithms are open-source. The current receiver version has a component cost of under \$30 (excluding enclosure) and can run for several years on two small LR44 coin cell batteries. SnapperGPS uses multiple GNSS constellations and our algorithms robustly provide median localisation accuracies around 10 m. In summer 2021, we deployed SnapperGPS on nesting loggerhead sea turtles (*Caretta caretta*) on the island of Maio, Cape Verde. We tagged 20 nesting females on the beach and recovered nine of the tags two weeks later when the individuals returned. Due to an issue with the surfacing detection mechanism some tracks were not captured or incomplete. However, this problem is unrelated to the snapshot GNSS technique. The three successful tracks demonstrate that the snapshot positioning worked reliably, providing a position fix whenever the receiver collected data at the surface. The recorded tracks revealed diverse behaviour among the population, with some individuals travelling around the island and others staying close to their nesting beach. We deployed SnapperGPS alongside Arribada Horizon v4.1 assisted GPS tags that utilise preloaded satellite data downloaded from the uBlox Thingstream service to speed up the time-to-first-fix. We deployed both tag types on the same population during the same nesting season. SnapperGPS was able to compute positions from significantly shorter signal captures than assisted GPS (milliseconds instead of seconds), whilst being more affordable to manufacture and more energy-efficient. Currently, we are working on making the SnapperGPS hardware open-source and available to other researchers.

## HABITAT USE OF NORTHWEST ATLANTIC LEATHERBACK SEA TURTLE

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Studying movement and habitat use is necessary to understand animals' biology and relationship with the environment. Marine animal movement occurs in response to biotic and abiotic factors including predation, prey distribution, and ocean circulation. Movement studies are especially important for highly migratory species such as the leatherback sea turtle (*Dermochelys coriacea*), which utilize large geographic regions over space and time. Declines in Northwest Atlantic Regional Management Unit (RMU) populations since 2009 have created an increasing need for knowledge on the location and physical properties of their critical nesting habitats. This research sought to explore protected species patterns in space by examining NWA leatherback movement in relation to environmental variables. The goal of this study was to understand how key oceanographic variables influence leatherback movement through use of telemetry data in conjunction with environmental data. Wildlife Computers SPLASH10 tags were deployed on three nesting leatherback turtles at Sandy Point National Wildlife Refuge, St. Croix, USVI, in May 2020. Location data were filtered and processed, and pseudoabsence data were generated using a Correlated Random Walk analysis. Thirteen environmental data variables such as ocean depth, Sea Surface Temperature (SST), and chlorophyll-A were sampled for each location, and statistical models (e.g., Generalized Linear Model and Random Forest Model) were used to determine which variables were important in driving species distribution. The results suggested that chlorophyll-A, mean SST, mean sea surface height anomaly, depth, and geostrophic velocity (u) were most the most important predictors of leatherback spatial distribution.

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## HOME RANGE AND CORE USE AREAS OF ADULT MALE LOGGERHEADS (CARETTA CARETTA) IN THE GULF OF MEXICO\*

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Little is known about the spatial distribution of adult male loggerhead sea turtles (*Caretta caretta*) because males spend their entire lives at sea, which presents logistical challenges for research. Considering that warming beach temperatures may result in the reduction of males in sea turtle populations, there is a growing need to better understand the life history and movement patterns of adult males for sea turtle conservation. Adult males that strand and are admitted to rehabilitation facilities present a unique opportunity to study male sea turtle movements after they are released back to the wild. Our study objectives were to examine the home range and core use areas of adult male loggerhead sea turtles after they were released from rehabilitation and to determine whether carapace size was correlated with total home range size. Satellite tags were deployed on nine adult male loggerhead sea turtles released from rehabilitation facilities in Florida between 2006 and 2021. Turtles ranged in size from 91.1 cm to 106.1 cm curved carapace length (notch-tip) and were tracked for 291-866 days. A switching state-space model was used to

filter individual tracks which produced two locations per day and assigned migratory and non-migratory behavior as the behavioral state for each location. Filtered locations were analyzed to determine the total home range of each male sea turtle using 95% kernel density estimation (KDE). Adult male loggerhead sea turtle total home ranges were 1,019.2 square kilometers to 98,802.8 square kilometers (median = 3,158.6 square kilometers). All total home ranges included the southwest Florida region of the Gulf of Mexico. Six males had total home ranges exclusively within the southwest Florida region and three males made excursions to the eastern coast of the United States. Core use areas were determined using a 50% KDE on the non-migratory locations for each male sea turtle. These core use areas ranged from 3.8 square kilometers to 10,103.4 square kilometers (median = 208.7 square kilometers). Multiple males had core use areas nearshore of Ft Myers, Florida (n=4 males) and near the mouth of Tampa Bay, Florida (n=4 males), suggesting these two regions are frequently utilized by male loggerhead sea turtles. Three males had core use areas offshore in the Gulf of Mexico; one was used exclusively, while the other two individuals had additional core use areas nearshore. Six males had multiple core use areas off the western and eastern Florida coasts and three males had one core use area off the western Florida coast. Male loggerhead sea turtle carapace size was positively correlated with total home range size (n=8 males,  $r=0.50$ ). Further investigations will examine how core use areas are associated with male loggerhead sea turtle mating and foraging behavior. Insight into the total home ranges and core use areas as it relates to migratory and non-migratory activities of male loggerhead sea turtles will better inform sea turtle management and conservation.

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## STABLE ISOTOPE ANALYSIS REVEALS DIVERGENT FORAGING STRATEGIES OF GREEN, OLIVE RIDLEY, AND LEATHERBACK TURTLES IN NORTHWEST COSTA RICA\*

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Knowledge of the foraging and migratory biology of sea turtles can help guide conservation management strategies. Here, we determined the stable isotopic profiles of three sea turtle species that nest in the Eastern Tropical Pacific to (1) identify potential differences in their foraging strategies; (2) elucidate their pre-nesting foraging habitats; and (3) determine the influence of body size on isotopic values. Upon encountering a nesting turtle, it was tagged using both metal and Passive Integrated Transponder (PIT) tags and measured for Curved Carapace Length (CCL) and Curved Carapace Width (CCW). We conducted a bulk stable isotope analysis ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values) on tissue samples from 52 sea turtles nesting on Playa Cabuyal, Northwest Costa Rica; 28 green turtles (*Chelonia mydas*), 20 olive ridley turtles (*Lepidochelys olivacea*), and 4 leatherback turtles (*Dermochelys coriacea*). Nine satellite transmitters were also deployed on green turtles to record their post-nesting migratory movements. Isotopic profiles and tracking data suggested that green turtles were coastal migrators feeding at a higher trophic level than green turtles from other regions globally. Olive ridley turtles exhibited similar nomadic behavior to conspecifics studied elsewhere and had minimal variance in their isotopic profiles. As body size increased for olive ridley turtles,

their  $\delta^{15}\text{N}$  values significantly decreased suggesting that larger turtles preferred deeper pelagic waters with less enriched N isotope concentrations. In addition to their pelagic nature, leatherback turtles showed a shift in their  $\delta^{13}\text{C}$  values compared to previous studies that could be indicative of coastal foraging due to the higher  $\delta^{13}\text{C}$  values found in this study. This project provides data that could aid in the development of isoscapes in the Eastern Tropical Pacific to help identify and prioritize high use foraging habitats and determine effective management practices for protecting these species and their habitats.

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## HIGH NUMBER OF LEATHERBACK TURTLE SIGHTINGS ON BRAZILIAN SUBTROPICAL COASTAL WATERS\*

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For many pelagic marine animals, the understanding of home range, distribution and seasonal movements is restricted. The limitations are a consequence of pelagic animal's remote and sparse distributions. The leatherback turtle is the largest and most widely distributed living sea turtle species. Often, the leatherback turtle migrates thousands of kilometres between tropical coastal nesting beaches and foraging habitats. The knowledge of leatherback turtle distribution in Brazilian waters is based on reports of incidental capture, strandings, and few telemetry studies. Herein, we report the first record of large numbers of leatherback sightings less than 3 km from the coast in Brazilian waters. Leatherback turtles were sighted during boat-based transects near the rocky shores of Arraial do Cabo (22°57'S, 42°01'W), on the south-eastern coast of Brazil. Arraial do Cabo is characterised by a change in the shoreline orientation from N–S to E–W, proximity to the 100 m isobath, and upwelling events. The study area is located within a highly touristic marine protected area with a recently elaborated management plan lacking no-take zones. This region has great ecological and biogeographic importance, harbouring tropical and subtropical species, including other species of sea turtles. Bimonthly surveys were conducted with a mean duration of 5 h (range: 4:00 – 6:00 h) from March 2019 to March 2020 (34 survey days) in a 9.6 m wooden vessel with a 3-cylinder WMW engine. The surveys followed a line transect route at approximately 6 km h<sup>-1</sup> to maximise study area coverage. Between November and December 2019, a total of 67 leatherbacks were sighted in an area with maximum depths of 60–87 m. There were no leatherback sightings before or after this period. A maximum of 20 individuals of leatherback were recorded in a single sampling day. All individuals were observed at distances not far from them 3 km from the coast. Individuals of leatherback were observed feeding on jellyfish (Cnidaria, Medusae) patches and resting near the seawater surface. The prevalence of strong north-easterly winds and low temperatures (<18°C) regimes were an indication of the upwelling events typical for the austral spring. Salinity was constant throughout the observations. We propose that leatherbacks are taking advantage of the nutrient-rich upwelling waters that boost the productivity of phytoplankton and zooplankton communities. The presence of a coastal aggregation of leatherback turtles in the region highlights the importance of this area as a marine hotspot for pelagic fauna and provides baseline information for guiding management strategies.

## INTER-NESTING HABITAT USE OF GREEN TURTLES FROM POILÃO ISLAND, WEST AFRICA, AND THE IMPORTANCE OF A MARINE PROTECTED AREA\*

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Understanding the spatial distribution of sea turtles across the habitats they occupy throughout their life cycle is essential to implement effective conservation measures. Most studies focus on hauled-out nesting turtles, as they are generally more accessible, with more recent research highlighting their spatial distribution at sea during migration, foraging and inter-nesting periods. Some regions of great significance for sea turtles remain, however, to date understudied. In particular, the extent of the inter-nesting movements of one of the world's largest green turtle (*Chelonia mydas*) nesting aggregations, taking place at Poilão Island (Bijagós archipelago, Guinea Bissau, West Africa), remains poorly known. Poilão Island is located within the National Marine Park of João Vieira and Poilão, which encompasses a central no-take zone where fishing activities are forbidden and a peripheral zone with regulated access. Whether this marine protected area (MPA) established in 2000 effectively enhances the protection of this population was uncertain, because knowledge related to the at-sea distribution of green turtles was not available when the MPA was designed. In order to evaluate the suitability of this MPA for breeding turtles, we assessed the inter-nesting habitat of 44 female green turtles equipped with satellite tags over the 2018, 2019 and 2020 nesting seasons. We found that the satellite-tracked females exploited a restricted habitat in the vicinity of Poilão Island, staying at  $1.96 \pm 4.24$  km from the coastline. Most of them remained within the protected area, with  $96.17 \pm 10$  % of satellite locations detected within the peripheral zone and  $86.45 \pm 14.49$  % occurring within the central no-take zone. Only two turtles performed significant excursions outside the protected area, one of them being the smallest turtle (curved carapace length = 78cm) and probably a neophyte. Additionally, we showed that nesting females exhibited strong fidelity to their at-sea habitat over consecutive inter-nesting intervals. This satellite-tracking study sheds light on the at-sea distribution of female green turtles during the previously overlooked inter-nesting part of their biological cycle. It further reveals the suitability of the National Marine Park of João Vieira and Poilão to support the conservation of female green turtles during the critical inter-nesting period. An extensive knowledge of the habitats exploited by green turtles throughout the year is essential to ensure the efficient protection of this charismatic species. Future work will focus on the at-sea distribution of male turtles during the mating period, for a synoptic view of the distribution of reproductive adults during the breeding season, allowing an assessment of possible threats.



## **POST-NESTING MIGRATIONS OF GREEN TURTLES (CHELONIA MYDAS) AT CAYO LARGO, CUBA**

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Historically, tagging has been one of the most valuable techniques to learn about sea turtles' distribution and migratory patterns. Although there have been other tools for years, such as genetic analysis and satellite tracking, tagging continues to have practical value for understanding the biology of these species. Taking this into account, we present the results obtained from the tagging-recapture, on the post-nesting migrations of the green turtle in Cayo Largo, the main nesting site of this species in Cuba, and one of the most important in the Caribbean Sea. Of 1077 nesting females tagged between 2005 and 2020, 20 have been reported in areas outside the Cuban shelf. Of these, 19 in coastal waters of Nicaragua at distances greater than 600 km from Cayo Largo, between 39 and 3375 days after tagged. The recaptured turtles measured between 96 and 118 cm (CCL) when they were tagged. Although the recapture percentage of the total number of tagged animals was low (1.8%), the fact that 95% of recaptures were reported in Nicaraguan waters, indicates post-nesting turtles from Cayo Largo go to these areas to feed in their extensive seagrasses between nesting seasons. Therefore, probably constitute the main feeding area for the green turtles that nest in Cayo Largo. On the other hand, these results confirm this region is a frequent destination for green turtles from different areas of the Cuban shelf (Moncada et al., 2006, 2016; Ruiz, 2017) as well as from other areas of the Caribbean Sea (Sole, 1994; Troeng et al., 2005) include green turtles that nest in Tortuguero, Costa Rica (Troeng et al., 2005).

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## **TRACKING POST-RELEASE MOVEMENT PATTERNS IN NEW YORK'S REHABILITATED COLD STUNNED SEA TURTLES**

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The aim of this project was to monitor the post-release movement pattern of 17 sea turtles rehabilitated at the New York Marine Rescue Center (NYMRC), formally Riverhead Foundation for Marine Research and Preservation, located in Riverhead, New York. Of the 17, 6 were Kemp's ridley (*Lepidochelys kempii*), 4 were Atlantic green (*Chelonia mydas*) and 7 were loggerhead (*Caretta caretta*) sea turtles. Every winter, sea turtles strand on beaches throughout New York as a result of climate-related cold snaps that typically occur quickly in the late fall and lead to cold stunning, a physiological temperature shock similar to hypothermia. The NYMRC is the primary rehabilitation facility for sea turtles in NY and responds to the second largest number of cold stuns in the Greater Atlantic Region. It has been suggested that rapidly decreasing water temperatures prevent these sea turtles from naturally migrating to warmer waters. In New

York, cold stunning events tend to occur November – January and typically affect juvenile Kemp’s ridley, juvenile Atlantic greens and sub-adult loggerheads. Between 2019 and 2021, NYMRC was able to attach satellite tags (Wildlife Computers SPLASH and SPOT tags) to 17 of the 52 (32.7%) NY sea turtles that were rehabilitated after cold stunning. All sea turtles were approved for release by NYMRC attending veterinarians and release locations were pre-approved by NOAA Fisheries Greater Atlantic Regional Fisheries Office Sea Turtle Stranding and Disentanglement Coordinator. Data collected from these tags will help support the rehabilitation efforts put forth by NYMRC and illustrate the post-release movement pattern of the once cold stunned sea turtles. The hope is that all released turtles will exhibit normal migratory behavior following release. Preliminary data showcases southern or offshore movement patterns which is associated with preferable post-release behavior. This data provides ample support for the rehabilitation efforts put forth by NYMRC indicating that sea turtles can return to normal behavior after receiving both short and long-term rehabilitation from cold stunning. Data from turtles monitored will also provide further insight regarding foraging areas used throughout New York state waters in the late summer and early fall.

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### **A COLLABORATIVE PHOTO-ID APPROACH TO DETERMINE SEA TURTLE PARAMETERS IN A ROCKY REEF FORAGING GROUND\***

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Sea turtles spend most of their life cycle in foraging grounds. Despite that, the bulk of studies were carried out in nesting grounds, sub-sampling non-reproductive females and males in foraging grounds. Cost-effective and non-invasive techniques allow public participation and can be useful to achieve data collection in foraging grounds. The present study aimed to use photographic-identification to investigate the spatio-temporal distribution and description of the presence of fibropapillomatosis in *Chelonia mydas* and *Eretmochelys imbricata*. This work was carried out at subtropical rocky reefs of the Brazilian southeastern coast in Arraial do Cabo (22°57’S, 42°01’W), within a sustainable conservation unit. The images were obtained through screening on social media, citizen science and intentional capture. A total of 641 photos (between 2006 and 2021) and 19 diving forms (November 2019 and March 2020) were obtained. All diving forms presented at least one turtle. The photo-id identified 174 individuals of *C. mydas*, with 45 resighted individuals (58.66% of the photos). *E. imbricata* had 32 individuals identified, and only 7 (71.57 % of the photos) individuals resighted. The median interval between the first and last individual sighting was 1.7 years for *C. mydas* and 2.4 years for *E. imbricata*. Fibropapillomatosis was only observed in *C. mydas*, with a prevalence of 13.99%, with only 2 individuals (10.00%) showing tumor regression. Citizen science and opportunistic data collection are efficient data collection tools, expanding the database in temporal and spatial scale. The results indicated Arraial do Cabo as an important development area for sea turtles with resident individuals for at least 6 years. As the region accumulates anthropogenic impacts it is critical to propose significant management measures to protect sea turtles. Such measures are essential to avoid continuous and synergistic impacts, mainly on resident individuals.



## UNDERWATER AND AERIAL OBSERVATIONS OF AGGRESSIVE INTERACTIONS AMONG LOGGERHEAD SEA TURTLES OVER FORAGING RESOURCES\*

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Despite progress in identifying foraging grounds frequented by sea turtles using remote monitoring techniques, elucidating how multiple individuals share the same space remains challenging. Yet, such information could reveal how sea turtles perceive resources and their social structuring, allowing the prioritisation of protected areas and optimising guidelines for wildlife viewing. Here, we evaluated the agonistic interactions of loggerhead sea turtles (*Caretta caretta*) foraging on sponges (*Chondrilla nucula*) at a reef on Zakynthos Island (Greece) over 11-years (2010-2021) using both underwater and aerial observations. Through photo-identification, we recorded 41 unique sea turtles, with 33 immature individuals and 8 adults. Of these, 25 were detected in 2–11 of the study years. During >900-min underwater observations, we recorded 95 interactions between 25 individuals. Interestingly, interactions represented just 1% of the activity budget (99% of time was spent swimming and foraging). However, >600-min aerial drone footage showed that individuals occupied distinct fine-scale patches (mean 50% KUD: 0.71 km<sup>2</sup>), with low overlap, likely delineated through these agonistic interactions. Interactions appeared to occur when turtles were close to the boundaries of patches of neighbouring individuals, or when passing through patches. Annual hierarchy rankings based on David's scores (dominance scores based on interaction outcomes) revealed the clear dominance of three individuals, consecutively across the study period. These individuals exhibited high levels of aggression, independent of body size and interannual site occupancy, and almost never lost fights with other turtles. Each individual dominated the reef for 2-3 years, (the first one was one of two foraging turtles killed by boat strike, the second was not sighted subsequently in our nearshore bay-wide surveys or social media records, and the third one was still present in 2021), with a new dominant turtle replacing it. Our work demonstrates how social interactions influence hierarchical structuring and resource acquisition in foraging loggerhead sea turtles, and how very small sites (such as reefs) can have very high resource value, with it being important allocate protection effort to such areas.

## THE BANC D'ARGUIN (MAURITANIA) IS A MAJOR FORAGING AREA FOR GREEN TURTLES IN THE ATLANTIC OCEAN\*

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The Banc d'Arguin is a vast (*ca.* 1 million ha) shallow marine area off the coast of Mauritania, known as a site of world importance for coastal migratory birds and other biodiversity, including extensive areas of seagrass. Sea turtles were reported to occur at this site and traditionally were regularly consumed by the local human population, but until recently nothing was known on their abundance, age-structure and origins, other than that some females nesting in the Bijagós archipelago (Guinea-Bissau) migrated to the Banc d'Arguin. Since 2018, we have extensively sampled adult and immature green turtles on the Banc d'Arguin with the help of traditional fishermen (the Imraguen) who operate sailing boats and gills nets targeting croakers and other large fishes. Turtles were captured using gill nets on feeding grounds, measured and sampled for genetics. Further, we extensively tracked adult turtles from the Bijagós archipelago, which is the only known significant green turtle nesting aggregation within 3000 km of the Banc d'Arguin. Based on satellite tracking of adult females (N=35), we estimate that 37% of the turtles from the Bijagós (estimated at *ca.* 25,000 adult females) migrate to the Banc d'Arguin post-nesting, which implies a feeding aggregation at this site of 9250 adult females. More limited tracking (N=9) confirms that adult males from Guinea-Bissau also use this site. Adult females represent only 8.6% of the turtles captured with the Imraguen (N=314). We know (from stranding data) that captures are biased against small turtles, so 8.6% is a minimum estimate for adult females. This implies that the number of turtles in the Banc d'Arguin likely exceeds 100,000 individuals. While we have no evidence for adult green turtles originating anywhere apart from Guinea-Bissau, genetic analyses (mtDNA control region – Bayesian many to many mixed-stock analysis; n=300 from Banc d'Arguin) suggest that immature turtles at the Banc d'Arguin include individuals originating from the Caribbean and South America (11%), and from Ascension Island or the Gulf of Guinea (3%) as well as a majority (86%) from Guinea-Bissau, West Africa. Most of the Banc d'Arguin enjoys effective protection by being included in a national park (the Parc National du Banc d'Arguin) where significant fisheries regulations are well enforced by an effective marine surveillance program. The vast majority of the turtles using the Banc d'Arguin forage in areas included in this national park. Our results suggest that the Banc d'Arguin represents one of the major foraging sites for green turtles of the Bijagós, and hence a critical site for the population using one of the major nesting aggregations for this species globally. Further, the Banc d'Arguin hosts turtles from widespread origins across the Atlantic, playing an important role for the species at the scale of the Atlantic Ocean basin, and likely of global importance.

## MOVEMENTS AND DISTRIBUTION OF FLATBACK TURTLES (*NATATOR DEPRESSUS*) IN WESTERN AUSTRALIA AND OVERLAP WITH MARINE PROTECTED AREAS\*

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Flatback turtles (*Natator depressus*) nest exclusively on remote Australian beaches that are logistically challenging to access, which has historically hampered efforts to effectively quantify movement patterns across genetic stocks. The proximity of industrial developments to key rookeries and improvements to satellite transmitter technology, however, mobilised research and monitoring efforts in Western Australia between 2005 and 2020. Here, we compile the largest satellite tracking dataset available to-date for a single marine turtle species. Tracked flatbacks (total  $n = 280$ ; 268 females, 10 males, 2 unknown sex) spent 99.5% of their time in Australian waters, where we quantified the extent of overlap of the five identified stocks (listed from west to east: North West Shelf, Eighty Mile Beach, Eco Beach, West Kimberley, East Kimberley) relative to inter-nesting, migratory and foraging behaviour (10, 10 and 80% of 288,075 records, respectively) and existing protective measures. Areas of importance for each behaviour were delineated relative to both the amount of time individuals spent within an area (i.e. occupancy index) and how many individuals visited each area. Flatbacks were tracked for a median of 275 days (range 11 – 820 days) and travelled a median distance of 2,832 km (range 66 – 11,158 km) within predominantly shallow, coastal waters ( $< 50$  m water column depth; 77.1% of records). Areas of importance identified for the species, particularly those relevant to multiple stocks, were used to inform review of existing reserve boundaries and the design of future spatial protections (e.g. marine reserves and Biologically Important Areas). Additionally, our extensive dataset was used to identify priorities for future research and monitoring efforts for the species at remote nesting beaches and offshore foraging grounds in northern Western Australia.

**IDENTIFYING PATTERNS IN FORAGING-AREA ORIGINS IN BREEDING AGGREGATIONS OF MIGRATORY SPECIES: LOGGERHEAD TURTLES IN THE NORTHWEST ATLANTIC\***

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Population assessments conducted at reproductive sites of migratory species necessitate understanding the foraging-area origins of breeding individuals. Without this information, efforts to contextualize changes in breeding populations and develop effective management strategies are compromised. We used stable isotope analysis of tissue samples collected from loggerhead sea turtles (*Caretta caretta*) nesting at seven sites in the Northern Recovery Unit (NRU) of the eastern United States (North Carolina, South Carolina and Georgia) to assign females to three separate foraging areas in the Northwest Atlantic Ocean (NWA). We found that the majority of the females at NRU nesting sites (84.4%) use more northern foraging areas in the Mid-Atlantic Bight, while fewer females use more proximate foraging areas in the South Atlantic Bight (13.4%) and more southerly foraging areas in the Subtropical Northwest Atlantic (2.2%). We did not find significant latitudinal or temporal trends in the proportions of NRU females originating from different foraging areas. Combining these findings with previous data from stable isotope and satellite tracking studies across NWA nesting sites showed that variation in the proportion of adult loggerheads originating from different foraging areas is primarily related differences between recovery units: individuals in the NRU primarily use the Mid-Atlantic Bight foraging area, while individuals from the three Florida recovery units primarily use the Subtropical Northwest Atlantic and Eastern Gulf of Mexico foraging areas. Because each foraging area is associated with its own distinct ecological characteristics, environmental fluctuations and anthropogenic threats that affect the abundance and productivity of individuals at nesting sites, this information is critical for accurately evaluating population trends and developing effective region-specific management strategies.

## DEVELOPING A STEREO-VIDEO CAMERA EQUIPPED UNOCCUPIED AERIAL VEHICLE FOR MEASURING SEA TURTLES, AND OTHER MARINE FAUNA\*

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The use of unoccupied aerial vehicles (UAVs) to study sea turtles *in situ* is a rapidly growing approach. While UAVs provide accurate visual data from an aerial perspective, these devices remain limited in their ability to accurately measure the size of smaller organisms (i.e. < 2 m). But, morphometric data provide valuable insights into a species' ecology and population status following disturbance. Single camera methods use the camera's field of view at a given altitude to determine the size of an object. However, single camera methods, such as altimetry-based, LIDAR, structure-from-motion, and real-time kinematic drones are useful for measuring marine megafauna, but they can have large errors if there is uncertainty related to the altitude of the drone relative to the water surface, or surveys are conducted in remote areas and exact locations cannot be detected (an issue for RTK specifically). Moreover, for these methods to be accurate the animal must be at the surface and orthogonal to the camera. As such, there is an opportunity to explore other, potentially more suitable to boat-based surveys, such as the use of UAV-mounted stereo-video cameras (SVCs). Here, we developed and tested a stereo-video camera (SVC) system that was mounted onto a commercially available UAV. We used the SVC-UAV to conduct remote body-size measurements for the green sea turtle (*Chelonia mydas*) and the nurse shark (*Ginglymostoma cirratum*) to test the validity of this approach. We initially tested the accuracy of the SVC to measure objects in the water from the air in a controlled indoor pool and related measurement accuracy with depth. The mean absolute error (MAE) was 2.12 cm and there was no relationship with depth, at least within ~1.07 m depth where objects were detectable, beyond that image refraction was too severe to identify measurement points. When comparing SVC measurements to those taken by hand, the SVC-UAV had a MAE of 4.44 cm (n=6) for green sea turtles and 7.16 cm (n=1) for the nurse shark. Using a linear model, we estimated the slope of the SVC versus hand measurements for green sea turtles to be 1.085 ( $\pm 0.099$  SE). Importantly, we achieved this level of accuracy for animals that were swimming and diving below the water's surface. Using model selection, based on a global model predicting MAE from animal distance to the SVC and body size, the top ranked model was the intercept-only model. This indicates that neither animal distance nor body size strongly influenced measurement error. The UAV-SVC may be a good survey option when traditional or in-water sampling is not feasible due to logistical or safety constraints, such as where water bodies are too shallow to access by boat, or in-water operations are not safe, or larger sample sizes are required. We are currently working towards lightweighting the SVC to be mounted to smaller drones, automating the calibration, and further reducing measurement error. To our knowledge, there is no other stand-alone SVC for UAVs available that offers similar accuracy and utility.

## **IN-WATER COMPUTER AIDED PHOTO-ID OF JUVENILE GREEN TURTLES (CHELONIA MYDAS) USING AFFINE TRANSFORMED FLIPPER SCALE PATTERNS**

**Kevin Pursley**

*Citizen Scientist*

In-water photo-ID techniques applied to *Chelonia mydas* flippers can be used for CMR. Flipper patterns can be used instead of or in addition to traditional head pattern matching. Application of affine transformations allows for a wide variety of viewing angles thus allowing minimally trained citizen scientist divers and snorkelers participate in CMR activities. Eight years of Photo-ID data collected in Bonaire of over 100 turtles shows the long-term viability of using flipper scales in a non-invasive manner to track sea turtle residency, local migration, tag loss/gain and injury recovery. This technique minimizes manual preprocessing work by substituting computer processing.

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## **GETTING THE MOST FROM SATELLITE TRACKING DATA: WHY TAGS FAIL, TAGGING SITE BIASES AND MAXIMISING DAILY LOCATIONS**

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Satellite tracking is very widely used for studying sea turtles, with around 1000 Argos tags deployed each year (Hays and Hawkes 2018, <https://doi.org/10.3389/fmars.2018.00432>), so the amount spent on satellite tracking is many millions of \$ a year. As the unit cost of tags remains relatively high, it is important to make the most of these deployments. We show how we are using diagnostic data relayed by tags to assess some of the possible reasons why tags stop relaying data, such as battery exhaustion and biofouling. For example, battery exhaustion was implicated as the reason why tracking data stopped being received in 68 of 78 (87%) satellite tags deployed on sea turtles between 2012 and 2019 (mean transmission duration = 267 days, *SD* = 113 days, range: 26–687 days, median = 251 days) (Hays et al. 2021a, <https://doi.org/10.1002/ece3.7558>). In this way we show how we are making informed decisions about the programming of tags and attachment methodology to optimise future deployments. We show how movement analyses are biased by where tags are attached (tagging location biases) (Hays et al. 2020, <https://doi.org/10.1111/1365-2664.13720>) and how these biases can be mitigated when interpreting tracking data, using a case study from the Indian Ocean. We demonstrate how estimates of space use are influenced by the accuracy of locations and hence how the data quality needs to be considered when comparing space use reported across different studies (Hays et al. 2021b). We describe approaches we are using routinely to increase the quality and quantity of locations received from Argos transmitters, including how tags are programmed as well as relaying data via ground receiving stations (Hays et al. 2021b, <https://doi.org/10.1002/eap.2418>).



## **JELLYFISH FOR DINNER? STABLE ISOTOPES REVEAL TEMPORAL TRENDS IN THE TROPHIC NICHE OF OCEANIC LOGGERHEAD TURTLES IN THE MEDITERRANEAN**

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Mobile marine predators such as sea turtles can act as bioindicators to monitor shifts in the pelagic environment and understanding their trophic ecology can provide insight on how marine ecosystems adapt to changes over time. In this study, trends in the isotopic niche of immature oceanic loggerhead turtles (*Caretta caretta*) which inhabited the waters around the Balearic Archipelago between 1997 and 2018 were investigated using stable isotope ratios ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of epidermis samples. More specifically, the response of loggerhead turtle foraging strategy in relation to the recent increase in jellyfish (*Pelagia noctiluca*) populations and to the deeper setting of longline fishing gear in the Western Mediterranean was assessed. The stable isotope results showed that oceanic-stage loggerhead turtles are generalists on the population level and consume a diversity of pelagic prey species. Nevertheless, the  $\delta^{15}\text{N}$  value of the turtle epidermis samples decreased steadily throughout time, suggesting an increased reliance on jellyfish as a food source. Bayesian mixing models supported this interpretation and also revealed a reduction in the isotopic niche breadth of the turtles when the abundance of jellyfish peaked. This indicates that loggerhead turtles are opportunistic predators and respond accordingly to fluctuations in prey populations. Finally, in the later years of the study a decrease in the contribution of fish to the turtle diet was observed, perhaps as a result of the deeper setting of drifting longlines following changes in fishing regulations. Overall, these findings show that long term data can be used to learn about the biology of the focal species, as well as about the environment they inhabit. In the future, consistent sampling and stable isotope analysis of sea turtles and their prey items combined with emerging telemetry technology can be used to monitor the effects of anthropogenic threats on marine ecosystems and inform conservation measures.

