ADVANCES IN THE BIOLOGY AND CONSERVATION OF MARINE TURTLES

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Mariana M. P. B. Fuentes and Richard D. Reina
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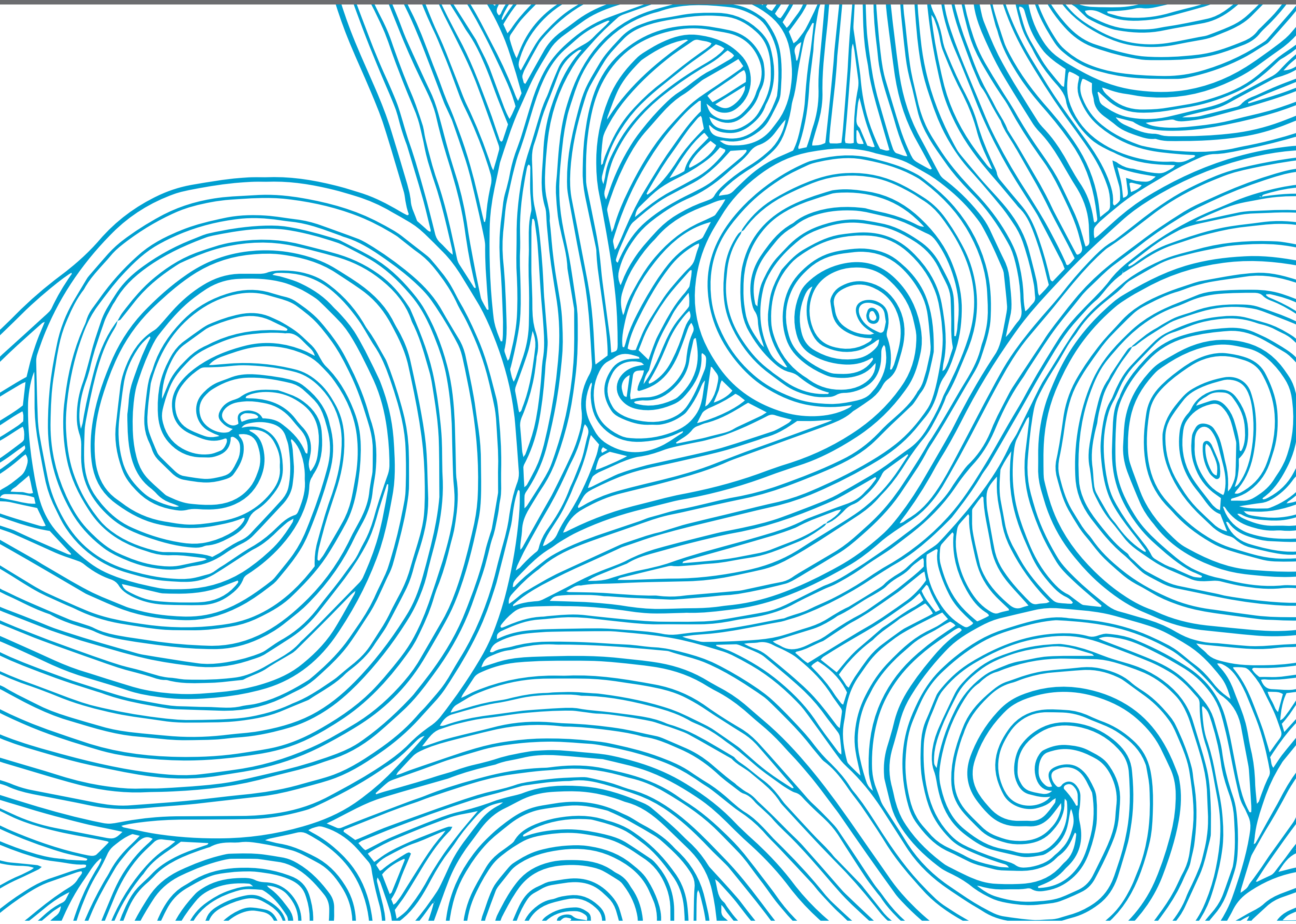

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ADVANCES IN THE BIOLOGY AND CONSERVATION OF MARINE TURTLES

Topic Editors:
Sara M. Maxwell, University of Washington Bothell, United States
Peter H. Dutton, National Oceanic and Atmospheric Administration (NOAA), United States
Sabrina Fossette-Halot, Department of Biodiversity, Conservation and Attractions, Australia
Mariana M. P. B. Fuentes, Florida State University, United States
Richard D. Reina, Monash University, Australia

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Editorial: Advances in the Biology and Conservation of Marine Turtles

Sara M. Maxwell[1,2]*, Annette C. Broderick[3], Peter H. Dutton[4], Sabrina Fossette-Halot[5], Mariana M. P. B. Fuentes[6] and Richard D. Reina[7]

[1] School of Interdisciplinary Arts and Sciences, University of Washington Bothell, Bothell, WA, United States, [2] Department of Biological Sciences, Old Dominion University, Norfolk, VA, United States, [3] Centre for Ecology and Conservation, University of Exeter, Penryn, United Kingdom, [4] NOAA Fisheries, Southwest Fisheries Science Center, La Jolla, CA, United States, [5] Marine Science Program, Department of Biodiversity, Conservation and Attractions, Kensington, WA, Australia, [6] Marine Turtle Research, Ecology and Conservation Group, Department of Earth, Ocean, and Atmospheric Science, Florida State University, Tallahassee, FL, United States, [7] School of Biological Sciences, Monash University, Clayton, VIC, Australia

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Graeme Clive Hays,
Deakin University, Australia

Reviewed by:
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***Correspondence:**
Sara M. Maxwell
smmax@uw.edu

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Editorial on the Research Topic

Advances in the Biology and Conservation of Marine Turtles

Marine turtles have been the subject of research over many decades, inspired by their unique life history and necessitated by their declining populations from a suite of human impacts including direct harvest, bycatch in marine fisheries, pollution, and climate change. Despite this, much about marine turtle biology has remained a mystery (Godley et al., 2008; Rees et al., 2016; Wildermann et al., 2018), but the rate of scientific discovery is increasing rapidly. As research techniques and conservation practices expand, the marine turtle research community has kept abreast of these developments and their application to marine turtles. In this special Research Topic, researchers submitted articles related to cutting-edge work in biology and conservation. The resulting 10 articles bring new insights across marine turtle movement, conservation, and methodological and analytical techniques, as well as other understudied areas and issues.

MARINE TURTLE MOVEMENT AND CONSERVATION

Contributing researchers filled critical knowledge gaps on movement of marine turtles across life history stages and sexes. Bond and James revealed the pre-nesting and mating movements and habitats of both male and female leatherback marine turtles (*Dermochelys coriacea*) in the North Atlantic Ocean. They identified both the timing and potential location of leatherback mating areas in coastal waters adjacent to nesting beaches and characterized a relatively confined geographic area likely used by both males and females to forage before the nesting season.

Dawson et al. used satellite tracking to reveal critical habitat for internesting olive ridleys (*Lepidochelys olivacea*) from a key nesting site located adjacent to an estuary heavily-used for shipping, fishing and other human activities. Diving data indicated that bottom-based fisheries were likely to pose significant threats as turtles rested on the estuarine bottom between nesting bouts, and satellite tracking data was used to design a marine protected area in the region to protect turtles during this important life-history stage.

Finally, Robson et al. developed a method to determine where and when to release rehabilitated turtles in relation to currents and ocean features to give turtles the greatest chance of encountering suitable habitat for survival. Using Western Australia as a case-study, the authors found a region along the northwest coast that was most conducive to release, as turtles would be quickly transported to deep, offshore waters for most of the year. Given the extensive resources put into rehabilitation—particularly of marine turtle species or populations—such information is critical for the success of rehabilitation efforts.

METHODOLOGICAL AND ANALYTICAL TECHNIQUES

Contributors to this topic explored new analytical techniques or new applications of existing techniques to better understand the biology of marine turtles, and thus improve conservation and management practices. Komoroske et al. conducted a thorough review of how genetic techniques have advanced our understanding of marine turtle population boundaries and connectivity, evolutionary history, phytogeography, life history, population vital rates, and human threats to marine turtles. Each of these aspects has the potential to contribute to the conservation of marine turtles. For example, increased resolution of genetic markers allowing differentiation between populations has been critical for defining population units to conserve, and genetic fingerprinting has been used as a tagging tool and to census the number of breeding males (Komoroske et al.). Additionally, transcriptomics and epigenetic markers help us understand responses to environmental perturbations such as rising nesting beach temperatures and environmental pollutants, and further highlight the heritability of such genetic responses (Gomez-Picos et al., 2014; Tedeschi et al., 2016).

Stable isotopes have become a useful technique to understand the biogeography and foraging habits of marine species, and Peavey et al. applied both bulk and compound specific stable isotope techniques to describe the generalist nature of olive ridley marine turtles in the Pacific Ocean. They demonstrated that plasticity in their foraging strategy has likely allowed increased populations despite the susceptibility of arribadas to be impacted by humans (Plotkin et al., 2012).

Finally Hoover et al. considered attachment techniques of transmitters to both hard- and soft-shelled marine turtle species. With increased hatchling tracking to better understand the "lost years," it is important to understand the potential impacts of transmitter attachment on small individuals. They demonstrated the efficacy of a new Velcro attachment of acoustic tags for tracking hatchling movement that avoids some of the negative impacts of harnesses.

INSIGHTS INTO UNDERSTUDIED AREAS AND ISSUES

Examples of emerging issues in sea turtle conservation biology include the impacts of ocean noise on marine turtle behavior and basic knowledge on the population demographics in understudied regions of their range. Summers et al. provided demographic information on a data-limited but endangered population of green turtles in the Northern Mariana Islands. Poaching contributed to reduced population growth and a female bias is suspected due to elevated nest temperatures. They also found lower population growth rates than expected, highlighting the need for continued conservation efforts in this region.

Anthropogenic noise is a major concern in the marine environment but understanding of impacts on marine turtles is limited. Tyson et al. used a novel sound-recording device to determine ambient noise and turtles behavioral responses. Using a three-axis accelerometer, gyroscope, and magnetometer to record the turtle's pitch, roll, and heading, behavior was determined in response to noise; in this case a green turtle responded to boat traffic by sitting on the seafloor bottom until the boat had passed. This is a major advance in understanding the ambient environment experienced by turtles—and the potential impacts of ocean noise.

Bycatch reduction techniques have been influential in reducing incidental capture of turtles in many fisheries, but basin-wide analyses are rarely conducted. Swimmer et al. compared bycatch rates in the longline fisheries in both the Atlantic and Pacific before and after the application of regulations and bycatch technologies and found that circle hooks and squid bait (instead of fish) were the most broadly successful techniques, reducing bycatch by between 40 and 95% across loggerhead and leatherback turtles. Other factors (i.e., light sticks, sea surface temperature) influenced bycatch rates in specific areas. This basin-wide insight is critical for development and support of bycatch reduction techniques.

Finally, Alexander et al. moved beyond traditional conservation techniques such as laws and economic incentives to explore the use of taboos and social pressure to increase turtle conservation in Ghana and beyond. Marine turtles have important cultural and spiritual connections in many Ghanian communities; as a result, Alexander et al. highlighted that in several communities, turtles are not targeted and are frequently released when caught incidentally. Social pressures can therefore be a key way to leverage conservation in many societies.

Collectively the studies in this Special Topic significantly advance our understanding of the current issues and solutions in marine turtle biology and conservation.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Endangered Green Turtles (*Chelonia mydas*) of the Northern Mariana Islands: Nesting Ecology, Poaching, and Climate Concerns

***Tammy M. Summers*[1], *Summer L. Martin*[2]*, *Jessy R. Hapdei*[3], *Joseph K. Ruak*[3] and *T. Todd Jones*[2]**

[1] Rainbow Connection Research, Saipan, Northern Mariana Islands, [2] Marine Turtle Biology and Assessment Program, Pacific Islands Fisheries Science Center, NOAA Fisheries, Honolulu, HI, United States, [3] Department of Lands and Natural Resources, Division of Fish and Wildlife, Saipan, Northern Mariana Islands

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Richard Reina,
Monash University, Australia

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Leslie Cornick,
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United States
Tammy Robinson-Smythe,
Stellenbosch University, South Africa

****Correspondence:***
Summer L. Martin
summer.martin@noaa.gov

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Marine turtles in the western Pacific remain threatened by anthropogenic impacts, but the region lacks long-term biological data for assessing conservation status and trends. The Central West Pacific (CWP) population of green turtles (*Chelonia mydas*) was listed as Endangered by the U.S. in 2016, highlighting a need to fill existing data gaps. This study focuses on the subset of this population nesting in the Commonwealth of the Northern Mariana Islands (CNMI). Using 11 years of nesting data, we (i) estimate reproductive demographic parameters, (ii) quantify abundance and trends, and (iii) estimate the impacts of anthropogenic threats, such as poaching of nesting females and increasing sand temperatures. In 2006–2016, nesting beach surveys, identification tagging, and nest excavations were conducted on Saipan, and rapid assessments of nesting activity were conducted on Tinian and Rota. On Saipan, temperature data-loggers were deployed inside nests and evidence of poaching (adults and eggs) was recorded. This study documents year-round nesting with a peak in March–July. Nester abundance for the three islands combined was 11.9 ± 5.7 (mean ± standard deviation) females annually, with at least 62.8 ± 35.1 nests observed per year. For 39 tagged individuals, straight carapace length was 95.6 ± 4.5 cm, remigration interval was 4.6 ± 1.3 years, and somatic growth was 0.3 ± 0.2 cm/yr. Reproductive parameter estimates included clutch frequency of 7.0 ± 1.3 nests per female, inter-nesting interval of 11.4 ± 1.0 days, clutch size of 93.5 ± 21.4 eggs, incubation period of 56.7 ± 6.4 days, hatching success of 77.9 ± 27.0%, and emergence success of 69.6 ± 30.3%. Mean nest temperature of 30.9 ± 1.5°C was above the pivotal threshold of 29.0°C for temperature dependent sex determination, suggesting a female bias may already exist. Model results suggest (i) hatching success decreases and embryonic death increases when nests experience maximum temperatures beyond 34.4°C and 33.8°C, respectively, and (ii) embryonic death increases in nests with mean temperatures beyond 31.1°C. On Saipan, 32% of nesters were poached, reducing the annual population growth rate from 11.4 to 7.4%. This study provides the first comprehensive assessment of a nesting green turtle population in the Mariana

Archipelago, as well as Micronesia, providing baseline data for the endangered CWP population. Our reproductive demographic data, abundance trends, and anthropogenic threat impact analyses are critical for endangered species management, including assessments of population status and fisheries impacts.

Keywords: Central West Pacific, sea turtles, population assessment, nesting demographic data, nest temperature, climate change, embryonic death

INTRODUCTION

Green sea turtles (*Chelonia mydas*) are ubiquitous throughout tropical and sub-tropical waters and have been of conservation concern for decades (Witherington and Ehrhart, 1989; Jackson et al., 2001; Broderick et al., 2006; Chaloupka et al., 2008; Wallace et al., 2011). Their life-history traits (e.g., long-lived, late maturation, highly migratory) make them vulnerable to anthropogenic impacts on land and at sea (Lutcavage et al., 1997; Jackson et al., 2001; Lewison et al., 2004; McClenachan et al., 2006). While many populations of green turtles have seen remarkable post-exploitation recoveries in recent decades (e.g., 285% increase at Ascension Island and 417% in Costa Rica; Troëng and Rankin, 2005; Broderick et al., 2006) some regions have sub-populations that are still in decline or are too data-limited to assess (McClenachan et al., 2006; Seminoff et al., 2015). A global review of green turtle populations (IUCN 2017, ongoing), recommendations to implement regional management units (Wallace et al., 2010), and a U.S. status review of green turtles (Seminoff et al., 2015) all suggest that distinct populations (e.g., geographically separated, genetically distinct) exist and have differing conservation status and trends based on nesting numbers. Specifically, the recent status review under the U.S. Endangered Species Act suggested that eleven distinct population segments (DPS) exist for green turtles worldwide (Seminoff et al., 2015). Several regions, such as the North Atlantic (i.e., Florida, Gulf of Mexico, and Caribbean) and Central North Pacific (i.e., Hawaii) have populations with recovering trajectories that are listed as Threatened; however, populations in a few regions, including the Central West Pacific (CWP; i.e., Micronesia to the Ogasawara Islands, Japan) are listed as Endangered and have declining or data-limited populations (Seminoff et al., 2015).

Like all marine turtles, green turtles are tied to land for ovipositioning, making them more readily accessible to observation on land than at sea. Counts of nesting turtles and the associated demographic parameters (e.g., remigration interval, clutch frequency, hatching success) are therefore particularly important in assessments of population status and trends. Nesting demographic parameters vary by region and population, but adult females typically return to their natal beach to nest every 2–6 years (Witherington and Ehrhart, 1989; Trono, 1991; Limpus, 2009; Balazs et al., 2015), replenishing energy stores at distant foraging grounds in non-nesting years. In one nesting season, they typically deposit three to six nests (Mortimer and Carr, 1987; Witherington and Ehrhart, 1989; Suganuma et al., 1996) over 2–3 months, returning to the water for 10–20 day inter-nesting intervals (Balazs et al., 2015) to rest and mate between nesting events. Nests contain roughly 80–120 eggs (Pilcher and Basintal, 2000; Limpus, 2009) and take 50–90 days to incubate (Balazs et al., 2015). Ovipositioning on land makes marine turtles highly susceptible to poaching and introduced predators (e.g., pigs, dogs; Lutcavage et al., 1997). The nest environment and sex-determination (i.e., temperature-dependence) makes them vulnerable to climate impacts through population feminization and embryonic death with increasing temperatures (Packard et al., 1977; Miller, 1985; Standora and Spotila, 1985; Mrosovsky, 1994; Ackerman, 1997; Fuentes et al., 2010). Understanding the nesting demographics and threats for each population facilitates assessment of its reproductive output, conservation status, and resilience to anthropogenic impacts.

Green turtles in the insular western Pacific remain impacted by anthropogenic threats (Seminoff et al., 2015), yet a lack of long-term ecological data in the region makes it difficult to assess conservation status and trends (Martin et al., 2016). The CWP population of green turtles was listed as Endangered under the U.S. Endangered Species Act (ESA) because it was data deficient in large portions of its range and exploitation of green turtles in the region was well-known (Seminoff et al., 2015). Prior to this study, the extent to which sea turtles used CNMI terrestrial habitats was based on short-term surveys conducted over 1 year or less (Pultz et al., 1999; Kolinski et al., 2001). Here, we summarize an 11-year research effort to characterize the nesting ecology of green turtles in the Commonwealth of the Northern Mariana Islands (CNMI).

The primary goals of this study were to (i) estimate reproductive demographic parameters of this nesting population (e.g., clutch frequency, remigration interval, and hatching success), (ii) quantify abundance and trends of nesting females, (iii) estimate the impacts of anthropogenic threats, such as poaching and increasing sand temperatures. This study provides the first comprehensive characterization of a nesting green turtle population in the Mariana Archipelago, as well as Micronesia more broadly, and provides a baseline for a portion of the Endangered CWP population. These demographic data serve a critical role in endangered species conservation and management, including assessments of population status, trends, and fisheries impacts.

METHODS

Study Area

The CNMI comprises 14 islands of the Mariana Archipelago, located in the western Pacific Ocean along the eastern boundary of the Philippine Sea (**Figure 1**). This study focuses on the southernmost islands of the CNMI (Saipan, Tinian, and Rota), comprising 6% of the nesting sites for the CWP DPS. These

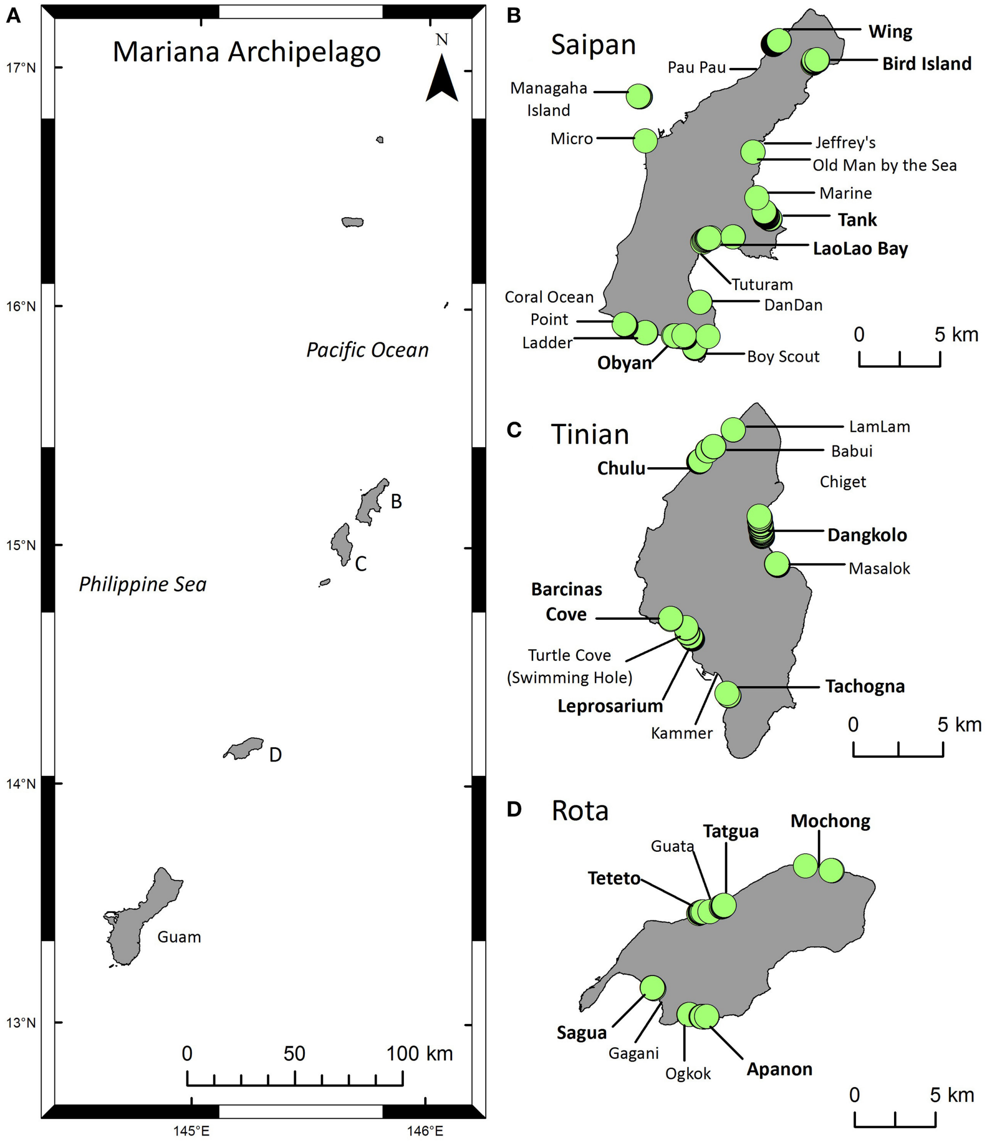

FIGURE 1 | Maps of the study area and green turtle (*Chelonia mydas*) nesting activity. **(A)** The Mariana Archipelago includes Guam at the southern end and the Commonwealth of the Northern Mariana Islands (CNMI) to the north. This study focuses on the southern CNMI islands of Saipan, Tinian, and Rota **(B–D)** where the majority of the CNMI human population lives. **(B–D)** Locations of index beaches (bold) and non-index beaches surveyed for nesting activity from 2006 to 2016. Green circles indicate locations of nests or other evidence of nesting activity recorded during beach surveys. The Supplementary Material contains a list of these sites.

tropical islands are located approximately 2,200 km east of the Philippines and 2,300 km southeast of Japan. Saipan (15.25°N, 145.75°E) is 122 km^2 and hosts 89% of CNMI residents (48,220 people; CNMI Dept. Of Commerce 2010). Tinian (15.00°N, 145.62°E) hosts the next largest population of 3,136 residents (CNMI Dept. Of Commerce 2010) on 102 km^2. Rota (14.15°N, 145.21°E) is 85 km^2 and supports 2,527 residents (CNMI Dept. Of Commerce 2010).

These islands have a distinct rainy (typhoon) season in July-November and a dry season in January-May (Carruth, 2003). Mean rainfall is 200 cm/yr and mean temperature is 20–32°C (Stafford et al., 2002). The beaches are primarily made of medium to coarse-grained calcareous sand, gravel, and coral rubble (Eldredge and Randall, 1980). In a few places, streams flow from high, interior lands to the coastline, where they deposit volcanic material (Eldredge, 1983) and create finer, darker beach sediments (e.g., Apanon beach on Rota). Beach length ranges from 0.01 to 3.35 km, and beaches are often narrow and segmented into small pockets by raised limestone (Eldredge, 1983). Saipan has 26 beaches of various sizes including the largest beaches of the three islands, Tinian has 13 beaches all < 0.5 km in length (Pultz et al., 1999), and Rota has 16 small pocket beaches. This study included five index beaches on each island, plus various non-index beaches per island (**Figure 1**, Supplementary Table 1).