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## **DENSITY ESTIMATES OF IMMATURE FORAGING TURTLES ASSESSED BY DRONE SURVEYS\***

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For sea turtles, the most widely used index of abundance comes from beach surveys of nesting numbers, which are then used to assess long-term changes in abundance and population status. While hugely valuable, these data ignore other parts of the population. Chagos Archipelago, in the Indian Ocean, has some of the most pristine tropical marine habitats and lies within one of the largest Marine Protected Areas (MPAs) in the World (<https://doi.org/10.1007/s00227-020-03776-w>). Green (*Chelonia mydas*) and hawksbill

(*Eretmochelys imbricata*) turtles have been protected by conservation legislation here since 1968 and 1970, respectively. In recent years, UAVs have been used in sea turtle research to investigate behaviour, operational sex ratio (OSR), density (mainly at breeding sites), abundance, and distribution (<https://doi.org/10.1016/j.biocon.2019.108214>). We are using UAV surveys to estimate the abundance and density of immature hawksbill and green turtles. We have combined UAV surveys with capture and marking of individuals with paint on the carapace, conducted in February and March 2021, so that the UAV survey results can be examined within a capture-mark-recapture framework. Satellite tracking information from 27 immature turtles (25 hawksbill and 2 green turtles) tagged at the study site were investigated to establish turtle movement and the probability of turtle availability for re-sighting over the survey period, which fed into our density estimate. Density was estimated using measurements of the area of available habitat at low, mid, and high tide in a shallow lagoon. For a shallow lagoon on the atoll of Diego Garcia, we estimated densities of turtles between 265 turtles/km<sup>2</sup> at high water and 499 turtles/km<sup>2</sup> at low water. We found more immature hawksbill (223-419 turtles/km<sup>2</sup>; 84%) than green turtles (42-80 turtles/km<sup>2</sup>; 16%) and we estimated one of the highest densities of foraging immature hawksbill turtles, ever recorded anywhere in the world. The importance of many decades of protection at this site are considered as the driver of these extremely high turtle densities.

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## **PATTERNS OF SEA TURTLE DIVING IN VERY SHALLOW WATER\***

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We examined diving behaviour of hawksbill turtles at a very shallow (generally < 2m) foraging site. Using high resolution time-depth recorders, we recorded the diving behaviour of individuals for up to several months. Even when diving to shallow depths in warm water, dive durations could be relatively long (up to 60 minutes). Across a broad range of air-breathing divers, including seabirds, marine mammals (e.g. seals and whales) and reptiles (e.g. turtles and sea snakes), depth loggers have transformed our understanding of patterns of depth use and the physiological ecology of diving. The vast majority of work has focused on animals diving to 10s or 100s of metres, to feed, rest or travel. For relatively shallow divers routinely descending to only a few 10s of metres, such as odontiid seals and hard-shelled turtles, the lungs may be an important oxygen store for dives. It might be thought that very shallow water diving imposes few physiological challenges as animals can quickly re-surface to breathe. However, foraging in shallow water may pose challenges for air breathing vertebrates if the lungs provide an important oxygen store, since they also generate buoyancy. During dives the lungs will compress with depth, reducing the buoyancy of the diver. For example, surface lung volume will reduce by 50% at 10m, 66% at 20m, 75% and 30m and so on, in accordance with Boyle's law. Diving vertebrates that rely on the lungs as the primary oxygen store have been shown to adjust inspired air volume according to the depth of the coming dive in order to regulate the buoyancy created by air-filled lungs. The oxygen store available therefore increases with depth, leading to greater dive durations at greater depths. Hence a specific issue with very shallow diving is that an animal may struggle to stay at the preferred depth due to being positively buoyant, or else have a limited oxygen store if it exhales prior to submergence. It could therefore be expected that very shallow water diving may be relatively inefficient. Yet to date there has been relatively little consideration of dive durations during very shallow water diving by marine species. Our finding of relatively long, shallow dives (average dive durations ranged between 11 and 32 min, in average depths of 0.5 to 1.8 m, at temperatures of 24-38°C) thus appears enigmatic. We conducted a review of published dive data and dive profiles specifically for U dives in cheloniid turtles. This comparative search has highlighted a clear inter-species difference in dive

durations at shallow depths, with hawksbill turtles consistently diving for longer at shallow depths than green turtles and loggerhead turtles. Adipose tissue has lower density than seawater and therefore provides buoyancy, and so differences in body composition between the species could potentially explain the difference in shallow dive performance.

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## USING MARINE TURTLES TO IDENTIFY HABITAT AND ASSESS CONNECTIVITY OF THE AUSTRALIAN NORTH AND NORTH-WEST MARINE PARK NETWORKS AND SEA COUNTRY\*

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Marine turtles predominantly feed on the benthos, consequently their foraging locations identified by satellite telemetry may be useful to identify benthic communities. Given turtles are highly migratory, their movements can also inform how connected marine ecosystems are across multiple spatial management units. We compiled satellite tracking data for four marine turtle species: green (*Chelonia mydas*, n=126), flatback (*Natator depressus*, n=164), hawksbill (*Eretmochelys imbricata*, n=59) and olive ridley (*Lepidochelys olivacea*, n=27) turtles from 2000-2018, and modelled benthic habitat data from Northern Australia. Tracking data were used to build species-level movement network models to identify connectivity among marine spatial management units (State, Indigenous and Commonwealth managed Marine Parks (AMPs) and nesting grounds) across the North (N) and North-West (NW) Marine Regions of Australia. We also quantified the foraging areas and overlaid them with the habitat data to assess how well turtle foraging areas can be used to identify benthic habitats by calculating simple spatial overlap as well as assessing statistical relationships using regression. Green and flatback turtles displayed a high degree of connectivity across all spatial management units between and within the N and NW, while hawksbill and olive Ridley turtle networks connected spatial management units within the N and NW only, with limited connection between them. There was only low overlap between turtle foraging and marine parks in the N but there was a paucity of turtle tracking data available for the N. The links between spatial management units suggests a need for collaborative management. The percentage overlap of the core foraging area (50% KUD) of the turtles with the habitat data ranged from 4.2 to 33% in the North-West and 11 to 42% in the North. Green and hawksbill turtle 50% KUDs overlapped hard coral, macroalgae, seagrass, filter feeders, turfing algae, and bare substrate habitats. This was in line with their documented habitat and diet. For olive Ridley and flatback turtles, their 50% KUD predominantly overlapped bare substrate habitat (78% and 56%

respectively) with much lower contributions of the other habitats. We conclude that turtle species with lower association with soft bottom habitats such as green and hawksbill turtles, are the most useful at indicating important habitat. While the spatial overlap analysis has shown that foraging turtles can inform on the occurrence of benthic communities, our regression analysis between the probability of green and hawksbill turtle foraging and proportion of underlying coral reef and seagrass habitat indicated positive relationships in only a few small areas. This was most probably due to a mismatch in the scales between the data types; km's for turtle tracking data compared to m's for the habitat maps. Areas within and adjacent to the Limmen and Gulf of Carpentaria AMPs within the North network, and Eighty Mile Beach, Roebuck and Kimberley AMPs within the North-West network had the highest turtle foraging activity and are thus likely locations that have important sensitive benthic communities (e.g., seagrass, hard and soft corals) and where future benthic habitat mapping can be targeted to validate foraging turtles as habitat indicators.

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## THE PRESENCE AND DISTRIBUTION OF CARETTA CARETTA IN AND AROUND THE MPA VENTOTENE AND S. STEFANO, WESTERN MEDITERRANEAN SEA

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The particular morphology of the volcanic seabed of Pontine Archipelago (central-eastern Tyrrhenian Sea), and the frequent sightings of loggerhead turtles in its pelagic waters, have given rise to the idea that this area could represent a crossing point along migrations of juveniles and adults in the Western Mediterranean Sea. Also, two recent nesting events that occurred on the beach of the Ventotene island have further highlighted the need to investigate this protected site and its adjacent waters for its potential role in marine turtle conservation. A pilot monitoring project has started in the area in 2019 thanks to the logistical and financial support of “Ventotene and S. Stefano” Marine Protected Area. The project aims to assess the temporal and spatial distribution of loggerhead turtles around the island of Ventotene and evaluate the demographic parameters and health state of individuals frequenting its waters. Dedicated small-boat surveys have been conducted at constant speed along fixed-transects designed to cover an effort area of approximately 10-15 km<sup>2</sup>. The animal's position, distance, body orientation and behavior were recorded for each sighting. Exploratory surveys were also performed to capture individuals using the turtle-rodeo technique and to assess the life stage and health status before releasing them. In addition, three selected individuals were equipped with satellite tags to investigate turtles' movement patterns. All field activities were conducted in spring-summer 2019, 2020 and 2021 for a total of 19 monitoring days. During the 3-year monitoring program, 50 turtles were sighted in the area, mainly in the southern and eastern sector within 10 nm from the Ventotene Island. Satellite positions of three tracked turtles suggested that turtles are not resident but pass the area in search of food during summer, while moving southwards during autumn-winter, using the whole Tyrrhenian Sea. Despite being mainly in good health conditions, most of the captured individuals showed signs of anthropic interactions, likely due to collisions with boats, fishing gears, or chemical pollution and two severely injured individuals needed to be retained and transported to the SZN rescue center in Portici (NA, Italy) for their recovery. The size of all sighted/captured animals ranged between post-hatchling to male and female adult specimens, indicating the co-presence of individuals at various life stages in the area. Investigations on the nutritional status of large juveniles and adults suggested the jellyfish *Pelagia noctiluca* as one of the primary prey. Results from the first years of activity were promising, encouraging us to implement them in the next years. Particularly, the potential

aggregation of turtles in the area during the summer period evidenced by our data should be investigated in relation to seasonal circulation patterns and food availability. In this perspective, the number of satellite-tagged turtles should be also increased adding depth, temperature and Chlorophyll sensors to the instruments investigating the role of environmental variables in determining spatial movements. All this information will help predict turtle habitat use and inform better proper conservation management of the species in the area.

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## **SPATIAL ECOLOGY OF THE HAWKSBILL SEA TURTLE IN AN UPWELLING REGION FROM THE EASTERN TROPICAL PACIFIC**

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Quantifying the spatial ecology of aquatic animals is crucial to develop effective management and conservation approaches, especially for highly threatened and mobile species like sea turtles. The hawksbill sea turtle (*Eretmochelys imbricata*) is a critically endangered species with a circumglobal distribution, and the East Pacific population is one of the most threatened. The north Pacific coast of Costa Rica is a region that is subject to a strong seasonal upwelling and has been previously identified as an important feeding ground for hawksbill turtles in the Eastern Tropical Pacific (ETP) ocean. However, knowledge of the spatial ecology and drivers that influence the movement patterns of hawksbill turtles in this region is lacking. Here, we used passive acoustic telemetry to investigate the residency, movement patterns, and habitat use of hawksbill turtles in two neighboring bays (Matapalito and Santa Elena) that form a marine management area located in the north Pacific coast of Costa Rica. An array of 11 acoustic receivers were deployed between October 2020 and November 2021 to monitor the movement of 10 acoustically tagged hawksbill turtles in both bays. These receivers covered a wide variety of habitats, including coral and rocky reefs, mangroves, and sandy/muddy bottoms. Residency index (RI; proportion of days individuals remain within the study system) of hawksbill turtles in Matapalito Bay (tagging site) was relatively low (RI = 0.13), with some individual spending up to 49% of the monitoring days inside the bay. Five individuals exhibited movements between both bays, spending a few days in Santa Elena Bay before returning to Matapalito bay or leaving the protected area. There was a clear difference in activity of the sea turtles along the day, showing a higher detection frequency during the nighttime. There is also a marked seasonal difference with the upwelling months having lower detection frequencies than during the non-upwelling season. These results show the importance a variety of habitats for hawksbill turtles in the region and suggest the impact that seasonal changes may have on how much they use specific habitats or need to find new ones. Identifying and fomenting connectivity between key coastal environments is crucial for the protection for hawksbill turtles in vulnerable stages and achieving population recovery.

## INSIGHTS INTO GREEN TURTLE ECOLOGY FROM COMBINING STABLE ISOTOPES WITH ACOUSTIC AND SATELLITE TAGGING\*

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We investigated green turtle ecology in the Ningaloo World Heritage Area, using a combination of stable isotope analysis with acoustic and satellite tagging. Studying wild animals in this way requires careful consideration of how to collect data humanely and according to sound ethical principles, which includes minimising the stress placed on the animals used. We tested a non-invasive method of collecting specimens from green turtles *Chelonia mydas*, using nail clippings as well as the more typically used methods of taking skin biopsies and blood samples. We compared skin, blood (including whole blood as well as red blood cells and plasma separated by centrifuge), and nail clippings from 148 green turtles. We found that using nail clippings as a non-invasive way of generating measurements of stable isotope composition worked extremely well, while skin did not. Combining data from nail clippings (inert) with data generated from red blood cells (relatively high turnover) of the same animals, we found that a turtle's stable isotope composition (and therefore diet) varied according to its size (and therefore age). The pattern tended to follow a trend consistent with a change to a seagrass-dominated diet after arrival of juveniles from their oceanic habitats to the coastal habitats of Ningaloo, followed by a seaward movement to the lagoon and reef to feed on seaweed and jellyfish as individuals grew larger than ~70 cm. These patterns were supported by acoustic tagging of a subset of 80 individuals, which found that smaller individuals occupied small home ranges closer to shore (where seagrass is more abundant), while larger individuals occupied larger home ranges in lagoon habitat further from shore (where macroalgae is abundant). High-resolution satellite locations also confirmed that individuals periodically left the lagoon for deeper water outside the reef, potentially to feed on jellyfish. Satellite tagging also confirmed that these turtles were resident, mostly moving no more than a few kilometres from place of capture after periods extending more than a year.



## ORIGIN OF GREEN (CHELONIA MYDAS) AND LOGGERHEAD (CARETTA CARETTA) TURTLES FORAGING IN KUŞADASI BAY, TURKEY

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Sea turtles are highly migratory species and thus feeding, and breeding sites can be very distant. Alternatively, sea turtles from the same breeding grounds can use different foraging grounds, which can be hundreds of km away from each other. Considering that sea turtles are species of conservation concern, it is essential to establish connections between foraging and nesting areas to link threats at seas, such as maritime traffic or fisheries impact, to the affected nesting populations. However, gaps in knowledge on the connections between nesting and foraging areas may jeopardize management and conservation efforts. Mixed Stock Analysis (MSA) based on genetic markers has been used worldwide to determine the populations of origin of marine turtles found stranded or in foraging areas. The eastern Mediterranean hosts nesting populations of the loggerhead (*Caretta caretta*) and green (*Chelonia mydas*) sea turtles. Previous studies on the loggerhead turtle have determined the origin of individuals on most known foraging areas and they have delineated the main migratory routes of juveniles from their areas of origin. However, the studies on green turtle foraging areas are much scarce. Still, the rise of new genetic markers, such as the mitochondrial STRs, makes it possible to perform reliable MSA in the Mediterranean. The origin of turtles foraging the Aegean Sea has not been addressed for either of the two species, leaving an important knowledge gap addressed in the present study. The Kuşadasi Bay is a foraging zone in the Aegean Sea inhabited by turtles from both species all year round. In this study, we genotyped 44 samples of green turtles (*Chelonia mydas*), with sizes ranging from 16 to 149 cm CCL, and using a pattern of four AT short tandem repeats (STRs) with varying copy numbers. We also genotyped 79 samples of loggerhead turtles (*Caretta caretta*), with sizes ranging from 60 to 115 cm CCL using a segment of the mitochondrial DNA (mtDNA) D-loop (control region). Samples from both species were collected around Kuşadasi Bay all year round from 2017 to 2021. The haplotypes for both species were compared with the available genetic data of Mediterranean and Atlantic nesting populations using a Bayesian MSA to obtain the origin frequency distribution of these two turtle species foraging in Kuşadasi Bay. The results of the mixed stock analysis, the genetic diversity of the Kuşadasi foraging area, and the genetic differentiation between foraging areas are discussed to aid the management and conservation of marine turtles in the Mediterranean Sea.

## 22 YEARS OF FLIPPER TAGGING PROGRAMS: GREEN TURTLE CONNECTIVITY IN FEEDING AND DEVELOPMENTAL GROUNDS OF SOUTHERN BRAZILIAN AND URUGUAYAN COASTAL WATERS\*

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Critical gaps of green turtle (*Chelonia mydas*) migratory behavior and distribution can be globally addressed by mark-recapture approach. Flipper tag is the most simple and low-cost method, being the technique most largely used for studies focusing on sea turtle migrations. Southern Brazilian and Uruguayan coastal waters present an important all year round feeding and developmental areas for juvenile green turtles in their southern distribution limit in the Atlantic Ocean. In the present study, we compile mark-recapture data from 2000 to 2021 of several organizations that are part of ASO-Network (Marine Turtle Specialist Group of Southwestern Atlantic) intending to describe the movements of juvenile green turtles among different feeding and developmental areas between Southern Brazil and Uruguay (only data from recaptures occurred in different country of original tagging are included). Flipper tags and recapture data for this study belongs to specimens originally tagged in areas monitored in Uruguay by Karumbé and in Brazil by NEMA, CRAM-FURG, Caminho Marinho, CECLIMAR/UFRGS, REVIS Ilha de Lobos, Fundação Projeto Tamar (Stations in Florianópolis - SC and Ubatuba - SP), UDESC, PBF-Instituto Australis, UNIVALI, LEC-UFPR, and IPeC. Karumbé and Tamar had a long-term in-water program which include intentionally capture, incidentally capture (fishing monitoring) and strandings monitoring. Other two organizations (LEC-UFPR and Caminho Marinho) started in-water programs in 2014 and 2016, respectively. The other organizations only perform strandings monitoring with different periodicity. All these organizations cover ca. 1800 km off the Southwestern Atlantic coast between Sao Paulo State, Brazil (23.7°S-44.7°W) and

Uruguay (34.9°S-55.2°W). A total of 79 recapture events of green turtles and three second recaptures were registered, of which 68 turtles were captured (by-caught or intentionally captured) and 14 were registered as stranding turtles within Brazilian and Uruguayan waters. Sources of tag recovery derived in 29.1 % (n=23) of cases were from in-water captured turtles (73.9% alive turtles) and in 70.9 % (n=56) from stranded animals (30.3 % alive turtles). The size at first release ranged from 32.2 to 59.2 cm CCL (mean±SD= 40.4 ± 6.2 cm). A total of 62 recaptured events were originally tagged within Uruguayan waters (total of turtles tagged, 2000-2021 = 2920) were recaptured in different Brazilian states: 37 in Rio Grande do Sul, 15 in Santa Catarina, five in Paraná and four in Sao Paulo. The 16 recapture events originally tagged in Brazil (12 in Rio Grande do Sul and four in São Paulo; total of turtles tagged, 2000-2019 = 11300) occurred in three Uruguayan departments: 13 in Rocha, two in Maldonado and one in Canelones. The maximum distance registered was 1700 km (straight coastline) and the maximum recapture intervals were 8.8 years. In recent years, more organizations started to conduct beach surveys and in-water captures, increasing the number of turtles tagged in the study area, probably increasing the recaptures in the South Atlantic. This collaborative effort will improve the knowledge about the green turtle connectivity in the South Atlantic, contributing to gain effective conservation measurements on a broad scale.

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## MASS STRANDING OF OVERWINTERING JUVENILE GREEN TURTLES (CHELONIA MYDAS) IN SOUTHERN BRAZIL AND URUGUAY (31° - 34° S)

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Green turtles (*Chelonia mydas*) use coastal waters of the Southwestern Atlantic as a feeding area and developmental habitat, also as a migration corridor, including Southern Brazilian States (São Paulo, Paraná, Santa Catarina and Rio Grande do Sul), Uruguay and northern Argentina. Stranding and bycatch records of green turtles were registered all year round in the coast of the Rocha Department-Uruguay [RO-UY] and in the coast of Rio Grande do Sul State-Brazil [RGS-BRA]. During the austral winter, a small proportion of this turtle aggregation overwintering in coastal habitats in this area instead to migrate to warmer waters. During overwintering, otherwise called “winter dormancy” or “brumation”, turtles reduce their activity, movements, and metabolism to tolerate low temperatures (11-12°C). Indirect evidence of this phenomenon was registered by the presence of unusual -benthic- epibionts on the carapace, i. e. not the common obligate symbiotic barnacles. Turtles with this lethargic behavior are exposed to intense colonization pressure by marine propagules. During this unique worldwide phenomenon turtles’ carapaces were covered with local benthic biota such as barnacles, seaweeds, mussels and polychaetes among others. Here we characterize the mass stranding event occurring during October and November 2019 between RGS-BRA from Parque da Lagoa do Peixe -Mostardas (31°21’S; 51°02’W) to Chui (ca. 355 km; 33°44’S; 053°22’W) and from RO-UY from Chuy to La Paloma (ca. 130 km, 34°39’S; 54°09’W). Stranded turtles were registered in Uruguay by the NGO Karumbé and in Rio Grande do Sul by the NGO NEMA. During the study period, a total of 380 green turtles were found stranded on beaches or found floating in shallow waters close to the shore. All of the specimens were juveniles (mean curve carapace length (CCL) = 40.9±6.5 range = 29.8-77.5 cm, n=351). Of the total, 171 were registered in RGS-BRA and 209 in RO-UY. Most of the turtles were

registered stranded and approximately half were alive; the latter were brought to rehab centers and released after rehabilitation procedures. Regarding the presence of unusual benthic epibionts, 173 turtles were registered with this epibiota, indicating the brumation during the winter in the area. Also, 98 of the total of the turtles get trapped among the “Canal Andreoni” (La Coronilla, RO-UY) discharge (including remains of vegetation, cattle carcasses, anthropogenic debris, among others), the beach and a sand bar along the mouth of the channel. The number of stranding green turtles during November-December of 2019 were significantly higher than the number of stranding registered for these months since 2001 in the study area. Probably, the higher number of turtles during these months in the study area were associated with favorable conditions. Indeed, after successive years with positive anomalies in SST, green turtles may have been influenced to stay in the area in higher numbers than usual, thus resulting in the mass stranding event reported here. This work highlights the importance of long-term monitoring programs to understand the threats affecting sea turtles and environmental changes affecting sea turtle aggregations in feeding areas far from the nesting zones.

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### **SUCCESS WITH PHOTO IDENTIFICATION SOFTWARE USING, HOTSPOTTER, IMPROVES GREEN TURTLE MARK-RECAPTURE EFFORTS\***

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Photo identification (PID) is an efficient tool to track wildlife. The issues of tag loss and capture stress associated with traditional capture-mark-recapture methods that require physically tagging individuals can be resolved using PID. This method creates a permanent record of digital identification data using an organism's unique markings. Data can be collected by both researchers and citizen scientists because of the accessibility of photo identification tools. Green turtles (*Chelonia mydas*) have highly migratory behaviors, thus tracking individuals across multiple life stages is difficult because external tags can foul or be lost and internal tags (PIT tags) can be cost prohibitive. We tested the applicability of HotSpotter, an automated photo identification software, for use on green turtles using photographs from a population of sub-adult individuals in Abaco, The Bahamas. We compared data from physical Inconel flipper tags to HotSpotter outputs across 324 captures of green turtles between 2013 and 2019. In addition to known recaptures, HotSpotter correctly identified an additional four unknown recaptures, including one turtle captured in two distinct habitats. HotSpotter results were verified manually and found a 92% match rate between the software output and a human observer for a subset of 90 test photos. Our results suggest that HotSpotter is appropriate for green turtle PID and will be useful in building a larger regional database that expands our capture-mark-recapture abilities throughout The Bahamas and neighboring countries. We anticipate our results will improve our ability to determine the interconnectedness of sites across green turtle ontogeny.

## **EVOLUTIONARY COMPARISONS OF CHELONID ALPHAHERPESVIRUS 5 (CHHV5) GENOMES FROM FIBROPA PILLOMATOSIS-AFFLICTED GREEN (CHELONIA MYDAS), OLIVE RIDLEY (LEPIDOCHELYS OLIVACEA) AND KEMP'S RIDLEY (LEPIDOCHELYS KEMPII) SEA TURTLES\***

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The spreading global sea turtle fibropapillomatosis (FP) epizootic is threatening some of Earth's ancient reptiles, adding to the plethora of threats faced by these keystone species. Understanding this neoplastic disease and its likely aetiological pathogen, chelonid alphaherpesvirus 5 (ChHV5), is crucial to understand how the disease impacts sea turtle populations and species and the future trajectory of disease incidence. We generated 20 novel ChHV5 genomes, from three sea turtle species, to better understand the viral variant diversity and gene evolution of this oncogenic virus. We revealed previously underappreciated genetic diversity within this virus (with an average of 2035 single nucleotide polymorphisms (SNPs), 1.54% of the ChHV5 genome) and identified genes under the strongest evolutionary pressure. Furthermore, we investigated the phylogeny of ChHV5 at both genome and gene level, confirming the propensity of the virus to be interspecific, with related variants able to infect multiple sea turtle species. Finally, we revealed unexpected intra-host diversity, with up to 0.15% of the viral genome varying between ChHV5 genomes isolated from different tumours concurrently arising within the same individual. These findings offer important insights into ChHV5 biology and provide valuable genomic resources for this oncogenic virus at the genome level.

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## **TRIALLING THE USE OF AN UNMANNED AERIAL VEHICLE AND VHF TECHNOLOGY TO TRACK HATCHLING SEA TURTLES**

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Given their small size, devices that have been developed to track marine vertebrates are too large to use on hatchling sea turtles. Published methodologies to track hatchling turtles have typically involved observations whereby the hatchling is 'tagged' visually, acoustically, or with a VHF transmitter. One method involves an observer equipped with a VHF receiver who directs a vessel in the bearing of a signal



received from a VHF transmitter towed by a hatchling turtle. Studies that trialled this methodology were able to detect signals up to 1200 m away, however, no study has used the received signals to determine the actual location of the hatchling (i.e. via triangulation) and instead relied on the location of the vessel as a proxy. In this study, we present the results of a proof of concept trial that combined an Unmanned Aerial Vehicle (UAV) with VHF technology to determine whether it could be applied to track the location of a sea turtle hatchling. The trial used specific criteria to assess the performance of three types of VHF receiver and five different VHF transmitters (weight range 0.3 g – 3.8 g). The trial was completed on-land and in-water using a DJI Matrice 210 multi-rotor UAV. VHF receivers were provided by Lotek (Pinpoint Commander Receiver), Wildlife Drones, and Drone Ranger and attached to the base of the UAV. To determine the limitations of each VHF receiver and identify the optimum UAV flight parameters for transmitter detection prior to an in-water field trial, the VHF transmitters were setup under different controlled condition scenarios. For the in-water field trial, transmitters were fixed to a float that was attached using monofilament line to a GPS-equipped remote-control boat that acted as a hatchling surrogate. Various flight scenarios were then undertaken to trial the performance of the receivers. The Pinpoint Commander Receiver successfully detected and downloaded GPS data stored onboard an in-water Pinpoint VHF-50 transmitter from up to 900 m away. The Wildlife Drone System was able to detect the signal of several VHF transmitters within wet controlled conditions. However, during the in-water field trial, the recorded signal strength was not sufficient for the system to estimate a location with suitable accuracy. The Drone Ranger System provided an estimate of the transmitter's location with an accuracy of <100 m when setup within wet controlled conditions. However, during in-water field trials, the location accuracy decreased further offshore and was likely due to a deterioration in sea state conditions. While there were aspects of the Wildlife Drones and Drone Ranger systems that were considered a success, the reliance on the UAV to continuously detect the transmitter to estimate its location over a broad geographical area and long duration, limits their use for tracking a hatchling. The Pinpoint Commander Receiver relied on the download of GPS location data that was being continuously recorded by the transmitter and could be downloaded by the UAV at a suitable range. However, the weight and cost of the Pinpoint VHF-50 transmitter (3.8 g; USD\$1,250) may limit its application.

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## SHOULD I STAY OR SHOULD I GO? MOVEMENTS OF SEA TURTLES DURING EXTREME WEATHER EVENTS

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Extreme weather events are becoming increasingly frequent worldwide, with environmental conditions such as temperature, precipitation and winds reaching or exceeding historical lower and upper levels. In the USA, the South Texas region is a hotspot for tropical storms during boreal summer, but also experiences extreme cold temperature spells during boreal winter. Such events can have a range of effects on marine megafauna, from none to minor disruptions to their typical behaviors to severe responses which can lead to massive deaths in the populations. In this study we will describe how sea turtles in Matagorda Bay, Texas, responded to two extreme weather events in 2021: (1) the 'Big Freeze', a two-week long temperature drop which started with winter storm 'Uri', and (b) hurricane 'Nicholas, a category 1 hurricane that made landfall in the Matagorda Peninsula approximately 80 km east of our study site during summer. We assessed changes in the individual behavior of sea turtles equipped with satellite transmitters during both events. In



addition, we extensively surveyed the study area during two weekends post-freeze to recover and record stranded sea turtles which enabled us to assess the effects of the extreme event at a larger scale. After the Big Freeze, we recorded a total of 62 dead green sea turtles along a 15 km stretch of coast. Nevertheless, both turtles tracked before and during the freeze left the area by the time temperatures dropped and migrated south to and past the border between the USA and Mexico. While seasonal migrations triggered by changes in water temperature are widely described in sea turtles, understanding the mechanisms of why many other individuals do not respond to such extreme temperature changes warrants further investigation. During the pass of hurricane Nicholas, we satellite tracked four green turtles which did not show any substantial changes in behavior other than momentarily moving into deeper waters, and one turtle that went outside the Bay and into the Gulf of Mexico after the storm only to return to the same area afterwards. Unfortunately, assessing changes in behavior at a finer scale was not possible given the lower transmission rate of GPS fixes due to dense cloud coverage. Understanding how sea turtles respond to extreme weather events at individual and population levels is important to create assertive response plans during the aftermath of future events, when there are widespread challenges in the human coastal communities and resources and time need to be wisely spent in a coordinated effort.

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## RESPONSE OF FLATBACK TURTLE HATCHLINGS TO LIGHT EMITTING DIODES AT SEA\*

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It is well known that light pollution disrupts the early dispersal of marine turtles. But now that light emitting diodes (LEDs) are rapidly replacing traditional lights, it is evident we know little about how they influence hatchling dispersal, or how modifying the intensity of the light affects their in-water behaviour. Here we used acoustic telemetry to assess the early in-water dispersal and predation rates of flatback turtle hatchlings (*Natator depressus*) in response to different intensities of white LEDs located on a boat 150 m offshore of a nesting beach on Thevenard Island in Western Australia. These lights were enriched in short light wavelengths which are known to disrupt the sea-finding ability of hatchlings. Hatchlings (n=68) were obtained from a total of seven nests and were released in experimental trials within 48 hrs of capture. Prior to release, an acoustic tag was glued to their undersides, and they were released at the waters' edge to swim through the nearshore zone where acoustic receivers had been deployed in an array totalling 21600 m<sup>2</sup>. This experimental setup allowed us to document the turtle movement paths as they dispersed through the nearshore zone in the presence and absence of LEDs of five different intensities (10, 30, 50, 70 and 120 watt). The experiment occurred over two nights, with the lights on the boat located either on the eastern or the western side of the array. We found no effect of LEDs on the bearing hatchlings took as they swam through the tracking array when lights were in the direction they dispersed under ambient conditions. When LEDs were not in their usual direction of travel observed under ambient conditions, variability in their mean bearing increased, and a change in bearing occurred with the highest light intensity. We found weak evidence that predation was also higher at this light intensity compared to ambient, and also in two of the lower light intensities (10 and 30 watts), but only on one of the experimental nights. We were unable to find a relationship between hatchling speed and time spent in the tracking area with light intensity. However reduced sample sizes (due to predation), and the fact that there was little difference in the measured intensity

between light treatments might have affected our ability to detect effects. Although more effort is required to increase the confidence in our findings, the use of white LED floodlights in coastal areas, even at low intensity, appears to pose a threat to hatchlings. We suggest light avoidance or other light management measures such as task lighting, shielding, and avoiding shining light directly on the water, might be more appropriate mitigation measures than simply reducing intensity.

## NESTING BIOLOGY

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### MODELING TEMPERATURE DYNAMICS IN A LARGE OLIVE RIDLEY BEACH HATCHERY: METABOLIC HEATING AND ESTIMATED SEX RATIOS

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Beach hatcheries in NW Mexico have become necessary to protect clutches from excessive temperatures using shading. Beaches hosting large nest abundances (>1000/season) require relatively large hatcheries. Monitoring sand temperature inside these hatcheries is imperative to ensure viable conditions and estimate sex ratios, yet extrapolating thermal regimes for all nests from limited sand measurements is challenging because clutches are translocated at different dates, temperatures vary extensively over the season, and there is limited knowledge on the extent and timing of metabolic heat (MH) for olive ridley embryos. To address current knowledge gaps, we (1) modeled the spatial and temporal temperature variation in the hatchery using an array of nest (NMS) and sand (SMS) temperature monitoring stations, 2) characterized the magnitude and timing of the MH in monitored nests to determine its impact on sex ratio estimates, and (3) used a combination of these results to extrapolate sex ratios of the entire set of nests. Fifteen temperature stations were distributed inside the covered 60 x 10 m beach hatchery at El Verde beach in Sinaloa, Mexico, where around 500 clutches were relocated Sept-Nov, 2018. Temperature was logged hourly in NMS and at the same depth in the sand 0.5 m away (SMS). MH was calculated as the difference between the NMS and SMS temperatures, and its variation during incubations was modeled from regression fits. Sex ratios were estimated from the mean nest temperatures during the second third of the incubation, using a pivotal temperature (30.46°C) and shape parameter (S=0.375) for the thermal reaction norm. Mean daily temperatures from the SMS were used to interpolate daily values for the entire hatchery. These were used to construct daily temperatures for each clutch according to their incubation duration dates. Although MH peaked during the final third of the incubation (mean=2.6°C; range= 1.3-4.5), the gradual increase during the second third (mean=0.54°C; range=0.16-1.36) had a significant impact on sex ratio estimates. MH was correlated with mean sand temperature and the number of hatchlings; however, both had low coefficients of determination ( $R^2 < 0.25$ ). Interpolated sand temperatures for the entire hatchery indicated a predominance of cooler values on the western edge and warmer on the SE sections with a mean difference of 2.8°C across the season (range= 1.0-4.2°C). This spatial temperature heterogeneity and variable climatic events (principally rain) was reflected by the large variation in estimated sex ratios for NMS (mean= 24.7% females; min=1.3%, max=85.5%). Combining the reconstructed sand temperatures for each nest in the hatchery and the MH modeling, estimated sex ratios for individual clutches indicated a mixture of sex ratios, with a global value of 42.2% females (min=9.3%, max=65.5%). The range of sex ratio obtained validates estimating individual nest values for large hatcheries with spatial and temporal temperature heterogeneity. Incorporating MH values is essential to adequately estimate correct temperatures in the nest during the thermosensitive period. However, the range of estimated MH values in our beach incubations indicates the need for a fuller understanding of influences by other variables to obtain robust estimates of sex ratios.

## UNDERSTANDING MULTI-SPECIES PREDATION ON EMERGING SEA TURTLE HATCHLINGS\*

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Two of the most vulnerable stages in a sea turtle's life are experienced when incubating as an egg and in the first minutes after emerging from the nest. These are highly vulnerable stages for all marine turtle species, particularly at rookeries with high densities of potential predators. Many animals have been implicated in the predation of hatchlings but there are few quantified observations to confirm these interactions. Over two summers, we used burrow densities as proxy to estimate the abundances of ghost crabs, did pre- and post-season nest inventories to estimate the number of predated eggs, and used infrared videography, filming nests continuously over several days, to capture both diurnal and nocturnal predator interactions with emerging loggerhead turtle (*Caretta caretta*) hatchlings at Bungelup Beach and Gnoraloo Bay, situated along the Ningaloo Reef on the west coast of Australia. Quantified observations of predation were derived from the examination of more than 7,500 hours of video footage. Overall, at Gnoraloo Bay ghost crab burrow densities were up to twice as high compared to Bungelup Beach. Generally, burrow densities were greater during the hatchling emergence period (February/March) compared to the egg laying period (December/January). Multiple species were observed predated sea turtle hatchlings, including ghost crabs (*Ocypode spp.*), silver gulls (*Chroicocephalus novaehollandiae*) and the invasive black rat (*Rattus rattus*). At Bungelup Beach, 36% of eggs were predated by ghost crabs and 44% hatched. Of the hatchlings that emerged, 43% were predated, primarily by ghost crabs but also by silver gulls. Quantified observations include incidences of klepto-parasitism, where silver gulls and a rat steal hatchlings from ghost crabs. At Gnoraloo Bay, 79% of eggs were predated by ghost crabs and for the 16% of eggs that hatched, there was no clear evidence that any hatchlings reached the water. To our knowledge, this study provides the most accurate hatchling predation estimates yet reported and the first real quantified data on hatchling predation by seagulls and of klepto-parasitism. These research findings increase the understanding of predator-prey interactions at sea turtle rookeries through well-established as well as novel methods, which could help determine appropriate intervention thresholds and management strategies for sea turtles.

## **DO SMALL-BODIED NESTING GREEN TURTLES (CHELONIA MYDAS) POSSESS THE CAPABILITY TO PRODUCE LARGE REPRODUCTIVE OUTPUTS?**

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Female green turtles can lay, on average, between 60-120 eggs per clutch and nest up to 8 different times during any nesting season. The ability to document morphometric data and the total number of eggs laid by an individual turtle can be important factors in assessing their nesting performance in terms of reproductive output during a single nesting season and throughout its lifetime. Furthermore, the long-term assessments of the reproductive biology and ecology of turtles at their natural nesting beaches will allow better estimations of their population dynamics in the future. Our study aimed to; 1) determine morphometric profiles (curved carapace length (CCL), curved carapace width (CCW), and body weight) and reproductive outputs (clutch size, mean egg mass, mean egg size, nesting frequency, and the total number of eggs per individual per season), 2) determine the relationship between maternal body weight and reproductive output, and 3) ascertain for optimality theory for the Chagar Hutang nesting green turtle population. The relationships between maternal body weight and reproductive outputs were analyzed using Pearson correlation coefficient and Spearman Rank correlation. We determined that morphometric profiles of 28 individual green turtles which were nested at Chagar Hutang beach, were relatively smaller in terms of CCL, CCW, and body weight when compared to other green turtle nesting populations elsewhere. The reproductive outputs such as clutch size, mean egg mass and mean egg size were consistent with other findings elsewhere, while data such as clutch frequency and the total number of eggs per individual per season were far lesser than previously recorded. Although the resulting data of clutch size, mean egg mass, and mean egg size showed a positive correlation with maternal body weight as expected, the nesting frequency and the total number of eggs per individual per season showed a negative correlation with maternal body weight. The results from this study are an integral component in elucidating the overall morphology and reproductive output profiles of nesting green turtle populations in the South China Sea and allow for further estimates of individual turtle's lifetime reproductive effort and the population's future reproductive viability.

## NESTING ACTIVITY AND REPRODUCTIVE OUTPUT OF LOGGERHEAD (*CARETTA CARETTA*) AND GREEN TURTLES (*CHELONIA MYDAS*) ALONG THE LEBANESE COAST, EASTERN MEDITERRANEAN SEA

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The two marine turtle species, the loggerheads (*Caretta caretta*-Cc) and the greens (*Chelonia mydas*-Cm) frequent the Lebanese waters. Those species are also nesting on many sites of the Lebanese coast. The survey results during the sea turtles females nesting/ and hatching season for both species (*Caretta caretta* and *Chelonia mydas*) over the last three years (2019-2021) are presented. As a result, the number of nests for both species is significantly increased from 2019 to 2021. Accordingly, 80 nests, corresponding to 74 Cc and six Cm, were recorded from 11 monitored sites along the Lebanese coast in 2019. While, the number of nests increased to reach 160 nests (107 Cc and 53 Cm) in 2020 and 218 nests (160 Cc and 58 Cm) in 2021, from 20 monitored sites of the Lebanese coast. Subsequently, 3940 hatchlings of Cc and Cm with 65% of success were recorded in 2019. In comparison, the number increased to reach 9903 hatchlings of Cc and Cm with 89 % success and 13692 with 78 % in 2020 and 2021, respectively. Based on the Marine turtles nesting frequency level, three sandy beaches are classified as important marine turtles nesting sites, five as moderate, three as low, nine as very low nesting sites, along the Lebanese coast. However, the marine turtles' nests along the Lebanese coast are under a complex mix of natural, human, and biological pressures. The natural pressures are more related to the humidity/ contamination/ rise temperature of the beach sand, inundation coming from the beach, and vegetation roots present on the beaches. While the human pressures are mainly represented by uncontrolled coastal development, the use of some beaches for livestock, tourism activity (significantly during summer and on the important nesting sites, including the Marine Protected Areas), often associated with the noise and light pollution and direct impacts on the sea turtles nesting sites (e.g., walking, camping, and using vehicles on the highly marine turtles nesting sites). In addition, illegal fishing methods and marine litter significantly impact sea turtles in the Lebanese waters, especially during mating season. As a result, a total of 122 sea turtle stranding incidents were reported over a year-long period (January 2021 – December 2021) across all the Lebanese coast. This is mainly due to illegal fishing methods and a collision with boats. Accordingly, the biological pressures are more represented by dogs, foxes, and crabs, especially the ghost crab (*Ocypode cursor*), and the river crab *Potamon* spp., as main predators of the marine turtles' eggs. In addition to the direct and significant impact of some terrestrial larval/ or adult macroinsectes (e.g., *Pimelia* sp., *Myrmeleon formicarius*, and *Tomarus subtropicus*) on the eggs of the sea turtle nests. From a conservation and protection point of view, an extensive successful awareness campaign was launched, along the Lebanese coast, to promote, motivate, and sensitize Lebanese fishers, the local community, and non-governmental organizations to help monitor and protect the marine turtles in Lebanon.



## GREEN TURTLE REPRODUCTIVE SUCCESS ON THE ISLAND OF MEIO, IN THE BIJAGÓS ARCHIPELAGO, GUINEA-BISSAU

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The green turtle, *Chelonia mydas*, is an oviparous species that seeks out sandy beaches to nest, where it leaves its eggs to incubate without parental care, exposed to the elements. During the incubation, the developing embryos face several risks of mortality, mainly due to predation, flooding of nests and overheating of eggs. Being philopatric, once they reach maturity, green turtles tend to return to the region where they were born to reproduce, which can present a problem if the reproductive success declines at natal sites. The Bijagós archipelago, Guinea-Bissau, is home to one of the largest populations of green turtles in the world, with most of the breeding females nesting on the small low-lying island of Poilão (ca. 25,000 clutches per year). Given its great magnitude, most of the nesting biology research in the region is conducted at Poilão, with fewer studies in other sites. Yet, significant nesting occurs in neighbouring islands, in some seasons numbering to thousands. In island that is closest to Poilão; Meio Island, 2,063 green turtle clutches were laid in 2016. Thus, this study aimed to evaluate the hatching success, predation and flooding rates of green turtle nests on Meio Island, and compare with the same parameters in Poilão Island. During this study, 98 nests were monitored on Meio Island and 95 on Poilão Island. On Meio island, the predation rate was very high (83.7%), and these clutches were predated up to a maximum of two days after laying. The predators identified were the monitor lizards (*Varanus niloticus*, 100% of preyed nests) and ghost crabs (*Ocypode cursor*, 7.1% of preyed nests). Additionally, 36.7% of the nests were subject to flooding. In Poilão there were no signs of clutch predation, and only about 4.2% of the monitored clutches suffered 100% mortality due to flooding. Unlike in Meio Island, hatching success in Poilão was quite high (77.8%  $\pm$  30.9 SD). We demonstrate here the urgent need to implement management strategies to protect green turtle nests at Meio Island, namely through clutch relocations and using strategies to deter predators. Although this island does not host the majority of the nesting, future changes at Poilão Island may increase the importance of Meio. For example, the high nest density at Poilão is leading to a decrease in hatching success due to clutch destruction by nesting females. Sustaining healthy nesting sites nearby is crucial for the persistence of this significant population.

## POPULATION GROWTH AND DYNAMICS IN ASSISTED COLONISATIONS OF GREEN SEA TURTLES IN THE CAYMAN ISLANDS\*

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Due to changing environmental conditions, many species will have to migrate or occupy new suitable areas to avoid potential extinction. Long-lived animals are especially vulnerable, and ex-situ conservation actions can provide solutions through assisted colonisations. However, there is little empirical evidence on the process of founding new populations for such species or on the feasibility of using assisted colonisations as a conservation measure. Monitoring and evaluation of assisted colonisation is crucial, as newly founded populations can suffer reductions of genetic variability due to the founder effect and reintroduced individuals can display reduced reproductive success. Here we combined genetics with reproductive data to study the rise of two wild populations of green turtles (*Chelonia mydas*) in the Cayman Islands as a possible outcome of a reintroduction program. This reintroduction was initiated in 1983 by the Cayman Turtle Farm (CTF) and was based on releasing captive-bred green turtles from the island of Grand Cayman, where the CTF is located. We genotyped hatchlings from 320 wild nests laid in the islands of Grand Cayman and Little Cayman at microsatellite loci and mtDNA markers, and compared them to 57 wild and 257 CTF captive female breeders genotyped in a previous study. We show that both populations are highly related to the captive population, with 88.1% of the nests related to the CTF. However, significant genetic differentiation was found between nests from the two islands, at the nuclear level and with shifts in haplotype frequency at the mitochondrial level. This indicates a rapid divergence, probably caused by the founder effect. The minimum genetically estimated breeding population size for the two islands was 121 females and 114 males, indicating unbiased sex-ratio. Individuals from the reintroduced populations showed high levels of nest fidelity, within and across nesting seasons, indicating that philopatry may help reinforce the success of new populations. According to hatchling genetic relatedness values, more than 60% of nests laid by the same female occurred within less than 1 km of each other. These results show that females in the Cayman Islands have a high degree of nest site fidelity despite coming from a reintroduction program. Additionally, to test the potential effect of the assisted colonisation on individuals' fitness (viability, fertilization success, clutch size and hatchling heterozygosity) we ran Linear Mixed Effects Models with particular focus towards individual heterozygosity, and relatedness to the CTF breeders. We found that larger females lay a significantly higher number of eggs per clutch, nests with larger clutches showed higher fertilization success, and nests with higher fertilization success showed higher viability. Female and hatchling heterozygosity and CTF relatedness had no significant effect on the fertilization success or viability, suggesting that individuals coming from the CTF program are not affecting the fitness of the new population in the short term. Sea turtle reintroduction programs can, therefore, establish new populations but require scientific evaluation of costs and benefits and should be monitored over time to ensure viability in the long term.

**METHODOLOGICAL STRATEGIES TO MINIMIZE IMPACT AND BIAS OF ORIENTATION EXPERIMENTS IN HATCHLING LEATHERBACK (*DERMOCHELYS CORIACEA*), GREEN (*CHELONIA MYDAS*), AND HAWKSBILL (*ERETMOCHELYS IMBRICATA*) SEA TURTLES**

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Orientation experiments on hatchlings as they orient from the nest to the water have been used to examine the response of sea turtles to a variety of environmental cues. In our orientation experiment examining hatchling response to sound and light cues present in the beach environment, we set out to develop methodological practices both minimally impacted behaviors post-release and produced actions within the testing arena that reflected innate responses. We examined the effects of habitation and holding time on hatchling movements within our testing arena, a circular platform surrounded by 36 converging bins within a light-proof tent. We placed a single hatchling in the middle of the arena and waited 5 minutes for it to crawl into one of the surrounding bins. Groups of hatchlings were subjected to 4 minute, 2 minute, and 0 minute habituation periods where they were able to explore the arena before the experimental treatments. In trials testing leatherback hatchlings to beach wave sounds, 4 minutes, 2 minutes and zero minutes of habituation did not result in a change in the final trial time or location of the turtles tested (time:  $p=0.808$ , location:  $p=0.40$ ). Similarly, trials testing leatherback hatchlings to simulated moonlight (4000K,  $>1$  lumens) with 4, 2 or zero minutes of habituation did not result in a change in the final trial time or location of the turtles tested (time:  $p=0.098$ , location:  $p=0.35$ ). To minimize our impact upon the sea turtles in our study, habituation was not tested on greens or hawksbills after it was determined to have no effect. Holding time, or the time between when the hatchling emerged from the nest and when they were tested within the arena, was examined for its effects on trial time or the time that it took for the hatchlings to move within the arena to an outer bin location. There was a weak positive relation between holding time and trial time for leatherback, green and hawksbills when examined together (Pearson  $r=0.089$ ,  $p=0.05$ ,  $n=484$ ). A weak positive relationship existed between holding time and trial time for each species individually (Leatherback: Pearson  $r=0.233$ ,  $p=0.014$ ,  $n=121$ ; Green: Pearson  $r=0.131$ ,  $p=0.064$ ,  $n=201$ ; Hawksbill: Pearson  $r=0.251$ ,  $p=0.001$ ,  $n=162$ ). We note that a stronger relationship was likely not found as we terminated the experiment for the night after several successive failures or trials in which hatchlings did not move to an outer bin within the allotted trial time. When successive failures occurred, we observed hatchlings began to display slower and more infrequent movements. We chose to release them while they were still active rather than persist in our experiment. The premise of our methods reflects the flexible actions necessary when working with live animals. Additional research is needed to further investigate the impact of holding time on hatchling experiments, especially over greater periods of time.

**USING IN SITU INCUBATION TIME TO VALIDATE MODELLING OF EMBRYO DEVELOPMENT DURING VARIABLE TEMPERATURES EXPERIENCED IN NATURAL NESTS\***

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The rate of development of sea turtle embryos is dependent on incubation temperature, higher temperatures cause faster development that result in shorter incubation times as indicated by laboratory experiments where eggs are incubated at constant temperatures. But incubation temperature is not constant in natural nests. In shallow nesting species, where nest depth is 20-30 cm, incubation temperature typically fluctuates on a daily basis, and in all sea turtle species incubation temperature typically increases towards the end of incubation because the clutch of growing embryos generates significant amounts of metabolic heat. To account for this variation in incubation temperature, researchers have used models based on data from constant incubation experiments to predict embryo development rates in natural nests. However, two problems arrive when formulating and validating such models, (1) sea turtle embryos fail to survive constant incubation temperatures above 33°C because early-stage embryos are killed at these temperatures, but in nature, late-stage embryos can continue to develop and hatch at temperatures between 33°C and 36°C, and (2) it is difficult to determine the exact time of hatching in eggs that are buried 30-80 cm below the ground surface in natural nests. Here, we use a novel 'hatching detector' to determine the time of hatching in beach nests and temperature data loggers placed in these nests to generate hourly temperature traces throughout incubation. We formulated predictive development rate models from constant temperature incubation data and then used these models along with temperature traces to predict hatching time in our beach nests. We then compared predicted hatching times with actual hatching determined by the hatching detector. We found that all of the models over-estimated the incubation period (defined as the time between egg laying and the time of egg hatching) compared to empirically measured incubation periods. At least part of these differences may be due to the assumptions made by the models on what happens to the rate of embryonic development when nest temperatures are greater than 33°C, temperatures where there is a lack of empirical data from constant temperature incubation experiments. Once reliable data on development rate at temperatures above 33°C is obtained these models could be refined and used in conjunction with real-time temperature data loggers to predict when the sex determining period begins and ends and hatching occurs in natural nests.

## SHADE MANAGEMENT: AN ALTERNATIVE TO MITIGATE THE EFFECT OF HIGH TEMPERATURES ON SEA TURTLE NESTS IN CUBA

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Vegetation has been shown to influence nest environment (temperature, humidity) affecting hatchling's development as well as emergence success and has also been used as a cue during the selection of a nesting site. For green turtles, *Chelonia mydas*, nest density is typically higher in the vegetated zones. Considering the effects of climate change on nesting beaches, the potential use of vegetation to reduce high temperature might constitute an alternative of great importance. Therefore, the present work evaluated the effect of the vegetation shade in the incubation period, hatching success and the incubation temperature in green turtle nests. The study was carried out between 2015 and 2019 at Antonio and La Barca beaches of the Guanahacabibes peninsula. The degree of shade significantly affected the three indicators evaluated. Regarding the incubation period (Kruskal-Wallis,  $H_{Antonio (14,67)} = 33,08$ ;  $p=0,03$  and K-W,  $H_{Barca (14,197)} = 27,39$ ;  $p=0,02$ ) and hatching success (K-W,  $H_{Barca (14,230)} = 44,42$ ;  $p<0,01$ ), the highest value of the median corresponded to the nests with total shade. On the other hand, the incubation temperature (K-W,  $H_{Barca (2,41)} = 12,97$ ;  $p=0,001$ ) presented the highest values in nests without shade. These results indicate that the use of vegetation as a low-cost alternative for shade management to reduce the impacts of climate change is possible. Although roots could affect embryos development and hatchlings emergence, the higher hatching success in nest with total shade implies that vegetation in the study site does not affect hatchlings emergence or development.

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## INCUBATION TEMPERATURES, HATCHING SUCCESS, EMERGENCE SUCCESS AND CONGENITAL ANOMALIES IN GREEN TURTLE NESTS FROM GUANAHACABIBES PENINSULA, CUBA

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Elevated incubation temperatures of sea turtle nests decrease hatching success and alter the resulting hatchlings' morphology. There is an absence of studies assessing the relationships between temperature and hatching success in Cuba, even when they could improve understanding the limits of thermal tolerance in these species. This study evaluated the influence of incubation temperature on hatching success and phenotypic malformations in green turtle hatchlings (*Chelonia mydas*); and analyzed the temporal variation in hatching and emergence success on the studied beaches. In 48 green turtle nests distributed along two beaches, incubation temperature and hatching success were recorded between 2014 and 2019. Increasing incubation temperature caused a decrease in the hatching success and an increase in the frequency of

supernumerary scutes. Despite the elevated temperatures (average > 30°C), hatching was higher than 80%. Significant differences in hatching success were only observed among seasons for nests in Antonio Beach (lower values in 2016 and 2019 compared to 2014). Hatching and emergence success was greater than 80% on both beaches.

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## THE PHYSIOLOGICAL EFFECTS OF SARGASSUM BEACH COVERAGE ON THREE SPECIES OF SEA TURTLE HATCHLINGS

**Abigail Marie Chaney and Sarah Milton**

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Sea turtle hatchlings face a variety of obstacles and threats as they crawl down the beach from the nest to the ocean. One of these obstacles is *Sargassum*, a floating brown macroalgae, that has been washing up in larger quantities on beaches from Florida to South America over the past decade. This study examined the physiological response (plasma glucose and corticosterone) and performance of three species of sea turtle hatchlings (*D. coriacea*, *C. caretta*, and *C. mydas*) as they crawled down a 10-meter runway on the beach and climbed over various heights of *Sargassum* for the final 2 meters. Treatment groups consisted of a control where hatchlings did not crawl, a crawl control where they crawled only on sand, a light treatment of *Sargassum* 7-9 cm in height, and a heavy treatment of *Sargassum* 17-19 cm. A blood sample was taken immediately after hatchlings reached the end of the crawl pathway. The light *Sargassum* treatment resulted in a 37.1%-115.7% increase in total time when compared to the control and the heavy *Sargassum* resulted in a 204.1-298.5% increase. In all three species, the heavy *Sargassum* treatment significantly increased the amount of time it took to crawl the entire length of the pathway. The number of inversions also significantly increased with the addition of *Sargassum*. There was no significant difference in blood glucose levels between treatments and further blood analysis will include examining corticosterone levels from blood plasma. During times when *Sargassum* accumulation on the beach is high, hatchlings will spend more time on the beach trying to navigate through the algae, leaving them vulnerable to predation for longer periods of time. However, *Sargassum* contributes to a healthy beach ecosystem, so many factors need to be considered when making management decisions about beach raking and other methods of *Sargassum* removal.

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## THE INFLUENCE OF NEST SITE SELECTION ON THE HATCHING SUCCESS OF GREEN TURTLE (CHELONIA MYDAS) NESTS LAID AT PLAYA TRES, CARIBBEAN OF COSTA RICA

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Hatching success is an important parameter of the sea turtle reproductive output and is crucial for survival of species. Sea turtle embryo development is regulated by environmental factor such as temperature and humidity, which influence hatching success. Metabolic heat and external factors, such as position of the egg chamber on the nesting beach in relation to the sea influence internal nest temperature and humidity.



In this study we tested whether hatching success is affected by how close green turtle (*Chelonia mydas*) nests are from the sea. As a part of Turtle Love's (<https://linktr.ee/turtlelovecr>) monitoring of sea turtle nesting activity at Playa Tres, on the Caribbean coast of Costa Rica, we marked and followed the fate of 95 green turtle nests from July through October 2021. However, nine of these nests were flooded, 31 were poached and 3 had not yet hatched by the end of monitoring. Thus, 52 nests, 25 relocated and 27 left *in situ*, were analyzed to test effects of distance from the tide line on green turtle hatching success. We measured distance from the nest to the tide line at days 1 and 20 of the incubation period and at excavation day. For each measure the tidal coefficient and relative water height in the port of Limón was reported and used as a covariate in the analyses. Nests were excavated to assess percentual hatching success, which was calculated as follows: Seventy five percent of studied nests were laid at an intermediate distance of the sea between 20 and 40m. There was no significant difference between the hatching success of relocated and *in situ* nests, which means Turtle Love relocation techniques are being effective. Hatching success ranged from 36 and 100% with an average of 86.6% ( $\pm 15.2$ ). Though we found hatching success slightly decreased and incubation periods slightly increases with the distance from the tideline, presumably due to drier incubation conditions, these tendencies were non-significant. Humidity is only one of many variables acting on green turtle embryo development and influencing hatching success. We concluded our sampling methodology did not capture the variability in the data, and we suggest further studies aiming to elucidate effects of nest site selection on sea turtle hatching success should measure temperature and humidity inside the nests.

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#### COMPARISON OF BEACH HATCHERY AND BOX NURSERY METHODS EFFECT ON HATCHLINGS AT AN OLIVE RIDLEY BEACH: HATCHING SUCCESS, PHYSICAL FITNESS, MORPHOMETRICS AND CONGENITAL MALFORMATIONS

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At the Litibu and Sayulita Sea Turtle Conservation Camps, Nayarit, Mexico, conservationists relocate all sea turtle nests found on their beaches to an enclosed beach hatchery or polystyrene boxes (used when beach conditions are unsuitable) to protect them from illegal egg collection, predation, beach erosion, and high sand temperatures. Between July and August 2021, we collected nest viability and hatchling quality parameters including hatching success, nest temperatures, incubation duration, and hatchling quality (locomotor performance, morphometrics, weight, and congenital malformations) for olive ridley turtles (*Lepidochelys olivacea*). We will present results from the comparative analysis between the beach nursery and polystyrene boxes (n=40). We will also present novel methods for collecting and calibrating sand moisture data using a readily available food dehydrator. Studies such as this are critical for conservation of sea turtles because the methods we tested are used worldwide and their effects on the quality of hatchlings may contribute to their ability to survive and reproduce in the future.

## **STOCK-WIDE ASSESSMENT OF COASTAL VULNERABILITY AT FLATBACK NESTING SITES IN THE PILBARA REGION OF WESTERN AUSTRALIA\***

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Sandy beaches are essential nesting habitats for sea turtles but their persistence and stability are threatened by rising sea levels and increases in the frequency and severity of storms, driven by climate change. Identifying which nesting beaches are at greatest risk from climate change is an important goal of sea turtle conservation globally. To date, efforts to identify at-risk beaches have been hindered by the ability to model complex processes and incomplete information on nesting distribution and abundance. This study explores the risk of beach erosion and inundation at sites utilised for nesting by flatback turtles (*Natator depressus*) in the Pilbara region of Western Australia, using the InVEST (Integrated Valuation of Ecosystem Services and Trade-offs) Coastal Vulnerability Model. A coastal exposure index was calculated for mainland and island shorelines in the Pilbara in terms of six bio-geophysical variables: (1) empirical wind- and (2) empirical wave- hindcast data to capture the effects of storms, (3) distance from the continental shelf to estimate storm surge potential, (4) coastal geomorphology which influences landscape stability, (5) coastal elevation, and (6) observed changes in sea level rise. Exposure was then coupled with published information on the distribution and abundance of *N. depressus* nesting activity, resulting in an exposure index for 402 sandy beaches, spanning more than 600 km of coastline. The majority of beaches (~70%) had an intermediate to high exposure index. In particular, 34% of beaches with the highest abundance of *N. depressus* nesting activity had the highest exposure. This suggests that coastal exposure is a key vulnerability for *N. depressus* reproductive success in this region. Promisingly, five beaches with the highest abundance of nesting activity also had the lowest exposure and these beaches may be critical for the long-term natural resilience of the stock against coastal exposure. Exposure varies across spatial scales and the approach presented here allows for a rapid and broadscale assessment of exposure and inundation risks at a scale most relevant to management.

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## **NEAR-SHORE MORTALITY'S ROLE IN SEA TURTLE HATCHLING EVOLUTION\***

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Sea turtle hatchlings experience high mortality during the first hour of entering the sea. This study aimed to determine if this form of intense natural selection has an evolutionary impact on body morphology or swim speed, using a novel method involving fitness landscapes, a tool from computational biology and

evolutionary computation. We used tracking and morphological data of leatherback turtle (*Dermochelys coriacea*) hatchlings on the Atlantic coast of Costa Rica from Hoover et al. (2016; n=34) and Barbour et al. (2018; n=34) and generated metrics of “simulated fitness” (probabilities of survival, independent of the actual survival status of the individual) for each hatchling under the hypothesis that spending time closer to shore decreases an individual’s probability of survival. Tracks that stayed close to shore were penalized with low fitness values and tracks that departed near-shore areas were rewarded with high fitness values. Using this metric and the morphological data, we constructed a corresponding fitness landscape and compared this with a phenotypic distribution ‘landscape’. These landscapes differed, so the metric of fitness (which prioritized escaping near-shore areas) was considered to not be defined by true or strong evolutionary pressures, since the frequency of a given phenotype should correspond to the fitness of that phenotype. 3D fitness and distribution landscapes revealed that first-hour mortality does not have a strong evolutionary impact on phenotype for sea turtle hatchlings, evaluated under the assumption that the mortality rate decays with respect to distance from shore. This indicates that random chance may play a strong role in survival during the first hour of sea turtle hatchlings’ lives after emergence, given that mortality rates tend to be very high during this period. Since our empirically-generated fitness landscapes differed from the phenotypic distribution landscapes, we rejected our hypothesis that near-shore mortality contributes to the evolution of sea turtle hatchling morphology via natural selection in this region. This insight may be consequential for conservation actions designed to increase juvenile recruitment (e.g. ex-situ conservation measures) in endangered, threatened, and internationally protected sea turtle species, since it indicates that artificially bolstering recruitment will not decrease the overall fitness of the new generation.

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## **RESILIENCE: NEST SUCCESS AFTER MID-INCUBATION DISTURBANCES**

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Incubating sea turtle nests are subject to invasive mid-incubation disturbances globally, including depredation, poaching and, tide/storm inundation. Such disturbances lead not only to the complete loss of nests, but also to the partial loss of nests or the need for emergency relocation outside of the recommended 12-hour post oviposition time frame. These disturbances leave managers with the decision of whether or not to continue monitoring and protecting these nests. Tide induced emergency relocations and coyote depredation are trending mid-incubation disruptions on Bald Head Island, North Carolina (BHI). Over the 2019, ‘20 & ‘21 sea turtle nesting seasons on BHI, the remaining eggs of 45 (12%) partially depredated nests produced 2,353 live hatchlings, 75% hatch success and 70% emerge success. Another 9 nests relocated after the recommended 12-hour post oviposition time frame produced 760 live hatchlings, 70% hatch success and 63% emerge success. Conducting the emergency relocations and cleaning and reburying partially depredated nests resulted in a 14.7% net increase in live hatchling production. Our results provide evidence that sea turtle nests are resilient enough to survive invasive mid-incubation disturbances. We hope this data will aid managers in their decision to continue monitoring and collecting data from nests that are disturbed mid-incubation. These results also provide an argument in favor of the relocation of imperiled nests in emergency situations after the recommended relocation time frame.

**MOONLIGHT SERENADE: THE ROLE OF SECONDARY ACOUSTIC CUES IN SEA-FINDING BY GREEN (*CHELONIA MYDAS*), HAWKSBILL (*ERETMOCHELYS IMBRICATA*), AND LEATHERBACK (*DERMOCHELYS CORIACEA*) SEA TURTLES\***

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Light and slope cues utilized by hatchling sea turtles during sea-finding have been well documented; however, the effect naturally occurring acoustic cues may contribute as an intervening factor have not been well explored. Hatchling leatherback (*Dermochelys coriacea*), green (*Chelonia mydas*), and hawksbill (*Eretmochelys imbricata*) sea turtles can detect aerial acoustic sounds between 50 and 1600 Hz and are most sensitive to sounds between 50 and 400 Hz. The highest sound energy of beach waves occurs at <1000 Hz which overlaps with their most sensitive hearing range. Beach wave sounds may serve as a secondary orientation cue for hatchlings. We measured the behavioral responses of hatchling leatherback, green and hawksbill sea turtles collected from nesting beaches within the St. Croix Sandy Point National Wildlife Refuge in the presence of recorded beach surf sounds (72.0 dB re: 20  $\mu$ Pa), along with simulated moonlight (4000K, >1 lumens). The speaker producing the recorded trial sound was located inside an enclosed light-proof tent and rotated corresponding to north, south, east and west compass bearings. The moonlight stimuli cues were oriented in three positions corresponding to the speaker: together at the same position (0°), at opposite positions (180°), and at 90° angles to each other. Hatchlings were placed in the middle of the tent on a level circular platform surrounded by 36 converging bins of equal length. The maximum trial time observed for each hatchling was 5 minutes or the lesser time it took for the hatchling to orient into one of the surrounding bins. We compared the location of the hatchling's destination bin to the stimuli location to determine whether the turtle oriented towards or away from the sound and light sources. When beach wave sounds were presented alone, leatherbacks ( $p=0.096$ ), greens ( $p=0.306$ ), and hawksbills ( $p=0.076$ ) oriented randomly in the arena. Leatherbacks and greens oriented toward the light source when moonlight was presented alone ( $p<0.001$ ), and when moonlight was located at both the same (0°,  $p<0.001$ ), opposite (180°,  $p<0.001$ ), or 90° locations to the speaker. Hawksbills did not exhibit a significant orientation toward moonlight when presented at the same (0°,  $p=0.175$ ), opposite (180°,  $p=0.927$ ), or 90° locations to the speaker; however, when moonlight was presented alone, they oriented toward the light ( $p=0.002$ ). These results suggest that beach waves sounds do not have an interactive orientation effect during sea-finding for leatherback and green hatchlings. Additional research is needed to further examine the significance of beach waves sounds as a secondary orientation cue for hawksbill hatchlings.