Nesting Surveys

Surveys to monitor nesting activity and nester abundance were conducted by local biologists at index and non-index beaches (**Figure 1**). A "survey" includes monitoring effort conducted on a single beach in a single day (or overnight); five beaches surveyed in 1 day represents five surveys. Non-index beaches were surveyed intermittently, primarily when there were public reports of nesting activity. On Saipan, where the field team was located, index beaches were surveyed as regularly as possible from 2006 to 2016, both diurnally to record all nesting activity and nocturnally to tag nesting females. Survey effort fluctuated annually, with diurnal surveys typically conducted 2–5 days per week over 6–12 months per year. Surveys on Rota and Tinian (2009–2016) were limited to diurnal rapid assessments (1–5 days each) conducted 1–2 times per year toward the end of the nesting season, and rare nocturnal tagging surveys. See Supplementary Table 1 for a summary of the years and locations of data collection incorporated into each metric described below.

Diurnal surveys were performed on foot during morning hours (06:00–11:30) along the edge of vegetation and high tide lines. All turtle crawls from the previous night(s) were recorded. Species and crawl identification followed Pritchard and Mortimer (1999) and the "Sea Turtle Identification Key" (www.seaturtle.org 2005). Nesting and non-nesting emergences were differentiated by examining crawl signs (e.g., presence/absence of escarpment, primary/secondary body pits, and thrown sand) and verifying the presence of eggs in suspected nests (Schroeder and Murphy, 1999). On Saipan index beaches, body pits and abandoned egg chambers were recorded as non-nesting emergences only if there were fresh tracks accompanying them. On Rota and Tinian, signs of non-nesting emergence were recorded even when fresh tracks were not present, as tracks become weathered over time and surveys were infrequent. Locations of nests and non-nesting emergences were recorded using a handheld GPS device.

Nocturnal tagging surveys were conducted during anticipated emergence periods. Nesters were tagged and measured after they completed oviposition and began backfilling the nest with sand. Turtles were double-marked with either titanium (Stockbrands Co. Pty Ltd, large size) or Inconel tags (National Band & Tag Co, 681C), with one tag attached proximally and adjacent to the first large scale on the posterior edge of each front flipper (Balazs, 1999). Passive integrated transponder (PIT) tags were applied from March 2009 onward. Turtles' flippers were scanned for the presence of PIT tags with a Biomark Pocket Reader© PIT tag scanner. If no PIT tags were found, then a single PIT tag was injected subcutaneously into one of the hind flippers using a 12-gauge disposable hypodermic needle and applicator.

Standard measurements were recorded. Straight carapace length (SCL) and curved carapace length (CCL) were measured from the anterior point at the midline (nuchal scute) to the longest posterior tip of the supracaudal scutes. Likewise, straight and curved carapace widths (SCW and CCW) were measured at the widest point (Bolten, 1999). Measurements were taken to the nearest millimeter using forester calipers (S-882 00 Haglof, Sweden) for straight measurements and a flexible tape measure for curved measurements. Prior to March 2009, only the curved measurements were taken. We converted CCLs to SCLs using a conversion equation specified for CNMI green turtles in Summers et al. (2017).

Nesting season length and peak were estimated on Saipan. Season length was determined through consistent survey effort on index beaches 2–3 days per week for 1 year (January 2012–January 2013). Dates of the first and last nests were recorded, along with the date of the last nest inventoried (see methods below). Season peak was evaluated using the mean number of nests laid per month across all years (except 2008 due to reduced effort during peak months).

Nesting periodicity was estimated at inter- and intra-annual scales on Saipan. Remigration interval, the number of years between nesting seasons, was calculated for recaptured turtles. Inter-nesting interval was estimated as the number of days between one successful nesting event and the start of the next nesting attempt within a season, even if the next landing was a non-nesting emergence (Alvarado and Murphy, 1999). This calculation only included data from 2011 to 2013, as monitoring effort during that period was intensified to ensure that nesters were directly observed each time they emerged.

Clutch frequency (number of nests per female within a season) was estimated using Saipan data from 2011 to 2016, when emergence schedules were tracked closely enough to attribute specific nests to individual nesters, even if the nester was not directly observed each time. Females included in this calculation were physically encountered at least twice in the season to confirm their emergence schedule and ensure particular nests were attributed correctly to specific individuals (Alvarado and Murphy, 1999). Turtles that were poached prior to their final nesting attempt were not included in this calculation.

Threats were assessed during surveys and nest excavations by quantifying: (1) nesters and nests poached (or nearly poached) by humans (details below), (2) nests depredated by predators (details below), (3) nests inundated by water caused by tropical storms, typhoons, and storm-water drainage from beach erosion, (4) nests with hatchling emergence success negatively impacted by roots of invasive vegetation and large pieces of coral rubble, and (5) human disturbance activities. We counted turtles as poached if at least one of the following lines of evidence were present: (1) crawl tracks emerging from the water but not returning, (2) human activity at the nest, such as digging, foot prints, and/or vehicle tracks, (3) a drag mark (indentation in the sand/vegetation) from flipping the turtle over at the nest and dragging it to the nearest parking lot or foot path, (4) discarded tools at the nesting site, including ropes and pallets, or (5) emergence tracks wiped away by humans, presumably to disguise a poaching event, typically in conjunction with missing return tracks and cessation of future nesting by a female that was expected to have additional nesting events in the season. Nests were considered poached if they were confirmed to be present and had evidence of human removal of eggs (e.g., digging and broken shells), sometimes accompanied by signs that the nester was also poached. For depredated nests, we determined predator type as follows: (1) monitor lizards on Rota left evidence of digging (or were observed digging), drag marks from their tails, large holes into nest chambers, egg shells strewn about on top of the nest, and/or eggs eaten within the chamber, (2) crabs left crab sized holes into the chamber and shells outside/on top of the nest, and (3) ants left tiny holes in the eggs, but the eggs remained in the chamber, and ants were sometimes observed in the nest.

Nest Excavations

Nests were marked for post-incubation triangulation and excavation by attaching labeled aluminum tags (Forestry Suppliers, 79260) and survey flagging to vegetation in two locations a measured distance away. Aluminum tags and triangulation methods began in 2012; prior to that, nests were marked using natural landmarks. Cryptic nest marking techniques are necessary in the CNMI due to the threat of egg poaching.

Nests were excavated 3 days after the first evidence of hatching was observed on diurnal surveys (except in rare cases when there was a 2–3 week logistics delay). When no hatching was evident, nests were inventoried on the 70th day after the original observation date. Only nests which showed obvious signs of hatching were inventoried during rapid assessments of Tinian and Rota. Incubation periods were estimated as the number of days between oviposition and hatching (the date primary hatching evidence was observed), using data from 2012 to 2013 on Saipan.

We examined and categorized the contents of each nest following Miller (1999). Categories included: (i) E = emerged hatchlings (departed/departing from nest); (ii) S = shells (empty shells counted if >50% intact); (iii) L = live hatchlings (remaining among shells; assumed to have missed the window for successful emergence); (iv) D = dead hatchlings (outside shell but dead); (v) UD = undeveloped eggs (unhatched with no obvious embryo); (vi) UH = unhatched eggs (with obvious embryo); (vii) UHT = unhatched term eggs (fully developed in egg shell or pipped); (viii) P = depredated eggs (open, nearly complete shell with egg residue). Reproductive output metrics were calculated as:

$$\begin{aligned}
\text{Total clutch size} &= \mathrm{E} + \mathrm{L} + \mathrm{D} + \mathrm{UD} + \mathrm{UH} + \mathrm{UHT} + \mathrm{P} \\
E &= S - (L + D) \\
\text{Hatching success (\%)} &= [\mathrm{S}/(\mathrm{S} + \mathrm{UD} + \mathrm{UH} + \mathrm{UHT} + \mathrm{P})] \times 100 \\
\text{Emergence Success (\%)} &= [(\mathrm{S} - (\mathrm{L} + \mathrm{D}))/(\mathrm{S} + \mathrm{UD} + \mathrm{UH} \\
&\quad + \mathrm{UHT} + \mathrm{P})] \times 100
\end{aligned}$$

Nester Abundance and Trends

We estimated annual nester abundance for Saipan using the nocturnal tagging survey data, as every nester was either tagged or identified through nesting activity. For Tinian and Rota, where only diurnal rapid assessments were feasible, we divided total observed nests by mean clutch frequency to estimate the number of nesters (Alvarado and Murphy, 1999). We summed these island-specific estimates for each year (except 2008 due to a lack of survey effort during peak nesting months) to estimate annual nester abundance for all three islands combined.

For Saipan, we estimated the population growth rate (PGR) using a log linear regression of annual nester counts (ln[nesters] as a function of untransformed year). We used the total number of nesters observed each year, regardless of whether they were eventually poached that year. Data from 2008 were excluded here as well (see above comment). We estimated the 95% confidence interval, *p*-value (alpha = 0.05), and R-squared goodness-of-fit.

Recognizing that poaching of nesters occurred throughout the study period, we let the analysis above represent the population trend with poaching and conducted a second analysis to estimate PGR without poaching. For nesters that were poached in a given year, we added them back into the annual counts for subsequent years as if they had not been poached, and ran the regression on the increased annual counts. Our analysis (i) assumes the poached nesters had the mean remigration interval determined by this study, (ii) ignores natural mortality, as annual survivorship for adult green turtles in the Pacific can be as high as 0.95–0.98 (Chaloupka, 2002; Seminoff et al., 2003) and the analysis only pertains to a short 10-year period, and (iii) assumes 100% of poaching was detected, which is likely true for this study period based on the overall low numbers, limited nesting habitat, small island community, and consistent monitoring efforts. We interpret the difference between the PGR estimates for the two scenarios—"with poaching" and "without poaching"—to represent the estimated impact of poaching on the nester abundance trend.

Climate Impacts

Nest temperatures were measured using HOBO temperature data loggers (Onset Computer Corporation, Pocassette, MA) on Saipan beaches (primarily index beaches) between mid-November 2012 and mid-September 2016. Loggers were set to record temperature every hour and deployed inside the egg chamber, with an identification tag attached by monofilament line or parachute cord (Layton, 2011). They were retrieved during nest excavations and transported back to the office, where the data were downloaded using HOBOware Pro software version 3.2.2 (Onset Computer Corp. 2002–2011). We removed the first and last 7 days of temperature data from each logger due to uncertainty associated with logistical field challenges (e.g., possible time lags between the logger being switched on and deployment, or retrieval and the logger being switched off after transport). This conservative decision minimizes the influence of temperature fluctuations associated with deployment and retrieval, and retains the most relevant incubation data.

We developed generalized additive models (GAMs; Hastie and Tibshirani, 1990) to explore the influence of nest temperature on reproductive success. We constructed several models, each with Hatching Success or Embryonic Death (proportion of eggs that were unhatched, UH, or unhatched term, UHT) as the response variable and combinations of Nest Temperature (mean and maximum), Beach, Year, and Month as predictor variables. All predictor variables were ordinal, except Beach was categorical. Although we were interested exclusively in Nest Temperature as a known mechanistic driver of reproductive success, the other predictors served to control other sources of variation in the response variables to isolate the influence of temperature as much as possible. We estimated the models using data from Saipan nests for which we had both temperature logger data (nest temperatures throughout incubation) and excavation data (hatching success and embryonic death).

We constructed and compared models in a forward step-wise manner in three stages, adding one predictor variable to the best model at each stage and selecting a new model to carry forward, similar to Ortiz et al. (2016). We included Beach in all models to capture inherent but unmeasured differences across sites (e.g., beach slope, length and width, sand grain size, rainfall, moisture, vegetation, shade, etc.) that may influence Hatching Success and Embryonic Death. We used standard selection criteria—Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC)—to evaluate competing models within each stage (Ortiz et al., 2016). In general, models with low AIC and BIC values and high Deviance Explained are considered the best. We selected the model with the lowest AIC and BIC values within each stage, without comparing it to previous stages. In the final stage, we confirmed that the selected model both included a Nest Temperature predictor and had higher Deviance Explained than models from previous stages with the same Nest Temperature predictor if the AIC/BIC values were similar (within 3 points for AIC and 7 points for BIC). We used this selection process because our intention was to examine the functional form of the relationship between Nest Temperature predictors and Hatching Success or Embryonic Death, not to develop the absolute best model for either response variable. We used the "mgcv" (mixed GAM computation vechicle) package in R for this analysis; "mgcv" is a routine that optimizes the degrees of freedom of the fitted GAM (Wood, 2017). We defined the models with the beta regression family of data distributions ("betar"), a "logit" link function appropriate for data in which the response is a proportion (0–1), and a cubic smoothing spline limited to 4 knots. We performed these analyses and produced figures in the R statistical computing environment (R Core Team, 2014; Version 3.1.2).

RESULTS

Nesting Ecology

Over 11 years (2006–2016), there were 5,427 diurnal surveys on index beaches on Saipan, 102 on Tinian, and 64 on Rota (**Table 1**). On Saipan, where effort was most intense, there was a mean of 493 diurnal surveys per year (*sd* = 217; range = 59–782), or 99 surveys per beach per year (*sd* = 43; range = 3–161). Nocturnal tagging surveys on index beaches totaled 467 on Saipan, 9 on Tinian, and 10 on Rota (**Table 1**). See Supplementary Table 2 for additional details on survey effort, including a summary by island, index beach, and year.

Green turtles nest year-round in the CNMI, as documented by observations of nests, hatchlings, and nesting females. Peak nesting occurred between March and July (91% of Saipan nests) with a mean of 6.5 nests laid per month (*sd* = 4.5; range = 1–18) during peak months. Nest deposition starts in mid-November of one calendar year and ends late August of the next, with hatchlings emerging into early November.

There were 364 total nests observed on Saipan (mean of 36 nests per year; *sd* = 15; range = 18–64; excludes 2008 due to missing survey effort in peak months), 156 nests on Tinian (22 nests per year; *sd* = 15; range = 1–42), and 113 on Rota (16 nests per year; *sd* = 12; range = 4–36) (**Table 1**). A total of 199 non-nesting emergences (NNEs) were observed on Saipan (22 NNEs per year; *sd* = 16; range = 2–48), 47 on Tinian (8 NNEs per year; *sd* = 6; range = 1–14), and 31 on Rota (6 NNEs per year; *sd* = 3; range = 4–11) (**Table 1**). Numbers for Tinian and Rota are likely biased low compared to Saipan due to lower levels of survey effort. For example, in years with frequent or intense tropical storms/typhoons we found little to no evidence of nesting on Tinian or Rota, as most signs of nesting had been inundated by high water by the time beaches were surveyed.

We tagged and measured a total of 39 nesters combined on Saipan (*n* = 34), Tinian (*n* = 3), and Rota (*n* = 2). Mean SCL was 95.6 cm (*sd* = 4.5; range = 81.0–103.6 cm; *n* = 39), mean SCW was 75.0 cm (*sd* = 4.6; range = 59.2–85.0; *n* = 29), mean CCL was 102.2 cm (*sd* = 4.7; range = 87.1–111.3; *n* = 38), and mean CCW was 92.5 cm (*sd* = 4.9; range = 76.7–103.2; *n* = 38) (**Table 2**). Ten nesters recaptured on Saipan in 2010–2016 (initial SCL: mean = 95.6 cm; *sd* = 2.6; range = 91.2–99.8) exhibited a mean absolute growth rate of 0.3 cm/yr (*sd* = 0.2; range = 0–0.7) (**Table 2**).

Remigration interval for the 10 recaptured nesters was 1.9–5.9 years (mean = 4.6; *sd* = 1.3) (**Table 2**). One turtle tagged on Obyan Beach in May 2012 was recaptured only 8.5 months later during in-water reef surveys at Balisa (west coast of Saipan) in January 2013, and then again during nocturnal nesting surveys in March 2014 (1.9 years from initial capture). These encounters suggest this turtle may be a resident forager. The short remigration interval may be explained by the lack of a long-range migration between nesting years and the associated conservation of energy.

Inter-nesting interval was 11.4 days (median = 11 days; *sd* = 1.0; range = 10–13; *n* = 16 nesters) (**Table 2**). Turtles whose normal nesting periodicity was disturbed by human activities (examples below) re-emerged post-disturbance 14–18 days after their last nesting event.

Mean clutch frequency was 7.0 nests per female per season (*sd* = 1.3; range = 5–10) observed across a mean 5 nesters per year on Saipan (*sd* = 2; range = 3–7; *n* = 28 nesters) for 2011–2016 (**Table 2**). Mean incubation duration was 56.7 days (*sd* = 6.4; range = 46–70; *n* = 41 nests); this estimate may be biased high since diurnal surveys were not performed 7 days a week.

We excavated 396 nests combined on Saipan (*n* = 291), Tinian (*n* = 49), and Rota (*n* = 56) and estimated a mean clutch size of 93.5 eggs (*sd* = 21.4; range = 32–186; **Table 2**). Hatching success was 77.9% (*sd* = 27.0; range 0–100) across the three islands but lower on Saipan (74.8%) where the sample size was highest (**Table 2** and Supplementary Table 3). Saipan also had a lower emergence success (64.0%) than the three-island mean of 69.6% (*sd* = 30.3; range = 0–100; **Table 2** and Supplementary Table 3). High variation in hatching success is possibly attributed to inundation and accompanying erosion from storms, depredation by crabs, ants, and monitor lizards, and temperature variations, as described below.

TABLE 1 | Summary of annual green turtle (*Chelonia mydas*) nesting activity on the islands of Saipan, Tinian, and Rota in the Commonwealth of the Northern Mariana Islands (CNMI), 2006–2016.

Year	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	Total
SAIPAN												
Diurnal surveys	59	316	782	505	309	404	425	645	692	726	564	5,427
Nocturnal surveys	4	13	2	29	15	49	79	68	74	54	80	467
Nests	24	29	*5	19	18	31	41	40	39	54	64	364
NNEs	0	2	*2	7	11	12	28	44	24	21	48	199
Nesters	8	4	*1	6	5	6	9	8	8	12	11	78
Poached nesters	4	1	1	4	2	1	6	2	1	1	2	25
Poaching attempt	0	1	0	0	0	0	0	0	1	0	0	2
New nesters	8	4	1	6	2	4	5	7	4	6	7	54
% New nesters	100%	100%	100%	100%	40%	67%	56%	88%	50%	50%	64%	69%
TINIAN												
Diurnal surveys	0	0	0	5	3	4	2	9	22	42	15	102
Nocturnal surveys	0	0	0	0	0	0	0	0	0	5	4	9
Nests	nd	nd	nd	38	0	1	22	16	10	27	42	156
NNEs	nd	nd	nd	1	0	0	8	13	1	10	14	47
Nesters	^1	nd	nd	5	0	1	3	2	2	5	6	25
Poached nesters	nd	nd	nd	2	0	0	0	0	0	0	1	3
Poaching attempt	nd	nd	nd	0	0	0	0	0	1	0	0	1
ROTA												
Diurnal surveys	0	0	0	3	3	10	8	12	8	14	6	64
Nocturnal surveys	0	0	0	0	0	0	0	0	0	10	0	10
Nests	nd	nd	nd	13	4	5	27	36	0	15	13	113
NNEs	nd	nd	nd	4	0	0	6	11	0	5	5	31
Nesters	nd	nd	nd	2	0	1	3	5	0	3	3	14

*Diurnal and nocturnal survey effort from index beaches is summarized for context. Survey, monitoring effort conducted on a single beach in a single day (or overnight); NNEs, non-nesting emergences (e.g., false crawls, body pits, and abandoned egg chambers); New nesters, neophyte nesters (untagged or first time observed nesting; Saipan only); % New nesters, percentage of annual nesters that were neophytes; Poaching attempt, harvest thwarted by researchers or enforcement; nd, no data collected. *no surveys between May 20, 2008 and July 13, 2008. ^nester reported by Tinian wildlife enforcement officials.*

Nest excavations revealed low levels of egg predation. Crabs depredated 5% of nests, destroying a mean of 3 eggs per nest (*sd* = 3; range = 0–13). Ants impacted 3% of nests, consuming a mean of 16 eggs per nest (*sd* = 32; range = 0–94). Monitor lizards depredated 2% of nests, devouring a mean of 17 eggs per nest (*sd* = 22; range = 0–57). One percent of nests showed evidence of egg predation by an unknown source, with 4 eggs lost per nest (*sd* = 6; range = 1–13).

Egg poaching accounted for the loss or partial loss of at least 8% of nests on Saipan, 4% on Tinian, and 2% on Rota. Direct take of a hatchling was also documented when a juvenile green turtle (CCL = 17.5 cm) was surrendered to wildlife enforcement by a Saipan resident in 2008. The turtle had been taken from a Saipan beach and raised as a family pet from hatchling size. These poaching observations should be interpreted as minimum values.

Inundation and accompanying erosion from tropical storms, typhoons, and storm-water drainage impacted 9% of nests excavated on Saipan, resulting in a low mean emergence success of 37% (*sd* = 34; range = 0–96). Inundation of excavated nests was not characterized on Tinian and Rota. Nests completely washed out by storms comprised 2% of observed nests on Saipan, 3% on Rota, and 8% on Tinian.

On Saipan, we observed hatchlings trapped inside the nest under introduced vegetation (i.e., *Leucaena leucocephala*, *Cassytha filiformis*, *Casuarina equisetifolia*, and *Cynodon nlemfuensis*) or coral rubble in 10% of excavated nests. This problem affected a mean 26 hatchlings per nest (*sd* = 24; range = 1–101) or 28% of the clutch (*sd* = 23; range = 1–92). Emergence success for these nests was low at 53% (*sd* = 26; range = 3–90). Entrapment observations were not typically possible on Tinian or Rota due to the lower survey frequency, but there was one documented case on Tinian.

Coral rubble, rocky substrate, and roots also impacted nesters by impeding their excavation of egg chambers. Abandoned egg chambers accounted for 29% of NNEs on Saipan, 40% on Tinian, and 32% on Rota. Two extreme cases were observed on Babui Beach, Tinian and Obyan Beach, Saipan where seven and eleven abandoned egg chambers (respectively) were documented within a single crawl each.

Human disturbance of nesters was observed in association with several activities, including camping, building bonfires, driving on the beach, using flashlights, and fishing nearshore with submersible lights. At least one type of disturbance was recorded during 8% of nocturnal surveys on Saipan. Typically, these disturbances prevented nesters from emerging on their

TABLE 2 | Reproductive parameters for green turtles (*Chelonia mydas*) nesting in the Commonwealth of the Northern Mariana Islands (CNMI) on Saipan, Tinian, and Rota islands.

CNMI			**Hawaii, USA**	**Rose Atoll, Am. Samoa**	**Turtle Islands, Philippines**	**Great Barrier Reef, Australia**	**Ogasawara, Japan**	**Malaysia**
References		**This study**	Balazs et al., 2015	**NMFS unpub. 2013**	**[1]** Trono, 1991 **[2]** Burton, 2012	**[1]** Limpus, 2009 **[2]** Limpus et al., 2003 **[3]** Fuentes et al., 2010 **[4]** Limpus and Chaloupka, 1997	**[1]** Suganuma et al., 1996 **[2]** Abe et al., 2003	Pilcher and Basintal, 2000
SCL (cm)	$n = 39$	95.6 ± 4.5 (81.0–103.6)	90.7 (75–106)	94.7	NR	NR	95.2 ± 4.5 (83–108) [1]	NR
CCL (cm)	$n = 38$	102.2 ± 4.7 (87.1–111)	97.0 (78–113)	NR	99.5 [1]	107 ± 5.5 (91–124) [1]	NR	98.5 ± 6.0 (68–121)
Remig. interval (yr)	$n = 10^{\dagger}$	4.6 ± 1.3 (1.9–5.9)	4 (2–9)	NR	2.5 [1]	5.8 ± 1.5 (1–9) [1]	3.7 ± 0.6 (max 6) [2]	2.4 ± 0.8 (1.0–4.3)
Clutch freq. (nests per year)	$n = 28^{\dagger}$	7.0 ± 1.3 (5–10)	4 (1–9)	NR	5 [2]	5.1 ± 2.0 (1–9) [1]	4.1 (max 6) [1]	2.7 ± 0.8
Inter-nesting Int. (days)	$n = 16^{\dagger}$	11.4 ± 1.0 (10–13)	13.2 (11–18)	NR	14.5 [1]	14.1 ± 1.7 (9–21) [1]	(11–12) [2]	15.5 ± 6.2 (5–30)
Incubation (days)	$n = 41^{\dagger}$	56.7 ± 6.4 (46–70)	64.5 (54–88)	NR	54.3 [1]	64.5 ± 6.1 (54–87) [1]	NR	53.1 ± 4.2 (40–69)
Clutch size (eggs)	$n = 396$	93.5 ± 21.4 (32–186)	104 (38–145)	NR	95.6 [1]	115.2 ± 27.9 (42–195) [1]	102 ± 26.2 (4–183) [1]	87.3 ± 21.9 (4–164)
Hatching success (%)	$n = 396$	77.9 ± 27.0 (0–100)	76.7 (0–100)	NR	87.1 [1]	79.6 ± 15.0 (20.4–100) [2]	NR	NR
Emergence succ. (%)	$n = 396$	69.6 ± 30.3 (0–100)	70.8 (0–97.6)	NR	85.7 [1]	78.6 ± 15.2 (19.4–100) [2]	NR	NR
Nest temperature (°C)	$n = 184^{\dagger}$	30.9 ± 1.5 (27.6–34.9)	(23.2–29.8)	NR	NR	29.0 ± 0.04* [3]	NR	NR
Somat. growth (cm/yr)	$n = 10^{\dagger}$	0.3 ± 0.2 (0–0.7) SCL	NR	NR	NR	0.12 ± 0.04 (SE) CCL [4]	NR	0.8 CCL

*Values are Mean ± SD (range). Values from other sites within the western Pacific are provided for context. Relevant regional locations include Hawaii (Balazs et al., 2015), American Samoa (NMFS unpub. data 2013), Philippines (Trono, 1991; Burton, 2012), Australia (Limpus and Chaloupka, 1997; Limpus et al., 2003; Limpus, 2009; Fuentes et al., 2010), Japan (Suganuma et al., 1996; Abe et al., 2003), and Malaysia (Pilcher and Basintal, 2000). SCL, straight carapace length; CCL, curved carapace length; Somat. growth, somatic growth rate observed or modeled from recaptured nesters; †samples were collected on Saipan only (vs. on all three islands for other parameters); NR, not reported by the study. *Fuentes et al. (2010) sand temperature value (measured at nest depth) for Raine Island, with 0.5°C increase added for metabolic heating in nests.*

expected return dates and caused them to nest after the activity was no longer a threat or to nest on adjacent (smaller) pocket beaches with sub-optimal habitat. On a few occasions, a nester was disturbed on the beach or could not find suitable habitat during nesting attempts (i.e., impedance by rocks or roots) and switched to a different nesting beach within a season. Most nesters, however, were only observed to nest on a single nesting beach.

Nester Abundance and Trends

Over 11 years, we recorded 78 nesters on Saipan (7.7 ± 2.5 annual nesters; range = 4–12), 25 on Tinian (3.1 ± 1.9 annual nesters; range = 1–6), and 14 on Rota (2.8 ± 1.3 annual nesters; range = 1–5) for a combined total of 117 nesters (11.9 ± 5.7 annual nesters; range = 4–20) (**Table 1**). Similar to the numbers of nests and non-nesting emergences, nester abundance estimates for Tinian and Rota are likely biased low due to the relative infrequency of survey effort. Poachers removed 25 females from Saipan (32%) and at least 3 from Tinian (12%); no poaching of nesters was documented on Rota (**Table 1**). Attempts to poach at least 2 additional nesters from Saipan and 1 from Tinian were thwarted (**Table 1**). Neophyte nesters (previously untagged) comprised 40 to 100% of annual nesters, with an overall mean of 69.2% across all years and 59.1% for 2010–2016 only, the period after one remigration interval had passed and thus remigrants could be expected. The estimated PGR for Saipan's nesting population was 11.4% when adding the poached nesters back into the population at the mean remigration interval of 4.6 years ($p = 0.001$; $R^2 = 0.74$) (**Figure 2A**). However, PGR decreased to 7.4% per year when accounting for observed levels of poaching for 2006–2016 ($p = 0.019$; $R^2 = 0.52$) (**Figure 2B**).

Climate Impacts

We deployed a total of 246 temperature loggers in Saipan nests in 2012–2016 and successfully retrieved 184 loggers that had at least 7 days of data each. This included 174 loggers from index beaches (**Table 3**) plus 10 loggers from non-index beaches (Supplementary Table 4). Mean nest temperature was 30.9 ± 1.5°C (index beach range = 27.6–34.2; non-index beach range =

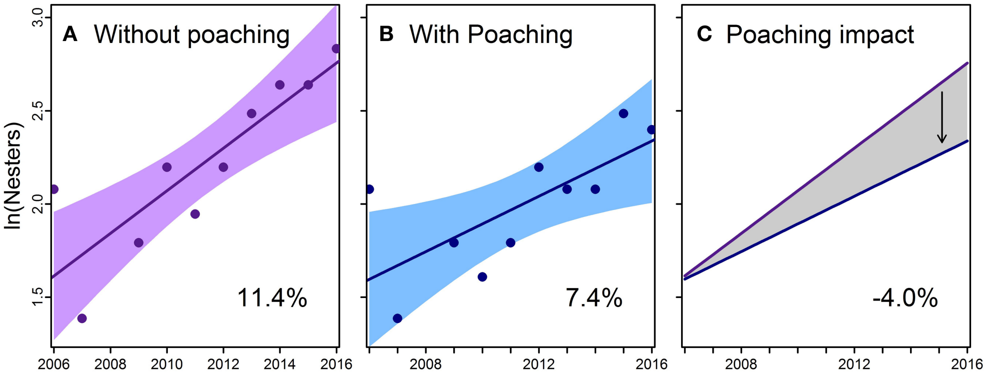

FIGURE 2 | Trends in abundance for green turtles (*Chelonia mydas*) nesting on Saipan, 2006–2016. **(A)** "Without Poaching" model scenario—theoretical nester abundance with poached nesters added back to observed annual counts using the estimated mean remigration interval of 4.6 years. Linear regression (solid line with shaded 95% confidence interval) through estimated annual nester abundance (points) suggests the population growth rate would be 11.4% without poaching ($p = 0.001$; $R^2 = 0.74$). **(B)** "With Poaching" model scenario—nester abundance (points) reflects actual levels of poaching, with a linear regression (line and 95% confidence interval shading) suggesting a 7.4% population growth rate ($p = 0.019$; $R^2 = 0.52$). **(C)** Difference between the two scenarios in **(A,B)** suggests poaching of nesters decreased the population growth rate by 4.0%, thereby slowing the recovery trend.

29.1–34.9; **Table 3** and Supplementary Material). Maximum nest temperature was 33.5 ± 2.0°C (range = 29.2–42.6).

For our modeling exercise, there were 89 nest temperature loggers with corresponding data on hatching success and embryonic death that were suitable for use. Results from our model selection process are summarized in **Table 4** and the smoothed responses to the predictors in the best models are illustrated in **Figure 3**.

For Hatching Success, the model with the lowest AIC and BIC was in Stage I with Beach and Year as predictors; however, adding Month to that model in Stage II increased Deviance Explained by 2% for a negligible increase in AIC and BIC (**Table 4**). In Stage III, adding Maximum Nest Temperature was better than adding Mean Nest Temperature, as evidenced by a 10% higher increase in Deviance Explained along with clearly lower AIC/BIC scores (**Table 4**). Although some models in Stages I and II had lower AIC/BIC values, we selected the model with Maximum Nest Temperature in Stage III as the best model because it had the overall highest Deviance Explained (57%) and included temperature, our variable of interest (**Table 4**; **Figures 3A–C**). This model ("Model 1") suggests (i) an annual decrease in hatching success until 2015 and a subtle increase thereafter, (ii) a slight maximum in hatching success in May-July, and (iii) hatching success increases with maximum nest temperature up to 34.4°C, after which it decreases (**Figures 3A–C**).

For Embryonic Death, in Stage I the model with the lowest AIC and BIC included Beach and Year as predictors; however, this model had the lowest Deviance Explained (**Table 4**). Adding Month in Stage II increased the Deviance Explained by 3% while maintaining similar values for AIC/BIC (**Table 4**). In Stage III, adding Mean Nest Temperature or Maximum Nest Temperature yielded similar results, with Mean Nest Temperature offering 1% higher Deviance Explained (29%) but almost identical values for AIC/BIC. For this reason, we considered both models as the best. The model with Mean Nest Temperature ("Model 2") suggests (i) a steady increase in embryonic death over the years, (ii) lowest embryonic death between May and July, and (iii) a pronounced increase in embryonic death with mean nest temperatures beyond 31.1°C (**Figures 3D–F**). The model with Maximum Nest Temperature ("Model 3") suggests (i) embryonic death has increased annually since 2012, (ii) highest embryonic death in February through April, and (iii) embryonic death decreases with maximum nest temperatures up to 33.8°C, and increases sharply beyond that (**Figures 3G–I**).

DISCUSSION

Nesting green turtles in the CNMI are part of the Endangered CWP DPS for which a major knowledge gap exists (Seminoff et al., 2015). Data on nesting ecology, population abundance and trends, direct human impacts, and climate impacts are critical for conducting population status assessments. Understanding these aspects of the population will facilitate science-based management and help direct conservation efforts both locally and regionally. Our 11-year study provides the first comprehensive characterization of this nesting population, filling in those major data gaps in demographic parameters and providing quantitative evidence of the current impact of poaching and the looming threat of rising temperatures.

Nesting Ecology

The reproductive demographic parameters estimated in this study fall within the ranges established for other green turtle populations in the western and central Pacific. Parameter estimates (mean ± *sd* and range) for several other nesting locations in the region (Hawaii, American Samoa, Australia, Philippines, and Malaysia) are provided in **Table 2** for context. Relative to those populations, CNMI nesters appear to be medium-sized with an intermediate remigration interval. Their SCL of 95.6 ± 4.5 cm and CCL of 102.2 ± 4.7 cm fall between low values observed in Hawaii (Balazs et al., 2015) and high ones

TABLE 3 | Temperature logger data (°C) from green turtle (*Chelonia mydas*) nests on Saipan index beaches, 2012–2016 ($n = 174$ loggers).

		2012	**2013**	**2014**	**2015**	**2016**	**All years**
Tank Beach	Mean	30.8	32.0	31.6	31.4	31.8	31.7
	sd	1.9	2.1	1.7	2.1	2.0	1.4
	Range	27.2–36.2	24.8–42.6	26.8–35.9	28.0–36.8	26.8–36.5	24.8–42.6
	N = loggers	7	24	14	3	24	72
	D = points	9,020	24,977	15,949	2,877	25,911	78,734
Obyan Beach	Mean	30.2	–	31.0	29.7	31.2	30.7
	sd	1.5	–	1.7	1.5	1.4	1.2
	Range	27.4–35.3	–	28.2–36.3	26.8–35.4	25.5–34.9	25.5–36.3
	N = loggers	10	–	15	5	3	33
	D = points	8,626	–	15,081	6,512	2,777	32,996
Bird Island	Mean	31.4	30.5	–	29.8	–	30.4
	sd	1.6	1.0	–	1.4	–	1.1
	Range	27.7–34.6	28.5–34.3	–	26.2–34.7	–	26.2–34.7
	N = loggers	7	4	–	14	–	25
	D = points	5,000	3,979	–	16,837	–	25,816
LaoLao Bay	Mean	–	–	29.5	30.0	31.5	30.7
	sd	–	–	0.7	1.3	1.8	1.2
	Range	–	–	25.5–30.3	26.7–35.1	24.5–36.2	24.5–36.2
	N = loggers	–	–	1	4	5	10
	D = points	–	–	1,179	4,194	5,077	10,450
Wing Beach	Mean	29.8	–	30.0	29.1	30.3	29.9
	sd	1.9	–	1.1	1.8	2.2	1.4
	Range	26.4–36.8	–	27.6–31.8	25.2–34.0	25.9–36.0	25.2–36.8
	N = loggers	11	–	2	9	12	34
	D = points	12,123	–	2,589	11,798	13,099	39,609
All Index Beaches	Mean	30.6	31.8	31.3	29.9	31.3	30.9
	sd	1.4	1.5	1.1	1.1	1.4	1.5
	Range	27.9–33.4	27.8–34.1	29.2–32.9	27.7–33.4	27.6–34.2	27.6–34.2
	N = loggers	35	28	32	35	44	174
	D = points	34,769	28,956	34,798	42,218	46,864	187,605

"–", no data; either no loggers deployed or loggers were lost or washed out by storms or tidal inundation. Summary data for loggers include (i) mean hourly nest temperature (standard deviation), (ii) range of nest temperatures, (iii) N = number of loggers, and (iv) D = number of hourly temperature data points. Mean and standard deviation values for "All Years" and "All Index Beaches" were calculated from the mean temperatures of individual loggers. See Supplementary Material for additional data from non-index beach nests and from loggers placed in the sand at nest depth to characterize ambient temperatures.

from the Great Barrier Reef in Australia (Limpus, 2009). Their remigration interval of 4.6 ± 1.3 yr falls between shorter intervals from the Philippines and Japan (Trono, 1991; Abe et al., 2003) and longer ones from Australia (Limpus, 2009).

Within one season, CNMI nesters lay a relatively high number of nests, deposit a low number of eggs in each nest, and spend a short period of time in the water between nesting events. Clutch frequency of 7.0 ± 1.3 nests per year for CNMI is the highest observed mean for the region (Suganuma et al., 1996; Limpus et al., 2003; Limpus, 2009; Burton, 2012; Balazs et al., 2015), but it falls within the ranges observed in Hawaii and Australia (Limpus, 2009; Balazs et al., 2015). Inter-nesting interval of 11.4 ± 1.0 days is on the short end of observed intervals from the other locations, and is most similar to Japan (Abe et al., 2003). Clutch size of 93.5 ± 21.4 eggs falls between smaller clutches observed in Malaysia (Pilcher and Basintal, 2000) and larger ones in Australia (Limpus, 2009).

Nests in the CNMI incubate relatively quickly and have hatching and emergence success comparable to other locations. The incubation period of 56.7 ± 6.4 days is low, similar to values observed in the warm climates of the Philippines and Malaysia (Trono 1991; Pilcher and Basintal, 2000), and shorter than those in the cooler climates in Hawaii and Australia (Limpus, 2009; Balazs et al., 2015). Hatching success of 77.9 ± 27.0% and emergence success of 69.6 ± 30.3% are within the ranges for the region (Trono, 1991; Limpus et al., 2003; Balazs et al., 2015), but potentially on the low end. Future studies could test whether these parameters are negatively impacted by (i) higher frequency

TABLE 4 | Generalized additive models (GAMs) exploring the influence of nest temperatures (mean and maximum during incubation) on hatching success and embryonic death for green turtle (*Chelonia mydas*) nests in Saipan, CNMI.

Model stage	Model	DE (%)	AIC	BIC
Hatching success				
Stage I	Beach + TempC.mean	40	−81.6	−57.7
	Beach + TempC.max	51	−98.4	−71.8
	Beach + Month.Exca	39	−169.5	−136.1
	Beach + Year.Exca	42	−173.8	−138.9
Stage II	Beach + Year.Exca + TempC.mean	45	−85.6	−56.9
	Beach + Year.Exca + TempC.max	56	−101.9	−69.1
	Beach + Year.Exca + Month.Exca	44	−173.1	−132.4
Stage III	Beach + Year.Exca + Month.Exca + TempC.mean	47	−85.6	−51.6
	Beach + Year.Exca + Month.Exca + TempC.max	57	−101.7	−64.9
Embryonic death				
Stage I	Beach + TempC.mean	27	−298.6	−271.3
	Beach + TempC.max	24	−295.2	−268.2
	Beach + Month.Exca	14	−389.6	−355.3
	Beach + Year.Exca	13	−391.2	−360.5
Stage II	Beach + Year.Exca + TempC.mean	28	−297.2	−267.5
	Beach + Year.Exca + TempC.max	26	−296.0	−266.4
	Beach + Year.Exca + Month.Exca	16	−390.8	−353.6
Stage III	Beach + Year.Exca + Month.Exca + TempC.mean	29	−294.9	−260.7
	Beach + Year.Exca + Month.Exca + TempC.max	28	−294.1	−259.4

DE, Deviance Explained, the amount of variation in the response variable explained by the predictor variables; higher values indicate better model fits to the data. AIC, Akaike Information Criterion; BIC, Bayesian Information Criterion. "Month.Exca" and "Year.Exca" are the month and year of nest excavation. AIC and BIC are goodness-of-fit performance metrics for which lower values are most ideal. Gray highlighting indicates models that were selected as final models.

and intensity of tropical storms and typhoons inundating nests in the CNMI (Shaw, 2013), (ii) warm temperatures experienced at low latitudes (Matsuzawa et al., 2002), and (iii) habitat factors, such as high instances of coral rubble and roots from non-native vegetation trapping hatchlings in nests (Zárate et al., 2013).

This population of nesting turtles exhibits a relatively low somatic growth rate. Although our recapture sample size was low (10 turtles), we documented a growth rate of 0.26 cm/yr for nesting green turtles in the CNMI. This is relatively low compared to a growth rate of 0.8 cm/yr from Malaysia (Pilcher and Basintal, 2000), the closest comparison in the region, and 0.5 cm/yr for nesters in Florida (Witherington and Ehrhart, 1989). High reproductive output during nesting seasons, combined with long-distance post-nesting migrations to foraging grounds, could potentially explain the low observed growth rate; growth rates of juveniles in this population range from 0.3 to 7.8 cm/yr (Summers et al., 2017).

Nester Abundance and Trends

Our results suggest that poaching is currently the greatest threat to this nesting population. The 32% harvest rate of Saipan's 7.7 ± 2.5 annual nesters potentially would be higher without the research efforts described in this study. Consistent monitoring often prevented or interrupted poaching and triggered enforcement efforts. Although we document other anthropogenic threats, adult females have a high reproductive value when compared to eggs and hatchlings, and thus their loss has the greatest impact to the population. For example, beach driving is one threat that impacts nests, hatchlings, and adults. The threat has largely been eliminated through beach barricades and a "walk it, don't drive it" campaign led by the CNMI Bureau of Environmental and Coastal Quality; however, on a few non-barricaded nesting beaches on Tinian and Rota and on Saipan beaches where barricades have been removed or recently washed away by super typhoons, there remains a "drive in" poaching opportunity to exploit nesting turtles.

The 4.0% difference between PGR estimates for the scenarios "with poaching" and "without poaching" represents the impact poaching has had on this population. In other words, the removal of nesting females from this small population has slowed its positive population growth trend. However, despite continued removal of reproductive females, this population is experiencing a positive trend in nester abundance with an annual PGR of 7.4%. This rate of increase is relatively high in comparison with the 6.8% estimate for green turtles in the Ogasawara Islands in Japan (Chaloupka et al., 2008; Seminoff et al., 2015), which belong to the same DPS. This rate is also steeper than the 5.4% observed in Hawaii (Balazs et al., 2015; Seminoff et al., 2015) and the 3.8% at Heron Island on the Great Barrier Reef (Chaloupka et al., 2008). While the CNMI nesting population is a small fraction of the larger DPS, the observed increase is a positive sign and suggests potential for recovery of this Endangered DPS.

We estimated a mean abundance of 11.9 nesters per year on Saipan, Tinian, and Rota combined. With a remigration interval of 4.6 years, this yields a total of 55 adult females. Total nester abundance for the CWP DPS is currently 6,518 individuals (Seminoff et al., 2015). While the CNMI portion represents < 1% of the total at present, a population growth rate of 7.4% suggests this population has not reached its carrying capacity. Observing a high percentage of neophyte nesters is expected at the start of any study as new individuals are tagged; however, our annual mean of 59% neophytes for 2010–2016 after one remigration interval had passed supports a hypothesis that newly mature adults continue to recruit into the breeding population. Alternative explanations may include much longer remigration intervals than estimated here, nesting at other sites (e.g., most likely within the Mariana Islands), or less than 100% detection of nesters during the study period. However, given the remigration intervals known for other green turtle populations, extreme nesting site fidelity, local knowledge and monitoring of nesting activity on Saipan, and a 20–40 year age at first reproduction, we find the neophyte hypothesis most plausible. New recruits are generally a good sign for the population, as long as they remain in the population and successfully reproduce. On beaches with saturation monitoring/tagging, the percentage of neophytes would be expected to stabilize at lower levels, assuming the previously tagged nesters are not being removed from the population through anthropogenic activities, such as poaching or bycatch.

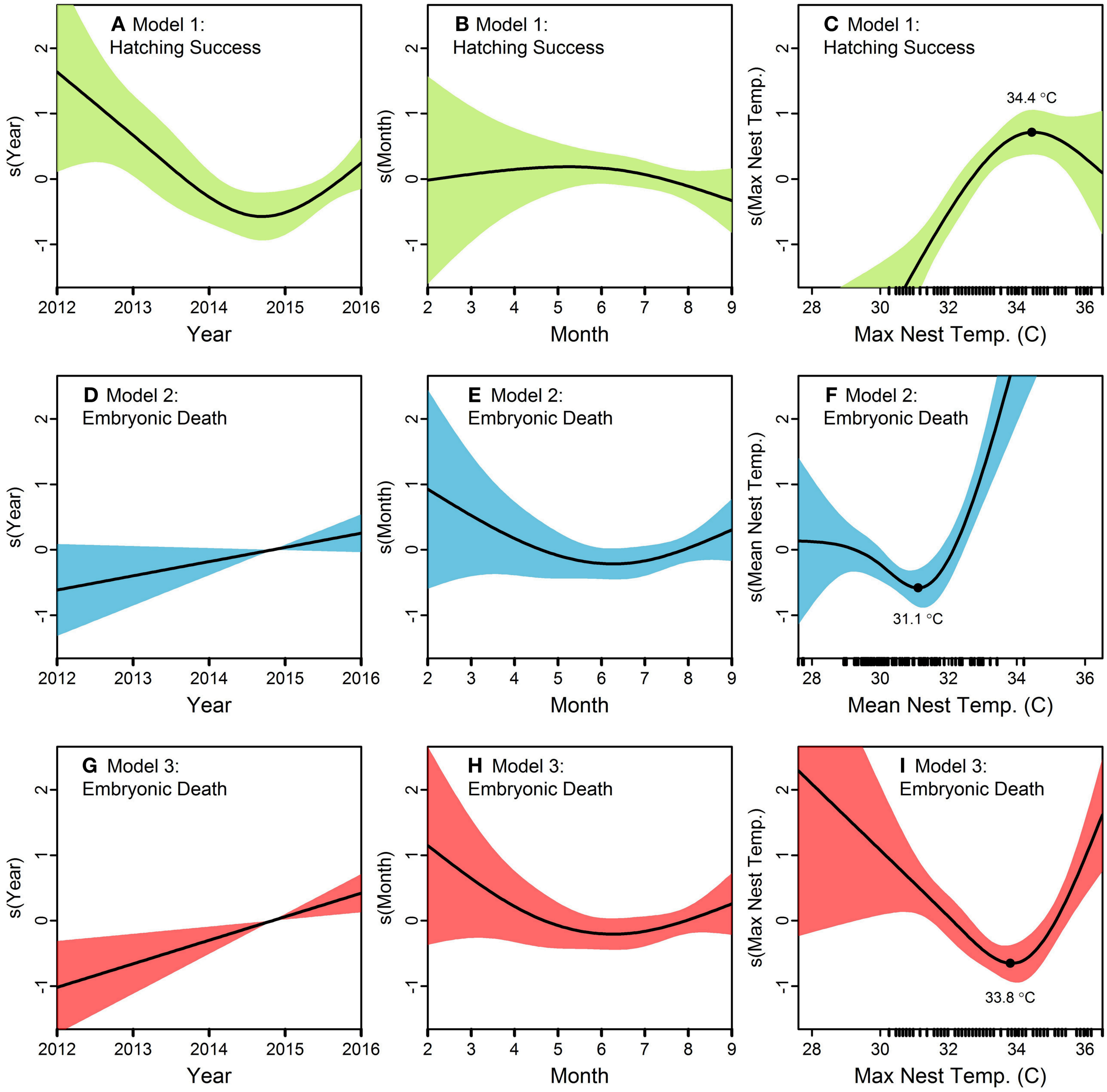

FIGURE 3 | Generalized additive models (GAMs) exploring the influence of year, month, and nest temperature (mean or maximum) on green turtle (*Chelonia mydas*) hatching success (Model 1) and embryonic death (Models 2 and 3) on Saipan beaches in 2012–2016. Nest temperature ($n = 89$ data loggers) is the predictor variable of primary interest, while beach (categorical variable; not shown here), year, and month are included to control other sources of variation in the response variables. Smoothed responses to individual predictors are shown on the y-axis of **(A–I)**. Rug plots along the x-axis of **(C,F,I)** show the distribution of raw data for the temperature variables. Model 1 suggests **(A)** an annual decrease in hatching success until 2015, **(B)** a slight maximum in hatching success in May-July, and **(C)** hatching success increases with maximum nest temperature up to 34.4°C, after which it decreases. Model 2, which uses mean nest temperature rather than the maximum, suggests **(D)** a steady increase in embryonic death over the years, **(E)** lowest embryonic death between May and July, and **(F)** a pronounced increase in embryonic death with mean nest temperatures beyond 31.1°C. Model 3 suggests **(G)** embryonic death has increased annually **(H)** highest embryonic death in February through April, and **(I)** embryonic death decreases with maximum nest temperatures up to 33.8°C, and increases sharply beyond that.

Climate Impacts

Our finding that the mean nest temperature of green turtles in the CNMI is 30.9 ± 1.5°C is concerning when compared to known pivotal temperatures for sex determination and embryonic death. This mean is above 29.0°C, the threshold beyond which a clutch becomes female biased (Standora and Spotila, 1985; Mrosovsky, 1994; Ackerman, 1997; Godfrey and Mrosovsky, 2006). Furthermore, it is above 30.3°C, a temperature which produces a minimum of 90% females in green turtle nests (Standora and Spotila, 1985; Spotila et al., 1987). Our results provide strong evidence that the current generation of green turtles produced in the CNMI is already female biased. This idea could be tested by determining sex for a sample of juvenile turtles on their foraging grounds (Allen et al., 2015). And while the expected female bias could boost the nesting population initially, this could become a problem if there are eventually too few males to sustain the breeding population (Layton, 2011).

The mean nest temperature of 30.9°C for CNMI green turtle nests currently falls below the pivotal threshold of 33.0°C for embryonic death (Packard et al., 1977; Miller, 1985). However, the maximum temperature of 33.5 ± 2.0°C across all nests in this study creates cause for concern and further investigation. Generally, it is the prolonged exposure to temperatures above 33.0°C that leads to increased hatchling mortality, but the exact critical temperature and exposure time needed to induce mortality in the CNMI is unknown. Projected increases in temperatures could lead to higher rates of embryonic death in the near future.

Our modeling results suggest a decrease in hatching success and corresponding increase in embryonic death beyond maximum nest temperatures of 34.4 and 33.8°C, respectively. Additionally, embryonic death appears to be triggered beyond a mean nest temperature of 31.1°C, which is only slightly higher than the mean nest temperature we measured. Warm temperatures resulting in embryonic death likely played a role in lowering hatching success from 100 to 77.9%. With rising global temperatures leading to rising sand temperatures, we can expect the hatching success of CNMI green turtle nests to continue declining.