## **SPATIO-TEMPORAL DIVERSITY OF NESTING BEACH ENVIRONMENT AND TEMPERATURE IN THE OGASAWARA GREEN TURTLE ROOKERY: IMPLICATIONS FOR HATCHLING SEX RATIOS**

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There are concerns over the sex ratios of sea turtles which have temperature dependent sex-determination due to global warming. Higher incubation temperature produces more females, and the skewed sex ratio may result in unsuccessful reproduction in the future. Therefore, it is important to understand the sex ratio of hatchlings in the population to establish appropriate management strategies. Importantly, incubation temperatures vary between and within nesting beaches due to various environmental conditions such as presence or absence of shade or vegetation, the color, grain size and albedo of the sand, and rainfall. Thus, investigating environmental diversity and spatio-temporal variations of sand temperature at nesting beaches are essential for better understanding of hatchling sex ratio in the population. The Ogasawara archipelago is the largest rookery of green sea turtles in western North Pacific, which consists of 50 small nesting beaches. However, little is known on the variation in incubation conditions at each nesting site and how these may contribute to produce different sex ratio at each location. In this study, major nesting beaches were selected within Chichi-jima, the largest island in Ogasawara, to study environmental diversity and estimate hatchling sex ratio. Each beach was spatially divided into two sections comprising nesting sites with and without vegetation, and their sand color and grain size were analyzed. Nest depth was recorded and sand temperature was measured at relevant depths (40, 60 and 80 cm) throughout the nesting season in representative areas using temperature data loggers. The sex ratios theoretically produced in each area were estimated using the logged thermal data and a tentative equation developed in previous studies (unpublished results). Nesting beach condition in the Chichi-jima rookery was found to be diverse, consequently resulting in marked sand temperature variation between beaches and even between sections of the same beach. Nest depth ranged from 30 cm to 70cm, and the nests in the vegetation area tended to be shallower. A maximum of 1.2°C difference was recorded depending on the depth. The sex ratios estimated from the sand temperature profiles differed spatio-temporally, producing both male- and female-biased nests. In summary, we found that the Ogasawara archipelago has high variation in environmental conditions between and within beaches. This high environmental diversity induces a wide range of sand temperatures, which theoretically results in a variety of sex ratios among hatchlings born across this rookery. Further studies are needed to corroborate these findings, such as by performing histological or hormonal analyzes to confirm the accuracy of sex ratio estimations based on sand temperature. Moreover, it is also necessary to discuss future operational sex ratio of the sea turtles by investigating the sex differences of biological features such as survivability and ages at sexual maturity.

## POPULATION GENOMICS OF THE LOGGERHEAD TURTLE COLONISATION IN THE WESTERN MEDITERRANEAN

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In recent decades, it has become clear that the planet is facing a biodiversity crisis. The loggerhead turtle (*Caretta caretta*) is particularly sensitive to climate change, due to temperature sex determination, hatchling mortality at high temperatures and the potential constraints to colonize new suitable areas due to philopatry. Despite these limitations, in the last decade this species has begun nesting with increasing numbers in the Western Mediterranean, which up to recent times presented unsuitable low temperatures. These growing nesting events might be the starting point for new stable populations, allowing the survival of the species despite global warming. As we are observing the initial steps of the colonisation process of this long-lived species, the understanding of its progress is fundamental for a scientifically based management. To study this unique process, we applied genomic approaches using a 2bRAD protocol optimized for the species by our research group. We genotyped between 2 and 10 individuals from 18 nesting events from 2016 to 2020 happened in the Mediterranean coast of Spain, for a total of 96 hatchlings. By performing pedigree analyses with the hatchlings genotypes we inferred the minimum number of breeders involved in these nesting events, discuss the frequency of multiple paternity, and the relatedness among nests due to female and male breeding mating behaviour. We identified the populations of origin of the colonising individuals, by comparing the hatchlings genotypes with previous data obtained from stable nesting populations using the same methodology. Our results show how population genomics is useful for investigation in biodiversity conservation. Moreover, this study has the potential to set the baseline for understanding the evolutionary consequences of the colonization in-action for a long-lived species. For this reason, we formulate scientifically based recommendations and strategies, by means of population genomics for biodiversity conservation to aid the viability and permanence of the loggerhead turtle (*Caretta caretta*) in the Western Mediterranean.

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## HATCHING SUCCESS AND PRIMARY SEX RATIOS: CURRENT STATUS AND A 30-YEAR PERSPECTIVE FOR A HAWKSBILL ROOKERY IN ANTIGUA, WEST INDIES\*

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Warming temperatures are predicted to drive increases in embryonic mortality and sex ratio imbalance in sea turtles. While much focus on this issue has been placed on long-term projections for the future, we would benefit from a better understanding of the current status of nesting populations. Here, we describe the results of a project using temperature data loggers to quantify relationships among incubation temperatures, hatching success, and estimated primary sex ratios for hawksbills (*Eretmochelys imbricata*)



nesting at Jumby Bay, Antigua. We then place our results in the context of over three decades of previous work at this location. Our findings suggest consistent hatching success and sex ratios over 30 years of nesting beach monitoring, coinciding with relatively stable atmospheric temperatures during annual nesting seasons. While we consider relatively high hatching success rates (75-80%) to be a positive trend, the possible effects of sustained female bias in primary sex ratios (estimated > 90%) merit further investigation. For example, inbreeding effects may represent a cause for concern. Our study highlights the utility of leveraging long-term monitoring datasets to reveal the contemporary status of populations amidst prolonged climate trends.

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## LONG-TERM CHANGES IN ADULT SIZE OF GREEN TURTLES AT ALDABRA ATOLL ACROSS 35 YEARS AND SEXUAL DIMORPHISM IN THE SOUTHERN SEYCHELLES\*

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Long-term changes in the carapace length and width of adult female green turtles (*Chelonia mydas*) at Aldabra Atoll, a UNESCO World Heritage Site in the Seychelles, are reported based on >4,600 measurements of individuals between 1982 and 2016. Over this 35-year period, both the mean length and width of turtles decreased significantly. Curved carapace length decreased by about 0.64 cm per decade, with the mean length of females decreasing from 112.2 cm in 1983 to 110.1 cm in 2016. Based on length versus weight measurements, the estimated decrease in the mean weight of adult female turtles between 1982 and 2016 was from 151.8 kg to 144.0 kg, a decrease of about 2.3 kg per decade. The decrease in female mean size has accompanied an increase in the number of turtles nesting annually. Based on 391 repeat measurements of the carapace length of individual nesting Aldabra females taken between 2.8 and 19 years apart, their mean growth rate was 0.14 cm. year<sup>-1</sup>. Adult females in southern Seychelles were larger than males, with the sexual dimorphism index (SDI), i.e., the ratio of size of larger sex to the smaller, being 1.09 and 1.10 for carapace length and width respectively, and 1.25 for weight. Smaller females tended to lay fewer eggs per clutch. An influx of smaller, first-time nesters is discussed as a potential reason for the decrease in the mean size of adults along with the possible reduction of growth rates and mean size at maturity that may be related to environmental and climatic factors.

## USING STABLE ISOTOPES AND SATELLITE TRACKING ON POST-NESTING GREEN TURTLES IN GUAM TO IDENTIFY FORAGING HOTSPOTS IN THE WESTERN PACIFIC

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Endangered green turtles (*Chelonia mydas*) from the Central West Pacific (CWP) distinct population segment (DPS) are susceptible to natural and anthropogenic threats found between and within foraging and reproductive habitats, making it important to protect these areas and migration routes. Resolving nesting green turtle foraging hotspots will be the first step towards identifying and addressing threats in these essential habitats. Foraging hotspots for nesting green turtles in Guam are unknown. The first aim of this project is to use satellite telemetry to identify the foraging areas used by Guam's post-nesting green turtles. We hypothesize that they will forage in the Western Pacific. Second, we aim to determine if stable isotopes can be used to identify and characterize key foraging areas that will be validated with satellite tracking. We hypothesize that stable isotopes can provide sufficient resolution to assign Guam's post-nesting green turtles to foraging hotspots linked to the coasts of Eastern Asia and islands in the CWP. From 2018 to 2021, we collected skin samples from 31 nesting females across six nesting beaches throughout Guam. Of these nesting females, satellite tags were attached to seven turtles. Bulk  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of skin tissue from tracked and non-tracked turtles were determined to compare potential differences in isotopic values between foraging areas. Satellite telemetry data was used to estimate the isotopic values characteristic of specific foraging areas. Tracking data demonstrated that post-nesting green turtles migrate to the Western Pacific to forage in varying Philippine coasts, Japan, and islands in the South China Sea. Isotopic values fall into three groups however these groups did not correspond to the three different tracked locations. Despite very large ranges in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , isotopic values from all three groups were tracked to the Philippines. Additionally, two similar isotopic values within the same group had turtles that were tracked to two different locations - Okinawa, Japan and Manamoc Island, Philippines. Principal component analysis (PCA) suggests that the isotopic values linked to these foraging areas are not distinct enough to assign non-tracked turtles to their foraging areas. These findings represent preliminary steps to evaluate whether stable isotopes can provide sufficient resolution to assign nesting green turtles to their foraging areas in the Western Pacific. If so, SIA could represent a cost-effective alternative to satellite telemetry that can facilitate larger sample sizes. We will expand our research to find alternative ways to characterize  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in the different geographic areas to further evaluate our approach.

## **DECREASED VIABILITY OF GREEN TURTLE (*CHELONIA MYDAS*) NESTS DUE TO SUN EXPOSURE AT PLAYA TRES, CARIBBEAN COAST OF COSTA RICA**

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A rapidly changing climate impacts sea turtle populations worldwide (eg.: nesting patterns, A rapidly changing climate impacts sea turtle populations worldwide (eg.: nesting patterns, decline in nest viability due to flooding, disease transmission, altered migratory behavior, skewed sex ratios, etc.). This highlights the importance of understanding parameters affecting sea turtle nest incubation and the production and survival of hatchlings. In this study, the effects of sun exposure on the nests of green turtles (*Chelonia mydas*) laid at Playa Tres, on the Caribbean coast of Costa Rica, were evaluated by assessing the relationship between the vertical position of the nests at the beach and the fitness of the hatchlings produced. Nests were classified in three categories according to the amount of sun exposure received: open beach (sunny all day), border (partially shaded during the day) or vegetation (shaded all day). Nests were monitored throughout the 2020 incubation period and exhumated after hatching evidence was found or after 70 days had elapsed since oviposition. Hatchlings were subjected to a fitness test, which consisted of measuring straight carapace length and width and body mass (biometric traits) and assessing locomotor capacity (behavioral trait). Data were obtained from 183 hatchlings belonging to 23 nests. Incubation period as well as hatchling biometric traits decreased in nests that were exposed to the sun (open beach), and there was a decrease in the hatching success of nests laid in this zone. This may be due to higher temperatures leading to a faster incubation and smaller rate of conversion of yolk into body tissue. An analysis of principal components shows that longer, wider and heavier hatchlings were also those with the best locomotor capacity. However, biometric and behavioral traits of hatchlings produced in the vegetation zone (shaded) present greater variation than at the other two zones. Thus, open beach and border zones result in hatchlings with more consistent traits. An increase in temperature during the incubation period will not only affect the hatching success but also the fitness of hatchlings.

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## **GEOGRAPHIC DISTRIBUTION AND INFLUENCE OF RIVER MOUTHS ON THE NESTING ACTIVITY OF LEATHERBACK (*DERMOCHELYS CORIACEA*) AND GREEN TURTLES (*CHELONIA MYDAS*) AT PLAYA TRES, NORTH CARIBBEAN OF COSTA RICA**

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Tortuguero is the most important nesting beach for green turtles (*Chelonia mydas*) in the Atlantic basin, whereas Bocas del Toro in northern Panama is the most important leatherback (*Dermochelys coriacea*) nesting beach in the Central America isthmus. Turtle Love is a nonprofit conservation organization that runs a community-based conservation project working to protect sea turtles nesting at Playa Tres, located

south of Tortuguero National Park (TNP) on the Caribbean coast of Costa Rica. Playa Tres is limited by the Jalova (north) and Parismina river mouths (south). The Parismina (or Reventazón) river is the second largest in Costa Rica and its movements influence the geomorphology of Playa Tres and, consequently, the selection of nesting site by female sea turtles. In this study, we aim to elucidate the influences of river mouth on selection of nest sites by green and leatherback turtles at Playa Tres. Understanding the geographic distribution of nesting activity per species would allow Turtle Love to use human resources more efficiently to protect sea turtles. We divided the beach into six 1-km sectors (0-5) which were marked in crescent order from north to south. Furthermore, from 2019 to 2021, we quantified nesting activity at each beach sector by counting sea turtle nests and false crawls per sector. We analyzed the variation in green and leatherback turtle nesting activity per sector Playa Tres. Overall, sea turtle nesting activity in Playa Tres is mainly concentrated on the northernmost half (sectors 1, 2, and 3) of the nesting beach, and nesting activity is significantly smaller close to river mouths (sectors 0 and 5). At the river mouths, beaches are more volatile and sea turtle nests laid in those regions are more prone to being lost. Thus, sea turtles may avoid nesting in these areas. Leatherback turtle nesting activity was more uniformly distributed at Playa Tres, which may be due to leatherbacks having higher motility during interesting intervals than green turtles. For green turtles, which present a high degree of fidelity to nesting sites, nesting activity was significantly higher on the northernmost portion of Playa Tres (closer to Tortuguero). Even though sectors 0 and 5 are located at similar distances from the river mouths at Playa Tres, sector 1 had significantly higher nesting activity than sector 4- 5, which may be due to its proximity to Tortuguero. Alternatively, it could be due to rivers of different magnitude have varying effects on nesting beaches. Thus, because Reventazón River is larger, a larger portion of the nesting beach may be under its influence.

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## STRATEGIC NEST SITE SELECTION IN ONE OF THE WORLD'S LARGEST LOGGERHEAD TURTLE NESTING COLONIES, MAIO ISLAND, CABO VERDE\*

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We conducted an eight-year (2012-2019) capture-mark-recapture study on the re-nesting behaviour of loggerhead turtles (*Caretta caretta*), to identify both inter- and intra-beach nest site selection patterns. Our study site, Maio Island in the archipelago of Cabo Verde, hosts one of the five world's largest loggerhead turtle nesting colonies. Out of 1,060 females analysed, 77% laid repeated clutches over less than 15 km both between and within nesting seasons. This site faithfulness was particularly high (64-71%) for turtles nesting on the east coast of Maio. In two areas (northwest and southeast), individual nesting zone consistency was extremely low (10-25%). Interestingly, in all cases, extra-zone re-nesting events were mainly directed towards the east coast. We also found that females avoided re-nesting near the shoreline, a particularly important feature especially in the face of rising sea levels. Overall, loggerhead turtles nesting in Maio are philopatric but are using a bet-edging strategy to distribute nests among several beaches, choosing the safest area within each beach to maximize their reproductive success. This study also reveals the priority sites for protection on Maio Island and can help optimizing capture-mark-recapture programmes and evaluating the potential for an adaptive response to projected sea level rises.

## HOW DO INCUBATION SUBSTRATES INFLUENCE HATCHING SUCCESS AND HATCHLING PHENOTYPE? A FIELD AND EXPERIMENTAL STUDY ON LOGGERHEAD SEA TURTLE EGGS

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We conducted a five-year field (2017-2021) and laboratory study of the relationship between type of substrate and hatching success, embryonic development, and the quality of hatchlings in loggerhead turtle nests. Our study site, the island of Maio in the archipelago of Cabo Verde, one of the world's largest loggerhead turtle nesting colonies, displays marked heterogeneity of sand coloration, with dark, mixed, and light sandy beaches. We experimentally incubated eggs, comparing different nesting substrates under standard temperature and humidity conditions. Females nest in all sand types without preference. However, both the field and experimental study revealed a significant difference in hatching success depending on the type of substrate. Substrate of volcanic origin, dark in colour, with a lower amount of calcium carbonate, had an average hatching success (HS) of  $30.3 \pm 20.2\%$ , producing higher and earlier embryo mortality than substrates of mixed (HS =  $46.1 \pm 26.5\%$ ) or light (HS =  $78.1 \pm 18.2\%$ ) colour. Conversely, eggs experimentally incubated in substrate that was light-coloured, with a larger grain size and higher calcium carbonate concentration, produced significantly more and larger offspring. Incubation temperatures were significantly higher in dark substrate, which partially explains the lower hatching success in this type of sand. However, experimental incubation with controlled temperatures consistently showed lower hatching success in dark sand. Thus, we found that not only the temperature, but also the specific characteristics of each substrate determine hatching success. The main predator of eggs and hatchlings (the ghost crab *Ocypode cursor*) showed no significant differences in abundance or size between different substrate types. Our results indicate that nest site selection between beaches or even within the same beach with different substrate conditions affects hatching success, hatchling physical condition and subsequently the reproductive success of each female. The results of this study can inform conservation programs with nest management and controlled incubation in the field and optimise adaptive nest management under future scenarios of rising global temperatures.

## **PATHOLOGY OF LEATHERBACK (*DERMOCHELYS CORIACEA*) EMBRYOS AND DEAD IN NEST HATCHLINGS IN ST. CROIX, U.S. VIRGIN ISLANDS**

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The North Western Atlantic population of leatherbacks (*Dermochelys coriacea*) is considered endangered by the International Union for Conservation of Nature. With leatherbacks having the lowest hatch success rate of all sea turtle species (~50%) (Bell et al., 2006) and nesting activity declining around the Wider Caribbean region (The Northwest Atlantic Leatherback Working Group, 2018) it is important to identify ways to improve hatch success. Assessment of pathology of embryos and hatchlings is a much needed first step towards determining causes of in-nest mortality and poor hatchling success. In St. Croix, the hatch success is around 47% (Garrett et al., 2010), which is higher than St. Kitts (20%) (Stewart et al., 2018) and Grenada (30%) (Choi et al., 2020). This study examines leatherback embryo/hatchling pathology in St. Croix on Sandy Point National Wildlife Refuge (SPNWR) from March to August 2019. One hundred and five early, late stage, and dead in nest hatchlings from 41 nests (mean [standard deviation] hatch success 63.59% [21.26]) were evaluated by comprehensive postmortem examination. Pathology was documented in 55/105 (52.4%) individuals, representing 21/41 (51.2%) nests. Gross abnormalities included coagulated yolk (25.7%; n=27), deformities of head and eyes (3.8%; n=4) and observations of twins (1.9%; n=2). Microscopic lesions included chorioallantoitis (19.1%; n=20), renal mineralization (11.4%; n=12), multi-organ mineralization (3.8%; n=4), skeletal muscle necrosis (3.8%; n=4), bacterial esophagitis (1.9%; n=2), fungal dermatitis (1.9%; n=2) and renal tubular degeneration and necrosis (1.9%; n=2). Renal mineralization and skeletal muscle necrosis were more frequently observed in St. Kitts and Grenada, chorioallantoitis occurred at a similar frequency, and malformations were more common in St. Croix (Hill et al., 2019; Choi et al., 2020). Chorioallantoitis was caused by intralesional bacteria (55%), fungal hyphae (10%), or both (35%) in affected embryos, indicating bacteria and fungi can penetrate the shell. Seven females were identified to having one or more nests with pathology. Sex was determined histologically, at the beginning of the season (May to Mid-June) the female to male ratio was 3:1 (females n=20 and males n=7). After Mid-June, sex was observed to be 100% female (n=46) for the rest of the season. It was hypothesized that maternal health, temperature, and other factors of the nest environment play a role in pathogenesis of lesions affecting embryos and hatchlings. Further research is needed to identify host and environmental factors associated with life-threatening lesions in perinatal leatherbacks such that hatch success can be improved.



**INTERESTING PERIOD AND NESTING FREQUENCY OF THE ENDANGERED POPULATION OF THE LOGGERHEAD TURTLE (*CARETTA CARETTA*) ON THE ISLAND OF BOA VISTA, CAPE VERDE\***

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Knowledge about the reproductive success of sea turtles is essential for their conservation and requires the estimation of nesting frequency, and often of other factors that can lead to variation in reproductive output, such as the female body size, previous experience, and environmental variables. If efficient techniques, such as satellite telemetry, are not available, the nesting frequency can be underestimated when calculated simply using the capture-mark-recapture method. This study aimed to estimate the nesting frequency, as well as the interesting period, of the threatened subpopulation of the loggerhead turtle (*Caretta caretta*) from the island of Boa Vista, Cape Verde, and specifically for two size/age classes - neophytes and re-emigrants. The effects of air and sea surface temperatures and precipitation on the reproductive effort of females were also tested. The mark-recapture data used was collected between 2013 and 2020 on João Barrosa beach, in the Turtles Natural Reserve. This population is apparently recovering, and the increase in the number of identified females over the years was reflected in the greater difficulty in recording all the nesting events of the same female. The average number of nests per female ranged between 1.4-4.0, with the minimum being a low value compared to other subpopulations. Calculating the corrected frequency of nests increased this value, albeit with a larger error. Neophyte and re-emigrant females differed in body size and nesting frequency, with no differences in the interesting period. Neophytes had a smaller body size, laying an average of 1.8-4.0 nests per female, and the re-emigrants 2.0-4.1 nests per female. Between 2013 and 2020 the duration of the nesting season increased significantly, due to the anticipation of the first emergences. Precipitation influenced the duration of the nesting seasons, with greater precipitation resulting in a longer duration. The nesting frequency has decreased significantly over the years, which may be due to the increasing proportion of neophyte females, that lay a smaller number of nests per female. Lower fidelity to the nesting beach and the overall increase in the number of breeding females that emerge, making it difficult to record all nesting events, may also contribute for the decrease in the nesting frequency. Furthermore, there has been a decrease in the body size of breeding females over the years. A negative relationship, although weak, was found between nesting frequency and air temperature. The interesting period decreased over the different nesting months, as the sea surface temperature increases. This study showed that the small body size of Cape Verde females may be reflected in a lower number of nests per female compared to other subpopulations, making it necessary to readdress this topic with more advanced techniques.

## **EVALUATION OF PUTATIVE HYBRID HATCHLINGS BETWEEN HAWKSBILL TURTLE (ERETMOCHELYS IMBRICATA) AND GREEN TURTLE (CHELONIA MYDAS) IN TORTUGUERO, COSTA RICA**

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Hybridization among the Cheloniidae family have been documented since the 19th century; however, reports of this phenomenon are scarce, therefore the record of a hybridization between green (*Chelonia mydas*) and hawksbill turtles (*Eretmochelys imbricata*) signifies an important event. In the present study, we report the third known record of hybridization between green and hawksbill turtles in the Caribbean Sea. In Tortuguero, Costa Rica, during the monitoring activities of the 2020-nesting season, we marked and monitored several nests from both species, the offspring from two *Ei* clutches in particular presented morphological characteristics corresponding to both species. We analyzed 20 individuals from each monitored nests, and we compared physical characteristics among them. For every hatchling we measured: carapace length, recorded their weight, and checked for morphological cues such as the scale patterns on the head to better identify the species. The analyzed individuals from these particular clutches presented different combinations of the representative characteristics of each species, so we concluded they represented hybrid individuals. Our finding provides a new record for a green x hawksbill crossbreeding.

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## **MOON PHASE AND NESTING ACTIVITY OF ENDANGERED GREEN TURTLES (CHELONIA MYDAS) DURING PEAK NESTING SEASON AT PLAYA TRES, CARIBBEAN OF COSTA RICA**

**Amelia Richardson, Gustavo Ortiz Lopez, and Renato Saragoça Bruno**

*Turtle Love, United States of America*

Gravitational pull from the moon affects tides throughout the Planet, which affects biology and ecology of marine animals. Female sea turtles lay eggs on sandy beaches during reproduction. Tides may affect the ability of a female sea turtle to emerge onto nesting beaches and the selection of a site for the nest and the consequent hatching success. The purpose of this study was to assess the effects of moon phases on the nesting activity of green turtles (*Chelonia mydas*) during peak nesting season at Playa Tres, Caribbean of Costa Rica. Playa Tres is a 5-km stretch of beach on the Caribbean coast of Costa Rica, south of Tortuguero. The nesting season for green turtles in the region goes from July to November, with a distinct peak between August and September. As a part of Turtle Love's (<https://linktr.ee/turtlelovecr>) monitoring of sea turtle nesting activity at Playa Tres, we conducted morning censuses to quantify green turtle nests and false crawls. For this study, we used data for the months of August and September collected from 2019 through 2021. As morning censuses were not conducted every day of the nesting season due to lack of resources, we used weekly nesting activity data for green turtles at Playa Tres. A period of 9 weeks was analyzed to understand the effects of timing of the season on the green turtle nesting activity at Playa Tres. We considered four moon phases as per NASA SkyCal: full moon, third quarter, new moon, and first quarter. Each phase was considered to be 5 days in length, two days prior to the phase, the phase, and two days after. Each of the 9 weeks in our samples were considered to be influenced by only one moon phase. Weeks

8 and 9 had significantly fewer green turtle nests than the other weeks, which means peak nesting season for green turtles at Playa Tres start dwindling then. Moon phase did not significantly affect green turtle nesting activity in Playa Tres. This holds true when weeks 8 and 9 were excluded from the analyses. This means that other factors, such as timing of the nesting season, tidal variations, and fidelity of green turtles to nest sites may be more important indicators of nesting activity. It is not yet possible for Turtle Love to prioritize the deployment of resources and personnel for green turtle conservation at Playa Tres based on the phase of the moon. It would be better to concentrate these resources based on the timing of the nesting season. We suggest further studies evaluating effects of moon phase on sea turtle nesting activity, should use daily nesting activity data and also take tidal coefficients and timing of the nesting season into consideration.

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## **COROZALITO: A NASCENT ARRIBADA NESTING BEACH IN COSTA RICA\***

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The olive ridley (*Lepidochelys olivacea*) is characterized by its mass-synchronous nesting behavior, known as arribada. Costa Rica hosts two renowned olive ridley arribada beaches: Nancite and Ostional. Nevertheless, very little information is currently available on other arribada nesting beaches in this country. Accordingly, we documented the arribada events in Corozalito, a beach located in the north Pacific of Costa Rica where arribadas seem to have started in the late 2000s. Specifically, we recorded the frequency of arribadas from 2008 to 2021, and the abundance of egg-laying females during twelve arribadas between 2019 and 2021. In addition, we estimated the nest incubation temperature, performed quadrat excavations, and marked nests to estimate the arribadas hatching rates. Moreover, we documented the turtles tagged from other sea turtle nesting projects laying in the arribadas. We documented 29 mass nesting events at Corozalito since 2008, all of which occurred between August and January with a mean duration of  $2.82 \pm 1.1$  nights. From 2019 to 2021, we estimated more than 150,000 egg-laying olive ridleys in the course of twelve arribadas. Both frequency and size of the arribadas seem to be increasing every year. We obtained a mean hatching success of 65% in the quadrat excavations and 59% in marked nests. The average arribada incubation temperature was 32.3 °C. We found Corozalito turtles tagged at other locations on the Pacific coast of Costa Rica, which is evidence of philopatric plasticity. We suggest that nascent arribada rookeries form rapidly via recruitment from regional turtle aggregations. Our findings show that so far, the incubation environment at Corozalito is conducive to a high hatching rate and embryo development, in contrast to the established arribada beaches of Nancite and Ostional. As such, Corozalito the third most important arribada beach in the country, and only the continued monitoring of nesting dynamics at this site will shed light on the fate of Corozalito as an arribada rookery, and perhaps on the mechanisms by which arribada beaches are born, maintained, and regressed. Moreover, constant monitoring is needed to assess nesting trends at this new arribada rookery and develop proper management plans, a task that could be facilitated by the official protection of this beach and associated marine protected area.

## **THE IMPACTS OF NEST TEMPERATURES ON LEATHERBACK (DERMOCHELYS CORIACEA) HATCHLING PERFORMANCE AND MORPHOLOGY\***

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Leatherback sea turtles are experiencing population declines due to various natural and anthropogenic threats. The beach is one environment that plays a crucial role in the survival of sea turtle species since reproductive success is related to the nest temperature, moisture levels, and gas exchange through the sand. As climate change threatens with sea-level rise and more storms, increased erosion could increase the need for beach nourishment. Alterations to sand characteristics (e.g., grain size, sorting, color) may result in changes to the nest microenvironment and impact the nests' temperature and gas exchange. These changes, in turn, may alter hatchling performance as well as morphology. This study examined the relationship between nest incubation temperatures and leatherback (*Dermochelys coriacea*) sea turtle hatchling self-righting and crawling performance. Two sites with different sand characteristics in Juno Beach, Florida, USA were selected for this study. HOBO U22 temperature data loggers were placed in 13 leatherback sea turtle nests on the day they were laid to record nest temperatures for nests laid in the early (laid 27 Mar – 18 Apr), middle (laid 3 May – 16 May), and late (laid 20 May – 14 Jun) South Florida nesting season. Upon emergence, hatchlings were tested for righting ability and crawling speeds. An inventory of the nests was conducted three days after the initial emergence to determine hatching and emergence success. Nest temperatures ranged from 28.97°C-32.51°C. Mean nest temperatures for early nests was 29.42°C; the hatchlings had a mean crawling speed of 0.029 m/s and a mean righting score of 4.5. Hatchlings laid during mid-season incubated at a mean temperature of 31.08°C; they had a slightly slower mean crawling speed of 0.026 m/s and a mean righting score of 4.025. The later season nests had a mean temperature of 32.07°C; the hatchlings had a mean crawling speed similar to the other nests with a speed of 0.027 m/s and a mean righting score of 0.92. Mean nest temperatures were significantly higher in the mid- and later season nests than early nests, but this was not correlated with a significant decrease in crawling speed. Righting response scores were significantly lower in late season, hotter nests, however. Thus there is a negative correlation between high nest temperatures and righting response, but not apparently between crawling speed and mean nest temperature for this species. The study results will allow for a better understanding of how the nest temperatures are impacting hatchling performance which may affect their survival.

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## **DEVELOPMENT OF SPECIFIC ENZYME-LINKED IMMUNOSORBENT ASSAY FOR DETERMINING FSH LEVELS IN THE GREEN TURTLE CHELONIA MYDAS, USING RECOMBINANT GONADOTROPINS**

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The present study reports the development and use specific competitive enzyme-linked immunosorbent assay (ELISA) for the Green Sea Turtle *Chelonia mydas* (cm) follicle stimulating hormone (FSH) for the measurements of FSH in the plasma. Detailed information on the nature and levels of circulating pituitary hormones and sex steroids is critical for understanding the true functional status of the reproductive system

and the mechanisms regulating reproductive cycles. When working with threatened or endangered wildlife, the tools for obtaining reproductive data are limited. Since the development of specific FSH and LH RIA's by Licht in early 1980, to date no specific ELISA's for turtle gonadotropins has been developed. We produced Green Sea Turtle, *Chelonia mydas* recombinant FSH as single-chain polypeptide in the methylotrophic yeast *Pichia pastoris*. Glycoprotein subunit alpha was joined with the beta subunit mature protein-coding sequences to form a fusion gene that encodes a tethered polypeptide, in which the gonadotropin beta-subunit forms the N-terminal part and the alpha-subunit forms the C-terminal part. The recombinant (r) *Chelonia mydas* gonadotropins were used to develop a specific and homologous competitive ELISA for the measurement of FSH in the plasma of green sea turtles using primary antibodies against rcmFSH $\beta$  and rcmFSH $\beta\alpha$  for the standard curves. Wells were coated with 1ng/well of rcmFSH $\beta$ . The final concentrations of the primary antibody (against cmFSH $\beta$ ) were 1:5000. The sensitivity of the assay was 334 pg/ml for measurements in the plasma samples. The reproducibility of the ELISA was relatively high, as shown by intraassay (8.7%) and interassay (14.5%) CVs. The linearized standard curves for cmFSH paralleled to serial dilutions of plasma samples of the green sea turtle and we also observed parallelism between the linearized standard curves and serial dilutions of loggerhead sea turtle (*Caretta caretta*) plasma samples, we currently testing more turtle's species to verify the expand of the ELISA use. The ELISA developed was used to study the plasma FSH profiles of males and females during the reproductive cycle kept under captivity. Nesting females showed an increase in FSH levels from April to June during the nesting season, another peak was shown from September to November. Additionally, it was noticed that after the oviposition stage the FSH levels of the females remained relatively high as opposed to the FSH levels of non-nested females that was relatively low. The specific ELISA we developed using recombinant FSH will increase our understanding of gonadotropins functions and effects on reproductive biology of green sea turtle kept in captivity and in the wild.