CONCLUSION

This study provides the first comprehensive assessment of nesting demographic parameters and abundance trends for nesting green turtles in the Mariana Archipelago specifically, and Micronesia more broadly. Furthermore, it quantifies major threats to the survival of the population. The CNMI nesting data suggest an annual increase in nesting females of 7.4% per year, which is corroborated by a 10% increase in foraging green turtles (mostly juveniles) estimated from aerial surveys in the southern portion of the archipelago (Martin et al., 2016). These positive trends are promising in light of the previous exploitation of nesting and foraging turtles throughout the region (Groombridge and Luxmoore, 1989; Seminoff et al., 2015); however, the trends may be slowed by continued poaching or offset by warming temperatures.

ETHICS STATEMENT

This research was performed under the Commonwealth of the Northern Mariana Islands Department of Lands and Natural Resources. This is a governmental institution which does not require an Institutional Animal Care and Use Committee (IACUC) protocol but does require that endangered species research is permitted through US Federal agencies: US Fish and Wildlife Service (USFWS) and National Marine Fisheries Service (NMFS). The work presented in this paper was performed under this US territorial government agency under the auspices of a US Endangered Species Act (ESA) valid permit issued by USFWS (permit no. TE-017352-17). All work was done under the USFWS ESA guidelines, permits, and certifications with experienced permitted personnel.

AUTHOR CONTRIBUTIONS

TS, JH, and JR designed the study and collected and maintained nesting data. TS, SM, and TJ quality-checked and analyzed data. TS, SM, and TJ prepared figures and tables and wrote the manuscript. All authors reviewed the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2017.00428/full#supplementary-material

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Opportunism on the High Seas: Foraging Ecology of Olive Ridley Turtles in the Eastern Pacific Ocean

Lindsey E. Peavey [1, 2*], ***Brian N. Popp*** [3], ***Robert L. Pitman*** [1], ***Steven D. Gaines*** [2], ***Karen E. Arthur*** [3], ***Shaleyla Kelez*** [4] **and *Jeffrey A. Seminoff*** [1]

[1] National Oceanic and Atmospheric Administration (NOAA), National Marine Fisheries Service, Southwest Fisheries Science Center, La Jolla, CA, United States, [2] Bren School of Environmental Science and Management, University of California, Santa Barbara, Santa Barbara, CA, United States, [3] Department of Geology and Geophysics, School of Ocean and Earth Science and Technology, University of Hawaii, Honolulu, HI, United States, [4] ecOceanica, Lima, Peru

Stable isotopic compositions in animal tissues have been widely used to gain insight into trophic dynamics, especially of mobile aquatic predators whose behavior and dietary preferences are difficult to directly measure. Olive ridley sea turtles (*Lepidochelys olivacea*) range across >3 million km^2 of the tropical and subtropical eastern Pacific Ocean and their trophic ecology in open ocean areas has not yet been adequately described. Individuals feed within biogeographic regions where varying nutrient cycling regimes result in phytoplankton with distinct δ^{13}C and δ^{15}N values that are assimilated by the turtles. We sampled 346 turtles at-sea between 2003 and 2009 and used bulk tissue ($n = 346$) and amino acid compound specific isotope analysis (AA-CSIA, $n = 31$) to empirically support the conventional understanding that olive ridleys are omnivores. Bulk δ^{15}N values did not significantly vary with carapace length, a proxy for age, or with putative sex of adults. We therefore hypothesize that trophic position (TP) does not vary across age or sex. In line with other isotopic studies of this biogeographic scale in the same region, we observed a trend of bulk tissue ^{15}N enrichment with increasing latitude. Using AA-CSIA to account for δ^{15}N baseline shifts among food webs (space), we estimated the TP of adult foragers using two methods. We found that across their eastern Pacific range, olive ridley δ^{13}C and δ^{15}N niche area varied, but median TP of adults remained constant (~3.1). Using a two-amino acid TP estimation method, we detected a small but notable elevation of TP for olive ridleys on the Costa Rica Dome. This study underscores the value of large-scale in-water olive ridley sea turtle research across oceanic foraging habitats to confirm or challenge anecdotal understanding of trophic roles, susceptibility to environmental change, and critical habitats. Further, it improves our understanding of why this species is now abundant in the eastern Pacific Ocean. A prey generalist with plenty of suitable foraging habitat can recover from the brink of extinction despite the presence of major threats. However, such foraging characteristics may require dynamic open ocean management approaches to meet conservation objectives if threats persist and/or increase.

Keywords: amino acid compound specific isotope analysis, niche area, trophic position, oceanic food webs, olive ridley, *Lepidochelys olivacea*

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Luis Huckstadt,
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Virginia Institute of Marine Science, United States

****Correspondence:***
Lindsey E. Peavey
lindsey.peavey@noaa.gov

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INTRODUCTION

The complexity of ecosystem function depends on food web biodiversity, predator-prey relationships, and the degree of consumer generalism (Duffy et al., 2007). Understanding the trophic role(s) of wide-ranging consumers can shed light on spatial variation in trophic structure and/or resource availability across oceanic food webs. For example, consumers may exhibit faster trophic responses to sudden changes in food supply or phenology in simple food webs vs. complex food webs (Suryan et al., 2009). A response such as an increase in trophic position may have demographic benefits, like increased survival.

Defined by their nutrient regimes, the three main types of pelagic systems are upwelling, oligotrophic oceanic, and eutrophicated coastal systems (Sommer et al., 2002). Regardless of the mechanistic drivers of the trophic structure within these systems [e.g., resource-driven (bottom-up) and/or predation (top-down)], it is well established that the biological characteristics of dominant species regulate biogeochemical cycling in spatially subsidized (i.e., patchy) open ocean food webs (Menge and Sutherland, 1976; Longhurst and Harrison, 1989; Polis et al., 1997). Today, humans are an important element of marine food webs by influencing both bottom-up and top-down controls via nutrient enrichment and resource extraction, respectively.

Mobile marine consumers like sea turtles can have variable foraging strategies. Omnivores feed on an array of prey within a food web and often span multiple trophic levels (Thompson et al., 2007). Omnivores are considered generalists when they access prey resources across food webs (Pillai et al., 2011). In contrast, specialists feed on a lower diversity of prey items within and across food webs, generally within only a single trophic level. Both types of consumers play important roles in top-down regulation of food webs. Phenology, physiology, and/or community structure can influence changes in trophic role across space (e.g., habitat) and time (e.g., with growth). Understanding the trophic position of a consumer, along with its ecological niche within a given food web, can provide insight into foraging preferences and/or response to ecosystem heterogeneity.

Stable carbon and nitrogen isotopic compositions have been used extensively to gain insights into foraging strategy, particularly for consumers whose behavior and dietary preferences are difficult to observe directly (Fry, 1988; Olson et al., 2010). δ^{13}C and δ^{15}N values vary across ecosystems and are tracers of metabolic and biogeochemical processes (Montoya, 2007). For example, the variability of primary producer δ^{13}C values is driven by physical forces of the carbon cycle, temperature and $[CO_2]_{aq}$, as well as biology (Laws et al., 1995; Cassar et al., 2006; McMahon et al., 2013). Coastal and benthic systems are typically nutrient rich compared to offshore and pelagic systems, and thus have phytoplankton more ^{13}C-enriched (France, 1995). As such, δ^{13}C values of consumer tissues reflect those at the baseline of the food web. Nitrogen integrated into consumer tissues is enriched in ^{15}N relative to prey, such that top predators have the highest δ^{15}N values (DeNiro and Epstein, 1981; Fry, 1988; Cabana and Rasmussen, 1996). Because the dominant nitrogen cycling regime in a region influences δ^{15}N values at the base of the food chain (Hobson, 1999; Vander Zanden and Rasmussen, 2001), spatially discrete food webs with differing nutrient cycling offer distinct biogeochemical frameworks to study animal movement and trophic ecology (Hobson et al., 2012). In practice, measuring δ^{15}N values of tissues from multiple individuals within a population can provide insights about local nitrogen sources, trophic level, and niche space (Thomas and Crowther, 2015).

When investigating wide-ranging consumers, it is important to consider natural variations of δ^{13}C and δ^{15}N values at the base of the food web, in addition to trophic fractionation. "Bulk" (whole) tissue δ^{13}C and δ^{15}N values reflect a composite view of all assimilated organic compounds (e.g., protein amino acids). Many marine studies of cryptic consumers have examined bulk tissue to infer diet composition and/or trophic interactions (Ruiz-Cooley and Gerrodette, 2012; Allen et al., 2013; Thompson et al., 2015), including isotopic niche space—a multi-dimension measure of all interactions between a consumer and its habitat and prey (Elton, 1927; Hutchinson, 1957; Bearhop et al., 2004; Newsome et al., 2007; Yeakel et al., 2016). However, the interpretation of bulk analysis results is constrained by an inability to discern trophic vs. baseline influences on δ^{13}C and δ^{15}N values of consumer tissues (Hussey et al., 2014; Bowes and Thorp, 2015). A second approach, amino acid compound-specific nitrogen isotopic analyses (AA-CSIA), can help overcome this limitation.

The δ^{15}N values of amino acids fall into two groups: "source" amino acids (e.g., phenylalanine, lysine) that minimally fractionate with trophic processing, and "trophic" amino acids (e.g., glutamic acid, alanine) that undergo ^{15}N-enrichment with trophic transfers (McClelland and Montoya, 2002; Chikaraishi et al., 2007; Popp et al., 2007). Source amino acids reflect the isotopic composition at the base of the food web, whereas trophic amino acids reflect the trophic level of the consumer. Examining δ^{15}N values of both types of amino acids can thus yield insights that cannot be gained with bulk-tissue analysis alone (Chikaraishi et al., 2009, 2010).

The integration of bulk-tissue stable isotope analysis with AA-CSIA has provided insights about several marine taxa, including sea turtles (Seminoff et al., 2012; Vander Zanden et al., 2013; Arthur et al., 2014). This combined approach is useful for studying the trophic ecology of cryptic species as well as those for which empirical dietary information is limited. The olive ridley sea turtle (*Lepidochelys olivacea*) is an example of both. It is an oceanic species, and individuals live offshore in waters largely inaccessible for research for the majority of their lives. Although long-term diet studies would be challenging, it is possible to collect tissue samples from individuals at sea.

Olive ridleys are the smallest and most abundant sea turtle species in eastern Pacific Ocean and are thought to mature at a younger age (∼13 years) than other turtles (Zug et al., 2006; Eguchi et al., 2007; Seminoff and Wallace, 2012). They range >3 million km^2 across this dynamic ocean basin and are thus present in multiple biogeographic regions (Pennington et al., 2006; Olson et al., 2010; Plotkin, 2010). As nomadic opportunistic omnivores, they don't undergo ontogenetic habitat shifts and may feed in both benthic and pelagic habitats (Bjorndal, 1997; NMFS and USFWS, 1998; Robins et al., 2002; Bolten, 2003; Whiting et al., 2007; Behera et al., 2015). Olive ridleys often forage via passive drifting rather than active swimming, meaning they eat within the same food web for many days (Block et al., 2002; Polovina et al., 2003; McMahon et al., 2007; Whiting et al., 2007; Plotkin, 2010). Their oceanic diet consists of mostly planktonic items or items living on or near flotsam including algae, crustaceans, and salps (Kopitsky et al., 2005; Jones and Seminoff, 2013; Wedemeyer-Strombel et al., 2015; Pitman, Kopitsky and Peavey, pers. comms.). Thus, the trophic position of the olive ridley may be different than for other large vertebrates such as marine mammals, sharks, and seabirds. Despite these insights, olive ridley open ocean trophic ecology has not yet been sufficiently investigated. However, their foraging behavior and temporal scale makes them perhaps the best large consumer to study spatial differences in trophic roles across oceanic food webs.

Here we apply bulk-tissue stable isotope analysis and AA-CSIA to olive ridley sea turtles foraging in the eastern Pacific Ocean. We quantitatively describe isotopic niche variation for olive ridleys across a large portion of their range, and estimate the trophic positions of adult olive ridleys. To our knowledge, this is the first account of the isotope ecology of olive ridley turtles in the Pacific Ocean. We discuss our findings as they relate to persistent but dynamic oceanic foraging habitats, conservation implications of potentially unique open-ocean foraging areas, and the olive ridley's resilience to climate, habitat quality and prey changes.

METHODS

Study Region and Sub-Regions

The study region spans the tropical and subtropical eastern Pacific Ocean, extending from ∼30° N (Gulf of California) to ∼16° S (Peru Current), and ∼76° W (west coast of the Americas) to ∼115°W (**Figure 1**). This region is oceanographically dynamic but has persistent and predictable areas of upwelling, warm pools, cold tongues, and boundary currents that support spatially explicit nutrient cycling regimes, such as nitrogen fixation and denitrification (Fiedler and Talley, 2006). Per these regimes, $\delta^{15}N$ values are distributed across the region's oceanographic features and distinct $\delta^{15}N$ baselines are assumed to represent distinct food webs.

We examined geographic variation of stable isotope compositions in olive ridleys relative to regions of varying biogeochemical processes at two different scales. First, we grouped samples by Longhurst biogeochemical provinces (VLIZ, 2009), and second, by oceanographic features described in Fiedler and Talley (2006). Longhurst provinces (L) were defined by the following numeric labels: 7 ("Coastal—Central American Coastal Province"), 8 ("Coastal—Chile-Peru Current Coastal Province"), and 35 ("Trades—North Pacific Equatorial Countercurrent Province") (VLIZ, 2009; **Figure 1A**). Alternatively, samples were grouped into five distinct sub-regions based on the following oceanographic features: the Gulf of California (GC), the North Equatorial Current (NEC), the Eastern Pacific Warm Pool (EPWP), the Costa Rica Dome (CRD, an oceanic thermal feature), and the Peru Current (PC) (**Figure 1B**). Spatial analyses across sub-regions were limited to individuals sampled within feature boundaries. Our analyses relied on the premise that stable isotope values of olive ridley skin reflect the local food web in which the turtle was sampled, considering the isotopic turnover of sea turtle skin tissue (ca. 40–50 d, Reich et al., 2008) and the passive movements of olive ridleys foraging at-sea.

Sample Collection and Preparation

From August to December 2006, in total, 320 olive ridley sea turtles were opportunistically hand-captured from a small boat deployed from the National Oceanographic Atmospheric Administration R/V *David Starr Jordan* during the *Stenella* Abundance Research (STAR) cruise (Jackson et al., 2008). Morphometric information was collected for all turtles, and putative sex was recorded for mature/adult individuals. Based on external morphology, individuals with straight carapace length $\geq$ ca. 56 cm are considered adult (NMFS and USFWS, 1998). Putative sex of adult-sized turtles was based on tail length; individuals with long tails (>20 cm length) were classified as males whereas those with shorter tails were considered females. For individuals sampled within the GC ($n = 29$), NEC ($n = 36$), EPWP ($n = 192$), and CRD ($n = 63$) sub-regions [alternatively: L7 ($n = 172$), L8 ($n = 21$), and L35 ($n = 151$)], a razor blade was used to collect epidermis ("skin") samples ∼2 mm from the dorsal neck surface, and samples were immediately frozen at −80°C and then stored at −20°C at the Southwest Fisheries Science Center (La Jolla, CA, USA) until laboratory analysis. All turtles were released unharmed within ∼20 km of where they were captured.

Prior to stable isotope analyses, samples were thawed and rinsed with distilled water, freeze dried for one 8-h cycle, and lipid-extracted using an Accelerated Solvent Extractor (ASE 200) according to previously published methods (Lemons et al., 2011; Allen et al., 2013). Lipid extraction is not known to significantly alter $\delta^{13}C$ or $\delta^{15}N$ values in sea turtle skin (Medeiros et al., 2015; Bergamo et al., 2016). All samples were analyzed for bulk-tissue stable isotope values ($\delta^{13}C$ and $\delta^{15}N$), whereas 4–14 samples in each sub-region were processed for AA-CSIA (**Figure 1**).

In total, 22 adult olive ridleys in the Peru Current were sampled from turtles incidentally captured by Peruvian longline fishing vessels. Using a 2-mm biopsy punch, skin samples were taken from the dorsal neck surface of adult olive ridleys in 2003 ($n = 3$), 2004 ($n = 5$), 2008 ($n = 10$), and 2009 ($n = 4$), preserved with salt, and archived at −20°C at the Southwest Fisheries Science Center (La Jolla, CA, USA) until laboratory analysis. These samples were lipid extracted using the same methods as above, and analyzed for bulk-tissue stable isotope values as described in Kelez (2011) and Arthur et al. (2014). AA-CSIA was completed for five samples from 2008 and 2009 using the same methods described below and in Arthur et al. (2014). Note that these samples were originally for a separate study, and as such they are unique in their collection year and method.

Bulk Tissue Analysis

i) Mass Spectrometry

For GC, NEC, EPWP, and CRD 2006 samples ($n = 320$), 0.7–1 mg of skin was homogenized with a razor blade and loaded into tin capsules. Samples were analyzed by a Costech

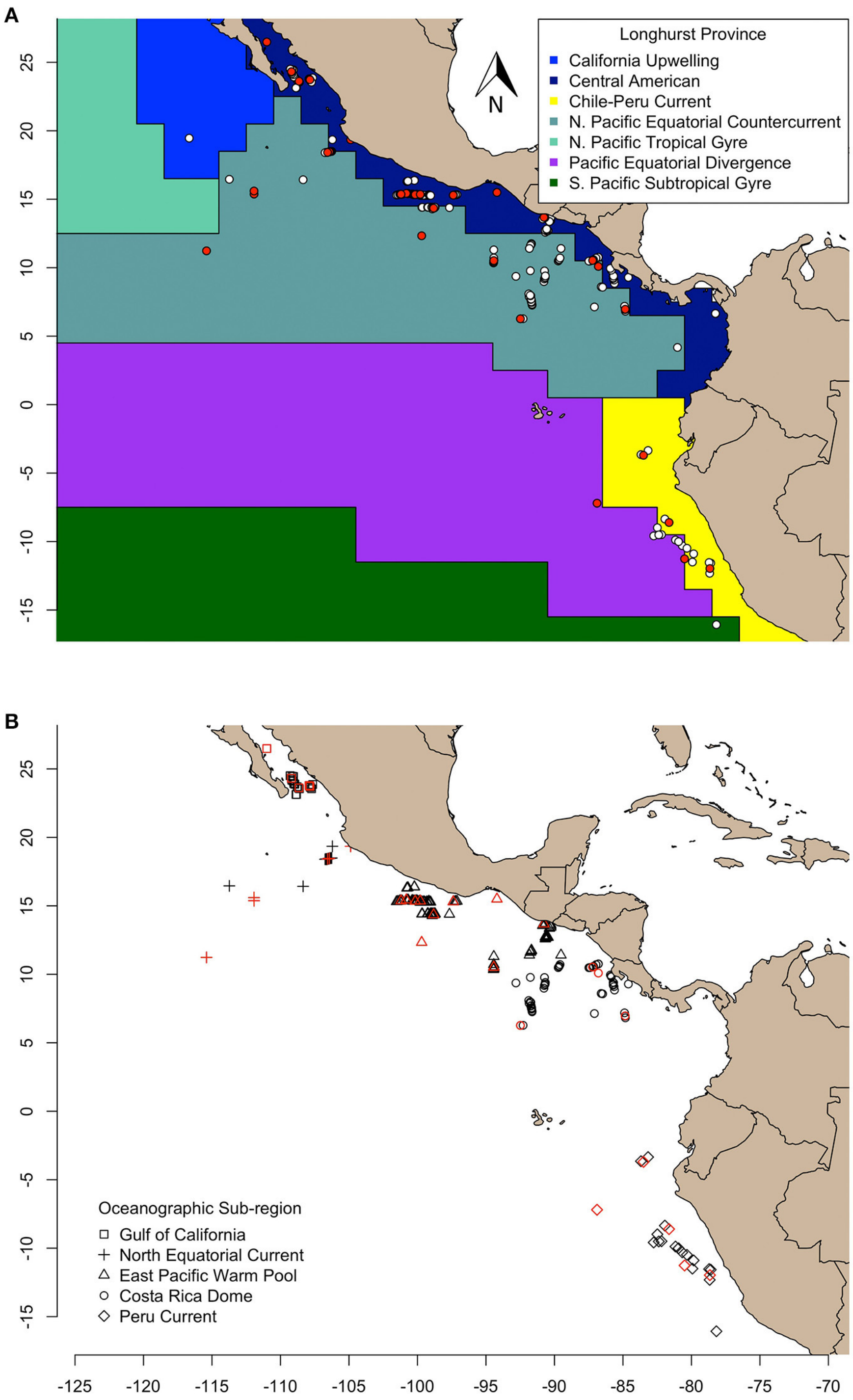

FIGURE 1 | Olive ridley turtles sampled in this study are represented as symbols and aggregated according to **(A)** Longhurst province (VLIZ, 2009), and **(B)** oceanographic sub-region (Fiedler and Talley, 2006). The δ^{13}C and δ^{15}N values of skin of all turtles were determined, and compound specific amino acid δ^{15}N values were measured for only those colored in red.

Instruments elemental combustion system (ECS4010) coupled to a continuous-flow Thermo Finnigan MAT Delta Plus XL isotope ratio mass spectrometer in the Stable Isotope Laboratory at the University of Florida, Gainesville. PC samples were analyzed in an analogous way in the same facilities, as described in Kelez (2011). Bulk isotope values are reported in standard delta notation (δ) in parts per thousand (‰): $\delta^{H}X = ([R_{sample}/R_{standard}] - 1) \times (1{,}000)$, where the superscript “H” is the mass of the heavy isotope, X is the element of interest, and R is the ratio of the heavy X isotope to the light X isotope (Fry, 2006). $R_{standard}$ was atmospheric N_2 and Vienna Pee Dee Belemnite (VPDB) for $\delta^{15}N$ and $\delta^{13}C$, respectively. Continuous calibration was completed using USGS40 (L-glutamic acid: $\delta^{15}N = -4.52‰$ and $\delta^{13}C = -26.39‰$) with an average precision of 0.07‰ for $\delta^{15}N$ and 0.10‰ for $\delta^{13}C$. To ensure accuracy, 1–3 blind sample duplicates were run per 30 samples with an average standard deviation of 0.14‰ for $\delta^{15}N$ and 0.27‰ for $\delta^{13}C$.

ii) Exploring $\delta^{15}N$ Shift with Size, Gender

Both curved carapace length (CCL) and straight carapace length (SCL) was measured for all STAR turtles. However, only CCL was measured for Peru turtles. In order to estimate SCL for Peru turtles based on the CCL measurements, we used the following model ($R^2 = 0.99$) derived from the linear relationship between SCL and CCL for STAR turtles ($n = 354$, see Supplementary Table 1):

$$y = 0.9417x + 0.1466 \tag{1}$$

Subsequently, a linear regression model ($\alpha = 0.05$) was used to explore if bulk $\delta^{15}N$ values in skin varied with SCL (cm; $n = 337$), after controlling for latitude by specifying sub-region as a factor:

$$\delta^{15}N \sim SCL + SubRegion + error \tag{2}$$

To explore if adult bulk $\delta^{15}N$ values in skin varied with gender (sex: female/male; $n = 185$) after controlling for latitude by specifying sub-region, a two-way ANOVA (Type III, $\alpha = 0.05$) was used:

$$\delta^{15}N \sim sex + SubRegion + sex * SubRegion + error \tag{3}$$

iii) Isotopic Niche Area

Standard niche width ellipse and convex hull areas were estimated using maximum likelihood, and Markov chain Monte Carlo (MCMC) credible intervals were generated to calculate uncertainty around ellipse estimates using Stable Isotope Bayesian Ellipses (SIBER) functions (Jackson et al., 2011) in the Stable Isotope Analysis in R (SIAR) package (Parnell et al., 2008, 2010). Probability of size differences between ellipses were calculated by comparing pairs of draws from the posterior MCMC distributions.

Amino Acid Compound-Specific Nitrogen Isotopic Analyses (AA-CSIA)

i) Mass Spectrometry

Of the 320 STAR samples used for bulk tissue stable isotope analysis, a subset of 26 samples from adults were dried and homogenized with a mortar and pestle and/or razor blade (2–10 mg). Samples making up the subset were chosen to cover the widest geographic area of each sub-region: GC ($n = 6$), NEC ($n = 6$), EPWP ($n = 10$), and CRD ($n = 4$); and alternatively, L7 ($n = 14$) and L35 ($n = 12$).

Samples were prepared (hydrolysis and derivatization) and analyzed for compound-specific isotopic composition of amino acids at the Biogeochemical Stable Isotope Laboratory at the University of Hawaii at Manoa following Popp et al. (2007), Hannides et al. (2009) and Décima et al. (2013). Briefly, samples were hydrolyzed (6N HCl, 150°C), the hydrolysate purified (0.2 μm pore size Polyethersulfone filters, cation exchange chromatography), the carboxyl terminus the amino acids esterified (4:1 C_3H_8O and CH_3COCl, 110°C) and the amino group acelyated (3:1 CH_2Cl_2 and 200 μl $C_4F_6O_3$, 100°C). A final solvent extraction assured that the sample derivatives were pure. Samples were stored frozen at −20°C until analysis in triplicate using a mass spectrometer (Thermo Scientific Delta^{Plus}V or MAT 253 interfaced with a Trace GC/GCIII; see Hannides et al. (2009) and Bradley et al. (2015) for further mass spectrometry details).