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## **RELOCATING GREEN TURTLE NESTS TO OPEN BEACH AREAS PRODUCE HIGHLY FEMALE-BIASED HATCHLINGS, IMPLICATIONS FOR SEA TURTLE HATCHERY MANAGEMENT\***

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The relocation of egg clutches into a localized hatchery site is a common conservation strategy used around the world to protect sea turtle eggs in areas where natural nests are vulnerable to poaching. However, due to the effect nest temperature has on sea turtle embryonic development the relocation of egg clutches into unshaded beach hatcheries may result in higher incubation temperatures that can threaten hatching success, skew natural sex ratios, and influence hatchling fitness and locomotor performance adversely. Our study measured the incubation temperature of both natural and relocated nests at two critically important sea turtle conservation sites in Terengganu, Malaysia; the Chagar Hutang Turtle Sanctuary on Redang island, and the Tiga Ruang hatchery site in the Perhentian Islands; to investigate the influence relocation of green turtle clutches has on incubation temperature and resultant sex ratio of hatchlings. We found that relocating nests raised incubation temperature by 1.8° C and were estimated to have increased the proportion of female hatchlings produced by 45% compared to nests that were left to incubate under natural conditions. Additionally, we performed the histological examination of deceased hatchling gonad tissues, collected at the Tiga Ruang hatchery, to verify our sex ratio estimates calculated from incubating nest temperature measurements. Hatchling gonad tissue samples were processed using standard histology procedures and the sex of each hatchling was determined using descriptive criteria distinguishing the sexes based on gonad

cellular characteristics previously described for green turtles. Sex ratio estimates were verified by the histological examination of deceased hatchlings' gonad tissue and confirmed our study's prediction of highly female-biased hatchling sex ratios. We identified the vast majority (97%) of deceased hatchling's gonads as ovaries indicating they were females, corroborating our sex ratio estimates from nest temperature measurements predicting highly female-biased hatchling production from relocated nests. If such practice continues, this lack of male hatchling production will cause the feminization of the population and thus the future extinction of the region's already threatened nesting sea turtle population. Ensuring the future production of some male hatchlings is crucial in the context of balancing out the female-biased hatchling sex ratios currently being produced from relocated nests at the Chagar Hutang Turtle Sanctuary and the Tiga Ruang Turtle Sanctuary hatchery site. If atmospheric temperatures are to increase in the near future as predicted, the finding of this study suggests that conservation management efforts must be taken to devise acceptable cooling strategies to increase the long-term recruitment of male turtles into the region's mating populations.

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### **DIFFERENCES IN VISUAL PERCEPTION ARE CORRELATED WITH VARIATION IN SEAFINDING BEHAVIOR BETWEEN HATCHLING LEATHERBACK (DERMOCHELYS CORIACEA) AND LOGGERHEAD (CARETTA CARETTA) MARINE TURTLES\***

**Samantha Elizabeth Trail and Michael Salmon**

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After completing embryonic development, marine turtle hatchlings emerge from their subsurface nest, generally at night, and exhibit a positive phototaxis by crawling towards the lowest and brightest horizon, which should lead them to the ocean ("seafinding"). That response depends upon the ability of the turtles to discriminate between the brighter seaward vs. a dimmer landward horizon. While the crawls of most marine turtle hatchlings are well oriented and straight, those of leatherback hatchlings are sometimes interrupted by bouts of circling. We conducted field experiments in Juno Beach, FL comparing the orientation and crawling behavior of leatherbacks to those of loggerhead hatchlings to determine why those differences occurred. These experiments included releasing hatchlings from their nest sites the night of a natural emergence and recording the time it took to reach the wrack line, the overall orientation angle, and the number of circles performed by each individual (33 leatherback hatchlings from seven different nests; 24 loggerhead hatchlings from 5 different nests). Additionally, we conducted lab experiments to determine the perceptual spectral sensitivity thresholds of leatherback hatchlings to light wavelengths between 340 – 580 nm. To do so, hatchlings were presented with near monochromatic wavelengths at 20 nm increments from one arm of a black Plexiglas y-maze while the other arm remained dark. Trials began using a stimulus bright enough to induce most (if not all) hatchlings to crawl into the illuminated arm. In subsequent trials (done with different hatchlings) light intensity was decreased in 0.5 log increments until the distribution of arm entries was statistically indistinguishable from random (by one-tailed binomial test). Finally, light intensity was once again increased to confirm the threshold (defined as the lowest stimulus intensity triggering a preference for the illuminated arm).



## **EXPERIMENTAL ANALYSIS OF WAVELENGTH PREFERENCES SHOWN BY HATCHLING LEATHERBACK SEA TURTLES (*DERMOCHELYS CORIACEA*)**

**Samantha Elizabeth Trail and Michael Salmon**

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In marine turtles it is well established that hatchlings exhibit a positive phototaxis during seafinding, crawling towards the lowest and brightest horizon. Additionally, the shorter light wavelengths provide more potent, and preferred, cues for nocturnal seafinding orientation than the longer light wavelengths. In this study we sought to determine whether that preference is based upon differences in light intensity, wavelength, or some combination of both variables in leatherback hatchlings (*Dermochelys coriacea*) from Juno Beach and Boca Raton, FL. In order to control for perceived intensities, phototaxis thresholds at near-monochromatic wavelengths were established in 20 nm increments from 340 nm – 580 nm by allowing hatchlings to crawl in a black plexiglas<sup>TM</sup> y-maze in which one arm was illuminated and the other arm remained dark. Thresholds were defined as the lowest intensity at which hatchlings still showed a significant preference for the illuminated arm (one-tailed binomial test). Wavelength preferences were then evaluated at a near-UV wavelength (380 nm) vs. a longer visible wavelength (500 nm) stimulus, selected because each represented a perceptual sensitivity peak within the threshold data. Wavelengths were presented simultaneously in a black plexiglas<sup>TM</sup> y-maze at perceptually equivalent intensities 0.3, 0.7, and 1 log unit above each wavelength threshold (n < 14 hatchlings at each intensity pairing for a total of n = 41 hatchlings). We found that under these relatively dim lighting conditions (closely mimicking those at the nesting beach during a new moon), the behavioral preference for the shorter light wavelengths was based upon intensity rather than spectral cues as the distribution of arm entries was statistically indistinguishable from 50:50 at all wavelength pairings. We speculate that under full moon illumination, or when hatchlings emerge before the onset of darkness (as commonly occurs in this species), wavelength cues might also be utilized.

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## **CLUTCH SIZE AND DEPTH OF GREEN TURTLE (*CHELONIA MYDAS*) NESTS LAID AT PLAYA TRES, CARIBBEAN OF COSTA RICA, DO NOT VARY BASED ON NEST PROXIMITY TO TIDE LINE**

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The largest Atlantic population of endangered green turtles (*Chelonia mydas*) reproduces in northeast Costa Rica. Female green turtles lay several egg clutches per season, and the position of each nest varies vertically on the nesting beach between the vegetation and the high tide line. Nesting site selection is an important factor of the reproduction process because factors influencing incubation success, such as temperature and humidity, also vary across the vertical gradient of the nesting beach. The objective of this investigation is to determine if nest depth, clutch size, or hatching success in green turtle nests vary based on the distance from the nest to the high tide line. We marked and followed the fate of 54 green turtle nests laid at Playa Tres, on the Caribbean coast of Costa Rica, south of Tortuguero National Park. We quantified clutch size

during oviposition or during nest relocation, measured the distance of the bottom to the surface of the egg chamber to the nearest centimeter, and calculated hatchling success by excavating the nests and counting empty eggshells. We also measured the distance between the nest and the surface of the sand. For 37 data points we measured nest depth when relocating the nest, which would have also accounted for the depth of the nest camouflage. For the remaining 17 data points, we measured nest depth prior to oviposition. These two datasets were analyzed separately. Neither clutch size nor nest depth varied significantly based on the distance of the nest from the tide line. This means that green turtles nesting at Playa Tres do not seem to compensate changes in temperature and humidity across the vertical gradient of the nesting beach by changing the depth of egg chambers. Neither does it seem that green turtles lay larger egg clutches in places where temperature would be more amenable to compensate for the metabolic heat produced during incubation.

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## **EXPOSURE AND CONSEQUENCES OF WAVE WASH-OVER FOR LOGGERHEAD SEA TURTLE NESTS IN THE NORTHERN GULF OF MEXICO\***

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Wave wash-over poses a significant threat to sea turtle nests, with sustained exposure to waves potentially resulting in embryonic mortality and altered hatchling locomotor function, size, and sex ratios. Identifying where and under what conditions wave exposure becomes a problem, and deciding what action(s) to take (if any), is a common issue for sea turtle managers. To determine the exposure of sea turtle nests to waves and identify potential impacts to hatchling productivity, we first modeled wave exposure across 40 nesting beaches used by the Northern Gulf of Mexico Loggerhead Recovery Unit from 2016-2019. Our models indicate that, on average, approximately 50% of the available beach area and 34% of nesting locations per nesting beach face a significant risk of wave exposure, particularly during tropical storms. Field data indicate that 42.3% of all nest locations reported wave exposure, which resulted in a 45% decline in hatching success. To investigate the effects of exposure frequency, duration, and timing on hatchling production, daily wave exposure was reported for all nests on St George Island – a priority location identified by the modeling effort – in addition to hourly inundation monitoring at a subset of loggerhead nests during the 2021 nesting season. Preliminary data suggest hatchling success declines an average of 71% (relative) with each subsequent wash-over event, with additional analyses ongoing including multiple years and nesting sites. This exercise offers a flexible approach for threat assessment integration into research and management questions relevant to sea turtle conservation, as well as for other beach species and human uses of the coastal environment.

## POPULATION BIOLOGY AND MONITORING

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### OPTIMISED 2B-RAD SEQUENCING PROTOCOL FOR GENOMIC STUDIES ON THE LOGGERHEAD SEA TURTLE (*CARETTA CARETTA*)

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High-throughput sequencing has revolutionized population and conservation genetics by providing thousands of genome wide markers. Furthermore, the use of RAD sequencing methods can be used on non-model species lacking a reference genome. Among all the RAD sequencing methods, 2b-RAD is a very promising technique for marine turtles as it can score thousands of marker loci across the whole genome, providing excellent results even with highly degraded samples. However, genotyping protocols need to be calibrated in order to obtain the expected number of markers, with enough sequencing depth for reliable genotyping and with the number of samples per lane optimised to adjust sequencing costs. Moreover, protocol sharing among different research groups is crucial to allow future cross comparisons among multiple studies carried out in the same or different laboratories, especially in highly migratory species such as marine turtles. Here we present our optimised 2bRAD protocol for genomic studies in the loggerhead turtle (*Caretta caretta*) that has been successfully used to study the permanent nesting populations in the eastern Mediterranean and the ongoing colonisation process of the western Mediterranean. In summary, extracted DNA is digested with the enzyme *AlfI* and libraries are constructed using a customised set of adaptors to perform a selective base ligation to adjust the number of loci for obtaining an adequate mean depth for a correct genotyping while remaining feasible cost wise. Illumina adaptors and barcodes are included by PCR amplification and the pooled libraries of 48 individuals are sequenced per lane of 200 million 50bp reads. This protocol provides a very cost-efficient way of genotyping high quality markers, completely comparable across laboratories, for population genomic studies in this species.

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### POPULATION GENOMICS OF NON-MODEL SPECIES: HELPING DECISION-MAKING FOR A RELIABLE AND COST-EFFECTIVE GENOTYPING

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High-throughput sequencing has set the pace for a new era of population and conservation genetics during the last 10 years by providing easy access to genomic data from virtually any taxonomic group. RAD sequencing methods, such as 2b-RAD, can be used on species lacking a reference genome by reducing the amount of the genome being analysed by the digestion with restriction enzymes. However, transferring protocols across taxa can potentially lead to poor results, such as low number of recovered markers or inadequate genotyping due to differences in the genome of the target species. Here we present the analysis of two different IIB enzymes (*AlfI* and *CspCI*) on two species with very different genome sizes (the loggerhead turtle *Caretta caretta* and the sharpshout seabream *Diplodus puntazzo*) designed to optimise the laboratory protocols for each species. Good results were obtained even with initially degraded samples,

showing the value of 2b-RAD in studies where DNA quality can be compromised, which is often the case in samples of endangered species such as sea turtles as sampling often derives from dead individuals. However, DNA quality after individual adaptor ligations was found to be a critical parameter on the number of reads and loci obtained for genotyping. Resampling analyses simulating different number of reads per individual showed a trade-off between the number of loci and the number of reads per sample. For both species, Alfl needed a much higher number of reads per individual than CspCI to reach the desired coverage of 20X, due to the higher number of loci obtained with this enzyme. To obtain this coverage for CspCI and Alfl the estimated number of reads for *D. puntazzo* was  $1.7 \times 10^6$  and  $3.5 \times 10^6$  respectively and for *C. caretta* was  $6.1 \times 10^6$  and  $13.5 \times 10^6$  respectively. We also demonstrated that selective-base ligation does not affect genetic differentiation between individuals, indicating that this modification of the 2bRAD protocol can be used in species with large genome sizes to adjust the number of loci to the study scope, reducing sequencing costs and maintaining suitable depth of coverage without compromising the results. Finally, we provide a set of guidelines to improve 2b-RAD protocols on non-model organisms with different genome sizes, to aid decision-making for a reliable and cost-effective genotyping.

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## GENOMICS ON A WARMING SEA: STRUCTURING AND ADAPTATION OF THE MEDITERRANEAN LOGGERHEAD (CARETTA CARETTA) NESTING POPULATIONS\*

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The population structuring of living organisms is shaped by the combination of different evolutionary forces such as migration, genetic drift or selection. The rise of genomic studies during the last decade has opened new research opportunities, including the analysis of outlier loci to look for genomic signals of local adaptation potentially associated with environmental cues. Thus, these new genomic tools have the potential to uncover adaptive patterns of structuring that are of great interest in species predicted to be heavily impacted by environmental changes, such as global warming. In the case of marine turtles, feminisation of the populations due to the rise of temperatures and a decline of hatchling survival rates due to excessive

warming threaten current nesting populations. The loggerhead turtle is one of the best oceanic migrators, distributed in tropical and warm-temperate regions and is the most abundant sea turtle in the Mediterranean Sea. Recent reports of the International Panel on Climate Change (IPCC) indicated that the impact of global warming in the Mediterranean area exceeds global trends. Conservation measures in the eastern part of this sea, where most loggerhead turtle rookeries are found, are crucial to support nesting populations. However, the population structuring of Mediterranean rookeries is not yet fully resolved and previous studies did not consider the role of local adaptation on population differentiation. We, therefore, genotyped by 2b-RAD a total of 243 individuals from 11 nesting populations from the Mediterranean Sea, and combined this genomic information (10,725 SNPs) with environmental (salinity and temperature), behavioural (hatchling dispersal patterns and adult foraging strategies) and reproductive data (clutch sizes) from the populations. Genomic data provided effective population sizes ( $N_e$ ) that strongly correlated to the number of estimated adult breeders and revealed a critical status of some of the studied populations. We found substantial genetic differentiation among most rookeries with a major break between Greek locations (including Crete) and the remaining populations. The general genetic differentiation confirmed male and female philopatry, resulting in reduced connectivity. Redundancy Analyses revealed significant genomic signatures associated with environmental, behavioural and reproductive population parameters, thus highlighting their importance in population differentiation in the Mediterranean Sea. Finally, we performed a conservation prioritisation analysis to identify the areas with higher priority for protection based on their global contribution in genomic variability and connectivity. These findings provide the baseline for future studies on sea turtle genomics for conservation since we were able to refine the population structure of this endangered species, detect signals of local adaptation and facilitate decision making for Mediterranean Sea turtle populations.

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## **ABUNDANCE MONITORING OF OCEANIC-STAGE LOGGERHEAD SEA TURTLES IN THE AZORES: THE IMPORTANCE FOR EFFECTIVE MANAGEMENT**

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Standardized boat-based surveys by fishing observers on-board the pole-and-line tuna fleet have been a key element in understanding the recruitment of juvenile sea turtles to the foraging grounds of the Azores. Due to the difficulty of studying the oceanic life cycle, very little information exists on the population dynamics of loggerheads during this stage. Thus, the data collected by the Azores Fisheries Observer Program (POPA) have been crucial in establishing the first correlation between the relative abundance of juvenile sea turtles in the North Atlantic and nest counts from the population's major source rookeries in Florida. We present updated abundance estimates based on 524 sightings recorded by fishing observers during 78,203km of standardized visual transects between 2001 to 2020, currently the longest time-series of its kind. Data were recorded on geographic location, time, Beaufort sea state, glare, visibility, and time of day of the transect. Sighting angle was calculated from the recorded compass heading of the vessel and the sighting, while distance from the vessel was estimated visually. The observer coverage of the pole-and-line fishery was 41-100% and provided usable coverage for 150,000km<sup>2</sup> of the Azores EEZ. The analyses



further revealed some limitations of the data, that allowed us to identify and discuss the challenges and advantages of the data collection program.

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## ANALYSIS OF LEATHERBACK AND GREEN TURTLE GENOMES REVEAL DIFFERENTIAL ADAPTIVE CAPACITY AND DEMOGRAPHIC HISTORIES\*

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Anthropogenic activities have resulted in global declines of sea turtle populations over the last two centuries. Effective conservation management of these declining populations relies on understanding the underlying genomic characteristics in order to enhance recovery efforts. Recent technological advances have allowed the development of high-quality reference genomes for non-model organisms, paving the way for in-depth genomic analyses to facilitate conservation. We leverage the chromosome-level reference genomes for the leatherback (*Dermochelys coriacea*) and green (*Chelonia mydas*) turtles generated through the Vertebrate Genomes Project (VGP) to conduct conservation genomic analyses and provide insights into differences between these two species. We demonstrate that the leatherback turtle has exceptionally low genome-wide diversity when compared to the green turtle, and to other non-avian reptiles, and shows a higher proportion of putatively deleterious variants within the genome. Despite this low diversity, the leatherback turtle genome shows a high number of short runs of homozygosity (ROH), suggesting sustained low effective population sizes ( $N_e$ ) and no evidence of recent inbreeding. In contrast, the green turtle has overall high diversity, but with relatively longer stretches of homozygosity, indicating that the individual used for reference genome assembly may have a recent history of inbreeding events. The observation of long-term low  $N_e$  is also reflected in the demographic history analysis, which suggests effective population sizes have remained relatively constant and less than 10,000 individuals over the last 5 million years, and much lower over the last 10,000 years. In contrast, the green turtle has shown much higher  $N_e$ , and wider fluctuations over the same period. Taken together, our results further concern for the persistence of the leatherback turtle under unprecedented climatic changes, with low diversity indicating a limited adaptive potential. Alternatively given that this species has persisted through multiple changes in climate with low diversity, this may offer hope for population recoveries should anthropogenic threats be mitigated.



## GEOMETRIC MORPHOMETRY AS A TOOL FOR THE CONSERVATION OF SEA TURTLES

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The use of computational tools that employ morphometric patterns to obtain quantitative results constitutes a modern non-aggressive and inexpensive tool for the individual characterization of organisms and reduces the time dedicated to photo-identification. For this reason, the objectives of this research are: To identify distinctive morphometric patterns of sex in green turtles' hatchling (*Chelonia mydas*) in the contours of the fore flippers and the cloaca, identify variations of the morphometry of facial scales among age groups, beaches and between mothers and hatchlings. A total of 425 photographs (167 mothers and 238 hatchlings) from three nesting areas in Cuba, two in Guanahacabibes and one in Cayo Largo, were analyzed. To identify the sex, gonadal histology was performed on 45 dead hatchlings. On this individuals, photographs of the fore flippers, both sides of the head, the carapace and the cloaca were taken in order to determine possible dimorphic characters in contrast to the sex determined by the morphology of the gonad. The greatest differences were found between individuals rather than among groups meaning that no similarity was found between mothers and their offspring and between siblings, so it is inferred that the morphometric patterns of the scutes is not heritable. It was also not possible to detect similarities in scutes patterns within the same beach. However, the use of geometric morphometry made it possible to effectively re-identify the same individual, which is why it can facilitate and validate the photo-identification process. The shape of the anterior flippers of the males was, in some cases, significantly different from that of the females, so it may be a trait that should have further evaluation for the possible identification of the sex by non-invasive techniques. It can be concluded that geometric morphometry can be a valuable conservation tool to identify individuals and recognize their sex using non-invasive techniques.

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## GENETIC STUDY OF OLIVE RIDLEY NESTS IN SOUTHERN SINALOA, MEXICO, REVEALS ONE OF THE HIGHEST LEVELS OF MULTIPLE PATERNITY AND LOW INCIDENCE OF DOMINANT MALES\*

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Multiple paternity (MP) is common in sea turtle species, with levels varying between populations and species due to poorly understood causes. Although MP is facilitated by the capacity for sperm storage in

female sea turtles, its frequency could be influenced by the selection of mates by the female, the operational sex ratio at mating sites, or post-copulatory competition between sperm from different males. We analyzed the mating system for a small olive ridley population laying on average 860 nests/year at Las Cabras-CIP Playa Espiritu, Sinaloa, Mexico. An average of 17 hatchlings/nest from 13 nests taken from the 2016 nesting season were assayed to estimate operational sex ratios and gain insights on the mating system from correlations between MP and female and clutch biometrics. Four nuclear microsatellites were used (OR-4, OR-11, CcP7D04, Cc1G02; with unbiased identity and exclusion probabilities of 0.0000001, and 0.999, resp.) to genotype hatchlings and COLONY software to estimate sires/nest. In contrast to most previous studies, MP was found in all nests and at a very high value of 2-7 sires/nest (mode=5; 31% of nests). Siring was not homogeneous; 67-90% of sires fertilized a single female, 6-31% two, and 2-4% three females. Interestingly, the proportional incidence by individual sires ranged 10-20%, reflecting a tendency towards even contributions by the different fathers. The estimated operational sex ratio was 4.2 males:females (i.e., 80% males), contrasting with beach primary sex ratios that are strongly female-biased. The correlations between MP and maternal and clutch biometrics were non-significant ( $P>.05$ ), although trends consistently indicated that increasing MP was associated with smaller females. When we grouped curved carapace widths of mothers associated with nests with fewer (2-4) vs. more (5-7) sires, there was a significant difference between the groups (Mann-Whitney  $U=36.0$ ;  $P=0.026$ ). This result could suggest that smaller females tend to be fertilized by more sires, perhaps due to having a lower capacity to defend themselves from males or being more easily controlled during copulation. The combination of the very high level of multiply sired clutches and a balanced contribution by the sires suggests the existence of highly competitive and promiscuous males at the mating grounds for this population, with low individual male dominance. A male-biased operational sex ratio at the breeding sites for this population is likely a significant factor contributing to such high MP values. Possible explanations include the presence of contrasting breeding patterns between the sexes, longer female than male re-migratory cycles, shorter durations of females than of males at the mating sites, and the participation of vagrant males from distant rookeries. Furthermore, the elucidated mating scheme should lead to a much higher allelic diversity in offspring, with increased adaptive and survival potential in future generations.

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## AI-BASED TECHNOLOGY FOR AUTOMATIC CLASSIFICATION OF SPECIES OF SEA TURTLES

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Currently, the world's population of sea turtles decreased dramatically due mostly to anthropogenic factors. Because Mexico like other countries receives several species of this reptile all along its coastal zones but is the only one that receives massively, *Lepidochelys kempii*, conservation, and preservation efforts are a must. To revert this problem the Mexican government created Protected Natural Reserves and repopulation programs involving diverse groups of both experts and volunteers. Furthermore, to contribute towards the conservation and identification of different species, new proposals must be submitted, one of which is the use of tools based on computer methods and algorithms. Deep Learning is an area of artificial intelligence that can identify animal species and individuals using images. This methodology has gained popularity in

monitoring and control of the wild animal populations, vis-à-vis invasive techniques such as physical tagging. This work presents the implementation of an optimized proprietary technology of convolutional neural network architecture. This technology can automatically classify six species of sea turtles with greater precision than that reported by literature. The designed neural network was trained with information representative of the main attributes of each six species, which were obtained from the digital processing of sea turtle images. These attributes were generated by digital characteristic's extraction techniques, such as SIFT, HOG, LBP, and the CIE 1976 L\*a\*b\* color space. Our database was created using more than 4,000 training images of sea turtles taken close-up close in different natural environments, of which 1,050 images were for validation, and 1,050 images for testing. It's important to mention that this database is *de novo* and never reported. The proposed neural network architecture obtained 87.9 % accuracy for the validation images and 86.9 % accuracy for the test images. A comparison was made with the typical pre-trained AlexNet neural network architecture, which obtained 85 % accuracy for validation and test images. Therefore, the results obtained by the proposed network are robustly achieving higher accuracy in the validation and test sets, surpassing the maximum obtained by other studies, permitting to differentiate, discriminate and identify all globally known sea turtles by means of their morphologic traits. This is a novel technology that enables the neural network to learn attributes and biometric aspects of sea turtles that are unique to each species, to obtain a high precision classification.

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## TRACING THE NATAL ORIGINS OF GREEN TURTLES FORAGING IN WATERS OFF SOUTHEASTERN AUSTRALIA\*

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Identifying critical habitats and links between breeding and non-breeding sites is a global priority for marine turtle conservation. Green turtles (*Chelonia mydas*) are impacted by a wide range of anthropogenic threats, with the severity depending on geographic region and the life-history stage of the individual. In Australia, the strengthening of the East Australian Current due to climate change is increasing ocean temperatures, particularly in southern New South Wales (NSW), and having large-scale impacts on the distribution and abundance of marine resources. Green turtles appear to be more frequently observed in the temperate southern NSW waters, but our knowledge on their habitat use, migration patterns and impact of threatening processes is limited. To assess the origins of green turtles foraging in southeastern Australia, samples were obtained from juvenile, sub-adult and adult green turtles that had stranded along an 800 km region of the NSW coast between 1997 and 2021. A ~770 bp mitochondrial DNA control region fragment was sequenced for 271 individuals and compared to 25 potential source breeding stocks in the Indo-Pacific region using mixed-stock analysis. A total of 24 haplotypes were identified in NSW, of which 13 had been previously observed at a rookery, seven have been identified previously, but not at a rookery (i.e. orphan haplotypes), and four were previously undescribed. Mixed-stock analysis revealed that NSW waters support multiple breeding stocks but are dominated by the Northern Great Barrier Reef (GBR), Southern GBR, Coral Sea and New Caledonia breeding stocks. Around 3% of green turtles originated from more distant stocks, including Micronesia, Marshall Islands, American Samoa, Borneo and French Polynesia. Understanding the connectivity between green turtle rookeries and foraging grounds provides an opportunity to assess the

impact of anthropogenic threats to turtle stocks, and in turn, prioritise management actions for the conservation of green turtles with regional, national and international partnerships.

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## UPWARD TRENDS IN NESTING ABUNDANCE AT AN IMPORTANT INDIAN OCEAN ROOKERY

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Sea turtles around the world have historically suffered population declines as a result of, for example, bycatch and harvesting of adults and eggs. These declines have motivated worldwide conservation efforts since the 1950s such as various beach protection measures, fisheries restrictions and the establishment of marine protected areas. While conservation concerns remain at many sites, encouragingly long-term increases in nesting abundance have been widely reported. Global assessments have highlighted the significance of the southwestern Indian Ocean (SWIO) where data gaps include the Chagos Archipelago, an important rookery of five atolls and 235 km coastline for hawksbill (*Eretmochelys imbricata*) and green (*Chelonia mydas*) turtles. To estimate the relative importance of the 67 islands of the Chagos Archipelago, we conducted track surveys on 90% of the coastline in 1996, 1999, 2006 and 2016 using standardised techniques and during the same season (February-April). To assess seasonality of nesting activities and long-term nesting abundance, we surveyed a 2.8 km index beach on the largest island (Diego Garcia) at two-weekly intervals during six nesting seasons (2006-2018). At a regional scale, the contribution of Chagos Archipelago egg clutch production to the southwestern Indian Ocean (SWIO) region was estimated for each country using information available in the literature including 50 publications from SWIO countries and SWOT ([www.seaturtlestatus.org](http://www.seaturtlestatus.org)) as well as previously unpublished data from Seychelles. Interannual and seasonal variation in nesting occurs; hawksbills have a distinct nesting peak (October – February) whilst green turtles nest year round (peak is June – October). We report increasing trends in nesting populations of hawksbill and green turtles over recent decades. Across an estimated 132 km of suitable nesting coastline, the nesting population size has increased to around 6300 hawksbill and 20,500 green turtle clutches laid annually (2011-2018) with an increase in abundance by 2-5 times for hawksbills and 4-9 times for green turtles since 1996 (<http://doi.org/10.1017/s0030605319001108>). Nesting numbers at the Chagos Archipelago rookery are now among the highest in the Indian Ocean. Satellite tracking has shown that green turtles that nest in the Chagos Archipelago migrate to foraging grounds across the SWIO (<https://doi.org/10.1016/j.cub.2020.05.086>), i.e., the Chagos Archipelago provides a nesting sanctuary for green turtles from across the region. SWIO regional estimates indicate the green turtle egg clutch production is ten times greater than that of hawksbills. But, this is offset by the fact that individual green turtles may produce almost twice as many egg clutches as individual hawksbills (<https://doi.org/10.1098/rspb.2016.2581>; <https://doi.org/10.2744/CCB-0830.1>). Importantly, the Chagos Archipelago nesting population accounts for 39-51% of estimated 12,500-16,000 hawksbill and 14-20% of estimated 104,000-143,500 green turtle clutches laid annually in the region.

## FROM A GRAIN OF SAND: MONITORING SEA TURTLES AND THEIR PATHOGENS VIA NON-INVASIVE ENVIRONMENTAL DNA ANALYSIS OF NESTING BEACH SAND TRACKS AND OCEANIC WATER\*

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Elusive aquatic wildlife, such as endangered sea turtles, are difficult to monitor and conserve. As novel molecular and genetic technologies develop, it is possible to adapt and optimize them for wildlife conservation. One such technology is environmental (e)DNA – the detection of DNA shed from organisms into their surrounding environments. We developed species-specific green (*Chelonia mydas*) and loggerhead (*Caretta caretta*) sea turtle probe-based qPCR assays, which can detect and quantify sea turtle eDNA in controlled (rehabilitation tank water and sand samples) and wild (free-ranging oceanic turtles and nesting females) settings from beach sand and water samples. We show that eDNA approaches can greatly complement traditional in-water monitoring of sea turtles - species-specific eDNA was successfully amplified from 100% of post-hatchling washback and juvenile rehabilitation water samples (with a positive correlation between eDNA concentration and turtle abundance), as well as 17/23 wild oceanic sites, using qPCR. Furthermore, we report the applicability of high throughput shotgun sequencing to eDNA sand samples enabling sea turtle population genetic studies and pathogen monitoring, demonstrating that non-invasive eDNA techniques are viable and efficient alternatives to biological sampling (e.g. biopsies and blood draws). Species-specific eDNA was detected in 100% of rehabilitation sand samples as well as maternal false crawls, maternal nesting crawls, nest emergences and single hatchling crawls on nesting beaches using qPCR. We were also able to determine the geographic origin and haplotype of nesting mothers from crawl sand alone, using Illumina shotgun sequencing and mitochondrial analysis. As genetic information can be obtained from sand eDNA without ever having to observe (or interact) with the target individual, such approaches can greatly reduce the sampling stress experienced by nesting mothers and emerging hatchlings (or the sacrificing of a viable egg per clutch). We also show that these eDNA approaches are viable even from maternal crawls that are over 24 hours old. In addition, the detection of sea turtle pathogens from crawl sand indicates significant potential for increased wildlife disease monitoring



capacity and viral variant surveillance, as we detected ChHV5 (the fibropapillomatosis-associated herpesvirus) in the crawl tracks of a single hatchling using qPCR and shotgun sequencing. Together, these results demonstrate the potential of eDNA approaches to ultimately help understand and conserve threatened species such as sea turtles.

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## SEA TURTLES ACTUAL STATE IN THE SAO TOME: A GENERAL OVERVIEW OF THEIR REPRODUCTIVE BIOLOGY AND CONSERVATION\*

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São Tomé and Príncipe unique oceanic islands from the Gulf of Guinea, West Africa, harbors five of the seven sea turtle species that exist in the world - *Eretmochelys imbricata*, *Lepidochelys olivacea*, *Chelonia mydas*, *Dermochelys coriacea* and *Caretta caretta* – and offers optimal conditions for these endangered species both on the nesting beaches and foraging sites at sea. Sea turtles have been extremely exploited since the 16<sup>th</sup> century in São Tomé and Príncipe until nowadays, but conservation and research initiatives in the past decade have improved our knowledge of these species, highlighted their importance regionally and globally and improved significantly their level of protection. For example, São Tomé and Príncipe is home to the last significant hawksbill rookery in the Eastern Atlantic, a genetically distinct population among the top 11 sea turtle conservation priorities worldwide; and the green turtles nesting in the country exhibit relatively high levels of genetic diversity, representing an important genetic pool in the region. Here, we present the results of the first seven years of systematic and consistent effort of data collection and analysis, in order to evaluate the success of sea turtle conservation actions and policies in São Tomé Island. Data collected from 2014 to 2020 were analyzed to determine the nesting ecology, population trends and identify events and policy decisions influencing these trends. Although, only seven years of data were analyzed, we observed a significant increase in all species nesting in São Tomé, mainly the Green Turtle, the Olive Ridley, the Hawksbill Turtle and the Leatherback. We suggest that local conservation actions that started in the 2000s, but reinforced in 2014, with the implementation of the national law and the establishment of a more consistent monitoring and protection program, have contributed to the gentle and progressive recovery of these sea turtles populations; however, given the small populations size and the persistence of various threats – such as intentional and opportunistic capture for human consumption, fisheries bycatch, climate change, pollution, unsustainable touristic and coastal development - these populations continues to be of conservation concern.



## INTEGRATING CLIMATE CHANGE AND MANAGEMENT SCENARIOS IN A POPULATION MODEL OF THE NORTHERN GREAT BARRIER REEF GREEN TURTLE GENETIC STOCK

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The green turtle (*Chelonia mydas*) population in the northern Great Barrier Reef (nGBR) is one of the world's largest, but there are several indicators that the population is under threat, largely due to climate change. Raine Island supports the largest rookery for the population but low nesting success and hatchling production has been observed for at least 30 years, which is influenced by increasing sea levels and temperatures due to climate change. At one foraging ground, sex ratios have become more female biased over time as expected due to temperature dependent sex determination, such that juvenile turtles from the nGBR were 99% female. These observations have prompted management actions to (1) alter the beach profile in some areas to increase the sand depth in preferred nesting habitat, (2) to reduce mortality of nesting females at Raine Island and (3) to work with Indigenous communities to consider what constitutes a sustainable legal harvest. To explore possible outcomes of various management actions we developed two density-independent, stochastic stage-structured metapopulation models, a "Moderate Climate Model" and an "Extreme Climate Model" using RAMAS software. The models were developed as a heuristic tool as they were necessarily based on limited demographic data, thus the results are qualitative and used to consider key features of population dynamics. Predicted climate change scenarios incorporated female hatchling sex ratios of 88% or 99%, and an estimated loss of nesting habitat due to sea-level rise of 7% or 15%. Males were included in the models for their potential to be the limiting sex and reduce female fecundity. The mating system assumed a female:male mating ratio of 10:1 or 30:1 in the two models. Harvest scenarios of 2000 or 6000 adult females were considered, as well as harvests that replaced a proportion of adult females with sub-adults. Population trajectories for the two models varied dramatically, with positive population trajectories under the Moderate Climate Model, and steeply declining trajectories under the Extreme Climate Model. Under the Moderate Climate Model, the population could be vulnerable to overharvest of adult females at levels that have been speculated to occur in the past. Replacing adult females with sub-adults lessened the severity of harvests, and population recovery was possible when harvests were reduced. Under the Extreme Climate Model, a steep population decline was due to the eventual lack of reproductive males. Population dynamics and the severity of any impacts depended largely on the ability of breeding male turtles to mate with a possibly unrealistic, large number of females. Management actions to reduce nesting female mortality at Raine Island had a small positive effect on population growth under the Moderate Climate Model, but no effect under the Extreme Climate Model. Management actions to increase the number of hatchlings produced had a small positive effect on population growth under the Moderate Climate Model. Under the Extreme Climate Model, a positive effect was only observed if nest temperatures were lowered to produce a large number of all male hatchlings.

## **CITIZEN SCIENTISTS REVEAL THE DISTRIBUTION, ABUNDANCE, AND MAIN THREATS TO THE FORAGING SEA TURTLES IN TAIWAN\***

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Photo identification has been reported as a helpful tool for identifying individual sea turtles by their facial scute pattern and has been demonstrated to provide valuable information for the sea turtle population. In Taiwan, scuba diving, freediving, and underwater photography have become some of the most popular outdoor activities in recent years. Therefore, we initiated a citizen science project called TurtleSpot Taiwan using social media to collect sightings and photographs of sea turtles from divers. Photos are then used to identify individual turtles manually and by a computer-assisted software HotSpotter (Crall et al. 2013). We have received 2035 records contributed by 383 citizen scientists, identified 581 individuals (547 of *Chelonia mydas*, 33 of *Eretmochelys imbricata*, and 1 of *Lepidochelys olivacea*) from June 2017 to May 2021. Sightings were from different places around Taiwan, and the top hotspot areas are Xiaoliuqiu, Kenting, and Lyudao. About 50.4% of individuals have repetitive sightings, and 33.6% are residents (stayed more than 1 year). In 16.4% of sightings, turtles suffered from fishing line entanglement, visible injuries or amputation, or were found stranded or bycatch. The percentage of injured individuals might be sampling biased because citizen scientists have a higher tendency to report when they encounter injured turtles. However, the types of injury and the recovery process of these injured individuals are critical for understanding the population status of sea turtles. We wish to raise conservation needs and awareness of sea turtles in Taiwan through this cooperation platform and encourage the public's immediate information exchange on sea turtles.

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## **GENETIC ANALYSIS OF THE HAWKSBILL SEA TURTLE (ERETMOCHELYS IMBRICATA) FROM THE MEXICAN CARIBBEAN SEA AND PACIFIC REGION USING CONTROL REGION SEQUENCES**

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Hawksbill turtles are distributed circumglobally throughout tropical and subtropical ocean waters. The important nesting beaches in Mexico are found in the Yucatan Peninsula, and sporadically in Jalisco, and Nayarit (Cuevas-Flores et al., 2016; Martinez-Estevez, L. et al., 2021). Genetic studies represent key tools for understanding the past, present, and future of the species, such information can be focused on the management of at-risk populations (Gaos et al., 2020). The aim of this work was to show a genetic analysis of mainly nesting females in the Mexican Caribbean Sea in contrast with Mexican Pacific and Pacific Hawksbill populations using the mtDNA control region (CR). Two sets of sequences from different origins were obtained, one from fieldwork and another downloaded from GenBank. All field samples were amplified by PCR using CR primers LCM15382/H950. All Sequence alignments were performed using ClustalW. A haplotype network was created using the Median-Joining algorithm in PopART. Haplotype and nucleotide diversity and average nucleotide differences were estimated in DnaSP. IQTree was used to infer phylogenetic tree and FigTree to visualize the tree. In this work, 251 hawksbill turtle sequences were analyzed. Ninety-eight field samples and 153 sequences were downloaded from GenBank. An average of 769 bp from the mtDNA CR for 98 hawksbills sampled in the Caribbean Sea (Campeche, Yucatan, and Quintana Roo) and Mexican Pacific (Jalisco, Nayarit, and Sinaloa) Ocean. From the GenBank sequences, an average of  $\geq 769$  bp sequences were obtained. All sequences were aligned and homogenized before any analyzes were performed. Phylogenetic analysis based on BIC was TIM+F+I+G4. DnaSP analysis showed variable ranges for  $h$  and  $\pi$  values for CR were 0.880 and 0.027, respectively, and varied depending on the ocean basin analyzed. For gene flow and genetic differentiation for CR, the sequences were grouped into 4 regions: the Mexican Caribbean Sea (MCS), and Eastern Pacific, Indo-Pacific, and the Mexican Pacific (MP) with a  $F_{ST}$  value of 0.684. The results expand the dynamic and genetic flow knowledge of nesting sites in the MCS and MP area and enable the comparison of Mexican populations' status with previously reported ones. We found values like previous studies (Gaos et al. 2016; Arantes et al. 2020; Espinosa and Zuñiga-Marroquin, 2017). However, we extended the study of MCS areas and present new sequences of different sites from MP.  $F_{ST}$  values clearly showed differences among the 4 groups of sequences based on the origins describes in this work in comparison with other works (Arantes et al. 2020) showing highest and lowers values are correlated among the farthest and nearest areas, respectively. These results support previous studies (Gaos et al. 2016) where there are two distinct evolutionary lineages in the Atlantic and the Indo-Pacific. These results increase our understanding of how this species is distributed around MCS in Yucatan Peninsula and the Mexican Pacific Ocean and how these nesting beaches add new genetic information to study hawksbill evolution and conservation in Mexico and worldwide.

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## MONITORING AND INTENTIONAL CAPTURE PROGRAM OF SEA TURTLES IN A FORAGING AREA IN THE STATE OF RIO GRANDE DO SUL, BRAZIL\*

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The Caminho Marinho Project is an initiative that seeks the harmonious coexistence between human beings and the marine environment, acting directly in the monitoring and conservation of sea turtles in the Southern region of Brazil. Through the intentional capture program for sea turtles, the project records important population data. The Molhes da Barra is a work of marine hydraulic construction of stones, inaugurated in

1915 in the city of Rio Grande, in Rio Grande do Sul, Brazil, formed by two kilometric structures, at the exit to the sea of Lagoa dos Patos (32°09'38" S and 52°05'54" W), largest coastal lagoon in the world. In addition to the economic and tourist importance, these rocky structures have strong ecological importance for several species, as they provide protection and support for the settlement of communities. The objective of the project was to evaluate the presence and abundance of sea turtles through the monitoring of artisanal amateur fishing (incidental capture) and intentional capture events. Artisanal fishermen use cross-mesh nets of 30 meters in length, acting individually along the breakwaters, ranging from 0 to 115 fishermen per day. As this is a fixed-net or cast net fishing gear, fishermen have the opportunity to remove the sea turtle from the net and get in touch with the Project team for stabilization, biometrics, and data collection. The intentional captures are carried out by a specialized team using special nets of 50 meters in length, 3 – 5 – 7 meters in height, and mesh of 30 centimeters. From 2016 to 2021, juvenile green turtles (*Chelonia mydas*) were recorded every year (n (2016) = 14, n (2017) = 66, n (2018) = 86, n (2019) = 72, n (2020) = 75, n (2021) = 73). Coming to a total of 389 registered sea turtles, of these, 279 were recorded through intentional capture and presented the following morphometrics: average size  $42,2 \pm 5,9$ , minimum 33,9 and maximum size 56,6. The total of individuals registered by incidental capture is 107 and presented the following morphometrics: average size  $41,3 \pm 7,0$ , minimum equal to 32.2 and maximum size equal to 69.8. The sea surface temperature ranged from 10° to 27°C, with 14°C being the thermal limit recorded with the presence of sea turtles. All animals underwent biometrics where information about the physical condition of the individuals and conditions of capture were recorded. In addition to an area established as development and feeding ground for the juvenile green turtle (*C. mydas*), the presence of loggerhead turtle (*Caretta caretta*) – 6 animals recorded and several sightings of individual of this species (sighting) – demonstrated the importance of the region for the conservation of sea turtles in the Western South Atlantic. Sea turtles play a significant role in the balance of the ecosystem contributing to the health and maintenance of their habitats. Currently, all species of sea turtles are included as vulnerable on the Red List of the International Union for Conservation of Nature, demonstrating the importance of monitoring their feeding areas.

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## THE VIRTUAL TURTLES PROJECT: A SOLUTION TO MONITOR MARINE TURTLES WITHOUT MARINE TURTLES

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Monitoring marine turtle populations is essential in many situations and in particular to define the conservation status of populations. However, this monitoring is made very complex by the peculiarities of the life history of these species: the juvenile stages and the males are only present at sea and the females are not seen annually on the beaches. The determination of demographic parameters was, until recently, complex and above all marred by biases related to the non-correction of the probability of observation or to the loss of identifiers. Recently new methods of assessing demographic parameters have appeared; one can, for example, determine the clutch frequency by the females while limiting the biases as well as the probability of remigration while taking into consideration habitat quality (and thus food availability). This has improved calculations of adult survival. Individual growth or sexual maturity can also be determined through extensive monitoring. At the same time, monitoring of individual movements by equipping individuals with tracking units has made it possible to better understand the use of space by individuals. Also, work on the nesting beaches has permitted a better understanding of the relationship between the parameters of beach habitat and successful incubation and sex determination. Taking advantage of these significant increases in knowledge, we have developed a spatially explicit model to estimate trends in

populations of marine turtles at the scale of all individuals in a population. In this model, the virtual eggs are incubated on a beach, some survive and give rise to male or female embryos. Young turtles enter the sea and drift with the currents, grow larger, and then reach sexual maturity. They then go to breeding or feeding sites. The females return to lay eggs and the cycle continues. The model reproduces the entire life cycle of tens and even hundreds of thousands of individual sea turtles over several generations. These virtual sea turtles provide a better understanding of the constraints on sea turtles in their natural environment. The model can be configured to incorporate known biological knowledge on these species and model remaining knowledge gaps. This model is now being used to develop risk assessments for sea turtles in the Pacific Ocean under By-catch and Integrated Ecosystem Management (BIEM) Initiative being implemented by the Secretariat of the Pacific Regional Environment Programme (SPREP) through the Pacific-European Union Marine Partnership (PEUMP) programme. Examples on the use of this model for leatherbacks will be presented.

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## REPRODUCTIVE SENESCENCE IN A CAPTIVE GREEN TURTLE POPULATION\*

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Many population models assume the physiological processes that underlie reproductive parameters in adult sea turtles occur independent of time. Under this framework, each adult has an equal contribution to population growth, and reproductive parameters are invariant. Senescence, which describes the negative effects of aging, proposes the contrary; it asserts that late-life is characterized by a progressive and irreversible deterioration in fitness. Changes in reproductive performance may occur in tandem with, or separate from, changes in survival. While birds and mammals have shown evidence of reproductive senescence, there is limited and, in some cases, contradictory information on reptiles, with no dedicated longitudinal study on sea turtles. To fill this knowledge gap, we used longitudinal data for a captive green turtle population over a 27-year period (1973-2001) to investigate the effect of aging on reproductive parameters. Undertaking such a study in wild systems is challenging. Without comprehensive monitoring on nesting beaches, sampling biases – like selective disappearance effects and observer error – can easily obscure any signal in the data. Given green turtles at the Cayman Turtle Center (CTC) are raised under similar conditions, individual variation is mostly driven by genetic factors rather than environmental stochasticity. This, in combination with the high detection rate of nesting females and known age of death for some turtles, which, in a natural setting, would ‘disappear’, make the CTC an ideal environment to begin assessing reproductive senescence. Of the 293 turtles included in our analysis, age was known for 35% of individuals. For the remaining 65% of turtles where exact age was not known, we used the time since first observed nesting as their starting point. We used nonlinear models to evaluate if the cumulative number of eggs per female increased linearly over breeding seasons or resembled a sigmoid curve, with a plateauing at older ages indicating reproductive senescence. We found that the adult age class forms distinct components that vary in their reproductive performance. First-time nesters lay fewer eggs than experienced turtles, but older age classes show a mixed response to aging, with some females capable of maintaining a high level of reproductive output and others laying significantly fewer and smaller clutches. Our results provide insights into how individual variation in reproductive parameters could translate into population-level effects. Although extrapolating our results to a natural system requires extreme caution, we provide a strong starting point to address the effect of aging in a wild sea turtle population. Understanding the



temporal viability of an adult turtle will guide demographic assessments, improve species management plans, and focus conservation efforts.

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## THE USE OF TIME-LAPSE CAMERAS TO DETERMINE PEAK NESTING ACTIVITY AT A REMOTE GREEN TURTLE ROOKERY IN THE NORTHERN TERRITORY, AUSTRALIA

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Turtles in the Northern Territory (NT), Australia, nest along the coast throughout the year but different species have their nesting peaks at different times. On the eastern side of the NT is the Gulf of Carpentaria, a significant area for nesting and feeding green turtles that has not been well-studied. The green turtle in the NT is a species of high cultural significance to many Indigenous communities. To the Mamarika people of Anindilyakwa Country (Groote Eylandt), it is a totem which ensues cultural responsibility. Their concerns about the long-term sustainability of the green turtle population in this region catalysed a project to investigate the phenology and status of green turtles on Groote Eylandt. Key to monitoring a nesting population in this region is understanding the nesting peak, so protection and monitoring efforts can be targeted. Peak nesting periods on beaches in this region are highly region specific, have not been defined, and are assumed from studies conducted elsewhere. We took a multi-pronged approach with the Anindilyakwa Land and Sea Rangers to learn more about the remote turtle population, this included grounding the study in place-based knowledge, tracking the movements of post-nesting turtles using satellite telemetry (n=10) to define their important habitats and daily track counts (up to n=10 days) on four key beaches. We also tested a new technique to remotely monitor turtle nesting by deploying time-lapse cameras (n=14) to record relative track density across multiple months. This method was used to trial an alternative to standardised track counts to determine the nesting peak for the green turtles. Given the remoteness of the nesting beach, the camera method may prove to be a more successful monitoring tool for ongoing nest monitoring than traditional labour-intensive methods, particularly in logistically challenging environments such as many parts of the NT. Tracking information provided key information on post-nesting movements of turtles nesting on Groote Eylandt. Post-nesting movements highlighted the importance of surrounding foraging habitats within the Anindilyakwa Sea Country and adjacent regions within the Gulf of Carpentaria. This study is a comprehensive initial step in understanding the green turtle nesting population in Anindilyakwa Country. It serves to address key questions raised by the local Aboriginal community and scientists about relative abundance and peak nesting times. We acknowledge a key limitation of this study is its brevity however, we conclude the following, the study highlights the potential for time-lapse cameras in understanding nesting population dynamics in areas where long-term monitoring is not feasible due to its remoteness, challenging logistics and limited capacity. By looking at the images over months and hopefully years, we will learn peak nesting periods for multiple turtle species which are critical knowledge gaps for improving turtle management.



## NESTING RANGE EXPANSION OF LOGGERHEAD TURTLES IN THE MEDITERRANEAN: PHENOLOGY, SPATIAL DISTRIBUTION AND CONSERVATION IMPLICATIONS

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Global warming is affecting habitat quality and availability on our planet and some species are predicted or already being observed to change their distribution range. Marine turtles are a particularly interesting case to study in this respect, since they have already survived and adapted to several important climate change events throughout their >1 million years of evolutionary history and they colonized tropical and subtropical nesting habitats around the world notwithstanding their natal philopatry. However, current climate change is happening at a much faster rate and is expected to have profound effects on the adaptability of sea turtles whose life history is characterized by longevity, late age of maturity and temperature dependent sex determination. It seems that loggerhead turtles have already started to expand their nesting range into the western Mediterranean, which has only been known to host sporadic nesting events but reports of nesting activity have been increasing since the 2010's. Here we compile information on nesting activity from four countries surrounding the Western Mediterranean and collected data on loggerhead turtle nests between 2010 and 2020 to provide an exhaustive overview on the phenomenon of emerging new nest sites for loggerhead turtles. The number of recorded nests has increased drastically since 2013 from 1-3 nests/year to a record number of 79 registered in 2020. The nests are unevenly distributed over the coasts of Spain, France, Italy and Tunisia with most nests occurring on the coasts of the Tyrrhenian Sea. A hotspot analysis identified beaches in SW Italy, SE Sardinia and NW Tunisia with statistically significant clustering of nests. Within these hotspots four beaches had nests regularly identified at least four out of the five last years, three in the Cilento Park (SW Italy) and one in Tunisia. Despite not showing hotspots yet, nesting events are increasing rapidly in Spain since 2014. Metadata for the nests were also collected and analysed and show mostly correspondence to nesting phenology of Eastern Mediterranean

rookeries whereby inferred sex ratios were balanced as opposed to the prospected feminisation of sea turtle nesting populations under climate change. Median hatching success of naturally incubating, non-manipulated nests was 78.1%, highlighting the suitability of the emerging nesting beaches. Yet these beaches are already under high tourist pressure and subject to intense coastal development, so that females have a hard time to select a suitable site to lay their eggs, nests are destroyed by mechanical beach levelling and emerging hatchlings are disorientated by artificial lights. Thus, while this study reveals the unique opportunity to witness and study an ongoing new colonisation process in loggerhead turtles, it also calls for urgent proactive conservation actions to mitigate these threats and allow the turtles to establish new nesting colonies.

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## GENETIC ASSIGNMENT OF JUVENILE GREEN TURTLES IN THE CENTRAL PACIFIC USING MIXED MOLECULAR MARKERS\*

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As adults, green turtles (*Chelonia mydas*) forage in shallow water and are the most commonly encountered and photographed of all sea turtles. However, as juveniles inhabiting offshore waters they are quite rare, even compared to other marine turtle species. Consequently, there are many unanswered questions about the distribution and ecology of the early life history stages of green turtles in the open ocean, and this is a major knowledge gap for a functionally important and endangered marine mega-herbivore. To better understand the stock composition of juvenile green turtles we obtained 46 young individuals, 27-78 cm in curved carapace length, caught by Hawaii- and American Samoa-based pelagic longline fishing vessels across large areas of the North and South Central Pacific. We genotyped these at nine microsatellite loci and one mitochondrial DNA marker, and used a baseline of 1,043 nesting female green turtles from beaches across the Pacific for population assignment and mixed-stock analysis. By analyzing both marker types jointly we were able to increase power and genetically resolve ten baseline stocks of nesting females with mean self-assignment and simulated accuracies of 75-97%. Overall, turtles originating from East, West, and Central Pacific breeding populations were major components of the bycatch, suggesting that the geographic ranges of these populations overlap across large tracts of ocean during their first years of life.

## **TAKING STOCK OF CRITICALLY ENDANGERED HAWAIIAN HAWKSBILL TURTLES: BREEDING SEX RATIOS AND FEMALE NESTING FREQUENCIES INFERRED FROM GENETIC RELATEDNESS**

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Hawksbill turtles (*Eretmochelys imbricata*) are endangered across their circumtropical species range. In Hawaii, the breeding population is particularly small, where there are typically less than twenty nesting females observed every breeding season, and less than fifty nests laid. The data for this small population is incomplete, however, because nesting beaches in the Hawaiian islands are remote, sparsely distributed, and hard for observers to access. Consequently, nests from unknown females are often discovered. Here, we used a custom PCR-based targeted genome resequencing assay and genetic relatedness analysis to better understand nesting trends, assigning unknown nests to known females. In addition to identifying unknown mothers, evidence suggests that some hatchlings from different mothers were half siblings. By reconstructing paternal genotypes we were able to indirectly count the number of breeding males and further corroborate this observation of polygyny, giving us a first indication of the breeding sex ratio for this population.

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## **SEA SCAPE GENETICS AND THE SPATIAL ECOLOGY OF JUVENILE GREEN TURTLES IN THE SOUTHWEST INDIAN OCEAN\***

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Understanding how ocean currents impact the distribution and connectivity of marine species provides vital information for the effective conservation management of migratory marine animals. Here, we used a

combination of molecular genetics and ocean drift simulations to investigate the spatial ecology of juvenile green turtle (*Chelonia mydas*) developmental habitats and assess the role of ocean currents in driving the dispersal of green turtle hatchlings. We analyzed mitochondrial(mt) DNA sequenced from 358 juvenile green turtles, and from eight developmental areas located throughout the Southwest Indian Ocean (SWIO). A mixed stock analysis (MSA) was applied to estimate the level of connectivity between developmental sites and published genetic data from 38 known genetic stocks. The MSA showed that the juvenile turtles at all sites originated almost exclusively from the three known SWIO stocks, with a clear shift in stock contributions between sites in the South and Central Areas. The results from the genetic analysis could largely be explained by regional current patterns, as shown by the results of passive numerical drift simulations linking breeding sites to developmental areas utilized by juvenile green turtles. Integrating genetic and oceanographic data helps researchers to better understand how marine species interact with ocean currents at different stages of their lifecycle and provides the scientific basis for effective conservation management.

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## **SLOW AND STEADY WINS THE IN-WATER PHOTO-ID CATALOG RACE TO 300 INDIVIDUAL HAWAIIAN HAWKSBILLS: IT ONLY TOOK NEARLY A QUARTER OF A CENTURY!**

**Cheryl S. King.**

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The Hawaiian archipelago, one of the most isolated island chains in the world, consists of 137 islands that span ~2,400 kilometers. There are primarily two species of sea turtles that inhabit nearshore habitats for foraging and that also nest in Hawai'i: the more common green sea turtles (*Chelonia mydas*) and critically endangered hawksbills (*Eretmochelys imbricata*). This study has utilized photo-identification (PID) methodologies to collect information on the population dynamics of these rare hawksbills for over twenty years, one of the longest-running PID projects worldwide. What makes this project different than most PID efforts is the analysis of both rear and front flipper scale patterns in addition to the head scales that are standard with sea turtle PID studies. This "every scale tells a tale" technique has been essential to allow the use of more photographers' diverse images that range widely in quality and views of the turtles. Citizen scientists (community members and visitors) have contributed a significant amount of sightings to add to our targeted research results. Sampling efforts over time, locations and photographers ranged widely, but the compiled results still provide the most significant amount of information known about Hawaiian hawksbills in their marine environment (1998-present). The number of individuals in the statewide catalog more than tripled after [www.HIhawksbills.org](http://www.HIhawksbills.org) was launched in February of 2016 and @hihawksbills social media started in November of 2019. Wildbook's Internet of Turtles, iNaturalist, SciStarter, and Anecdota online platforms also help promote this Hawaiian Hawksbill Conservation project and gather sightings. Through 2021, >400 photographers have contributed >2,500 confirmed sightings of 301 unique hawksbills, all with various metadata: date, time, location, depth, habitat, behavior, and reaction to human presence. Since our Turtle Team members reside on Maui, the majority of the individuals have been documented there (Maui= 117, O'ahu= 71, Hawai'i Island= 60, Kaua'i= 32, Lana'i= 14, Molokai= 2, Kaho'olawe= 1, Ni'ihau= 1, and the Northwestern Hawaiian Islands= 3). All individuals from each island were given numbers, plus each photographer who submitted a unique individual to the catalog was given the opportunity to choose a name for it. Sighting rates of individual hawksbills ranged widely from 1-447 within <1-21 year spans. Size class compositions varied by island. Statewide, 184 (61.1%) were juveniles, 58 (19.3%) were subadults, 57 (18.9%) were adults (29 females, 28 males), and 2 (0.7%) were unknown. Hawksbills were sighted in diverse habitats foraging on a variety of species of algae, coral, sponges,

crustaceans, invertebrates, and fish. Some hawksbills displayed no visible reaction to humans, while others exhibited flight behavior. Survival threats include fishing gear interactions, harassment, habitat degradation, marine debris entanglements, shark bites, and boat strikes. Twelve catalogued individuals were known to have died, with others suspected to be deceased due to their poor physical condition or lack of being re-sighted after a long period of time. Despite some data collection limitations, the discovery curve continues to rise; therefore more knowledge will be gained as this insightful 24-year collection expands with the goal of assessing population status and trends for management purposes.

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## STATISTICAL ESTIMATION OF STAGE-AND-SEX-STRUCTURED POPULATION DYNAMICS MODELS FOR THE GREEN SEA TURTLES IN THE OGASAWARA ISLANDS, JAPAN\*

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The Ogasawara islands in Japan are important rookeries for green sea turtles in the northwest Pacific Ocean, and the local people have traditionally harvested them. Although the Ogasawara population of green sea turtles had dramatically declined due to past overexploitation and it had once faced a risk of extinction, some protection and management measures seems to have led to a steady recovery in the population. In order to achieve a balanced management between conservation and sustainable use, it is necessary to carry out population assessments and develop resource management strategies based on these assessments. However, the quantitative evaluation of population status and associated modelling work have not yet been undertaken. To this end, we developed a stage-and-sex-structured population model by taking account of three life stages (recruitment as hatchlings, juvenile and adult stages) as well as the density-dependent stock-recruitment relationship. For statistical model fitting, we employed time series data of green turtle nests and hatchlings from coastal surveys conducted continuously during the breeding season since 1975. Biological parameters such as initial depletion level, resilience, a recruitment survival rate and proportional parameters related to monitoring were estimated as free parameters using a composite likelihood function, while the carrying capacity and reproduction rate at the pre-exploitation were estimated by solving mathematical equations and juvenile and adult survival rates were assumed as 0.9 and 0.95, respectively. The estimation results showed that the model used in the analysis fitted the data well. Although the adult population is estimated to exceed at least 50% of the environmental carrying capacity, the extent of recovery depends on the assumptions of the model, including the assumed survival rates between the juvenile and adult stages. The model is able to incorporate a possible reproduction characterized by the temperature-dependent sex determination to account for a possible impact of environmental changes and by density-dependent encounter rates between adult male and female individuals. Furthermore, the model can be used as an operating model for stochastic simulations to develop candidate management procedures under the framework of management strategy evaluation.

## **BIOELECTRICAL IMPEDANCE ANALYSIS ACCURATELY ESTIMATES ADIPOSE TISSUE MASS IN GREEN TURTLES (*CHELONIA MYDAS*)\***

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Sea turtle populations are endangered worldwide due to anthropogenic impacts. The loss of fat reserves (i.e., adipose tissue mass) in individual turtles is linked to environmental stress and diseases, and is considered an indicator for population health. Bioelectrical Impedance Analysis (BIA) has emerged as a portable technology that accurately estimates adipose tissue mass in fishes and humans, showing potential for field-based studies in sea turtles. Our aim was to adapt BIA as a field-based method for adipose tissue mass estimation in juvenile green turtles (*Chelonia mydas*). To do so, we assessed precision (i.e., reproducibility) and the impact of likely field variables on BIA measurements, and determined device accuracy using computed tomography (CT) scans as a comparison method. Measurement reproducibility and the effects of temperature (20-30°C), time post-prandial (i.e., time after feeding), and time spent out of the water were assessed on 35 juvenile turtles using multi-frequency BIA. BIA measurements were then used to estimate fat-free mass, which is used to calculate adipose tissue mass. Adipose tissue mass estimations were compared to those determined from CT scans on 49 additional turtles. A deep learning model, based on convolutional neural networks, was trained for automated CT image processing. Results showed that BIA measurements were accurate and precise. Precision was highest (0.9-2.3% median coefficient of variance) when standardising the technique to the turtles' preferred thermal range (25-30°C). A Bland-Altman plot (95% levels of agreement) revealed a mean difference (i.e., error range) between the predicted and the measured adipose tissue mass of 0.1-0.6%. In comparison, weight as the sole predictor for adipose tissue mass resulted in a mean difference of 17.8%. We present the first BIA calibration study in sea turtles, and our standardised protocol provides a reliable estimation of adipose tissue mass in green turtles. This valuable information on individual health status can inform conservation management decisions on a population level.



## IDENTIFYING NESTING STOCK ORIGIN OF HAWKSBILL TURTLES (*ERETMOCHELYS IMBRICATA*) THROUGH DNA EXTRACTION OF ILLEGAL TORTOISESHELL PRODUCTS\*

Erin L. LaCasella<sup>1</sup>, Michael P. Jensen<sup>2,3</sup>, Christine A. Madden Hof<sup>3</sup>, Ian P. Bell<sup>4</sup>, Amy Frey<sup>1</sup>, and Peter H. Dutton<sup>1</sup>

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For centuries, hawksbill turtles (*Eretmochelys imbricata*) have been exploited for their beautiful shell, known as bekko or tortoiseshell, to make products such as earrings, bracelets and hair combs. Over the last 150 years, studies have estimated that millions of hawksbills have been harvested, resulting in detrimental declines in populations across the globe. With the widespread movement of such products, it is important to trace back where the turtles originated from and identify which nesting populations are most affected by this trade. In this study (LaCasella et al. 2021), we developed an effective and standardized method to extract DNA from tortoiseshell products, which are typically a difficult tissue type to successfully obtain quality DNA. Using a commercially available kit, this new extraction method ensures consistency that that can be easily followed and scaled up for conservation forensics. While following detailed procedures and implementing quality control, DNA was extracted from 13 tortoiseshell products acquired from markets in Papua New Guinea and the Solomon Islands. We successfully amplified ~800 base pairs of the mitochondrial DNA (mtDNA) control region with primers LCM15382 and H950g (Abreu-Grobois et al. 2006) using PCR methodologies. Each sample was then sequenced on a genetic analyzer, aligned and edited using the software Geneious. Haplotypes were determined for each sample by comparing the produced sequences to a reference library of published and unpublished haplotypes identified throughout the Pacific. Six haplotypes were identified within these tortoiseshell products. We directly compared our haplotype frequencies with potential source populations identified throughout the Indo-Pacific, to determine the stock origin of the samples. Our results indicate that the majority (73%) of our samples originated from turtles from the Solomon Islands. Three of the haplotypes identified have only been found in foraging populations, which indicates there are undocumented nesting populations that exist in the region. Extensive sampling of all potential source populations is extremely important to fill baseline information gaps which will allow for more precise statistical analysis to be conducted. Our study demonstrates the effectiveness of using a standardized protocol to extract DNA from tortoiseshell products to determine the nesting stock origin of these turtles. We are encouraged these methodologies can be used worldwide to help inform management and conservation efforts to help tackle the illegal tortoiseshell trade.