The $\delta^{15}N$ values of 13 amino acids [alanine, glycine, valine, serine, leucine, isoleucine, proline, glutamic acid, phenylalanine, lysine, tyrosine, and norleucine (Nor) and aminoadipic acid (AAA)], measured against internal Nor/AAA reference material of known isotopic composition, were quantified in each sample. Every block of three sample measurements was bookended by a suite of amino acids with known $\delta^{15}N$ values (alanine, threonine, isoleucine, proline, glutamic acid, and phenylalanine). Suite/samples were co-injected with Nor and AAA with known $\delta^{15}N$ values serving as internal reference material and to control for errors due to sample loss, injection variations, and variability in dilution preparations. Sample $\delta^{15}N$ values for 11 amino acids were normalized using regression (typically $R^2 > 0.9$) of either the Nor/AAA or suite standards. Accuracy was maintained to within 1‰ of the known value, and the average standard deviation of $\delta^{15}N$ across all 2006 samples and amino acids was 0.75‰.

The Peru Current samples were prepared and analyzed separately but in the same lab and with the same protocol at the University of Hawaii at Manoa. These were grouped as PC ($n = 5$), and alternatively L8 ($n = 5$), and had an average standard deviation of $\delta^{15}N$ across all samples and amino acids of 0.56 and 0.63‰, respectively (Arthur et al., 2014).

ii) Exploring Variation of Bulk $\delta^{15}N$ Values

To test our hypothesis that the variation in bulk $\delta^{15}N$ values in olive ridley sea turtle skin [standard deviation (SD) = 0.8] is driven by $\delta^{15}N$ values of source nitrogen, we built the following Deming regression (Type II, $\alpha = 0.05$) using the "mcr" package. Deming regression is an extension of simple linear regression that compares two estimation methods by accounting for measurement errors along both the x- and y-axis, instead of only along the y-axis. If two methods are parallel, a slope of one is expected.

$$\delta^{15}N\ skin \sim \delta^{15}N\ source_{aa} \tag{4}$$

We ran the regression two ways across the 31 samples that had both bulk and amino acid $\delta^{15}N$ values, one using phenylalanine ($SD = 0.5$) as the source amino acid (ratio of variance = 1.6), and one using the weighted mean of three source amino acids [glycine, lysine and phenylalanine] ($SD = 0.4$, ratio of variance = 2).

iii) Trophic Position Estimations

We compared two approaches to estimate trophic position (TP) according to Chikaraishi et al. (2009, 2010) and Bradley et al. (2015). We used $\delta^{15}N$ values for either phenylalanine (Phe) or the weighted mean of three source amino acids (glycine, lysine, and phenylalanine), and glutamic acid (Glu) or the weighted mean of three trophic amino acids (alanine, leucine, and glutamic acid) to estimate olive ridley TP in each sub-region. If samples had missing values for any of these amino acids, they were excluded from the weighted mean trophic position approach and the method comparison. All samples had values for Glu and Phe. We propagated error to calculate *SD* (see Dale et al., 2011; Choy et al., 2012; Bradley et al., 2015 for details). The two approaches were compared with a two-sided, paired Wilcoxon Signed-Rank test.

The following TP equation shows Glu and Phe as placeholders but were replaced with weighted means for the second approach (see Equation 2 in Nielsen et al., 2015). Trophic discrimination is reasonably predictable and can be accounted for with an enrichment factor (DeNiro and Epstein, 1981; Minagawa and Wada, 1984; Bradley et al., 2014). Estimates of ^{15}N enrichment among amino acids in primary producers ($\beta_{\mathrm{Glu-Phe}} = 3.6‰$) and for each trophic level ($\Delta_{\mathrm{Glu-Phe}} = 5.7‰$) were recommended by Bradley et al. (2015) and were equivalent across the two approaches. For trophic enrichment, others suggest $\Delta_{\mathrm{Glu-Phe}} = 7.6‰$ (Chikaraishi et al., 2009), 6.6‰ (Nielsen et al., 2015), or other values (see Lorrain et al., 2009; Dale et al., 2011). We chose values vetted in the literature ($\beta = 3.6$, $\Delta = 5.7$), and because they resulted in more reasonable omnivorous TP estimates considering the range of known $\delta^{15}N$ values for eastern Pacific olive ridley prey (Supplementary Tables 1 and 2).

$$TP = \frac{\delta^{15}N_{Glu} - \delta^{15}N_{Phe} - \beta}{{}_{Glu-Phe}} + 1 \quad (5)$$

Due to the small AA-CSIA sample sizes, we did not assume that TP estimates are normally distributed, and thus used the Kruskal–Wallis non-parametric statistical test to compare TP distribution across sub-regions. Exploratory analysis of $\delta^{15}N$ probability densities, median TP, and confidence intervals of each sub-region prompted a pooled pairwise comparison (Mann–Whitney *U*-test) of trophic position estimates between the Costa Rica Dome and all others. We treated sub-region as a blocking factor in the Mann-Whitney rank sum test, and $\alpha = 0.05$ for both tests. All statistical tests were performed in R (www.r-project.org/).

RESULTS

Bulk $\delta^{13}C$ values across the study region ($n = 346$) ranged from −17.8‰ (Gulf of California) to −14.5‰ (East Pacific Warm Pool), and 8.8‰ (Peru Current) to 15.6‰ (Gulf of California) for $\delta^{15}N$ (**Table 1**). We observed a general trend of ^{15}N enrichment with increasing latitude (Supplementary Figure 1). Within the northern latitudes, the turtle sampled at the lowest latitude (4.17°N) had a $\delta^{15}N$ value of 11.9‰ and the turtle sampled at the highest latitude (26.48°N) had a $\delta^{15}N$ value of 15.6‰ (**Figure 2**). Bulk $\delta^{15}N$ values were not correlated with the $\delta^{15}N$ values of phenylalanine (Deming regression slope ≈ 2.7, 95% confidence bounds: 1.8, 5.6) or the weighted mean $\delta^{15}N$ values of source amino acid (Deming regression slope ≈ 2.9, 95% confidence bounds: 1.4, 12.1).

After controlling for sub-region, SCL was only a significant predictor of bulk skin $\delta^{15}N$ values when the model included PC turtles ($p = 0.02$). When PC turtles were excluded, SCL was not

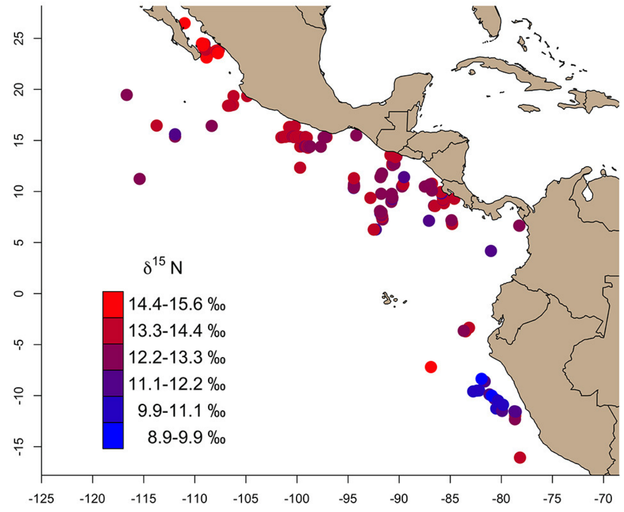

FIGURE 2 | The gradient of bulk skin tissue $\delta^{15}N$ values from olive ridley turtles sampled across the study area ranged from 8.9 to 15.6‰. In general, $\delta^{15}N$ values increased from south (Peru Current) to north (Gulf of California).

TABLE 1 | Bulk $\delta^{15}N$ and $\delta^{13}C$ (‰) information summarized by oceanographic sub-region, listed in latitudinal order from north to south.

Sub-region	Range $\delta^{15}N$	Mean $\delta^{15}N$	*SD* $\delta^{15}N$	Range $\delta^{13}C$	Mean $\delta^{13}C$	*SD* $\delta^{13}C$
Gulf of California	12.9, 15.6	14.4	0.71	−17.8, −15.2	−16.2	0.47
N. Equatorial Current	11.5, 14.5	13.6	0.57	−16.9, −15.1	−15.7	0.42
E. Pacific Warm Pool	11.9, 15.0	13.3	0.54	−16.2, −14.5	−15.4	0.34
Costa Rica Dome	11.4, 14.0	13.0	0.57	−16.1, −14.8	−15.4	0.29
Peru Current	8.8, 15.2	11.7	1.68	−16.7, −14.6	−15.9	0.96

See Supplementary Data Table 1 for the complete dataset, including sampling location, turtle size and sex.

a significant predictor of δ^{15}N values [$p = 0.06$; Supplementary Table 2; Supplementary Figure 2]. After controlling for sub-region, adult gender was not a significant predictor of bulk skin δ^{15}N values [$F_{(1)} = 0.2$, $p = 0.70$; Supplementary Table 4].

Maximum likelihood sub-region ellipse area estimates (with small sample size corrections in parentheses) are as follows: L7 = 0.7(0.7) ‰^{2}, L35 = 0.9(0.9) ‰^{2}, L8 = 2.7(2.8) ‰^{2}, CRD = 0.5(0.5) ‰^{2}, EPWP = 0.6(0.6) ‰^{2}, GC = 1.0(1.1) ‰^{2}, NEC = 0.7(0.8) ‰^{2}, PC = 2.7(2.8) ‰^{2} (**Figures 3A,B**). In comparing niche area across Longhurst provinces (**Figure 3C**), olive ridley niche area is much larger in L8 than in either L35 or L7 (>99% probability). Further, their niche in L35 is significantly larger than in L7, even though the two are much more similar in size compared to in L8.

Comparison of niche area across oceanic food webs defined by oceanographic features (**Figure 3D**) shows that olive ridley niche in the Peru Current is over two and half times larger than it is in the Gulf of California (>99% probability). Further, niche is larger in the Gulf of California than in the North Equatorial Current and the Costa Rica Dome (>89% and 99% probabilities, respectively). However, there is only moderate (~60%) confidence that niche is larger in the Costa Rica Dome than in the East Pacific Warm Pool, as their ellipse areas are quite similar.

Trophic position estimates using $\delta^{15}N_{Glu} - \delta^{15}N_{Phe}$ vs. weighted means of multiple trophic and source amino acids were not significantly different [$V = 231$, $p = 0.98$; Supplementary Table 3]. Median TP across the entire eastern Pacific seascape was 3.14 ± 0.2 *SD* using the $\delta^{15}N_{Glu} - \delta^{15}N_{Phe}$ approach ($n = 31$), and 3.18 ± 0.2 *SD* using the weighted mean approach ($n = 29$).

When comparing median TP across sub-regions, there was no significant difference across Longhurst provinces [$\chi^2(2) = 1.83$, $p = 0.40$], and no significant difference across oceanographic regions GC, NEC, EPWP, CRD, and PC [$\chi^2(4) = 5.52$, $p = 0.24$], regardless of TP estimation method. We see a significant difference in median TP between the Costa Rica Dome and all other oceanographic sub-regions pooled [$W = 20$, $p = 0.046$] when using the TPs estimated with $\delta^{15}N_{Glu} - \delta^{15}N_{Phe}$. Using this

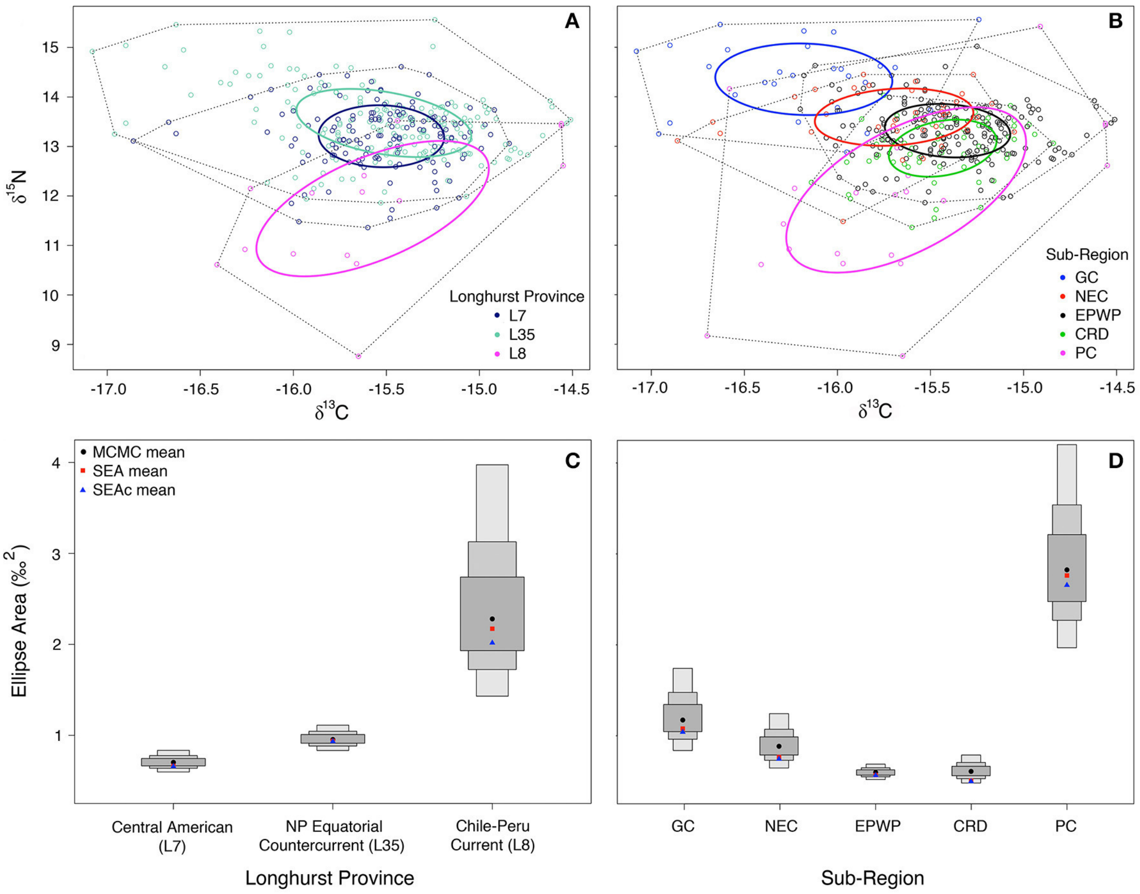

FIGURE 3 | Maximum likelihood standard ellipse area (‰^{2}) estimation is plotted for **(A)** (L7) Central American ($n = 186$), (L35) North Pacific Equatorial Countercurrent ($n = 137$), and (L8) Chile-Peru Current ($n = 15$) Longhurst provinces; and **(B)** Gulf of California ($n = 29$), North Equatorial Current ($n = 36$), East Pacific Warm Pool ($n = 192$), and Costa Rica Dome ($n = 63$) oceanographic sub-regions. Plotted in **(C,D)** are the Bayesian inference credible intervals (CI) around the Markov chain Monte Carlo (MCMC) ellipse area means, standard ellipse area (SEA) means, and the small sample size-corrected standard ellipse (SEAc) means (Parnell et al., 2010) for the Longhurst provinces and oceanographic sub-regions, respectively. The smallest/darkest gray box around the MCMC means (black circles) represents the 50% CI, and the next largest the 75% CI, and the largest the 95% CI.

method, we find that individuals on the Costa Rica Dome were feeding at a 0.36 median TP higher than in other oceanographic sub-regions. However, we do not see a significant difference in median TP between the Costa Rica Dome and all other sub-regions pooled [$W = 45$, $p = 0.70$] using the TPs estimated with weight means of δ^{15}N source and trophic amino acids. See **Figure 4** and Supplementary Table 3 for results for both approaches.

DISCUSSION

This study is the first to quantify olive ridley isotopic niche and trophic position. Whereas trophic level estimates based on bulk tissue isotopic composition exists for other wide-ranging marine consumers, few studies compare trophic position across a species' range by accounting for baseline δ^{15}N differences using AA-CSIA. We found that olive ridley trophic position is consistent across the eastern Pacific (∼3.1), apart from perhaps on the Costa Rica Dome where it may be slightly elevated. Our AA-CSIA results also indicate that trophic position does not differ between adult males and females. Trophic consistency among olive ridleys within a given region is further supported by finding that bulk skin δ^{15}N values do not vary with size (i.e., age), and this lack of diet shift may explain the lack of an ontogenetic habitat shift, as found in other sea turtle species (Turner Tomaszewicz et al., 2015). Together, these findings demonstrate the value of combining bulk-tissue analysis and AA-CSIA and highlights the status of the olive ridley as a unique oceanic consumer with relatively uniform foraging strategies among individuals, regardless of age, sex, or oceanic food web.

Isotopic Niche Space and Omnivory

The large ranges of δ^{13}C [−17.08, −14.51‰] and δ^{15}N [11.36, 15.56‰] values support the notion that olive ridleys are generalist omnivores, preying on a variety of primary producers (basal carbon sources) from primary and secondary trophic levels and in different areas of the ocean. Our findings suggest that despite potential individual foraging differences (e.g., prey species), the functional role of olive ridleys as omnivores, albeit largely planktivorous, remains consistent across oceanic food webs separated by thousands of kilometers. This is consistent with the few empirical dietary data for olive ridleys in this region (Bjorndal, 1997; NMFS and USFWS, 1998; Holt et al., 1999; Supplementary Tables 1, 5).

By estimating isotopic niche ellipse areas for each food web we are able to think critically about why the niche space of an

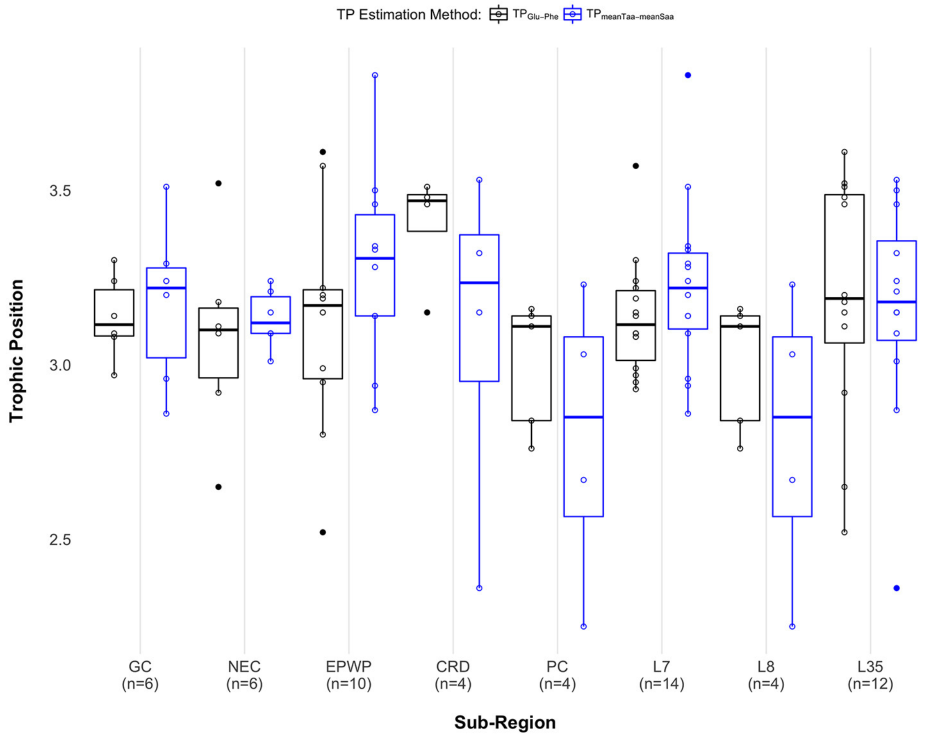

FIGURE 4 | Paired boxplots show individual trophic position estimates as open black circles for the two-amino acid approach ($TP_{Glu-Phe}$) and open blue circles for the weighted mean approach ($TP_{meanTaa-meanSaa}$). Median trophic position for each method is indicated as a dark black ($TP_{Glu-Phe}$) or a blue ($TP_{meanTaa-meanSaa}$) band within each box, first and third quartiles as the lower and upper box sections, respectively, and the minimum and maximum estimates as whiskers. Outliers are displayed as solid circles. Sample sizes are labeled along the x-axis as follows: GC, Gulf of California ($n = 6$); NEC, North Equatorial Current ($n = 6$); EPWP, East Pacific Warm Pool ($n = 10$); CRD, Costa Rica Dome ($n = 4$); PC, Peru Current ($n = 5$ for the two-amino acid method; $n = 4$ for the weighted mean method); L7, Central American Coastal ($n = 14$); L8, Chile-Peru Current ($n = 4$); L35, North Pacific Equatorial Countercurrent ($n = 12$). Note that only the pair PC and L8 include the exact same data points. See Supplementary Material Data Table 2 for complete AA-CSIA data, including error propagation.

omnivore may vary across a species' range. Since baseline δ^{13}C and δ^{15}N values and their within-food web variation influences niche space, we discuss each ellipse estimate in relation to the biological and physical forces acting in each food web. Although, Longhurst province boundaries are defined by physical forces that regulate the distribution of phytoplankton in the ocean, grouping foraging turtles accordingly was too coarse a scale for this investigation. While these ecological partitions are useful in guiding large-scale biogeochemical (e.g., isotope) studies concerned with nutrient cycling, they are static and quite large compared to the sub-regions that have been defined based on persistent oceanographic features with distinct biogeochemical cycling. Given this, we focus our discussion on the latter.

Ellipse area estimates suggest that the olive ridley's isotopic niche is larger in the Gulf of California and the North Equatorial Current than on the Costa Rica Dome or in East Pacific Warm Pool. Further, their Peru Current isotopic niche space is roughly three times the size of any eastern Pacific food web that we examined north of the equator, driven by large variations in both δ^{13}C and δ^{15}N values.

One explanation for a broader isotopic niche ($\sim$1‰^{2}) for olive ridleys in the Gulf of California compared to the other northern sub-regions might be that the majority of turtles were sampled in the Gulf entrance, where some hypothesize regional coupling of denitrification-nitrification occurs (Mee et al., 1984; White et al., 2007). There, nitrogen inputs come from a mix of ^{15}N-enriched (denitrified) subsurface water from the Eastern Tropical North Pacific (ETNP) transported via the California Undercurrent; relatively ^{15}N-depleted warm surface water from the west; and ^{15}N-enriched terrestrial inputs (sediments, guano, runoff). Whereas N_2-fixation in the Gulf of California appears to be episodic and seasonal (White et al., 2013), all the Gulf of California olive ridleys were sampled during the month of August. During summer months, the water column is stratified and Gulf of California surface waters, including entrance zone waters, are dominated by picocynaobacteria and modest N_2 fixation (White et al., 2013). We suggest turtles could have conceivably fed offshore or in deeper entrance zone waters within the month prior to sampling, retaining the "offshore" δ^{13}C signal as they moved into the Gulf (Hobson et al., 1994; Hill et al., 2006; Miller et al., 2008), or perhaps turtles fed in both pelagic and benthic habitats across the entrance zone and lower Gulf (France, 1995; Hill et al., 2014). Both scenarios could result in the observed δ^{13}C range [−17.08, −15.24].

The East Pacific Warm Pool supports an impressively small isotopic niche area for olive ridleys (0.6‰^{2}) considering it is the largest sub-region by geography, spanning 10° of latitude and 16° of longitude. N* values (a metric used to trace nitrate deficit relative to phosphate) in this sub-region indicate a high degree of denitrified waters that are likely a dominant control on the δ^{15}N values of bioavailable nitrogen. As such, across the entire subtropical and tropical eastern Pacific, nitrate concentrations are lowest in the East Pacific Warm Pool, as are N* concentrations, modeled downwards of < -20 (μM; Rafter et al., 2012). Further, characteristics that influence the bioavailable nitrogen in the euphotic zone such as temperature, pycnocline, and mixed layer depth are particularly stable and reliable during the season in which our sampling was conducted (Fiedler and Talley, 2006). The Gulf of Tehuantepec and the Gulf of Papagayo are two especially productive gulfs (relatively higher chlorophyll and NO_3^- concentrations) within the East Pacific Warm Pool due to wind-driven upwelling. Many of the samples we examine within the East Pacific Warm Pool were in or near those two gulfs because encounter rates were so high there.

The Costa Rica Dome supports an isotopic niche area almost equal in size (0.5‰^{2}) to the East Pacific Warm Pool, but is considerably smaller in geographic size (we consider it 800–1,000 km in diameter). It is the most seasonally dynamic oceanographic feature in the eastern tropical Pacific (ETP) with a predictable strong and shallow thermocline (15 m at the peak of the dome, shoaling off to 50 m to the N and S) (Fiedler, 2002). Both the East Pacific Warm Pool and the Costa Rica Dome are within the Tropical Surface Water mass (Fiedler and Talley, 2006) and have high concentrations of chlorophyll and nitrate compared to other areas in the eastern Pacific (Pennington et al., 2006). While driven by different physical sources, the gulfs of Tehuantepec and Papagayo and the Costa Rica Dome share characteristics, such as upwelling, nitrate concentrations, and denitrification, that determine basal isotopic signals, which is why we believe these areas support a similar isotope niche for olive ridleys.

The Peru Current is one of the strongest upwelling regions in the world, particularly during austral winter, and thus denitrification is dominant and CO_2 efflux is high. This creates large variation in δ^{15}N, upwards of 11‰, and δ^{13}C, upwards of 5‰, just south of the equator and along the equatorial belt (Farrell et al., 1995; Arguelles et al., 2012). The pattern of low δ^{15}N values in olive ridley skin in this food web, specifically between 5° and 15° S, follow observations of low δ^{15}N values of particulate organic material in surface waters. This suggests that the observed variation of bulk skin δ^{15}N values was caused by baseline shifts, not trophic shifts. The large Peru Current isotopic niche area estimate ($\sim$2.7‰^{2}) suggests there may be unique food web dynamics in the south equatorial region.