## **ASSESSING SEA TURTLE INCUBATION TEMPERATURES OVER 100-YEARS OR MORE**

**Jacques-Olivier Laloë<sup>1</sup>, Nicole Esteban<sup>2</sup>, and Graeme C. Hays<sup>1</sup>**

<sup>1</sup>*Deakin University, Australia*

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There is widespread interest in sea turtle incubation conditions because the combination of temperature-dependent sex determination and climate warming may lead to highly female-biased hatchling sex ratios (Hays et al., 2017). Adding to these concerns is the fact that warmer incubation temperatures may increase hatchling mortality (Laloë et al., 2017). We show how incubation conditions can be assessed at nesting sites across breeding seasons and how these data can be used to hindcast likely incubation conditions over the last century (Laloë et al., 2021a) as well as predict future conditions based on IPCC climate warming scenarios. We show the importance of nest-shading and rainfall in cooling nests (Laloë et al., 2021b) and we consider how extreme marine heatwaves can impact incubation conditions (Hays et al., 2021). We highlight how we are using these methodologies in ongoing monitoring work around the world. Long-term monitoring of incubation temperatures and hatchling success may be a simple procedure for assessing when increased temperatures are starting to negatively impact populations.

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## **FORTY YEAR ANALYSIS OF SEA TURTLE NESTING BEHAVIOR ON A LARGE GULF OF MEXICO ROOKERY**

**Jake Lasala, Kristen Mazzarella, and Melissa Macksey**

*Mote Marine Laboratory, United States of America*

Longitudinal data sets are essential to the study of imperiled organisms. This is especially true for species that have long life history stages that are largely difficult to study due to their location, such as marine turtles. Examining trends over time can reveal changes that might not be evident from a standard census. These data are critical for conservation managers to properly assess recovery. For marine turtles, one of the best ways to estimate population size is to examine nesting numbers and extrapolate how many individuals are actively nesting within a community. Further productivity metrics examine nest success (successful nests / not-successful attempts), nest hatch success (number of eggs hatched) and emergence success (number of hatchlings leaving the nest). These values frequently fluctuate from year to year due to nest placement, predation, and storm activity. The Sea Turtle Conservation and Research Program of Mote Marine Laboratory has been monitoring nesting turtles on 35 miles of coastline on five islands off the Florida Gulf coast since 1982. STCRP has documented over 133,000 turtle crawls and almost three million hatchlings leaving the beach. In this talk, we will compare nesting success and nest productivity across the five islands over this 40-year dataset and discuss how nesting trends in this region are actively contributing to this imperiled population.

## **OVERVIEW OF MARINE TURTLES NESTING AT THE JEEN WOMOM COASTAL PARK, TAMBRAUW REGENCY, PAPUA BARAT**

**Deasy Natalia Lontoh<sup>1</sup>, Yairus Swabra<sup>1</sup>, Petrus Batubara<sup>1</sup>, Abraham Leleran<sup>1</sup>, Johni Mau<sup>1</sup>, Siis Werimon<sup>1</sup>, Tonny Duwiri<sup>1</sup>, Arfiandra Wanaputra<sup>1</sup>, Manjula Tiwari<sup>2</sup>, and Fitriyanti Pakiding<sup>1</sup>**

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The northwest coast of the Bird's Head region of West Papua provides important nesting habitat for leatherback, hawksbill, olive ridley, and green turtles. Historically, over 20,000 nests were laid annually in the region. In the last few decades, however, Bird's Head's marine turtle population has declined sharply. Updated information on marine turtle nesting numbers and patterns is needed. Nesting activities are concentrated at Jeen Yessa (formerly Jamursba Medi) and Jeen Syuab (formerly Wermon) Beaches. Both nesting beaches are part of the Jeen Momom Coastal Park; an MPA established in 2017 to protect these critical nesting areas. Based on daily nest count data between 2018 and 2021, we determined each species' average nest numbers and spatial and temporal nesting patterns. The Coastal Park remains a critical nesting area, with 3767 marine turtle nests on average per year. Most are leatherback nests (60.5%), followed by olive ridley (28.8%), while green and hawksbill turtles make up approximately 8.0% and 2.7% of the total nests, respectively. Between the 1980s and 2011, the leatherback population declined by 78 % at Jeen Yessa and has continued to decline. Compared to historical nest numbers, the number of green turtle nests has declined by 98%. Although the number of hawksbill nests has increased steadily in the last four years, the number of hawksbill nests has declined by 93% from the 1980s. Historical olive ridley nest numbers for Jeen Yessa and Jeen Syuab are not available; however, compared to nests numbers reported in the early 2000s, we found that olive ridley nest numbers were 1.2 times greater. Spatial and temporal distributions of nesting activities vary among species, but nesting occurs year-round. The Jeen Momom Coastal Park is the most important nesting site for western Pacific leatherbacks. Currently, the State University of Papua (UNIPA) leads a holistic leatherback conservation effort in the Coastal Park. UNIPA partners with the local community and beach owners to protect nesting leatherbacks and maximize hatchling production by implementing science-based nest protection methods.

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## **ADAPTIVE IMMUNE GENE EVOLUTION AND FIBRO-PAPILLOMATOSIS IN JUVENILE GREEN TURTLES (CHELONIA MYDAS) AND LOGGERHEADS (CARETTA CARETTA)\***

**Katherine R. Martin, Katherine L. Mansfield, and Anna E. Savage**

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Characterizing polymorphism at the major histocompatibility complex (MHC) immune genes is key to understanding the vertebrate immune response to disease. Despite being globally afflicted by the infectious tumor disease fibropapillomatosis (FP), immunogenetic variation in sea turtles is minimally explored. We sequenced the  $\alpha_1$  peptide-binding region of MHC class I genes (162 bp) from 268 juvenile green (*Chelonia mydas*) and 88 loggerhead (*Caretta caretta*) sea turtles in Florida, USA. We recovered extensive variation (116 alleles) and trans-species allelic polymorphism. Supertyping analysis uncovered three functional MHC

supertypes corresponding to the three well-supported clades in the phylogeny. We found significant evidence of positive selection at seven amino acid sites in the class I exon. Random forest modeling and risk ratio analysis of *C. mydas* alleles uncovered one allele weakly associated with smooth FP tumor texture, which may be associated with disease outcome. Our study represents the first characterization of MHC class I diversity in *C. mydas* and the largest sample of sea turtles used to date in any study of adaptive genetic variation, revealing tremendous genetic variation and high adaptive potential to viral pathogen threats. The novel associations we identified between MHC diversity and FP outcomes in sea turtles further highlight the importance of evaluating genetic predictors of disease, including MHC and other functional markers. Furthermore, our study provides a baseline of immunogenetic diversity in sea turtles that can inform future studies and management of FP-impacted populations.

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## CRITICAL NEST PROTECTION OF LEATHERBACK TURTLES ON TWO REMOTE ISLANDS IN SOLOMON ISLANDS

**Cameron Masakolo<sup>1</sup>, Johnson Haro<sup>1</sup>, Alec Hughes<sup>2</sup>, Robert Howard<sup>2</sup>, John Read<sup>3</sup>, and Katherine Moseby<sup>4</sup>**

<sup>1</sup>*Tetepare Descendants Association*

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<sup>3</sup>*Ecological Horizons*

<sup>4</sup>*University of New South Wales*

Solomon Islands is a key foraging and nesting site for the critically endangered population of Pacific leatherback turtles (*Dermochelys coriacea*). The species have seen a dramatic decline over the past 40 years with the Western Pacific population declining 80% since the 1980s due to threats from bycatch, direct harvest, coastal development, and climate change. The latter being notably felt in Solomon Islands on the remote islands of Rendova and Tetepare, where, according to rangers on the ground inundation of nests from rising sea levels has increased over the past decade and is having a devastating impact on nests. Community rangers on both islands have therefore been working to protect the nests for almost 20 years, given the importance of Solomon Islands as a key nesting area. In 2002, the Tetepare Descendants Association (TDA) was established by the community with tasks including protecting nesting leatherbacks. This soon extended to the nearby island of Rendova, where Baniata and Havila communities also took a strong interest. While the work over the years depended on available funding, community rangers, during the peak nesting season from November-January and off season in May-July have been patrolling the beaches nightly for nesting females, which they tag, measure, count the eggs and move to a hatchery due to the threat of flooding. On Tetepare, since 2002 rangers recorded an average of 30 nests per year, (excluding 2013-2017 and 2018-2019), ranging from 2-82 nests. On Rendova, between 2003-2004, 235 nests were recorded on Baniata and Havila beaches combined, and from 2012 to 2015, an average of 59 nests were observed. In more recent surveys on Rendova (2018-2021), community rangers have reported an average of 192 nests annually. Since 2018, on both Tetepare and Rendova islands, hatching success in insitu nests averaged 46.6% and in hatchery raised nests average 51.6%. Hatching success in nearby PNG varies from 37-87% and in Indonesia 9.3-44.7%. While the past several years have been very positive, in terms of the numbers of nests, especially on Rendova, the threat of nest flooding and beach erosion still remains, requiring almost all nests having to be relocated to hatcheries. This in itself can bring risks, with potential damage to the embryo from movement to the issue of temperatures in the nest chamber not mimicking natural nests, which can affect the sex ratios of the clutch or if too hot, be lethal to the embryos. Community rangers have recently installed temperature loggers in the hatcheries and will be analysing the data at the end of the 2021/2022 peak nesting season to ensure the nest environment in the hatchery is

natural as possible. In addition, rangers will look at beach temperatures to monitor any impacts climate change may have on the temperature of in-situ nests. More work is also required on establishing robust figures on the number of abundance of nesting females and not just number of nests.

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## AGE PREDICTION OF MARINE TURTLES WITH AN EPIGENETIC CLOCK\*

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Age is a fundamental life history trait that correlates with fecundity and mortality rate. The distribution of ages in wild animal populations is therefore an important determinant of population growth rate. Unfortunately, a practical or non-lethal method to determine the age of most animals, including marine turtles, is not available yet. In this study, we present an epigenetic clock for marine turtles. This was developed using DNA extracted from skin biopsies of 63 known age (1-43 years) green sea turtles (*Chelonia mydas*) from the Cayman Islands and Réunion and further validated using wild recaptures of known time intervals. The model uses the collective DNA methylation at 18 CpG sites, also known as an epigenetic clock, and estimates age with a median absolute error of 2.1 years (2.8% of lifespan). We further used the epigenetic clock to predict the age of 250 green sea turtles across a wide range of carapace lengths (36-108cm) at Ningaloo, Western Australia. These turtles were found to be between 1-49 years of age. This enabled us to develop a population specific growth curve with predicted ages and lengths of the turtles. This study is the first to develop an epigenetic clock in a reptile and further demonstrates age can be predicted from DNA methylation across a broad variety of vertebrate species. It is also the first to use a molecular based method to characterise an age profile for a population.

## SEA TURTLE OF THE WORLD GENOME PROJECT\*

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The seven sea turtle species inhabit all ocean basins except the polar regions, and are classified as threatened or data deficient by the IUCN. This is overwhelmingly due to human activities. Conservation efforts have demonstrated that sea turtle populations can be restored through evidence-based management. Genomic tools offer a growing source of evidence for sea turtle management, including provenance of illegally traded sea turtle products, estimating population abundances, age determination, adaptive capacity, and identifying distinct population segments. However, the lack of authoritative reference genomic resources for most sea turtle species means we cannot take full advantage of this rich source of biological information. The Sea Turtles of the World Genome Project is constructing high-quality, annotated reference genomes for all sea turtle species. Building on our recent completion of leatherback and green turtle genomes, we have again joined forces with the Vertebrate Genomes Project, with support from the Revive & Restore Wild Genomes Grant, to create analogous high quality, publicly available reference genomes for the five remaining sea turtle species. Sequencing for this project commenced in late 2021, and we predict that all genomes will be ready for public use by early 2023. These genomes will serve both to amplify the utility of existing genetic resources for conservation (e.g., identifying stock structure and human impacts), and create new applications that are currently not possible (e.g., tracking abundances and age structures, developing in-situ and ex-situ interventions to mitigate nest destruction and climate change impacts). We aim to directly engage the global community to ensure the resources are equitably accessed and employed for conservation applications through the existing Marine Turtle Genomics working group and organization of a special workshop in a future ISTS edition.

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## NOAA FISHERIES NATIONAL PROTECTED SPECIES TOOLBOX INITIATIVE

**Erin McMichael<sup>1</sup>, Mridula Srinivasan<sup>2</sup>, and Patrick Lynch<sup>3</sup>**

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Recent technological advances allow a significant increase in our understanding of the biology and conservation needs of many marine taxa, including sea turtles. Data collected with these new technologies often require new methods of analysis and novel ways to combine the data with existing data sets to allow a more comprehensive assessment of sea turtle populations and/or management units. Recognizing the need for new user-friendly approaches that can utilize disparate data sources from multiple platforms and new technologies, NOAA Fisheries has supported efforts to develop, evaluate, disseminate and utilize new analytical and diagnostic tools that improve protected species assessments, inform management decisions,



and aid in the recovery and conservation of sea turtles and other marine taxa protected under the Marine Mammal Protection Act and Endangered Species Act. Since its inception in 2013, this effort, known as the National Protected Species Toolbox (NPST) initiative, has supported 19 projects, including many that are multidisciplinary, multi-species, and multi-taxa, to address NOAA Fisheries regional and national priority areas of concern for protected species within the US Exclusive Economic Zone. Here, we highlight 12 projects that focus on sea turtle population assessments, abundance and density estimations, habitat and environmental characteristics, spatial distribution and movement, and human impacts. We also highlight selected tools created through the initiative. These tools are housed within NOAA's Fisheries Integrative Toolbox, a centralized, interdisciplinary, web-based portal that also contains toolboxes for fish, ecosystem, human dimensions, and other protected species that can be used for a variety of applications including data preparation, modeling, and forecasting. Projects supported by the NPST initiative have resulted in the creation of a variety of products and applications that expand sea turtle assessment capabilities and ultimately support the recovery and conservation of these protected species.

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## **SURVEYS OF THE ANGOLAN COAST UNCOVER THE LARGEST OLIVE RIDLEY NESTING POPULATION IN THE ATLANTIC**

**Miguel Veríssimo Morais<sup>1</sup> and Manjula Tiwari<sup>2</sup>**

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Understanding the abundance and distribution of species is important for designing and prioritizing conservation and management activities. Despite numerous existing studies on the distribution and status of sea turtles, we still lack knowledge about certain populations, especially in geographically data-poor regions such as Angola, which is considered to be the southernmost range for nesting sea turtles in the eastern Atlantic. This study provides an overview of the status, size and distribution of the olive ridley *Lepidochelys olivacea* population nesting along the coast of Angola, and its relevance in the context of other olive ridley turtle nesting populations in the Atlantic. Aerial and ground surveys were conducted along 1,410 km of Angolan coastline and daily beach monitoring over 53.9 km of seven permanent study sites at a range of latitudes during 2011–2020. Angola was found to host the largest olive ridley turtle nesting population in the Atlantic, and the largest non-arribada population globally. Although the population appeared relatively stable, the pressures from various threats on land (e.g. consumption of turtles and eggs) and at sea (captures in fishing gear) necessitate the development of a comprehensive management plan, improved and strengthened legislation and law enforcement, and a cohesive approach to conserving all sea turtle species in Angola.

## POPULATION VIABILITY ANALYSIS TO EXPLORE SOURCES FOR DEMOGRAPHIC RESCUE OF THE IMPERILED EAST PACIFIC LEATHERBACK SEA TURTLE

**Anna Antonia Ortega<sup>1,2</sup>, Nicola J. Mitchell<sup>1</sup>, Philip S. Miller<sup>3</sup>, Sean A. Williamson<sup>2,4</sup>, and George L. Shillinger<sup>2</sup> Antonia**

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The East Pacific subpopulation of leatherback sea turtles (*Dermochelys coriacea*) is Critically Endangered as a result of historic unsustainable egg harvest and ongoing fisheries bycatch. Despite extensive and sustained conservation efforts, the most optimistic prediction is that the subpopulation will be functionally extinct by 2080. While population viability models have suggested that the population might recover with a reduction in fisheries bycatch and improved protection of nesting beaches, complementary actions such as egg translocation and headstarting may also be needed. Crucially, the opportunity to implement egg translocations into the East Pacific hinges on whether any *D. coriacea* subpopulation outside of the East Pacific can withstand large egg harvests over at least several decades. Demographic and life history parameters for eighteen global *D. coriacea* stocks were sourced from the 2020 ESA Status Review for *D. coriacea* and inputted into stochastic population viability analysis models using Vortex software (Version 10.3.6.0). Each stock was subject to 25 years of annual harvest of 2000, 4000, or 6000 eggs to simulate levels needed to recover the East Pacific subpopulation through egg translocations. Results indicated that no stock could withstand egg harvest at these levels, but doomed nests from up to four stocks could provide an alternate source for egg translocations and headstarting actions. The success of any identified source will depend upon consideration and further research surrounding egg transportation, genetic compatibility, local adaptations, best practices for egg translocation and headstarting, cost, and sociological factors. By continuing this work to answer knowledge gaps involved in the implementation of additional ex situ conservation actions, the Eastern Pacific leatherback and other similar imperiled species may be stabilized and recovered before extirpation.

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## EXPLORING THE GENETICS OF ‘LOST YEARS’ SEA TURTLES IN THE GULF OF MEXICO\*

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Genetic analyses have greatly enhanced our understanding of sea turtle populations, though the majority of our baselines to date have focused on nesting beaches. While rookery sampling is invaluable for defining management units and serves as a reference for mixed in-water aggregations, we still lack genetic data from across life stages to evaluate connectivity from nesting beaches to offshore habitats and eventual foraging sites. Here, we leveraged a unique sampling opportunity for oceanic-stage juvenile sea turtles in the northeastern Gulf of Mexico to 1) identify the ~800-bp long-fragment mtDNA haplotypes represented in these ‘lost years’ individuals; 2) estimate the source rookeries contributing to the region; 3) examine the haplotypes we found in a broader phylogenetic context. We genotyped 35 oceanic-stage juveniles (straight

carapace lengths: 14.7-24.5 cm) from three species: green turtles (*Chelonia mydas*; n=30), hawksbills (*Eretmochelys imbricata*; n=3), and loggerheads (*Caretta caretta*; n=2). We estimated green turtle rookery contributions using many-to-many Bayesian mixed stock analyses (MSA) that incorporated probabilities based on rookery size and transport via ocean currents. The majority of the green turtles sampled were Cm-A1.1 (n=20) and Cm-A3.1 (n=7), with lower frequencies of Cm-A18.1 (n=2) and Cm-A28.1 (n=1). Our MSA results indicate a high likelihood that oceanic-stage green turtles we sampled originated from nesting beaches along the coast of Mexico, with smaller contributions from Costa Rica and Suriname. For the other two species, the Ei-A23 haplotype we identified for all three hawksbills is found in source rookeries in Mexico and the Greater Caribbean, while the loggerhead haplotypes included one in the CC-A4 lineage which suggests connectivity with rookeries in Brazil. Finally, we assembled a phylogeny based on the long-fragment mtDNA haplotypes available in the literature and on GenBank for all seven sea turtle species, totaling over 700 sequences, to characterize the lineages found in oceanic-stage turtles in the Gulf of Mexico and assess gaps in life-stage information across ocean basins (Atlantic, Mediterranean, Pacific). The species-level relationships we found are consistent with those presented by other researchers. Outside of the single-basin Kemp's ridleys (*Lepidochelys kempii*) and flatbacks (*Natator depressus*), there were few basin-specific clades when all life stages were included, and several haplotypes were identified in juveniles dispersing outside of the basin that was associated with rookery samples. Most of the long-fragment haplotypes we encountered in Gulf of Mexico oceanic-stage turtles have only been observed in the Atlantic basin to date, with the exception of one loggerhead haplotype that has also been identified in Mediterranean juveniles. Our results highlight the need for updated genetic analyses across nesting beach projects to refine mixed stock analyses, as well as a broad-scale lack of data from in-water juvenile sites and their corresponding genetic composition, especially for the oceanic stage.

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## DIVERSITY AND NATAL ORIGINS OF OLIVE RIDLEY SEA TURTLE (*LEPIDOCHELYS OLIVACEA*) IN SOUTHERN BRAZIL\*

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The olive ridley sea turtle (*Lepidochelys olivacea*) is the most abundant sea turtle in the world. It is classified as Vulnerable by the IUCN Red List, and as threatened by the Brazilian government's environmental agency. In Brazil, the main rookery of this species are located at Sergipe and north of Bahia state (both in northeast Brazil), sporadic nesting has also been observed in Espírito Santo, Ceará, Rio de Janeiro and Rio Grande do Norte. Olive ridleys feed along the entire Brazilian coast and over the last ten years the number of *L. olivacea* strandings has increased in Rio Grande do Sul – located in the southern region – likely due to rising population numbers and fisheries bycatch. Although it was reported that the number of nesting olive ridleys in Brazil is increasing, it has been shown that the genetic diversity of rookeries remains low, and this might have a limited impact on adaptive potential compared to MHC and other nuclear genomic regions of populations to accelerated environmental changes. Despite its high abundance, little is known about the genetic characteristics of olive ridleys in the Atlantic, and there is no information on feeding grounds; such information is fundamental to support management and conservation plans of this species. The present work aims to evaluate the genetic diversity, population structure and possible natal origins of

olive ridley turtles from a feeding area located in South Brazil. Tissue samples were collected from fifty-nine *L. olivacea* specimens between 2013 and 2021, from Lagoa do Peixe (31°15'S; 50°58'W) to Chui (33°44'S; 53°22'W), at the southern portion of Rio Grande do Sul. Genomic DNA was extracted and an 800bp fragment of the mitochondrial DNA control region was amplified via Polymerase Chain Reaction (PCR). Haplotypes were classified according to previously described haplotypes available in GenBank. Haplotype and nucleotide diversities were calculated in Arlequin and a haplotype network was constructed using the median-joining network method in PopArt. Probable natal origins were estimated through Mixed Stock Analysis (MSA) using Bayes. Source populations considered possible contributors to the studied aggregation correspond to all Atlantic olive ridley rookeries with haplotype frequencies described in literature. The olive ridley aggregation from South Brazil presented low nucleotide ( $\pi = 0.000142$ ) and haplotype ( $h = 0.0982$ ) diversities; only two haplotypes, F ( $n = 56$ ) and E ( $n = 3$ ), with 1 bp difference between them, were found, both already reported for this ocean. The MSA showed almost equal contributions from rookeries at Sergipe, Bahia, and Surinam (17%), but standard deviations were very high and indicate uncertainty in these estimates. This could be due to the lack of information on the genetic characteristics of other nesting grounds, and we suggest that future studies improve rookery baseline data for olive ridley MSA.

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## OPTIMISING METHODOLOGIES FOR ASSESSING TURTLE NUMBERS IN DRONE SURVEYS\*

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Unmanned Aerial Vehicles (UAVs) facilitate observation of sea turtles in remote locations, and are increasingly used to assess in-water population densities at nesting and foraging grounds, estimate operational sex ratios, or observe breeding behaviour. Using straight-line transect surveys by UAVs in the Chagos Archipelago (hereinafter Chagos) in the Indian Ocean, we assessed density and distribution of green (*Chelonia mydas*) and hawksbill (*Eretmochelys imbricata*) sea turtles across ocean and lagoon habitats, and considered how survey-value could be optimised. Operational protocols followed established methodologies: surveys were conducted at 30 m altitude and 5 m/s speed; transects ran parallel to the shore at increasing distances of 50 m, 150 m, 250 m, and were 1 km in length with 4 km buffer intervals; and three repeats were conducted in fine weather and sea-state only. For image analysis, customised hardware optimise image resolution of 4K footage (computer with graphics cards and 4K monitor). Software was trialled to identify that most-suited to manipulating footage to assist with turtle identification, e.g. changing playback speed, extracting frames. Analysis protocols were developed: carapace morphometrics (straight carapace length and width) were utilised to define set length-to-width ratios of green and hawksbill turtles based on mark-recapture data of genetically-linked turtles in the Seychelles, and applied to turtles to identify species; the smallest adult green and largest immature hawksbill were utilised to differentiate life-stages; and sex was determined by tail length (male tails are notably longer). Species allocation was validated by viewing turtles and accepting or rejecting the assigned species, with 75% accuracy. Sex was only determined for individuals larger than the smallest nesting size. Turtles were classed by certainty of observation (definite, probable or possible) and final analyses were based on definite-only observations.

Population estimates based on visual observation can be affected by availability bias i.e. target species not available to be observed. Environmental conditions that hindered the availability of target species were quantified: turbidity and depth were noted on a scale (1 - 4); and glare and wave swell as percentage cover of area, utilising a known distance-per-pixel from preliminary calibration flights. Of 660 frames which contained turtles, 89% were in shallow, clear environments, and mean glare was 8% and wave swell 3.6%. Availability bias was therefore not corrected for. For population density calculations, the total number of turtles in all repeats was divided by the total available area. The total available area for each transect was determined by removing unavailable area due to glare or unavailable habitat utilising an orthomosaic approach: at 5m/s and 30 m altitude, a resulting 5.4 seconds-travelled moved one frame-of-view. Unavailable area was measured and removed from the total area (transect length x width). UAVs can be used for remote surveys to assess population trends of sea turtles in nesting and foraging sites. We highlight the value of UAV surveys for turtle population assessment, while considering variables to optimise survey techniques and data analysis protocols.

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## USING A PHOTOGRAMMETRIC MARK-RECAPTURE APPROACH TO DENOTE SEA TURTLE ENCOUNTERS WITHIN THE NORTHERN GULF OF MEXICO

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Identifying sea turtles individually is vital to understand their ecological importance within shallow environments and aids towards conservation efforts for these endangered species. In-water intraspecific surveying indicates re-sightings, which allows us to understand site fidelity and habitat preference. Photogrammetry is one method that intraspecifically identifies individuals by denoting unique markings along their body and facial scales. In this study, we used photogrammetry to target loggerhead (*Caretta caretta*), green (*Chelonia mydas*), and Kemp's ridley sea turtles (*Lepidochelys kempii*) from November 2018-July 2021 to contribute to a non-invasive mark-recapture database, as these species are commonly encountered during Stereo-Video Camera System Surveys (SVCS) in the northern Gulf of Mexico. We conducted in-water surveys along 13 shallow artificial reefs (ex: piers, jetties, and cement module pilings) ranging from 6-29 feet within the shelf of the Florida Panhandle via snorkeling using a handheld action camera to collect footage of facial scales to establish individual identity. We concurrently conducted SVC surveys to estimate straight carapace length (SCL) using a specialized software (EventMeasure). Still images of each individual's facial profile were obtained to characterize scale shape, size, and pigmentation. We utilized freely available online programs for photo identification (ex: I3SP). The resighting data was analyzed to understand site fidelity, how individuals may be using multiple sites, and to detect differences in habitat type usage. Out of 107 turtle encounters from November 2018-July 2021, a total of nine turtles were re-sighted: six individuals were re-sighted once, and three individuals were re-sighted with two encounters occurring at Navarre reef, Park East reef, and Navarre Pier. Based on SCL measurements, which ranged between 34.2-59.5 cm, all turtles re-sighted were juveniles. Seven of the individuals were green sea turtles, which were re-sighted at the same artificial habitat in which they were originally encountered. However, the timespan in which each individual demonstrated site fidelity ranged from one day to 5 months. The two other re-sighted individuals were Kemp's ridleys; notably, one turtle migrated 12.2 miles between Navarre reef to Park East reef within one day, whereas another turtle demonstrated site fidelity at Topsail Hill 38 days apart. The Navarre area appeared to be a favored site, as six out of the 21 re-sighting events occurred at Navarre reef and seven of the encounters were observed at Navarre Pier. The ratio of turtle encounters to re-sighted individuals (107:9) demonstrated that site fidelity was low within the artificial



habitats of the Gulf of Mexico. However, the high number of new encounters indicated juvenile turtles were ontogenetically shifting to various habitats. This photogrammetric method has been used by other scientists within the Florida Panhandle for intraspecific identification. Furthermore, it is beneficial for both the researcher and marine turtle, as it is non-invasive and does not require direct in-water capture, which can impose handling stress on both individuals. As few turtles appeared to be residents at the artificial reefs in northwest Florida, it seems likely that juvenile sea turtles were using this habitat as transient resources for rest, foraging, and self-cleaning purposes.

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## NUCLEAR DNA MARKERS PROVIDE FURTHER INSIGHT INTO PACIFIC GREEN TURTLE POPULATION STRUCTURE

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This study builds upon the current understanding of green turtle population structure based on mtDNA in order to more holistically characterize rookeries, examine regional connectivity, and expand our knowledge of green turtle population structure in the Pacific. A total of 1,111 nesting green turtle samples were analyzed with 10 nDNA microsatellite markers from rookeries throughout the Pacific Ocean. Spatial genetic patterns were assessed with discriminant analysis of principal components (DAPC) and isolation by distance was evaluated with estimated effective migration surfaces (EEMS). The data indicated defined structure throughout the Pacific largely in agreement with stock structure and natal homing signals defined



by mtDNA studies, except for some areas including the Central American and Australian continental shelf rookeries, providing evidence of potential male-mediated gene flow. The broad scale pattern for the DAPC analysis showed Hawaii as a distinct central Pacific cluster distinguishing from the eastern and western Pacific rookeries, and revealed the connectivity of rookeries along continental shelves in the eastern Pacific and southwestern Pacific. EEMS results suggested that rookeries from the same continental shelf are more genetically similar to each other than to those on nearby oceanic islands. For example, along the coast of Central America, the genetic dissimilarity between rookeries increased at a rate expected, or less than expected, given the distance between them, while many of the models tested indicated a weak genetic barrier between the Central American continental shelf and the Galapagos and Revillagigedo Islands. In summary, the results of our study provide an ocean-wide rookery baseline dataset for green turtles in the Pacific and prompt reconsideration of how some population units for conservation have been characterized for green turtles by incorporating nDNA variation with previous mtDNA assessments.

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## GENETIC DIVERSITY OF *LEPIDOCHELYS OLIVACEA* IN THE NESTING COLONY LA IXTAPILLA, MICHOACAN

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The Olive Ridley turtle has a pantropical distribution and is the most abundant sea turtle in the world. In the Mexican Pacific there are several beaches of solitary nesting and, until the end of the last century, only in the state of Oaxaca rookeries with “arribadas” were present. But, in 1997 began the first documented arribada of *L. olivacea* in La Ixtapilla at Michoacan state. Currently, this colony is the most recent with an arribada behavior and the third in importance of Mexico, with 30.525 nests on average per year. Since 1997 all the conservation activities are conducted by the Nahuatl indigenous community of La Ixtapilla who wonder, from where those turtles are from. There are no previous genetic approximations from La Ixtapilla that allow establishing its diversity or its possible origin. In this study, genetic diversity analysis was carried out from mitochondrial DNA control region sequences and compared with the genetic data reported of rookeries from Mexico and the world. We found six haplotypes of 400 bp on 42 samples ( $h = 0.598$ ,  $\pi = 0.0028$ ). One haplotype present on two samples is private to La Ixtapilla and contrary to our expectations is one of the oldest (diverged 3.4 million years ago) of the Pacific Ocean. 62% of the individuals of La Ixtapilla have the most frequent haplotype globally, which is found in the North Pacific and the Indian Ocean. Although it is not possible to infer the origin of La Ixtapilla, our results encourage keeping the genetic analysis to monitor the genetic diversity of this species (the basal component of biodiversity), which still has gaps despite the number of turtles sampled around the world (>500).