Trophic Position

Our understanding of olive ridleys as opportunistic foragers implies that their trophic role (i.e., trophic position) would not differ across their range. This study design provided an opportunity to test that assumption, as well as to compare the results of estimating TP using the weighted mean approach with the approach of using just two amino acids (phenylalanine and glutamic acid). Recent studies by Bradley et al. (2015) and Nielsen et al. (2015) show that using weighted means to estimate marine teleost TPs resulted in more precise estimates across taxa and trophic levels. Using the weighted mean approach, we did not find any statistical difference in TP across food webs; but using the two-amino acid approach, we detected a 0.36 TP elevation on the Costa Rica Dome.

Similar to other organisms in the eastern Pacific (e.g., copepods, laternfishes and tuna; Popp et al., 2007; Olson et al., 2010; Hetherington et al., 2017), olive ridley bulk skin δ^{15}N values showed a tendency to be more ^{15}N-enriched in higher latitudes. Often, differences in δ^{15}N values of consumers may be driven by trophic differences among spatially discrete foraging populations (Vander Zanden and Rasmussen, 2001). However, the TP of adult olive ridleys was remarkably constant (3.1 ± 0.2 *SD*) across a variety of oceanographic settings in the eastern Pacific, including the Peru Current. With this, we are confident that variation of bulk $\delta^{15}N$ values was indeed driven by the shifting $\delta^{15}N$ baseline across food webs.

TP estimates from both methods, ranging from ∼2.4 to ∼3.6, make biological sense and reflect the expected omnivory. Other sea turtles studies that have estimated TP have raised concern over the reasonability of their TP estimates, driven by the beta and trophic discrimination factors used, and/or the TP estimation method (Seminoff et al., 2012; Vander Zanden et al., 2013). It is important to note that these constants can be somewhat arbitrary for species that have not been the focus of controlled feeding studies, and varying them can noticeably change TP estimates. In this study, constants from Bradley et al. (2015) were chosen carefully; they did not vary between TP estimation methods and appear to have performed well.

Source amino acids grouped nicely from trophic amino acids across samples, and the bulk skin $\delta^{15}N$ values reflected a composite of both groups. For each group of amino acids, there was a median $\delta^{15}N$ spread of about 7‰. Among the source amino acids, the largest spread was between lysine (low) and tyrosine (high), and among the trophic amino acids the largest spread was between aspartic acid (low) and alanine (high). No amino acids appeared to be intermediate. Regardless of the robustness of this study's amino acid isotope data, across taxa, to date the amino acids that seem to most reliably estimate TPs indicative of ecological expectations are phenylalanine and glutamic acid (Chikaraishi et al., 2009). Quantification of amino acid-specific incorporation rates and trophic discrimination for multiple taxa and tissues types would provide clarity as to which approach (two-amino acid vs. weight mean) is most reliable.

Costa Rica Dome—A Unique Food Web?

There was one exception to the trophic position consistency for olive ridleys: on the Costa Rica Dome, individuals fed at a 0.36 median level higher than in any other sub-region (**Figure 4**). As stated above, this conclusion can be drawn from estimating TPs using $\delta^{15}N_{Glu} - \delta^{15}N_{Phe}$, however it does not hold when using weighted means. While the former result seems reasonable, as many high-level consumers (cetaceans, seabirds, tuna) consistently aggregate to feed on the Costa Rica Dome's standing stocks of zooplankton and other prey (Reilly and Thayer, 1990; Sissenwine et al., 1998; Ballance et al., 2006), this discrepancy highlights the need to continue to advance the application of stable isotopes to understand sea turtle ecology.

To our knowledge, all AA-CSIA studies of wide-ranging consumers, both generalists and specialists, find the same TP consistency that we found for adult olive ridleys. For example, TPs of tuna, adult leatherbacks, lanternfishes, dragonfishes, and zooplankton do not change across ocean basins (Popp et al., 2007; Olson et al., 2010; Choy et al., 2012; Seminoff et al., 2012; Hetherington et al., 2017). This underscores the uniqueness of detecting a potential trophic shift on the Costa Rica Dome. Such a shift could be a reflection of a diet of a slightly wider variety of prey items, or a diet of a relatively larger proportion of high order prey (see Supplementary Table 5).

The benefits of using stable isotopes are many; the analyses can be cost effective and integrated with other techniques like telemetry and genetics. However, limitations can cloud our ability to interpret results and/or can produce conflicting results, as in this case. Limitations include the lack of accurate and species-specific discrimination factors, and our understanding of what might drive variation across groups of amino acids (source, trophic). The approach used to estimate TP seems to matter, however more research on this is needed for sea turtles.

Conservation Implications

Olive ridley subpopulations in the eastern Pacific are genetically distinct from subpopulations in the western Pacific breeding population (Bowen et al., 1997; Shanker et al., 2004; Wallace et al., 2010; Jensen et al., 2013). While individuals have the ability to migrate >1,500 km, as nomads they do not have consistent home ranges (Pandav and Choudhury, 1998; Polovina et al., 2003; Whiting et al., 2007; Plotkin, 2010). Rather, they continuously forage opportunistically on a wide variety of prey. The empirical characterization of open ocean foraging ecology via stable isotope analyses has provided insight into why this turtle species has been successful compared to other, coastal species. We believe that foraging plasticity combined with relatively fast generation time allowed olive ridleys to recover quickly in the eastern Pacific, compared to other depleted turtle species, after near extinction from over-harvest in the 1960s (Abreu-Grobois and Plotkin, 2008; Plotkin et al., 2012).

The olive ridley's generalist foraging strategy is advantageous for survivorship and suggests that they may be resilient to disturbance (Heppell et al., 2005; Plotkin, 2010; Clavel et al., 2011). However, they have other biological characteristics such as low metabolism, narrow thermal niche and nesting dichotomy that may make them vulnerable to environmental change unless they are able to adapt on relevant time scales (Merchant-Larios et al., 1997; Lutz et al., 2003; Polovina et al., 2003; McMahon and Hays, 2006; Plotkin et al., 2012). Climate change may be particularly problematic for sea turtles given population sex-ratios are temperature-dependent, and they nest on beaches that may be impacted by sea level rise (Hawkes et al., 2009). As ocean temperature, chemistry, circulation, and species distributions are changing with climate (Doney et al., 2012), it is a critical time to broaden our understanding of phenology, demography, trophic roles and function of mobile consumers so that vulnerable species and their oceanic habitats can be better managed in the face of environmental variability (Micheli, 1999; Edwards and Richardson, 2004).

Since olive ridleys encounter spatially-explicit resources and threats, innovative management approaches may be necessary to achieve modern conservation objectives. A promising dynamic ecosystem-based management approach would be to predict areas where suitable foraging habitat overlaps with other areas of interest, or the presence of threats (e.g., fishing) (Howell et al., 2008; Maxwell et al., 2014, 2015; Scales et al., 2014). Given their enormous eastern Pacific range across swaths of unproductive warm waters, where they feed largely on things humans are not yet interested in harvesting, strategic conservation measures (e.g., protecting key nesting beaches) may enable olive ridleys to be a global warming winner.

CONCLUSIONS

This study demonstrates the value of large-scale, in-water research across different foraging habitats to understand the foraging ecology of highly migratory marine species, such as the olive ridley sea turtle. As the frequency and durations of costly research cruises decrease, the value of taking advantage of research platforms of opportunity to study oceanic species like olive ridleys increases. Further, we have presented yet another example of how stable isotope analyses provide a relatively non-invasive and cost-effective analytical approach to describe the trophic ecology of a cryptic, mobile species with a large oceanic distribution. We used bulk tissue and compound-specific analyses to develop the hypotheses that in the ETP, the trophic position of the omnivorous olive ridley sea turtle remains constant with ontogenesis and sex.

We conclude that olive ridley turtles exploit persistent but dynamic oceanographic features as distinct food webs. Using AA-CSIA, we did not detect a shift in the trophic position of adult olive ridleys across any of the identified sub-regions using the weighted mean approach, indicating their energetic requirements are comparably met throughout their range. The isotopic data generated in this study adds to the growing body of work describing stable isotope baseline data for marine organisms in the eastern Pacific Ocean (Arthur et al., 2008; Olson et al., 2010; Ruiz-Cooley and Gerrodette, 2012; Seminoff et al., 2012). A natural future direction would be to estimate marine isoscapes across taxa and trophic levels to provide a systematic framework for stable isotope ecological applications, as well as empirical studies of trophic dynamics (Somes et al., 2010; Ceriani et al., 2014; Vander Zanden et al., 2015; Magozzi et al., 2017; Kurle and McWhorter, 2017). A central repository of stable isotope data for sea turtles would support the advancement of this field and reduce duplication of efforts (Pauli, 2017). Refinement of species-specific diet-tissue δ^{15}N values and other parameters will minimize assumptions, improve interpretation, and aid our understanding of oceanic food webs.

ETHICS STATEMENT

Peruvian research and protocol were reviewed and approved by the Duke University Ethics Committee and all necessary permits were obtained from Peruvian authorities (Peruvian Government Authorization No. 177-2008-IRENA-IFFS-DCB).

AUTHOR CONTRIBUTIONS

LP, RP, and JS conceived of and designed this study. LP, BP, KA, and SK completed the laboratory analysis. LP completed the quantitative analysis. LP and JS wrote the paper with input from all authors. LP, BP, SG, and JS provided financial support.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2017.00348/full#supplementary-material

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Informing Marine Protected Area Designation and Management for Nesting Olive Ridley Sea Turtles Using Satellite Tracking

Tiffany M. Dawson[1]*, Angela Formia[2], Pierre D. Agamboué[2], Georges M. Asseko[3], François Boussamba[4], Floriane Cardiec[2], Emmanuel Chartrain[2], Philip D. Doherty[5,6], J. Michael Fay[7], Brendan J. Godley[5], Francis Lambert[7], Brice D. Koumba Mabert[8], Jean C. Manfoumbi[9], Kristian Metcalfe[5], Gianna Minton[10], Ivan Ndanga[2,4], Jacob Nzegoue[2], Carmen K. Kouerey Oliwina[2], Philippe Du Plessis[11], Guy-Philippe Sounguet[4,7], Dominic Tilley[5], Matthew J. Witt[6] and Sara M. Maxwell[1]

[1] Department of Biological Sciences, Old Dominion University, Norfolk, VA, United States, [2] Wildlife Conservation Society, Gabon-Program, Libreville, Gabon, [3] Agence Nationale des Pêches et de l'Aquaculture, Libreville, Gabon, [4] Aventures Sans Frontières, Libreville, Gabon, [5] Centre for Ecology and Conservation, College of Life and Environmental Sciences, University of Exeter, Penryn, United Kingdom, [6] Environment and Sustainability Institute, University of Exeter, Penryn, United Kingdom, [7] Agence Nationale des Parcs Nationaux, Libreville, Gabon, [8] Centre Nationale des Données et des Informations Océanographiques, Libreville, Gabon, [9] Ibonga-ACPE, Libreville, Gabon, [10] WWF Gabon, Libreville, Gabon, [11] Fondation Liambissi, Port-Gentil, Gabon

Edited by:
Ellen Hines,
San Francisco State University,
United States

Reviewed by:
Mariana M. P. B. Fuentes,
Florida State University, United States
Mario Santoro,
Istituto Zooprofilattico Sperimentale
del Mezzogiorno, Italy

***Correspondence:**
Tiffany M. Dawson
tdaws001@odu.edu

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Understanding the horizontal and vertical habitat of olive ridley sea turtles (*Lepidochelys olivacea*), a threatened species, is critical for determining regions for protection and relevant gear modifications that may effectively reduce bycatch, the largest threat to this species. Satellite transmitters were used to determine the movement and dive behavior of 21 female olive ridley turtles tagged in Pongara National Park, Gabon during the 2012, 2013, and 2015 nesting seasons. A switching state-space model was used to filter the tracking data and categorize the internesting and post-nesting movements. Gridded utilization distribution (UD) home range analysis of tracking data revealed that the entire core habitat occurred in the Komo Estuary during the internesting period. Within the Komo Estuary, 58% of this core UD occurred in shipping lanes. Dive data from the 2015 tagging season revealed that during the internesting period, turtles spent the majority of their time resting on the estuary seabed. Approximately 20% of all dive time was spent on the bottom and all maximum dive depths corresponded to the depth of the seabed, indicating that bottom set gear during the internesting period may pose the greatest potential for fisheries interactions. National parks currently protect many of the nesting sites and the Gabon Bleu initiative has formally designated 10 new marine parks and a network of community and industrial fishing zones; this data was a layer used in determining the park and zone boundaries. Shared use of the estuary by fisheries, shipping, and olive ridley turtles creates a need for management measures to reduce interactions. Thus, the results from this study can further provide detailed information that can be used to support the development of evidence-based management plans.

Keywords: home range, marine protected area, shipping, bycatch, dive behavior, Central West Africa

INTRODUCTION

Protecting long-lived, highly migratory marine species is challenging since they encounter multiple threats across broad areas and in different life stages (Witt et al., 2011; Maxwell et al., 2013; Lascelles et al., 2014), often requiring multi-faceted and multi-national conservation efforts (Blumenthal et al., 2006; Gore et al., 2008; Maxwell et al., 2011; Croxall et al., 2012; Pikesley et al., 2013b; Doherty et al., 2017). Several studies have used satellite telemetry to describe the horizontal movement data of large marine vertebrates (Godley et al., 2008; Block et al., 2011; Hawkes et al., 2011; Hazen et al., 2012; Yurkowski et al., 2016; Citta et al., 2017; Vaudo et al., 2017) and to determine overlap with anthropogenic threats such as fisheries (seabirds: Suryan et al., 2007; Bugoni et al., 2009; Žydelis et al., 2011; sea turtles: da Silva et al., 2011; Witt et al., 2011; Revuelta et al., 2015; marine mammals: Geschke and Chilvers, 2010; Rosenbaum et al., 2014; sharks: Holmes et al., 2014), shipping (marine mammals: Mate et al., 1997; Schorr et al., 2009), and in-water habitat degradation (seabirds: Montevecchi et al., 2012; marine mammals: Johnson and Tyack, 2003; Rosenbaum et al., 2014). Satellite telemetry has been critical in evaluating threat exposure for marine species (Witt et al., 2008; Maxwell et al., 2013; Lascelles et al., 2014) and assessing how efficient conservation boundaries, such as Marine Protected Areas (MPA), are at encompassing the wide ranging habitat distribution of migratory species (Hart et al., 2010; Scott et al., 2012; Young et al., 2015; Maxwell et al., 2016).

Olive ridley turtles (*Lepidochelys olivacea*), like other sea turtle species, lay multiple clutches of eggs in a nesting season (Plotkin, 2007; Maxwell et al., 2011). Therefore, they remain in the vicinity of nesting beaches for several weeks at a time (Whiting et al., 2007; Hamel et al., 2008; da Silva et al., 2011; Maxwell et al., 2011), termed the internesting period, during which they are susceptible to additional anthropogenic impacts (Pikesley et al., 2013a). This period also makes sea turtles ideal candidates for conservation measures because both sexes congregate in the same waters to breed and females typically return to the same beaches to nest multiple times in the same season (Van Buskirk and Crowder, 1994).

Olive ridley turtles are a vulnerable species as defined by the International Union for Conservation of Nature and Natural Resources (IUCN) Red List due to over-exploitation and characteristics such as slow growth rate, late reproductive maturity, and low hatchling survival rate (Abreu-Grobois and Plotkin, 2008). These factors make it difficult to maintain sufficient populations in the face of anthropogenic influences (Abreu-Grobois and Plotkin, 2008). In the East Atlantic, one of the largest threats to olive ridley turtles is fisheries bycatch, including trawls and gillnets (Frazier et al., 2007; Parnell et al., 2007). In this region, olive ridley turtles are distributed across multiple commercial and artisanal fishing hotspots as well as political boundaries, making conservation practices difficult (Godgenger et al., 2009; Wallace et al., 2013; Metcalfe et al., 2015b), however MPAs are being developed in some parts of the East Atlantic, such as in Gabon, Africa.

Horizontal movement and habitat use are important measures for conservation but dive behavior can also be critical to understanding in-water threats to marine species. Understanding water column use can influence fisheries management strategies such as gear set depth, soak time, and gear type (Lewison et al., 2013). For example, in the North Pacific, Polovina et al. (2003) used turtle dive data to determine that banning shallower set longlines would decrease the amount of bycatch for sea turtles, specifically loggerheads (*Caretta caretta*) and olive ridley turtles, because they spent most of their time in the upper water column. Presently, little is known about dive behavior of olive ridley turtles and even less is known about their dive behavior during the internesting period. The only olive ridley turtle dive studies are from the West Atlantic and focus on post-nesting dive behavior (Plot et al., 2015). Understanding how olive ridley turtles use the water column is crucial to developing evidence based management plans within the MPA fishery zones.

In 2014, the President of Gabon announced and initiative known as "Gabon Bleu," a marine spatial planning process that included the creation a network of MPAs in Gabonese waters. The final boundaries were released in June 2017. In order to inform this process, planners required the distribution of species as well as putative threats (Roberts and Hamann, 2016). Similar to other Central African countries, Gabon has more small-scale fishing than industrial fishing and a majority of fishermen use purse seine, drift gillnet, and bottom set gillnet techniques (Metcalfe et al., 2015b). Through field surveys, Metcalfe et al. (2015b) found that 62% of the 1,831 boats were working out of Libreville and the Komo Estuary, which is also adjacent to Pongara National Park, one of the largest olive ridley nesting beaches in Gabon (Metcalfe et al., 2015a). Artisanal fishing was also found to be heavily aggregated in estuaries, and river and lagoon mouths (Metcalfe et al., 2015b). Based on a study of sea turtle bycatch from industrial fisheries in Gabonese waters, olive ridley turtles were the most impacted sea turtle species, representing 80% of the total sea turtle bycatch (Casale et al., 2017). Furthermore, a study on beached carcasses has indicated that bycatch of sea turtles, particularly olive ridley turtles, is appreciable in Gabon (Parnell et al., 2007). The Komo Estuary is also a major shipping region, however, the impacts of shipping on sea turtles have not been well studied. Impacts may occur in the form of pollution from vessels via discharge of oil, fuel, organic pollutants, sewage, and debris, noise pollution, or through direct ship strikes (Abdulla and Linden, 2008; Maxwell et al., 2013 and references within). Even when the magnitude of impacts is unclear, understanding where and when turtles and threats overlap is necessary to inform conservation strategies.

To inform the Gabon Bleu initiative, we undertook satellite tracking of olive ridley turtles in Northern Gabon to determine distribution of sea turtles within Pongara National Park and the adjacent Komo Estuary, a heavily used waterway, and compared this distribution to threats in the region. We collected location and dive depth data from nesting female olive ridley turtles to better understand their vertical and horizontal habitat movement along the Gabonese coast. We further consider the movements of olive ridley turtles in relation to the boundaries of shipping lanes and the boundaries of newly designated MPAs. Movement and dive data provided a basis to inform the boundaries of

MPAs and other marine zones and can be used to further inform management plan for zones in the area.

METHODS

Ethics Statement

All federal, international, and institutional guidelines were followed, and this study was approved by and carried out in accordance with the recommendations of University of Exeter's College of Life and Environment Sciences Ethics Committee and the Institutional Animal Care and Use Committee at Old Dominion University (IACUC Permit 15-016). Permissions to work within park boundaries and with the study species were issued by the Gabon Agence Nationale des Parcs Nationaux (AEl5025, AR0010/12, AE140003).

Study Area

Gabon, Central West Africa, is home to the largest olive ridley rookery in the Atlantic (Metcalfe et al., 2015a), however, even with global decline and their vulnerable status this region remains data deficient. Previously, an expansive network of national parks protected 81% of sea turtle beach nesting sites (Metcalfe et al., 2015a), but in-water protection only existed in southern Gabon via Mayumba National Park. However, the government recently designated a new network of MPAs that consist of 10 marine parks covering 23% of the Gabonese Economic Exclusive Zone.

One of the newly created MPAs is contiguous to Pongara National Park. It is an important area for both people and marine life, adjacent to the Komo Estuary and proximate to Gabon's capital city, Libreville, one of the main anthropogenic use regions in Gabon and most populated city in the country with ∼800,000 inhabitants. The Komo Estuary is the location of a major port and is also an important artisanal fishing region.

Satellite Transmitter Deployment

The internesting movements of 21 olive ridley sea turtles were monitored from Pongara National Park, one of the largest nesting beaches in Gabon (Metcalfe et al., 2015a), over three nesting seasons (2012, 2013, 2015). Satellite transmitters were attached to nesting females early in the nesting season to capture internesting movements (Maxwell et al., 2011). Olive ridley turtles at Pongara National Park nest between October and January, peaking in late October to early November (Metcalfe et al., 2015a). SPOT-293A transmitters (Wildlife Computers, Redmond WA, USA) were deployed between 26th October and 5th November 2012 ($n = 6$) and 12nd October and 13th October 2013 ($n = 5$). SPLASH10-351C (Wildlife Computers, Redmond WA, USA) were deployed between 27th October and 31st October 2015 ($n = 10$). Attachment followed techniques described in Maxwell et al. (2011), and did not start until the female began oviposition and care was taken not to disturb the nest during attachment. Transmitters were attached to the flattest portion of the two front most vertebral scutes of the carapace using either T308+$^{\text{TM}}$ Epoxy (Powers Fastners, Brewster NY, USA) in 2012 and 2013 or Superbond 1:1 Expoxy Adhesive (FiberGlass Coatings, Inc. St. Petersburg FL, USA) in 2015. First, the carapace was cleaned of algae and smoothed using sandpaper and sea water; once cleared the area was dried with acetone. The transmitter was placed with the antennae pointing toward the head and perpendicular to the top of the carapace and the epoxy was molded to ensure attachment around the edges. To minimize stress and handling, a wooden box was placed around the female after she finished covering the nest. Once the epoxy set, the female was released and monitored to make sure she returned to the water. Before tag attachment curved carapace length (CCL) and curved carapace width (CCW) were measured, and metal flipper tags Monel 1005-62 (National Band and Tag Co., Newport, KY) were attached to the front flippers. Satellite data were collected via the Argos Satellite System.

Track Analysis and State-Space Model

All Argos location quality points except for 0 and Z were used in the movement analysis (i.e., location classes 3, 2, 1, A, B) and a hierarchal state-space model (hSSM) with a time interval of 3.5 h was used to filter the location data and determine internesting behavior (Breed et al., 2009; Jonsen et al., 2013). SSMs use parameters associated with an animal's position (Jonsen et al., 2003) and determine a behavioral state for each point in time (Breed et al., 2009; Jonsen et al., 2013). Through the use of a two part model, an equation is used to determine how behavioral states change randomly in time and previous locations are used to estimate the next location based on an independent random walk model and biological constraints of the animal, such as how far they can travel in a day (Jonsen et al., 2013). SSMs are used to draw inferences from data on behavioral changes through time and how it can be related to actual biological processes (e.g., foraging, breeding, or migrating). The model also accounts for observation error between the satellite-produced location and the unseen true location and credible limits for the predicted locations (Breed et al., 2009). A hSSM structure was used since we had multiple tagged animals and wanted to focus on population level dynamics instead of individual movement. A short track, run individually through the model, could not produce accurate behavioral states but when run with other tracks in the hSSM, behaviors can then be categorized (Breed et al., 2009). The hSSM is ideal when behaviors cannot be directly observed throughout the track, such as with the olive ridley turtles, therefore data such as speed and turn angle are used to delineate probable behavior (Jonsen et al., 2013).

We used the hSSM model to run 2 Markov Chain Monte Carlo simulations for 10,000 iterations with a burn-in of 15,000 to generate 10,000 posterior samples in the "bsam" package in R (Jonsen et al., 2005; Jonsen, 2016). We used a thinning factor of 10 to minimize the within-chain sample autocorrelation. We used a time step of 0.15, creating a 3.5-h time interval between points; time step determined by finding the average time between existing points, following Maxwell et al. (2011). Trace plots were examined for autocorrelation and to ensure that they converged on a mean density, and resulting tracks were examined to determine if tracks made biological sense, the most important validation metric (G. Breed, pers. comm.). Due to the coastal and estuarine nature of our study area, resulting hSSM locations occasionally occurred on land. These locations were shifted, such that previous and post-locations were used to determine directionality of movement. The inland location was then manually moved to the closest in-water location. If a relatively confident location could not be estimated then the location was discarded from analysis.

The hSSM also assigned a behavioral estimate between 1 and 2 to each location point based on the mean turn angle (Θ) and the autocorrelation between speed and direction (γ). We defined the "transiting" behavior state as behavioral estimates between 1.0–1.39 and "internesting" behavioral state as 1.6–2.0, and only internesting locations were used for the remainder of the analyses. Behavioral estimates between 1.4 and 1.59 were considered behavioral transitions from internesting behavior to transiting; comprising only 2.4% (47 locations) of the data, and transition locations were not considered in the analysis.

We also identified renesting events within the tracks. Renesting events were inferred by consecutive high quality location points directed toward land and consistent with the known re-nesting interval of 17–30 days for olive ridley turtles (Miller, 1997; Maxwell et al., 2011).

Comparison of Sea Turtle Distribution, Zone Boundaries, and Commercial Shipping

Home range analysis, in the form of a gridded utilization distribution (UD) was used in the software R to determine the core use area, where 50% or more of the hSSM locations occurred during the internesting period (Maxwell et al., 2011). Using custom scripts in R 3.0.1 (R Core Team, 2003) we visually determined a base grid size of 0.015° ($\sim$1.5 km^2) which allowed for the best spatial resolution within the restricted area of the Komo Estuary without oversmoothing as a turtle moved between cells (Maxwell et al., 2011). To aid in future zoning of the Komo Estuary, we quantified the percent of the internesting locations inside of the Komo Estuary. Additionally, we divided the estuary into 6 zones based on draft management zones in the Gabon Bleu initiative and nautical landmarks within the estuary. Zones 1, 3, 4, and 5 represent sections of current community fishing areas within the estuary, Zone 2 is the newly-designated MPA boundary adjacent to Pongara National Park, and Zone 6 is the current Pongara National Park and Buffer boundary. Zone 4 was further divided into three subsections, A-C, to examine whether turtle movement occurred equally across the zone or closer to Zone 2, the MPA boundary. The percentage of internesting locations that fell within each zone were then calculated to identify high use areas that could be used to inform development of management plans for the regions.

The UD was also compared to a UD of commercial shipping location data collected by the Automatic Identification Systems onboard vessels and downloaded from ExactEarth. Under Regulation 19 of the Safety of Life at Sea: Chapter V by the International Maritime Organization, location data, transmitted every minute at variable intervals, for shipping vessels are recorded globally for all vessels greater than 300 tons if traveling internationally and greater that 500 tons if localized (IMO, 2001). We used available vessel location data along the Gabonese coast from October and November 2012 to mirror the internesting period of the olive ridley turtles. All anchored or moored data points were removed from analysis (1.6% of all data points), such that only transiting data were used. The shipping UD was created using the same base grid as the olive ridley UD. The shipping and turtle UDs were then compared to determine the percent of overlap within the internesting habitat.

Dive Depth Analysis

Dive depth was collected in 2015 only ($n = 10$), with depth recorded every 2.5 min. The transmission of dive data, however, was variable and dependent on satellite availability. Additionally, the transmitted dive data did not necessarily correspond to known locations as dive data was recorded continuously while location can only be determined by satellites when turtles are at the surface. To determine dive behavior near known locations, dive depths within a 1-h window before and after a high-quality Argos location (location quality 3, 2, and 1 only) were extracted. The maximum dive depth within this 2-h window was compared to a gridded bathymetric map of the Komo Estuary to determine if turtles likely used the estuary bottom. The gridded bathymetric map was created from *Bahia de Corsico to Estuaire du Gabon, Nautical Chart 1356*, a nautical chart of the estuary, rasterized using the Raster package in R (Hijmans, 2016) with the same resolution and extent as the grid used for the UDs.

Using all of the dive data available from the 2-h windows, regardless of location quality, we determined the portion of the water column used by the olive ridley turtles. In this analysis, we included all depth locations that were recorded along the course of the dives. We normalized the dive data from each 2-h window previously described by dividing each depth by the maximum depth of that window creating a proportion between 0 (representing surface use) and 1 (representing bottom use). This allowed us to determine where in the water column turtles spent time throughout the internesting period across all dive data. Using the proportion created for each dive depth, we compared the surface and bottom use to the time of day to look for diel dive patterns.

RESULTS

Satellite Transmitter Deployment

Twenty-one satellite transmitters were attached to nesting female olive ridley turtles in 2012 ($n = 6$), 2013 ($n = 5$), and 2015 ($n = 10$) in Pongara National Park (Table S1, **Figures 1A–D**). The overall mean track duration was 102.8 days (±58.4, min = 29, max = 203) with an average duration of 82.4 days (±53.9, min = 29, max = 151) in 2012, 169 days (±36.4, min = 112, max = 203) in 2013, and 79.9 days (±45.5, min = 34, max = 184) in 2015. The mean overall internesting period was 15.6 days (±12.2). An approximate re-nesting event was identified for turtles G, H, and, P and each event was an estimated 15, 15, and 18 days after the transmitter deployment date, respectively. Turtle M from the 2015 season stopped transmitting before switching from internesting to post-nesting movement but remained in the Komo Estuary for 43 days and was therefore retained in the analysis. Turtle B from the 2012 season was discarded from further analysis because the Argos data returned

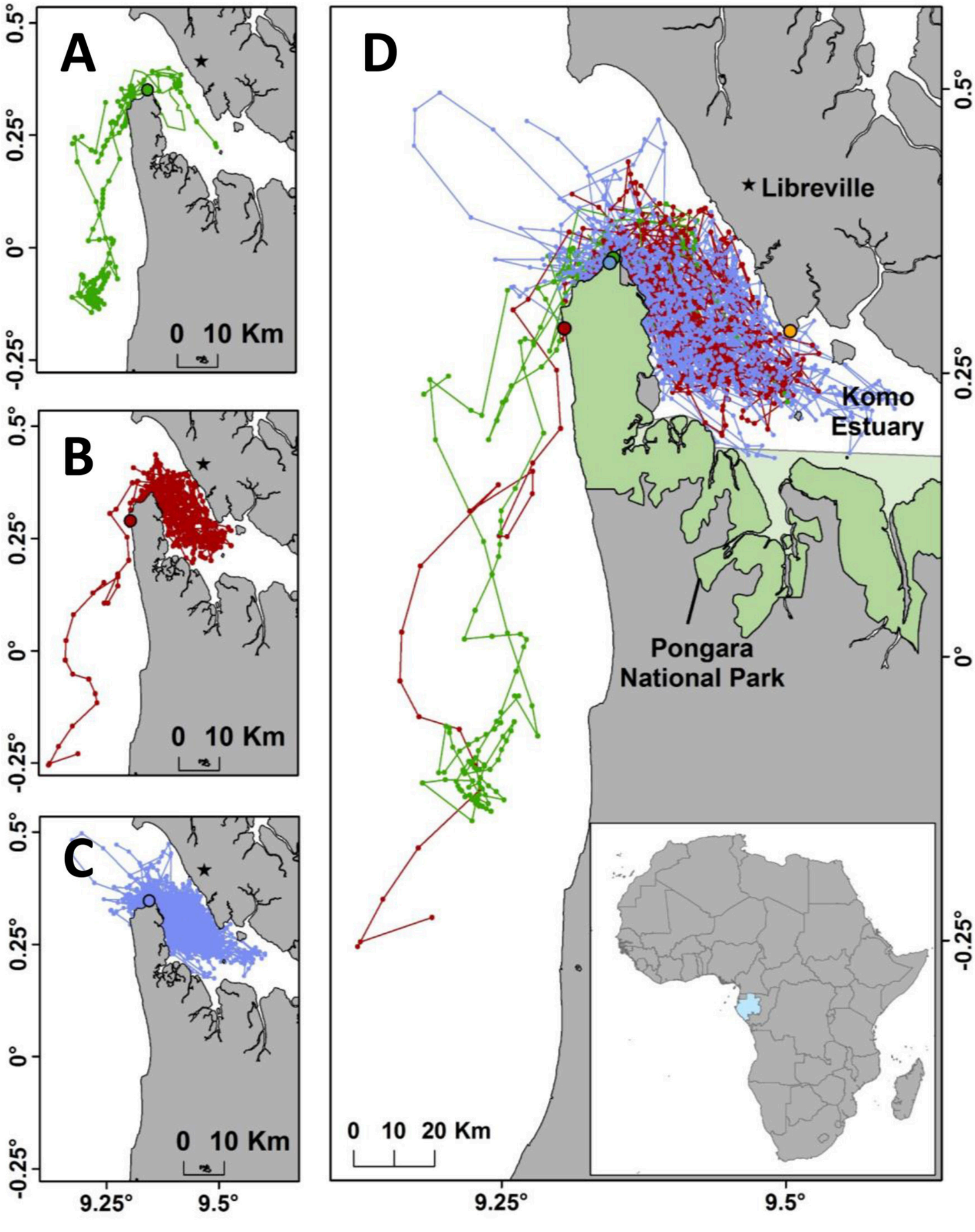

FIGURE 1 | (A) State-space modeled internesting tracks of female olive ridley sea turtles tagged from Pongara National Park, Gabon from the 2012 field season ($n = 5$). The green dot represents the tagging location for this season. **(B)** Tracks from the 2013 field season ($n = 5$). The red dot represents the tagging location for this season. **(C)** Tracks from the 2015 field season ($n = 10$). The blue dot represents the tagging location for this season. **(D)** Combined tracks from all three field seasons ($n = 20$). Green, red, and blue tracks represent 2012, 2013, and 2015 data respectively along with the coordinating colored dots representing the tagging locations. The orange dot represents the Owendo commercial shipping port in the Komo Estuary.

improbable locations and movement patterns thought to be from transmitter failure. Turtle P had location points within the internesting period that demonstrated an unexpected full behavioral switch from internesting to transiting then back to internesting. During the switch, the turtle remained in the Komo Estuary and later we saw the anticipated long-term behavioral switch to transiting along with a southward movement toward the foraging grounds (Pikesley et al., 2013b). Therefore, we included the short behavioral change in our internesting period analysis.

Comparison of Sea Turtle Distribution, Boundaries, and Commercial Shipping Data

The full (100%) UD demonstrated that olive ridley turtles remained in the Komo Estuary and within 20 km of the coast south of the estuary during the internesting period. This includes use of the waters off the nesting beach of Pongara National Park (**Figure 2A**). The core UD highlighted that the high use areas are entirely within the Komo Estuary and at the mouth of the estuary near to where much of the nesting occurred and where most tags were deployed. Using the mouth of the estuary as an arbitrary divide, 89% of the hSSM internesting points were within the estuary and 95% of the points were in the mapped zones. Zone 2, the new MPA, and Zone 4, a new community fishing area, had the highest density of points, with 45 and 40% respectively. Zone 4A contained the highest percentage of the three subsections with 17% of the points. Zones 4B and 4C contained 11 and 12% of the internesting points, respectively. Zones 3 and 5 each had 5% of the points, Zone 1 contained 0.5%, and Zone 6, the in-water portion of the current Pongara National Park, only encompassed 0.21% of the points (**Figure 2B**).

The UD of the commercial shipping data demonstrated the highest ship traffic in the mid estuary from the Owendo port and out along the Gabonese coast line. The full shipping (100%) UD overlapped with 34.1% of the olive ridley full UD and 48.5% of the core UD (**Figure 2C**).

Dive Depth Analysis

The dive depth data from the 10 satellite tags deployed in 2015 provided a mean of 185.3 h of data across all turtles (±136.3, min = 32.0, max = 403.4) during the internesting period. The mean maximum depth recorded was 33 meters (±10, min = 19, max = 51) with mean dive depths ranging between 7 and 12 m (mean = 9 ± 2) (Table S1).

Of the transmitted dive data that correlated with high quality locations ($n = 89$), 100% of the maximum dive depths were found to coincide with depth of the seabed (**Figure 3**). Normalized depths (the proportion of time spent at depths within each dive normalized across all dives) demonstrated that 19.7% of the recorded depths occurred on the bottom and 7.8% were at the surface, with the remainder in the water column (**Figure 4**). Both surface and seabed use occurred at all hours of the day. However, 64% of surface time, represented by a proportion of 0–0.2 from the normalized depths, occurred at night between 6:00 p.m. and 6:00 a.m. The seabed use, proportions 0.8–1.0, was more evenly dispersed throughout the day with 53% of time spent during the day, 6:00 a.m.–6:00 p.m., and 47% at night, suggesting that turtles avoided coming to the surface during the day.

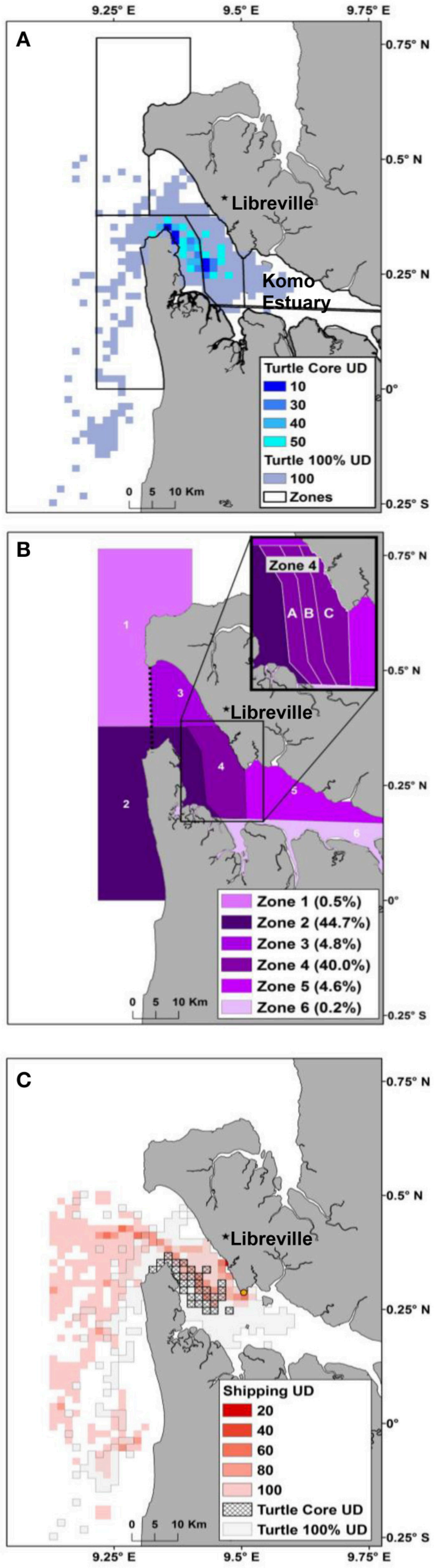

FIGURE 2 | (A) Utilization distribution (UD) of all female olive ridley sea turtles during the internesting period tagged in Pongara National Park, overlaid by

(Continued)

FIGURE 2 | Continued
zones. Zonation of the Komo Estuary is based on newly designated MPA boundaries and community fishing areas as well as nautical landmarks. The turtle core (50%) UD represents the most used areas, dark blue representing the most intensely used, by the olive ridley turtles. **(B)** Zones are colored based on utilization by the tagged olive ridley turtles during the internesting period with darker areas more heavily used and Zones 2 (the new MPA) and 4 (community fishing area) being the most utilized areas, implying areas of conservation potential. The dotted line represents the boundary used to calculate density in and out of the estuary. **(C)** UD of October 1—November 30, 2012 transiting shipping location data (red) compared to the olive ridley full UD (dotted) and the core UD (hashed) within the Komo Estuary. The orange circle represents the Owendo Port, the majoring shipping port in the Komo Estuary.

DISCUSSION

In 2014, the President of Gabon announced plans to create a network of marine protected areas (MPAs) in Gabonese waters and the final boundaries were released in June 2017. The planning process explicitly incorporated the habitat utilization data of sea turtles, including the olive ridley turtles tagged in this study, and as a result an MPA has been designated is located off of Pongara National Park matching Zone 2 (**Figure 2B**). To examine estuary use and inform the process, we created zones similar to those being considered within the planning process and our results indicate that the area around the mouth of the estuary, Zone 2, is a critical area in need of conservation strategies and will be protected as a marine protected area under the newly established Nature Preservation Agency, and likely designated as an expansion of Pongara National Park. The beaches within Pongara National Park are the most heavily used nesting beach for olive ridley turtles in northern Gabon, hence where most tagging occurred. As a result, close to 45% of the tracking locations occurred within Zone 2 and with its protection, particularly during the nesting season, it is more beneficial than the existing park boundaries (Zone 6), which encompass 0.2% of the locations. This reaffirms the benefit of the new network of marine parks that have recently been implemented.

Internesting Estuarine Movements

The female olive ridley turtles that nest in Pongara National Park are an example of marine vertebrates whose habitat use is understudied and are at risk from fishing pressures in the adjacent estuary. The telemetry tracks illustrate that females remain in the vicinity of the nesting beach (<20 km), and spend a majority of their time within the Komo Estuary (89% of locations, **Figures 1A–D**). There can be considerable variability in internesting movements among species at different nesting beaches and within the same nesting population (Godley et al., 2008). In most leatherback populations, females cover extensive areas between nesting events, though normally stay within the continental shelf (Eckert et al., 2006; Georges et al., 2007; Hitipeuw et al., 2007). Despite some loggerhead, olive ridley, and green sea turtle (*Chelonia mydas*) populations undertaking oceanic loops during internesting periods (Blumenthal et al., 2006; McMahon et al., 2007) and flatback sea turtles (*Natator depressus*) demonstrating variable behaviors in Western Australia (Waayers et al., 2011; Whittock et al., 2014), most hard shelled turtles tend to stay close to their nesting beaches (loggerheads: Godley et al., 2003; greens: Hays et al., 1999; Craig et al., 2004; Troëng et al., 2005; Fuller et al., 2008; hawksbills (*Eretmochelys imbricate):* Troëng et al., 2005; Whiting et al., 2006; Hoenner et al., 2016; Kemp's ridley (*Lepidochelys kempii*): Seney and Landry, 2008; Shaver and Rubio, 2008). This intense habitat use allows for more targeted conservation measures (Godley et al., 2008).

Some of the tracked females remained in the area for over a month before switching to the transiting phase and moving south, likely to known foraging grounds off the coast of Angola (Pikesley et al., 2013b). This is the first noted occurrence where olive ridley turtles almost exclusively used estuarine habitat during their internesting period. Green sea turtles nesting in French Guiana similarly used the Maroni River Estuary during their internesting period (Chambault et al., 2016) but additional occurrences are not known. In the Indian Ocean, female olive ridley turtles tagged on nesting beaches in Oman display consistent coastal use (Rees et al., 2012) but other populations of olive ridley turtles have been seen to move to offshore waters adjacent to the nesting beaches or immediately end the internesting period after their last nesting event and migrate toward foraging grounds (Whiting et al., 2007; Maxwell et al., 2011; Plot et al., 2015).

Within the estuary both the shallower coastal and deeper mid-estuary areas were used, highlighting the importance of this enclosed area to the internesting females. There are several hypotheses for estuarine use. Shallower depths in the estuary may result in reduced energy expenditure by turtles coming to the surface to breathe as they wait for egg development between clutches. During their time in the estuary, the olive ridley turtles appear to be utilizing the bottom habitat with U-shaped dives, which are likely related to resting or foraging (**Figures 3**, **5**, Plot et al., 2015). In addition, turtles may find increased foraging opportunities in the estuary; whether olive ridley turtles forage during the internesting period is unclear, but some turtles remained in the estuary long after nesting was completed, suggesting that they were likely taking advantage of available resources, as has been hypothesized in other regions (Rees et al., 2012).

Turtle-Threat Overlap and Conservation Measures

Using a multi-use human area, we showcase an example where data collected on a marine vertebrate species overlaps with putative threats in the habitat. We further augment with diving data, a behavioral component, to evidence where bycatch and boat interaction risk might be high and where management interventions may lead to improved conservation outcomes. The newly designated MPA boundary, Zone 2, is an example of management intervention for the marine species utilizing the Komo Estuary. The Gabon Bleu initiative also took socioeconomic impacts into consideration in designating

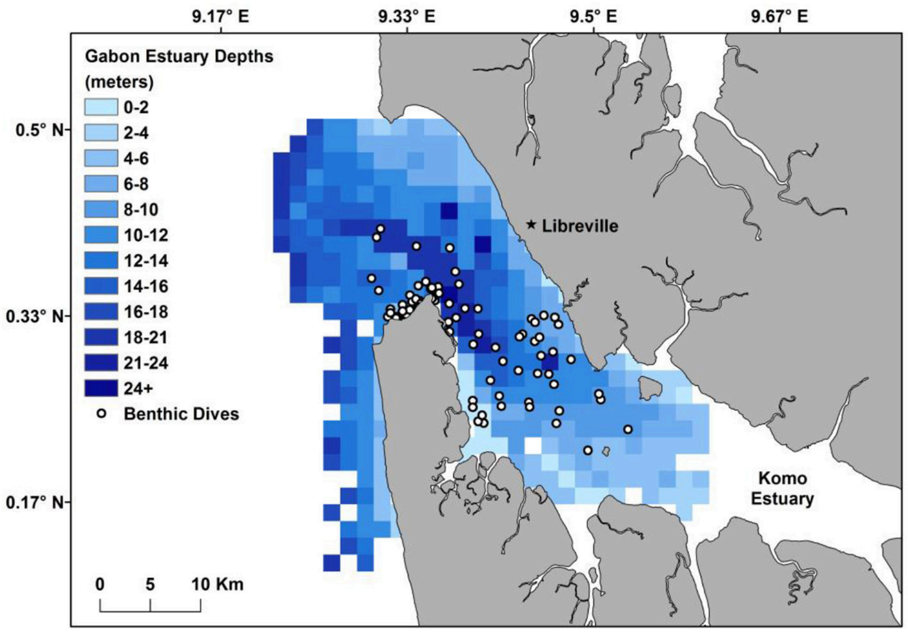

FIGURE 3 | Benthic grid of the Komo Estuary highlighting locations where olive ridley turtles location data correlated with dive depth data (white points) providing a comparison between max dive depth and the estuary bottom.

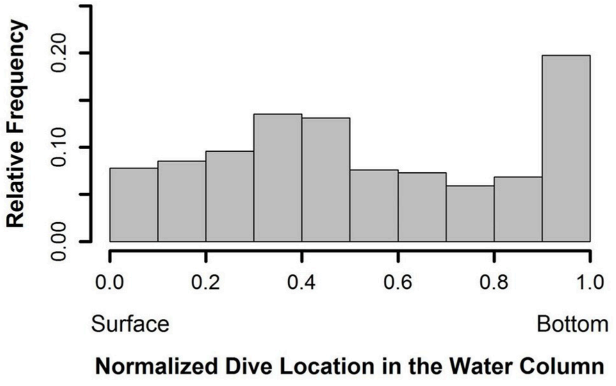

FIGURE 4 | Proportion of time spent at dive depths, normalized across all dives. Dive data was extracted from the 2-h window surrounding each location where dive data was transmitted and normalized based on the maximum depth of the respective window.

the zones and MPA, resulting in the community fishing areas where industrial fishing will be prohibited but artisanal fishing is allowed. While artisanal fishing can result in bycatch, commercial fishing gears such as trawls are also responsible for considerable bycatch (Metcalfe et al., 2015b) so the restriction of commercial vessels in this region will be beneficial to turtles.

Dive data indicated that olive ridley turtles spent a large portion of their time on the estuary bottom during all hours (**Figures 3**, **4**). Bottom set gillnets, which are widely used by artisanal fisheries in Central Africa (Metcalfe et al., 2015b, 2016), pose a direct threat to sea turtles resting on the bottom and studies have highlighted the depth at which fishing occurs can influence the bycatch rates of sea turtles (Polovina et al., 2003; Gilman et al., 2006). Outside of the MPA, the estuary has been designated for community fishing activities; management plans may consider seasonal fishing restrictions during the internesting period, particularly for bottom-set fishing gear, which restricts turtles' ability to breathe at the surface. Midwater or surface gear also has the potential to result in interactions with turtles, particularly as our data demonstrates that the olive ridley turtles spend more time at the surface between the hours of 6:00 p.m. and 6:00 a.m. Gear set at the surface and frequently monitored for capture of turtles could be an alternative to bottom-set gear to at least increase the potential for turtles to be released alive if captured.

The overlap between the core turtle UD and the shipping lane poses a potential threat, however the new MPA also overlaps with the deepest part of the estuary and necessary depths for a shipping channel. While management plans for the new MPA have not been completed, the new MPA is likely to restrict fishing but regulations on shipping are unclear. With a major port located in the Komo Estuary, heavy ship traffic occurs throughout the year in addition to coinciding with the internesting period (**Figure 2C**, **Figure S1**). Management, however, could include speed restrictions or designated shipping lanes during the internesting months.

CONCLUSIONS

There are examples of marine vertebrates from all ocean basins that are under threat throughout their range as seen by large-scale declines in recent decades (Baum et al., 2003; Myers and

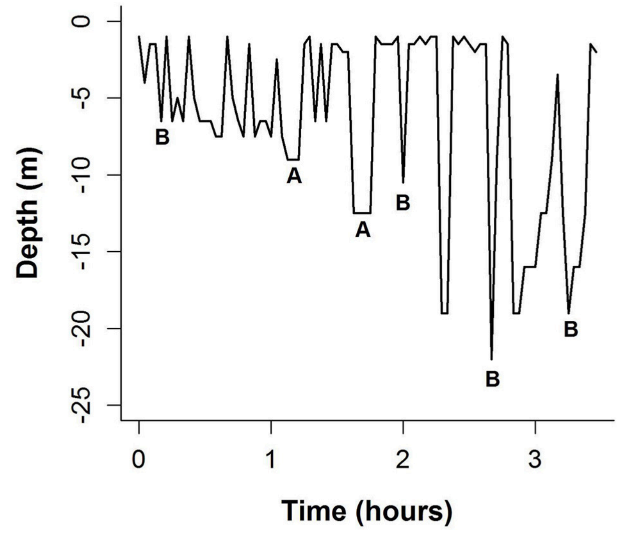

FIGURE 5 | Dive profile of Turtle U during the internesting period. "A" represents a U-shaped dive indicative of resting or foraging and "B" represents a V-shaped dive indicative of exploratory dives.

Worm, 2003; Heithaus et al., 2008). Red List Status might be improving for some species (Balazs and Chaloupka, 2004; Dutton et al., 2005; Simpfendorfer and Dulvy, 2017), highlighting that improved knowledge can lead to better management and conservation outcomes. Nonetheless, threats are highest where species aggregate such as to breed or forage (Edgar et al., 2008; Hays et al., 2010) and where species and humans frequently overlap; for marine species this is often in coastal zones (Maxwell et al., 2013). Quantifying specific threats can be difficult due to limited data, largely because of the feasibility of collection, but quantitative data is necessary to undertake comprehensive assessments and quantify impacts on marine vertebrates. With robust data on human impact and species distribution it becomes possible to accurately gauge threats in space and time, and then appropriately manage human activities for the preservation of species of conservation concern. Here, as the first study to look at internesting movements at this site, we were able to quantitatively determine fine-scale movements of olive ridley sea turtles and compare them with anthropogenic activities, and—critically—this data was used to development of an MPA designated off of Pongara National Park.