## USING DATA FROM NESTING BEACH MONITORING AND SATELLITE TELEMTRY TO IMPROVE ESTIMATES OF MARINE TURTLE CLUTCH FREQUENCY AND POPULATION ABUNDANCE\*

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Population abundance data are often used to define species' conservation status. Ideally, abundance estimates should consider all life stages from individuals within a population and be determined for both sexes. However, this can be difficult, particularly for species with high dispersal, and when different life stages and sexes are not equally available for counting. This is the case for marine turtles, which are highly migratory and utilize a variety of areas within a region, being residents to core areas (e.g., foraging areas), but being transient in some locations (e.g., often only being observed once at specific locations), biasing counts, and abundance estimates. As long-lived animals, determining marine turtle abundance across life stages is challenging, especially for those where limited information is available, such as the first few years following hatching. Juveniles and adult males are also more difficult to encounter and count as they remain in the marine environment for their entire life, and do not come ashore like nesting female turtles. As a result, abundance of marine turtles is typically estimated using nesting beach monitoring data such as nest counts and clutch frequency (CF, i.e., the number of nests female turtles lay within a nesting season). However, studies have shown that CF determined solely from nesting beach monitoring data can be underestimated, leading to inaccurate abundance estimates. To obtain reliable estimates of CF for hawksbill turtles in northeastern Brazil, the region with the highest nesting density for the species in the South Atlantic, data from beach monitoring and satellite telemetry were combined from 2014 to 2019. Beach monitoring data with intensive tagging effort indicated the date of first nesting event, while state-space modeling of satellite telemetry data indicated the departure date of turtles, allowing calculations of residence length at breeding site and CF estimates based on internesting intervals. On average, 42 individual nesting hawksbill turtles were encountered each season during the beach patrols with an average of  $124 \pm 8$  (range 114–138) nests per season. In average 41.2% of nesting females were transient (seen nesting only once during the nesting season within our study site). Females were estimated to nest up to six times within the nesting season with CF estimates between 4.5 and 4.8 clutches per female. CF estimates were used to determine the number of nesting females at the study site based in two approaches: considering and not considering transient turtles. We used the total number of nesting females observed by intensive beach monitoring to compare with the estimates based on CF. Our estimates that accounted for transient turtles were much closer to the number of females observed nesting during beach monitoring than the estimates did not account for them. Our study highlighted that the use of CF data to estimate abundance of nesting females based on nest counts should only be used when the whole nesting population assemblage is considered, which should include transient turtles. When sampling a fraction of the population, there is the need to account for biases from transient turtles.

## GENETIC STRUCTURING AND DIVERGENT MEXICAN ORIGINS OF NORTHERN GULF OF MEXICO JUVENILE GREEN TURTLE FORAGING AGGREGATIONS SPANNING TEXAS TO THE FLORIDA KEYS\*

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Mitochondrial DNA markers have elucidated patterns of connectivity between green turtle populations (rookeries) and neritic nursery habitats. However, missing rookery baseline data and haplotype sharing among populations have often impeded inferences, including addressing origins of Gulf of Mexico (GoM) juveniles. We sequenced the mitochondrial control region and informative mitogenomic single nucleotide polymorphisms of juveniles foraging in Port Fourchon, Louisiana (LA, n = 127) and Santa Rosa Island, Florida (SRI, n = 38), along with collecting additional genetic data for previously characterized neritic aggregations in southern Texas (TX, n = 167), St. Joseph Bay, Florida (SJB, n = 174) and southwestern Florida (SWFL, n = 96). We assessed genetic structure among these, incorporating published data from a surface-pelagic aggregation offshore of Louisiana to northwestern Florida (SP, n = 121) and a neritic aggregation from the Big Bend region of northwestern Florida (BB, n = 177). We estimated source contributions to aggregations with new genetic data using a Bayesian many-to-one mixed stock analysis (MSA) approach. Western (TX, LA) and eastern (SJB, BB, SWFL) neritic aggregations were significantly differentiated. SRI had intermediate haplotype frequencies and was not significantly different from aggregations to the west or east. Nonetheless, the strongest shift in haplotype frequencies among proximal sites occurred between SRI and SJB, separated by only 150 km. This reinforces the lack of a geographic yardstick for predicting genetic structuring between foraging aggregations as well as implicating Loop Current dynamics and directed swimming behavior in shaping the distribution of neritic juveniles. Tamaulipas/Veracruz contributions dominated the western aggregations. MSA results indicated more diverse, and disparate, Mexican origins for SJB and SWFL aggregations, with smaller Tamaulipas/Veracruz contributions, but larger inputs from other Mexican nesting populations in Campeche, Yucatán, and Quintana Roo. In contrast to previous results from SJB and SWFL using different baseline populations and sequence data, there was no signal of Atlantic Florida juveniles in any GoM foraging aggregation. This research demonstrates the significance of the entire GoM coast of the United States as nursery habitat for green turtles of Mexican origin and highlights the need for international coordination for management of these populations. Additional mitogenomic sequencing of Mexican, Texas, and Florida nesting populations is warranted to improve resolution in future MSA.

## MITOCHONDRIAL CONTROL REGION AND REPEAT SEQUENCES RESOLVE POPULATION STRUCTURE OF FLORIDA'S GULF OF MEXICO GREEN TURTLE ROOKERIES

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Most green turtle nesting in Florida occurs on the Atlantic Coast. Nonetheless, over the last few decades, surveyors have recorded small but apparently increasing numbers of green turtle nests on the Gulf of Mexico (GoM) Coast of Florida and in the Keys. The genetic and demographic structure among these rookeries is unclear. We sequenced the mitochondrial control region (CR) and the mitochondrial short tandem repeat (mtSTR) in 243 samples from the Dry Tortugas National Park, Marquesas Keys, Sanibel Island, Manasota Key-Sarasota County beaches, and northwestern Florida. Samples yielded 9 CR haplotypes and 19 combined CR-mtSTR haplotypes. Pairwise haplotype frequency differentiation and AMOVA supported the presence of at least five genetic groups: 1) Dry Tortugas National Park, 2) Marquesas Keys, 3) southwestern Florida, 4) eastern panhandle (Tyndall Air Force Base and eastward), 5) western panhandle (Panama City Beach and westward). The presence of two distinct genetic clusters in northwestern Florida with minimal haplotype sharing was unexpected given a single cohesive loggerhead turtle subpopulation across this region. Biopsies collected between 2010 and 2016 (3 from the western cluster and 6 from the eastern cluster) matched 2017 nest samples from each respective region and were absent in the other. Several of the most common Gulf Coast barrier island haplotypes also occur among Atlantic Coast rookeries. This haplotype sharing invokes the possibility that GoM beaches were colonized by Florida Atlantic Coast females and is supported by telemetry data indicating intra-seasonal nesting on both the Gulf and Atlantic Coasts. Two haplotypes found in 10 Gulf Coast nests have yet to be detected on the Atlantic Coast of Florida and may represent colonization from Mexican GoM rookeries. Continued genetic sampling and telemetry data collection are needed to test the consistency and range of female nest site fidelity and potential management unit boundaries. Mitochondrial repeat data resolved some population relationships that were ambiguous based solely on control region sequences. We recommend utilizing the repeat array where control region haplotype sharing has interfered with inferences of stock structure and population origins in foraging aggregations.

## **GENETIC CHARACTERIZATION OF A PREVIOUSLY OVERLOOKED GULF OF MEXICO GREEN TURTLE (*CHELONIA MYDAS*) NESTING POPULATION ALONG THE PADRE ISLAND NATIONAL SEASHORE (PAIS) IN TEXAS, USA**

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Five marine turtle species are known to nest in the northwest Gulf of Mexico along the southern coast of Texas, including the surrounding areas of the Padre Island National Seashore (PAIS), an undeveloped barrier island in the southeastern USA. This important nesting habitat has been surveyed and monitored since 1986 and yet nesting trends and genetic population stock structure are poorly understood for green turtle (*Chelonia mydas*) populations along the Texas coast. Although nesting is infrequent, it has increased in recent years. Without historical data, it is unclear whether this nesting population is recovering from an exploited, once abundant nesting population or represents a spread of nesting from Mexico. Green turtle tissue samples were collected from nesting females, dead-salvaged hatchlings and unhatched embryos from individual nests during each nesting season consistently between 2002-2020 and sent to the Southwest Fisheries Science Center for genetic analysis and long-term storage. DNA was extracted from 73 tissue samples and the mitochondrial DNA control region was PCR amplified and sequenced. Sequences were aligned and edited using the software Geneious and haplotypes were identified for each individual by comparing resulting 817 bp standardized sequences to a reference library of published and unpublished haplotypes identified throughout the Atlantic and Gulf of Mexico (GOM) (<https://accstr.ufl.edu>) to characterize genetic diversity and stock structure. Six haplotypes were identified within the dataset. Of note was the presence of CmA8 in Texas, absent from other rookeries in the GOM but common and characteristic of rookeries in the South Atlantic. Results of ongoing stock structure analysis provide insights into demographic and historic connectivity with other rookeries in the region. Our results also fill an existing gap in current rookery baseline data, which are required to identify nesting origin of foraging aggregations, strandings and fishery by-catch. This study is the first genetic characterization of a previously overlooked green turtle nesting population along the Texas coast in the northwest Gulf of Mexico.

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## **USING DISTANCE METRICS AND TEMPORAL TRENDS TO REFINE MIXED STOCK ANALYSES**

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Sea turtle foraging areas are often composed of multiple source populations, resulting in mixed stock aggregations. The distribution of individuals among foraging areas can be influenced by source population distance, oceanographic currents, seasonality, and stochasticity. An important variable to consider is the size of source populations, used to estimate the likelihood of contribution to mixed stocks. For the green sea turtle (*Chelonia mydas*), several nesting sites in the Northwest Atlantic and the Caribbean have seen increases in the number of nests laid over the past three decades, while other sites have experienced different

trajectory patterns. Though previous studies assessed how contributions from source populations may fluctuate over time, it is still unclear how variation in rookery size impacts site-specific contributions to mixed stock analysis. This study presents how changes in source population size affect the estimates of two mixed stock aggregations over a 15-year period. We used samples collected from juvenile green turtles in 2003-2005 and 2016-2018 at two foraging sites on the east-central coast of Florida, USA. We amplified a fragment of the mitochondrial DNA and compared haplotype frequencies from each site to available data from other mixed stocks and nesting populations in the Northwest Atlantic and the Caribbean. We modified the code from the 'mixstock' package in R to simultaneously incorporate the relative geographic distances among all sites along with the rookery sizes into a single many-to-many mixed stock model. This is a critical advance in mixed stock analyses as previous studies could only incorporate distances into many-to-one models. We identified 11 haplotypes in samples from the Indian River Lagoon (IRL), with CM-A03 (45.8%) and CM-A01 (30.6%) being the most common, and six haplotypes in the Trident Basin with CM-A01 (53.1%) and CM-A03 (34.6%) also as the most common. Compared to the standard many-to-many model using only source size to our modified model, estimates from Tortuguero decreased from 18% to 8% for the IRL and from 17% to 6% for Trident. In comparison, South Florida estimates increased from 8% to 16% and 42% to 55% for IRL and Trident, respectively. Results from our modified model evaluating variation in source size over time indicated that mean estimates increased from 2003-2005 to 2016-2018 for the IRL from Campeche (12% to 25%) and Tamaulipas in Mexico (8% to 20%) and decreased from Tortuguero, Costa Rica (27% to 8%). For Trident, there was an increase in contributions from South Florida (28% to 55%) and a decrease from Tamaulipas (18% to 11%) and Quintana Roo, Mexico (21% to 10%). Our analyses highlight the need to incorporate weight by distance (or similar weighting metrics) and temporal resampling in addition to source size in many-to-many models. Moreover, we emphasize the need for consistent and periodic reports of basic biological data from index nesting populations (e.g., annual number of nests, area surveyed).

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## **WHERE ARE THE LEATHERBACKS? NEST NUMBERS AT SANDY POINT PLUMMET TO LOWEST LEVEL IN 40 YEARS\***

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<sup>3</sup>*National Oceanic and Atmospheric Administration, La Jolla, CA, United States of America*

Since 1977, research and monitoring of the leatherback population has occurred at Sandy Point National Wildlife Refuge, St. Croix, Virgin Islands. The intensive program has been conducted at this northern Caribbean Index Site by many dedicated volunteers, researchers and local management agencies over the years; it is now managed by the US Fish and Wildlife Service. In 2020, the lowest number of leatherback nests was recorded at the refuge since the program began, reinforcing concern that leatherbacks are declining here and elsewhere in the Western Atlantic. During the 1980s, efforts became consistent with each turtle being identified with tags and tracked through the nesting season. Nest numbers in that decade averaged 146 nests per year. The number of nests continued to rise through the 1990s (avg = 389 nests/year) and reached a high in the 2000s (643 nests/year). Since 2010, nest numbers (and the number of individual turtles) began gradually declining to the lowest number recorded – 31 nests in 2020 laid by 5 individuals. There was a slight rebound in 2021, to 98 nests, and 27 individual turtles. Here we investigate life history parameters of leatherbacks nesting at Sandy Point, and find that an increasing remigration interval (now averaging 4.5 years), and potential lowered reproductive output (fewer nests per female) may be contributing to this trend. However, the recruitment of neophytes has not diminished – approximately 25%



of all turtles encountered are first-time nesters; this has been consistent over the years. In addition, in 2019, we observed two turtles that first nested 31 years ago – a global record for leatherbacks for reproductive longevity. We are still actively researching age to maturity using genetic methods (since 2008), and once that analysis is complete, we may see increased time to maturity in recent years. We compare trends at Sandy Point with other leatherback rookeries, and offer reasons for the decline. These include lowered forage quality and changing oceanographic conditions, migration to other nearby nesting beaches, and increased mortality on migration routes and in foraging grounds. We thank all volunteers who have helped track Sandy Point leatherbacks over the years.

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## **A NEW MOLECULAR METHODOLOGY FOR MONITORING THE MEDITERRANEAN GREEN TURTLE POPULATION AND THE ISRAELI BREEDING STOCK**

**Yaron Tikochinski**

*Ruppin Academic Center, Israel*

The threatened green turtle population of the Mediterranean has been monitored in the past two decades by molecular means, mainly the genetic variation of the mtDNA control region. A new method of haplotypic determination enabled an international collaboration identifying four management units in the region's population. However, since this approach ignores the male contribution to the population variability, we had to develop a nuclear-based DNA typing. RAD-sequencing of 50 Mediterranean green turtles, including the members of the Israeli breeding stock, yielded about 30,000 informative SNPs. This vast amount of genetic variation enabled calculating the genetic distance between any two individuals, identifying relatives like siblings and half siblings. Next, we have developed an algorithm to select for the 40 SNPs that generated the best representation of the genetic distances. We could design a Mass-Array chip for rapid and inexpensive individual genotyping. We have already used our findings for paternity determination of hatchlings in the breeding stock. We intend to use the chip methodology for understanding and monitoring the Mediterranean population gene flow for management and conservation purposes. The same method can be later applied for sea turtle populations worldwide.

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## **INVESTIGATING THE RELATIONSHIP BETWEEN CHELONIID HATCHLING SEX AND GROWTH RATE IN CAPTIVE CONDITIONS**

**Emily Turla and Jeanette Wyneken**

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This project investigates whether sex influences initial size and growth rates of loggerhead (*Caretta caretta*) and green sea turtle (*Chelonia mydas*) hatchlings. Emergent hatchlings were collected from in situ nests during the 2012-2020 nesting seasons and were raised in captivity for a minimum of 11 weeks until their sex could be identified by laparoscopic examination. All hatchlings were reared in the same conditions (e.g., water quality, amount and type of species-specific food offered) and measured upon collection and again at weekly intervals until offshore release. Measurements taken were straight carapace length notch-to-tip (SCL), straight carapace width (SCW) and mass. It is well documented that initial carapace dimensions of turtles are influenced by incubation conditions in that warmer conditions typically produce smaller hatchlings and cooler conditions typically produce larger hatchlings. Initial mass of hatchlings has

previously been found to be similar between hatchlings with different carapace dimensions and can be explained by the difference in residual yolk mass. Interestingly, our results found that for both loggerhead and green turtles, male hatchlings are larger in SCL, SCW, and mass. Analyses of growth rate data found that green turtle males grow faster in mass, females grow faster in SCW, and there is no difference in growth rate for SCL. Male loggerhead hatchlings grow at a faster rate than female hatchlings in all measurements. Increased size and growth rate could result in a reduced risk of predation by gape limited predators and may increase the chance of survival during critical periods when mortality risk is high. These findings reveal that there are species-specific differences in early growth and there are sex-specific differences in initial size and growth rates, which may result in trade-offs for survival between sexes.

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## FIRST AGE-SPECIFIC VITAL RATE ESTIMATES FOR AUSTRALIA'S ENDEMIC FLATBACK SEA TURTLE (*NATATOR DEPRESSUS*) BY SKELETOCHRONOLOGY\*

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Here we present the first-ever skeletochronology-derived age and growth data to address a major data-gap for flatback turtles. Using a valuable collection of bone samples gathered from across northern Australia, we applied skeletochronology with the goals of characterizing the length-at-age relationship, establishing baseline growth rates from the hatchling to adult life stages, and producing empirical estimates of age-at-and size-at-sexual-maturation (ASM, SSM) for the flatback species. We analyzed 74 flatback humeri and report all values as the range, mean and SE; 27 hatchlings/post-hatchlings (CCL: 6.0 to 17.0 cm,  $9.4 \pm 0.64$  cm), 15 neonatal to juvenile stage (CCL: 17.6 to 36.6 cm,  $26.6 \pm 1.5$  cm), and 32 putative adults (CCL: 78.8 to 96.0 cm,  $87.5 \pm 0.8$  cm). Turtles were recovered from Western Australia (n=48), Eastern Australia (n=13), the Gulf of Carpentaria (n=5), Northern Territory (n=3), and unknown locations (n=5). We identified and measured a total of 690 lines of arrested growth (LAGs) from among the 73 fully processed humeri, with turtles retaining between 0 to 42 ( $9.3 \pm 1.3$ ) LAGs. We identified rapprochement growth patterns in 29 of the 73 turtle bones, thus indicating the onset of sexual maturity. Estimates for ASM ranged from 12.0 to 23.0 years ( $16.3 \pm 0.53$ ), and SSM ranged from 76.1 to 94.0 cm CCL ( $84.9 \pm 0.90$ ). The maximum observed reproductive longevity, estimated as the number of LAGs observed beyond the onset of maturity (rapprochement) was 31 years for a male flatback estimated to be 45 years old. The growth of flatback turtles, modeled by both a GAMM smoothing spline and a von Bertalanffy curve, showed similar patterns of monotonic growth, slowing around age 10, and further as turtles approached maturity at a range of body sizes. Mean SSM obtained from rapprochement (84.9 cm CCL) corresponded with a spline-predicted ASM of 18 (95% CI: 16 to 24) years. The total range of mean ASM estimates corresponded to the mean nesting sizes reported in the literature and ranged from 86.4 to 94 cm CCL, yielding ASM estimates of 24+ years. The bootstrapped von Bertalanffy growth model-estimated the ASM, at the mean rapprochement SSM, to be  $16.3 \pm 0.05$  years (95% CI: 12.8 to 27.7 years), the estimated upper size limit,  $L_{inf}$ , was  $89.2 \pm 0.04$  cm (95% CI: 85.5 to 95.9 cm), and the intrinsic growth rate parameter,  $k$ , was  $0.185 \pm$

0.0004 (0.16 to 0.22). To test back-calculated ages against known chronology, deceased adult females (n=4) were recovered from rookeries with mark/recapture studies. The paired-samples Wilcoxon rank sum test found that the LAG-back-calculated CCL estimates and observed CCL measurements (mean value of all paired samples used for each individual turtle) were not significantly different ( $p = 0.875$ ), providing initial validation for the use of LAG-based skeletochronology size estimates, as well as supporting the assumption of annual LAG formation in flatback humeri. This first skeletochronology study for flatback sea turtles has generated valuable empirical estimates for ongoing conservation and management efforts nationwide. Additional specimens are in prep to consolidate with the present findings.

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## SEA TURTLE HYBRIDS: ANCIENT AND RECENT HYBRIDIZATION PATTERNS IN THE SOUTH ATLANTIC OCEAN FROM WHOLE GENOMES\*

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Reconstructing past events of hybridization and population size changes are required to understand speciation mechanisms and current patterns of genetic diversity, and ultimately contribute to species' conservation. Five of the seven extant sea turtle species are known to currently hybridize, especially along the Brazilian coast where some populations can have ~32%–42% of hybrids. Although observed today, it is not clear what role hybridization played in their evolutionary diversification. Here we will report phylogenomic results and preliminary population-level analysis from whole genome resequencing data for four sea turtle species from two populations from the South Atlantic Ocean (Brazil and São Tomé e Príncipe) to understand the role of hybridization in shaping the genomes of these ancient species. We will report past and current hybridization patterns, demographic changes, and estimate population's genetic load which may influence future conservation planning. Our results raise questions whether current hybridization events should be considered as a part of these species' evolutionary history.

**OVERVIEW OF A FACILITATED DECISION-MAKING WORKSHOP PROCESS, INFORMED BY POPULATION VIABILITY ANALYSIS, TO PROVIDE RECOMMENDATIONS ON IMPLEMENTING EX SITU CONSERVATION ACTIONS TO PREVENT THE EXTINCTION OF THE EASTERN PACIFIC LEATHERBACK POPULATION**

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The Eastern Pacific Leatherback Population (EPLB) is considered critically endangered and current model projections predict functional extinction by 2080. The known key threats are; primarily, fisheries bycatch of adult and sub-adults, and secondarily, reduced hatchling output from nest loss. An action plan to address these two threatening processes has been developed. However, the situation for the EPLB is yet to improve, as cumulative human impacts from the aforementioned threats and others continue to increase. Although it is critical that conservation efforts to address the threats continue and are further reinforced, we compiled information on potential complementary actions that could be implemented to attempt to prevent extinction of the population. The potential additional actions include (1) translocation of eggs from other subpopulations and/or (2) captive rearing and release (head-starting) of juvenile turtles into the Eastern Pacific. In collaboration with the International Union for Conservation of Nature (IUCN) Conservation Planning Specialist Group (CPSG), we designed a two-step workshop process to develop recommendations around whether or not these ex-situ actions should be implemented. More than 50 stakeholders participated in the workshop process. Population viability analysis (PVA) models were constructed using Vortex software to simulate the current trajectory for the population and future trajectories under different conservation scenarios. We will present the key outputs of these models. Stakeholders developed a shared set of recommendations using model outputs to inform their decisions. The key recommendation was that, given current uncertainties concerning the practicability and likely impact of ex situ management activities on EPLB recovery, such actions should not be embarked upon at the current time, though they merit further examination and study. The stakeholders identified a range of research questions and overarching research themes for further investigation that could reduce uncertainties surrounding the ex situ management approaches proposed. These research questions will be discussed in the context of their utility in improving the ability of conservation practitioners to assess potential benefits of various sea turtle conservation actions.

## SOCIAL, ECONOMIC AND CULTURAL STUDIES

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### RETOMALA ON-LINE: A PERTINENT SOLUTION DURING PANDEMIC OUTBREAK\*

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In 2020, RETOMALA – the Latin American Sea Turtle Specialist Meeting (in Spanish: Reunion de Especialistas Latinoamericanos en Tortugas Marinas) was organized to be part of the annual International Sea Turtle Symposium in Cartagena, Colombia. However, due to the outbreak of the pandemic the ISTS Board of Directors took an appropriate decision to postpone our annual in-person meeting. Under an environment of uncertainty the Latin American Sea Turtle community decided to keep in touch during those times. We used the social platform provided by Instagram® (@Retomala2021), using the Live-IGTV tool, to carry out 16 interactive conversations with sea turtle experts or researchers between April and July-2020. Under a relax environment and few pre-determined questions and live-questions generated by the audience, the conversations in Spanish were transmitted weekly using mentioned platform and were no longer than 60 minutes. Varied themes were covered and were prioritized topics that have links with Latin American Sea Turtle Projects. In total, the RETOMALA Instagram account increased its audience from 183 followers to 1,205. The 16 interactive conversations reached in total 1,450 likes, 2,994 views, and 10,011 of engagement based on their posts. We reached an audience that were not aware about RETOMALA before these conversations, and to date are very responsive followers and supporters. This latter among general (non-turtle researchers or activists) and youth audience. These sessions were very welcomed among the usual RETOMALA's attendees and among new followers. Some limitations of our initiative were identified. For example, in-person meetings allow us to record the presentations and plenary to be further discussed with our community. However the participation during these on-line conversations was limited to "live viewers" that coincided with the transmission. This latter was identified as a strong limitation because of poor internet access and reach of social media platform in working areas (e.g. nesting beaches in Latin America) is very limited and with poor quality (we had in the past illiterate participants). Time difference also impacted our initiative, as some comments in the posts were about questions that were not answered during the transmission. Covid-19 pandemic has changed our society and the ways that we interact as communities. Indeed, this is a learning process which motivated us to find different and better ways to keep in touch as community. We, as ISTS, need to face this challenge and provide our best responses to cope this pandemic and worldwide new scenario. We thank the presenters who generously provided their time and expertise during our on-line sessions.

## **INVESTIGATING BEHAVIOURS FROM ILLEGAL SEA TURTLE TRADE IN CABO VERDE BY ADAPTING A COMPREHENSIVE BEHAVIOURAL MODEL\***

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Successful conservation outcomes often depend on influencing human behaviours that negatively impact biodiversity, such as unsustainable and/or illegal wildlife trade (IWT). Inclusive psychology models that examine what motivates these behaviours have been underutilised in IWT contexts. This research aimed to understand the drivers of illegal harvesting and consumption of sea turtles on Maio, Cabo Verde (West Africa), by combining broad semi-structured interviews (N=20) with questionnaires (N=325) adapting the Comprehensive Action Determination Model, a theoretical framework developed in environmental psychology. Local behavioural motivations seem to have changed over time, but some key beliefs remained intact. Structural equation modelling showed intention to consume turtles has always been influenced by positive attitudes towards consumption, but interviews suggest personal and social norms may also become relevant to mitigating this behaviour. The same seems true of harvesting, reportedly performed mostly by young men looking to sell turtle by-products. Beliefs underlying both behaviours are different, so outreach should carefully address each. These results further demonstrate how conceptual models developed in other scientific areas can be adapted to expand the interdisciplinary tools available to conservation practitioners. Embracing such approaches is crucial towards addressing the socially intricate and contextual behaviours of illegal wildlife trade.

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## **ADDRESSING GAPS IN SOCIO-ECONOMIC AND NATURAL ASSETS TO HALT MARINE TURTLE EGG POACHING: A LIVELIHOOD FEASIBILITY STUDY IN THE TURTLE ISLANDS WILDLIFE SANCTUARY, PHILIPPINES**

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Straddling the border between the Philippines and Malaysia, the six islands of the Turtle Islands Wildlife Sanctuary (TIWS) form part of the largest green turtle nesting ground in ASEAN (UNESCO World Heritage Centre, 2015). Due to various identified vulnerabilities, such as an increasing human population within TIWS, the severe impacts of the monsoon season, a perceived decline in natural resources, and the remote geographical location, the Turtle Islands municipality is highly susceptible to external shocks. To increase the resiliency of community members, livelihood interventions took place in the islands in the late 1990s and early 2000s, however, none of these were sustainable in the long-term: International NGOs took



on the difficult task to substitute the income people lost when the Philippine Wildlife Resources Conservation and Protection Act was passed in 2001, prohibiting the previously regulated egg trade. Livelihood interventions, such as a live fish food project, were introduced. However, with a municipal poverty incidence of 35.1% (2015) (PSA, 2019) and a pressing need for basic services, these interventions were set up to fail in the long-term. This paper analyses the current status of socio-economic and natural assets in the TIWS to identify the gaps in basic services and sustainable livelihood interventions. The Sustainable Livelihood Approach Framework was selected as the basis for assessments of existing Assets, the Vulnerability Context, Structures, Processes, and Livelihood Strategies. By drawing on rare empirical data from local communities in the TIWS, the study also provides unique insights into current practices of illegal wildlife trade as a result of livelihood constraints and a lack of enforcement. In total, 87 respondents were interviewed between November 2018 and June 2019, rating the five Assets of the framework from 0-100%, with 100% signifying complete availability of and access to these assets. For the entirety of TIWS, Human Capital was rated with 43%, Natural Capital with 49%, Financial Capital with 19%, Physical Capital with 49%, and Social Capital with 46%. However, these values differed significantly among the islands. On average, Taganak Island displayed the strongest and Lihiman Island the weakest set of Assets. Moreover, the majority of respondents confirmed not to have access to loans or micro-credits, offering little opportunities for business development. Current livelihood strategies identified were fishing and small-scale trade, with a high potential for eco-tourism, as the year-round nesting of green turtles makes the TIWS a prime turtle watching site. The results of the Anonymous Survey revealed that the illegal turtle egg trade is still on-going, driven by poverty. Numerous recommendations were given per Asset on how to close identified gaps in basic services in the long-term, of which many have great potential to be turned into livelihoods. This study contributes to the research on the effects of poor health and a lack of education on livelihoods (Hossain, 2013; Mattos et al., 2021; Mphande, 2016; Tran et al., 2020), addressing precarious socio-economic conditions to curb illicit activities (Lamichhane et al., 2020), and advancing marine turtle conservation (Mejías-Balsalobre et al., 2021; Veríssimo et al., 2020).

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## STILL SWIMMING WITH TURTLES – THE IMPACT OF COVID-19 ON TOURIST-TURTLE INTERACTIONS IN THE MALDIVES\*

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The Republic of Maldives is a small island nation in the Northern Indian Ocean famous for its marine biodiversity. With over one million visitors per year in 2018 and 2019, more people than ever were visiting the country. With them came an increase in activities such as snorkeling and diving, which was abruptly reduced to zero in 2020 due to travel restrictions related to the COVID-19 pandemic. Marine megafauna such as whale sharks, manta rays and sea turtles are usually major attractions for many visitors. In this study we recorded sea turtle behaviour during in water surveys and analysed photographic snapshots and video footage of sea turtles taken for a photo identification study in 15 locations in Lhaviyani atoll over a time period of 11 months pre- and 12 months post-pandemic related lockdown. We recorded habitat use, intra-species interactions as well as the reaction to the presence of humans. This reaction was classified based on the estimated distance of observer to the turtle, and the observation in behavioural response of the turtle ranking from no change of behaviour, to alertness and avoidance behaviour to flight. The overall reaction was assigned to one of six categories (1 = no reaction, 6 = most severe reaction). Results from the pre-pandemic phase showed a significantly lower number of severe behavioural responses in locations with a

high daily probability of human-turtle interactions, pointing towards a habituation effect. Post-pandemic a significantly higher number of adverse reactions was recorded in these previously high interaction/low reaction areas. Several scenarios are discussed as a potential cause for the observed change, including the overall reduction in interactions, an increase in negative interactions related to illegal take of turtles, as well as a turnover in the resident turtle population.

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## **REPRESENTATION OF TOURISM EXPERIENCES AT THE VELAS KASAV MAHOTSAV (TURTLE FESTIVAL), INDIA, ON MOBILE SOCIAL MEDIA**

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The Velas Kasav Mahotsav (Turtle Festival) is an annual event showcasing the nesting and hatching of olive ridley turtles on the Konkan coast of Maharashtra, India. Olive ridley turtles are classified as a vulnerable species globally and are listed on Schedule I of the Indian Wildlife (Protection) Act, 1972 in India. Threats to olive ridley turtles nesting in Maharashtra include bycatch, illegal take of eggs, and coastal development. A joint venture among the local NGO Sahyadri Nisarga Mitra, state Forest Department, village of Velas, and Gram panchayat (village governing council), the Kasav Mahotsav is promoted as community-based ecotourism with benefits for sea turtle conservation and local livelihoods. However, ecotourism in India is poorly regulated and receives little institutional support, making it challenging for initiatives to fulfill the principles of ecotourism as defined by The International Ecotourism Society (TIES). To assess the promotion and experience of the Velas Kasav Mahotsav through the lens of ecotourism, we used qualitative content analysis to examine the features of social media posts made from public accounts of ecotourism providers and tourists using the hashtag #velasturtlefestival in the year 2019. Tour providers portrayed the Festival as an opportunity to observe sea turtles and experience nature (images), but were often self-promotional (captions) and did not highlight other ecotourism experiences such as engaging with local communities. Tourists showcased their experiences waiting at the hatchery and while exploring the beach and surrounding environment (images) and shared information about sea turtle biology and threats (captions), with the majority of posts demonstrating a positive sentiment. Our findings indicate that tourists represent the Velas Kasav Mahotsav as a memorable and educational experience, despite relatively few people personally experiencing a hatchling release, on Instagram. However, some of the ecotourism principles are not reflected on social media posts by tourism providers or tourists. The local economy and longevity of turtle conservation efforts in Velas may rely on tourists visiting the region for reasons other than the Kasav Mahotsav alone.

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