While the insights gained from this study greatly improve knowledge on the movement of female olive ridley turtles during the internesting period, there are still critical data gaps for this population. We know very little about male olive ridley turtle movement in general (Godley et al., 2008) and also in this area and they could also be at risk from fishing and boat strikes if they aggregate in the area to breed. Coastal waters could be even more of a conservation concern if the males are shown to utilize coastal waters such as zone 2, as has been demonstrated in other regions (James et al., 2005; Shaver et al., 2005; Hays et al., 2010), but this is entirely unknown in Gabon. Additionally, we are lacking data on the seasonality of males in the region. As they are likely to arrive at breeding grounds prior to females (Plotkin, 2007), they are potentially exposed to threats before the internesting period highlighted in this paper. Understanding this seasonality is critical to ensuring that management measures align with when turtle abundance occurs across sexes. Furthermore, while our data spans three nesting seasons, looking at long-term interannual data could reveal different patterns and habitat use of this area. There may be interannual variability in habitat utilization as well as variability in the distribution of threats such as fishing and shipping. More information on threats would also better aid in designing effective conservation measures. While there is the potential for sea turtle-ship interactions, little is known about how likely these interactions are to occur, or what the impact is on either individuals or populations. More detailed fishing data on the bycatch rate of sea turtles, specifically within the artisanal fisheries, in the estuary or off the coast could guide conservation strategies for restrictions or gear modifications in the area that best suit both the species and fishermen. Though more knowledge is needed, the current data we provide can lead to beneficial conservation strategies for the nesting females from Pongara National Park, Gabon, and provide a framework for studies in other regions.

AUTHOR CONTRIBUTIONS

TD, PDD, AF, BG, KM, DT, MW, and SM conceived the study and conducted data analyses. All authors contributed to the data collection and logistics and writing the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fmars.2017.00312/full#supplementary-material

Figure S1 | AIS location points of the transiting ships from October 1 to November 30, 2012 illustrating the shipping channel within the Komo Estuary. The orange circle represents the Owendo Port, the majoring shipping port in the Komo Estuary.

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Sea Turtle Bycatch Mitigation in U.S. Longline Fisheries

Yonat Swimmer[1]*, Alexis Gutierrez[2], Keith Bigelow[1], Caren Barceló[3], Barbara Schroeder[2], Kenneth Keene[4], Keith Shattenkirk[5] and Daniel G. Foster[6]

[1] Pacific Islands Fisheries Science Center (NOAA Fisheries), Honolulu, HI, United States, [2] Office of Protected Resources (NOAA Fisheries), Silver Spring, MD, United States, [3] College of Earth, Ocean and Atmospheric Sciences, Oregon State University, Corvallis, OR, United States, [4] Southeast Fisheries Science Center (NOAA Fisheries), Miami, FL, United States, [5] Oceans Program, Leonardo DiCaprio Foundation, Los Angeles, CA, United States, [6] Southeast Fisheries Science Center (NOAA Fisheries), Pascagoula, MS, United States

Capture of sea turtles in longline fisheries has been implicated in population declines of loggerhead (*Caretta caretta*) and leatherback (*Dermochelys coriacea*) turtles. Since 2004, United States (U.S.) longline vessels targeting swordfish and tunas in the Pacific and regions in the Atlantic Ocean have operated under extensive fisheries regulations to reduce the capture and mortality of endangered and threatened sea turtles. We analyzed 20^{+} years of longline observer data from both ocean basins during periods before and after the regulations to assess the effectiveness of the regulations. Using generalized additive mixed models (GAMMs), we investigated relationships between the probability of expected turtle interactions and operational components such as fishing location, hook type, bait type, sea surface temperature, and use of light sticks. GAMMs identified a two to three-fold lower probability of expected capture of loggerhead and leatherback turtle bycatch in the Atlantic and Pacific when circle hooks are used (vs. J hook). Use of fish bait (vs. squid) was also found to significantly reduce the capture probability of loggerheads in both ocean basins, and for leatherbacks in the Atlantic only. Capture probabilities are lowest when using a combination of circle hook and fish bait. Influences of light sticks, hook depth, geographic location, and sea surface temperature are discussed specific to species and regions. Results confirmed that in two U.S.-managed longline fisheries, rates of sea turtle bycatch significantly declined after the regulations. In the Atlantic (all regions), rates declined by 40 and 61% for leatherback and loggerhead turtles, respectively, after the regulations. Within the NED area alone, where additional restrictions include a large circle hook (18/0) and limited use of squid bait, rates declined by 64 and 55% for leatherback and loggerhead turtles, respectively. Gains were even more pronounced for the Pacific shallow set fishery, where mean bycatch rates declined by 84 and 95%, for leatherback and loggerhead turtles, respectively, for the post-regulation period. Similar management approaches could be used within regional fisheries management organizations to reduce capture of sea turtles and to promote sustainable fisheries on a global scale.

Keywords: sea turtles, longline fishing, observer data, statistical models, bycatch reduction

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Mariana M. P. B. Fuentes,
Florida State University, United States

Reviewed by:
Marc Girondot,
Université Paris-Sud, France
Brett W. Molony,
Department of Fisheries,
Western Australia, Australia

***Correspondence:**
Yonat Swimmer
yonat.swimmer@noaa.gov

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INTRODUCTION

It is well-established that fisheries bycatch poses a significant threat to numerous sea turtle populations worldwide (Kaplan, 2005; Wallace et al., 2010, 2013). Pelagic longline fishing, a gear type present in all the world's oceans, is directly associated with high rates of bycatch and variable rates of mortality of sea turtles (Camiñas et al., 2006; Swimmer and Gilman, 2012). Sea turtle vulnerabilities to longline fishing gear are dependent on gear configuration as well as the species' geospatial, temporal, and vertical depth distributions (Wallace et al., 2013). Previous assessments of sea turtle bycatch in longline fisheries indicate significantly higher catch rates in fisheries setting gear at shallow depths (<60 m), typically targeting swordfish (*Xiphias gladius*), compared to most deep-set fishing targeting tuna (Lewison et al., 2004; Kaplan, 2005). Numerous investigations indicate a high percentage of turtles are released alive from shallow-set fishing gear (e.g., Swimmer et al., 2006, 2011, 2013; Piovano et al., 2009; Sales et al., 2010; Swimmer and Gilman, 2012), however a proportion of these turtles are assumed to subsequently die as a result of injuries, with likelihood of mortality a function of anatomical hooking location and degree of gear removal (see Ryder et al., 2006; Carruthers et al., 2009). Mitigating the effects of fisheries bycatch is a conservation priority worldwide, yet both research and managements actions are hindered by statistical challenges when analyzing rare and episodic events, as in common many examples of fisheries bycatch. Despite this, the magnitude of fisheries effort worldwide results in a cumulative negative effect on threatened populations, such as sea turtles, and therefore these challenges must be addressed for effective management.

Commercial longline fishing operations in United States (U.S.) Exclusive Economic Zone are regulated by the National Marine Fisheries Service, which aims to address the conservation needs of highly migratory populations of threatened and endangered marine species such as sea turtles while simultaneously managing domestic fisheries. U.S. fisheries must be in compliance with the Endangered Species Act (ESA) [as amended, 16 U.S.C. 1539(a) (2)] that requires federal agencies to ensure that any action they authorize (such as commercial fisheries), fund or carry out is not likely to jeopardize the continued existence of any endangered or threatened species or result in the destruction or adverse modification of critical habitat of such species. The ESA, in addition to other statutes such as the Magnuson-Stevens Fisheries Conservation and Management Act and the Marine Mammal Protection Act, provide the regulatory regime for U.S. Federal fisheries.

In U.S. waters, pelagic longline fishing (PLL) involves the setting of a mainline to which baited hooks are attached by gangions (or branchlines), occurs in the Atlantic Ocean, Gulf of Mexico and Pacific Ocean. The fisheries in the Atlantic, Caribbean and Gulf of Mexico (herein "Atlantic data") are managed according to 11 distinct statistical areas (**Figure 1**). The sea turtle species most commonly captured as bycatch in both ocean basins are leatherback (*Dermochelys coriacea*) and loggerhead (*Caretta caretta*) turtles (Witzel, 1999; Lewison et al., 2004; Gilman et al., 2007; Zollett, 2009), both of which are listed on the ESA as either endangered or threatened.

Starting in 2000, the Northeast Distant (NED) statistical area (8.9 million km^2) of the U.S. Atlantic PLL fishery, a highly productive area that includes the Grand Banks, was partially closed and then fully closed in 2001 in response to legal action aimed to reduce bycatch of endangered sea turtles (July 6, 2004, 69 *FR* 40734; U.S Dept. of Commerce, 2001; NMFS, 2004b). The NED portion of the Atlantic is primarily a swordfish-targeted fishery and was previously determined to have high rates of sea turtle bycatch (Witzel, 1999). Around the same time, in the U.S. North Pacific PLL fishery, the fishing grounds north of Hawaii (north of 28°N and between 150 and 168°W) were partially closed beginning in December 1999, and the entire longline swordfish fishery was closed in 2001 due to sea turtle bycatch. Beginning in April 2001, a spatial and seasonal closure off the U.S. North Pacific PLL fishing grounds south of 15°N during April-May was also implemented (NMFS, 2004a,b).

These temporary fisheries closures in both the Atlantic and Pacific Oceans lasted for ∼3 years. During this time, U.S. government-sponsored research was conducted in the NED that provided evidence that the use of a relatively large (18/0) circle hook in combination with ∼200–500 g Atlantic mackerel (*Scomber scombrus*) bait could significantly reduce bycatch rates of both loggerhead and leatherback sea turtles (Watson et al., 2005). Additionally, hook-and-bait combinations were also found to decrease the proportion of deeply ingested hooks in loggerhead turtles (Watson et al., 2005), thereby presumably increasing the rates of post-interaction survival. Based on these findings, both previously closed areas in the North Pacific and NED area of the Atlantic were re-opened with required use of circle hooks with minimum width dimensions equivalent to an 18/0 size hook (∼4.9 cm). In the Hawaii shallow-set-permitted fishery, bait type is limited to fish-only, whereas in the NED bait type allowances are made for use of squid bait in addition to fish bait. In both regions, additional regulations also included variations of limited entry and fishing effort, turtle bycatch limits, requirements for sea turtle education and outreach efforts, as well as increased on-board scientific observer coverage [NOAA, 50 *CFR* Part 660 (Pacific); NOAA 50 *CFR* Parts 223 and 635 (Atlantic)].

In this investigation, we used long-term fisheries observer data to assess the efficacy of regulatory measures on the probability of sea turtle bycatch in two U.S. pelagic longline fisheries. Specifically, we tested the null hypothesis that bycatch per unit effort (BPUE) was the same before and after regulations. Additionally, we used various statistical models to identify explanatory variables associated with the probability of sea turtle bycatch in both ocean basins, thereby providing further insight into the regulatory measures as well as new information on turtle species' vulnerabilities and responses to specific mitigation methods.

METHODS

Data Sources

Observer data for the analysis originated from the Pelagic Observer Program (POP) and the Longline Observer Data System for the Atlantic and North Pacific, respectively. POP data are maintained by the NMFS Southeast Fisheries Science

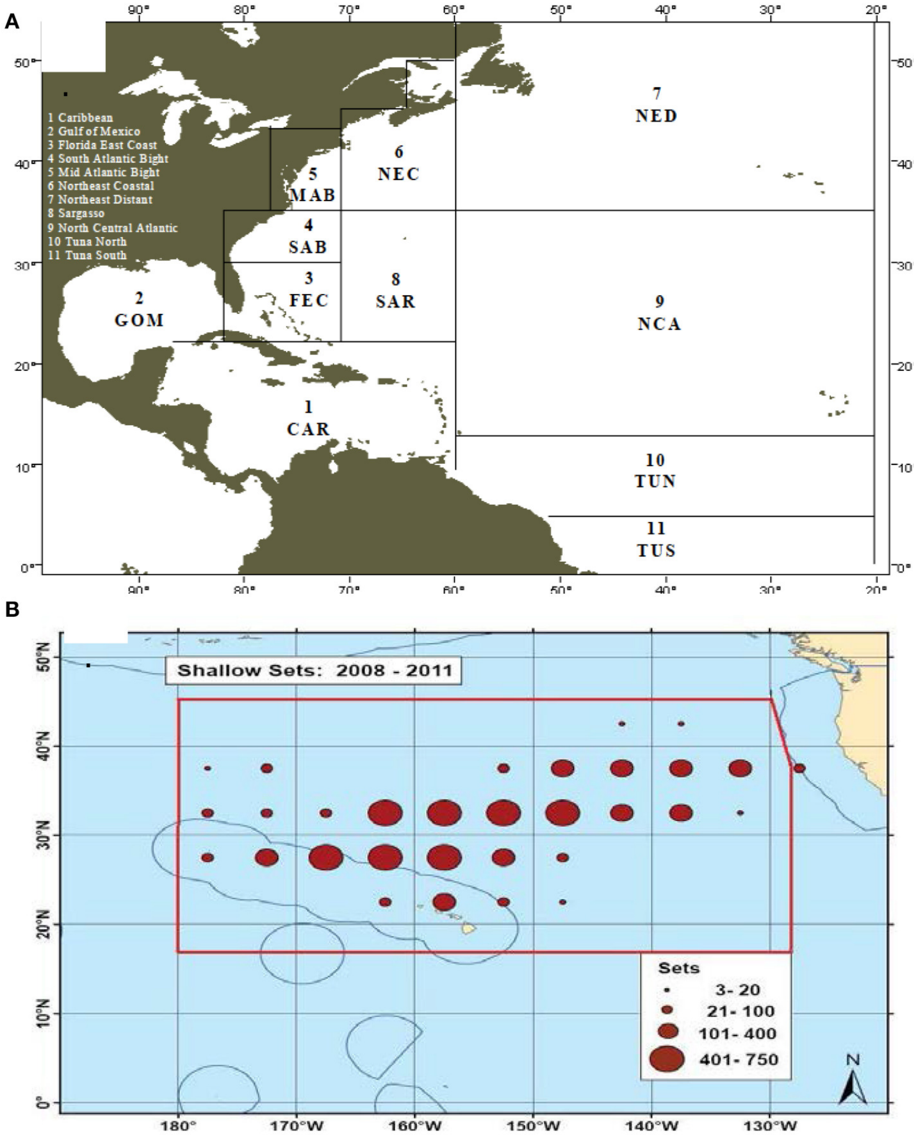

FIGURE 1 | United States **(A)**. Atlantic pelagic longline fisheries statistical reporting areas as recorded by NOAA NMFS Southeast Fisheries Science Center. CAR, Caribbean; GOM, Gulf of Mexico; FEC, Florida East Coast; MAB, Mid-Atlantic Bight; NCA, North Central Atlantic; NEC, Northeast Coastal; NED, Northeast Distant; SAB, South Atlantic Bight; SAR, Sargasso; TUN, Tuna North and TUS, Tuna South. **(B)** Location of shallow sets made by the Hawaii shallow-set longline fishery from 2008 to 2011 (source: NOAA Fisheries Pacific Islands Regional Office).

Center and have been previously described (Keene, 2016). For use in this study, POP data were limited to trips that targeted both swordfish and a mix of swordfish and tunas from 1992 to early 2015, which were analyzed jointly. These analyses omitted data from experimental operations when vessels used modified gear to test various outcomes (e.g., during the NED closure from 2001 to 2003). Pre-regulation data are defined as years 1992–2001, and post-regulation data start in mid 2004 after the fishery was re-opened. These data were combined and analyzed jointly. Observer coverage varied during this time, ranging between ∼3 and 5% of total fleet effort from 1992 to 2003 (Beerkircher et al., 2002) followed by a mandated minimum 8% coverage of the fleet beginning in 2004 (NMFS, 2004b).

The NMFS Pacific Islands Fisheries Regional Office maintains the Pacific observer data. Pacific data analysis was limited to the specified shallow-set (swordfish-target) sector of the fishery from 1994 to 2014. Pre-regulation data include data prior to February 2002, and the post-regulation period after May 2004. Between 1994 and 2000, observer coverage ranged from 3 to 10% (mean ∼5%) and increased to 20.5% in 2001. Observer coverage became mandatory (100% coverage) for all Hawaii-permitted pelagic longline vessels targeting swordfish since the fishery re-opened.

Data Caveats

General observer data characteristics and turtle bycatch specific to the different targeted sets from the Atlantic and Pacific Oceans are in **Table 1**. For both combined Atlantic and Pacific data, the number of observed sets analyzed is heavily skewed post-regulation vs. pre-regulation, which is a function of the mandated increased observer coverage when the fisheries were re-opened in 2004. For all data, nominal bycatch per unit effort (BPUE) was calculated as individual loggerhead and leatherback turtles caught for each unique set per 1,000 hooks. In certain situations, we collapsed categorical variables to achieve sufficient sample sizes and statistical rigor (see **Table 2**). Turtle size measurements are only available for loggerheads, as leatherback turtles were not boarded due to their size.

Analysis of sea surface temperature (SST) data obtained from both regions indicated a high degree of discrepancies when compared with satellite-derived data at a slightly broader scale and time span encompassing fishing location coordinates. This is largely due to the frequent collection of SST using unstandardized methods. Based on these findings, our statistical analyses included SST data derived from 5-day composites from AVHRR Pathfinder v4.1 (1985–2003). These SST data were continued by the AVHRR Global Area Coverage dataset (January 2003–April 2016) with a spatial resolution of $0.1^\circ \times 0.1^\circ$. Analyses included the weekly values when available, otherwise monthly data were used.

Analytical Methods

All analyses were conducted separately for the two regional data sets (Atlantic and Pacific) as well for each turtle species, leatherback, and loggerhead. On a few occasions, a subset of the Atlantic data, specifically the NED region, was analyzed separately given the enhanced regulatory requirements in this area (e.g., 18/0 circle hook). Turtle catch probability was uniquely referenced at the level of longline set, which refers to the individual mainline set (or haul) with baited hooks that remain soaking in the water for $\sim$8–12 h. Spatial statistics were used to generate spatio-temporal kernel density maps to visualize longline sets that captured one or more turtles. Finally, we used statistical models that incorporated a suite of variables that help explain and predict the probability of sea turtle bycatch on longline fishing vessels in the U.S. Atlantic and Pacific fleets.

TABLE 1 | General characteristics and sample sizes for observer data from the Pacific Hawaii-based shallow-set longline fishery, the Atlantic swordfish-set longline fishery, and Atlantic mixed-set longline fishery from ∼1992 to 2014.

General data characteristics	Pacific data (swordfish)	Atlantic data (swordfish)	Atlantic data (mixed-fishery)
Approximate time of initial set	Sunset	Sunset	Sunrise
Number of hooks between floats	Majority 4–5 (range: 3–21)	Majority 3–5 (range: 1–12)	Majority 4–5 (range: 2–10)
Mean number of light sticks per hook (all years)	0.57	0.92	0.38
Hook preferred pre-regulations	J 9	J 9	16/0
Hook regulations (type and minimum size)	Circle 18/0	Circle 16/0 or 18/0*	Circle 16/0
Bait preferred pre-regulations	Squid	Squid	Squid
Bait regulations	Fish only	Fish or squid**	Fish or squid
Sets with fish-only bait (all years)	13,713	890	262
Sets with squid-only bait (all years)	1,532	2,268	3,566
SEA TURTLES CAPTURED (ALL YEARS):			
Leatherback	105	415	429
Loggerhead	222	672	230

**In NED, hook must be 18/0.*

***In NED, squid bait is only allowable if using a non-offset hook.*

TABLE 2 | List of explanatory variables for Atlantic and Pacific observer data used in generalized additive mixed models.

Explanatory variable	Type	Description
Target species	Categorical	Atlantic data: mixed (swordfish and tuna) and swordfish. Hawaii: swordfish-target only.
Maximum hook depth	Continuous	Atlantic data: sum of lengths of floatline, branchline, dropline length. Does not account for mainline sag, sheer, or other factors. Hawaii: not consistently recorded.
Sea surface temperature (SST) °C	Continuous	Weekly SST were obtained from NOAA Pathfinder SST data by location (average of initial set and end of haul locations). When weekly was not available, monthly data were used.
Hook type	Categorical	Atlantic data: 9/0 J hook was predominant hook type pre-regulation, circle hooks (16/0 and 18/0) were used exclusively post-regulation. Pacific data: nearly 100% use of 9/0 J hooks pre-regulation and 100% use of circle hooks (18/0) post regulation.
Hook size	Categorical	Atlantic data: inclusive of circle hooks 16/0, 17/0, 18/0, 20/0, with sizes 16/0 and 18/0 represented in 66% of data. Very few 17/0 and 20/0. For analysis, data collapsed so that small circle hooks were sizes 16 and 17, large circle hooks were sizes 18 and 20. Small J hooks were sizes 7, 8, 9, and large J hooks were 10 &11. However, sample sizes in general were too small to make appropriate comparisons. Pacific data: nearly 100% use of size 9/0 J hooks prior to 2002 and 100% use of circle hooks size 18/0 after 2003.
Bait	Categorical	Atlantic data: Three categories-fish, mix of fish, and squid. Pacific data: Three categories—fish, squid, other (unknown). Mackerel is the most common fish species. Squid used nearly exclusively prior to regulations.
Soak duration	Continuous	From initial set time to end of set (haul).
Lightstick to hook ratio	Continuous	Ratio of total number of light sticks to number of hooks per set.
Number of hooks between floats	Continuous	Atlantic data: Mixed fishery range: 2–10 (majority 4 or 5 floats). Swordfish fishery range: 1–12 (nearly all between 3 and 5). Hawaii data: range: 3–21 (nearly all were 4 to 5).

Assessment of Regulations

In order to assess the efficacy of the conservation measures as a whole, we used non-parametric statistics (Mann-Whitney, due to lack of normality and homogeneity of the variances) to test the null hypothesis that sea turtle BPUE was similar before and after the regulations in mid 2004. Specifically, we compared BPUE for time periods before and after the regulations for leatherback and loggerhead turtles in the Atlantic and Pacific.

Identification of Spatio-Temporal Bycatch Patterns

Spatial kernel density maps were created for the locations of fishing effort and sets with turtle bycatch (>0) using the *"kde2d"* function in the *"MASS"* package in R (v.3.3.1).

Generalized Additive Mixed Models to Determine Probability of Turtle Bycatch

Longline observer data were analyzed to determine the probability of catching leatherback and loggerhead sea turtles using binomial GAMMs. We modeled the presence or absence of either sea turtle species within a single longline set. GAMMs are a non-linear regression technique in which the relationships between the dependent and the independent variables are modeled with non-parametric smooth functions and make allowances for complex relationships (Hastie and Tibshirani, 1990; Wood, 2006). A random intercept mixed models was used with individual vessel ID to account for repeated longline trips by individual vessels.

Species (leatherback, loggerhead) and region-specific (Atlantic, Pacific) full models were constructed that had the following generalized relationship (Equation 1). Specifically, given a dependent variable y and a set of *x* independent covariates, the relationship between them is established by:

$$y_{s,r} = \alpha + \alpha i + \sum\nolimits_{j=1}^{m} g_i \left(x_{ij}\right) + \varepsilon_i \qquad (1)$$

The dependent term (y) in our models was binomial set data (0 = no turtle caught on a set; 1 = one or more turtles caught per set) and was modeled with a logit link function (Wood, 2006). α_i is the variance component around α associated with the vessel effect, g_i are one-dimensional cubic spline smoothing functions for each independent continuous covariates, x_{ij} were independent covariates that included distinct variable combinations dependent on region. Full species-specific models in both regions included independent covariates described in **Table 2** and **Table A1**. All models met the assumptions of constant variance and normal residuals.

Year as a variable was confounded with gear changes and thus omitted from the models. Maximum hook depth [sum of gangions (branchlines), droplines, and leaders] was only recorded by observers in the Atlantic and thus was not included in Pacific models. Additional predictor variables used in all full models included month, SST, bait type (categories: squid, fish, other), hook type (circle, J, other), light stick to hook ratio, soak duration of gear, and number of hooks between floats. Hook size, bait size, or hook offset were not analyzed due to limited sample sizes before and after regulations. A backward selection approach was used to identify the best model. We determined the best-fit models by minimizing the Akaike Information Criterion (AIC). Model selection **Appendix** and terms specific to turtle species and region are outlined in **Table 2**. All GAMM analyses were carried out using the "mgcv" package in R (v. 3.3.1).

RESULTS

Descriptive Summary of Atlantic Sea Turtle Bycatch Data

In total, Atlantic data in our analyses included 11,982 unique sets conducted on 1,762 trips from an approximately equal number of swordfish and mixed target trips. Throughout the 1992–2015 period, 844 leatherback and 902 loggerhead turtles were captured. Turtle bycatch per unit effort (BPUE; # individuals caught per 1,000 hooks, ±SD) rates in each of the statistical reporting areas within the Atlantic PLL fishery for all the years are reported (**Figure 2**), with clear distinction of the NED region, which had the highest turtle BPUE of any statistical reporting area within the Atlantic PLL fishery. BPUE tended to be higher for years prior to 2001. Bycatch rates are reported by year for all areas combined in **Figure 3**.

Ninety-four percent (94%) of all Atlantic sets showed zero leatherback turtles recorded. A single leatherback was caught on 611 sets, 2 or 3 were caught on 92 sets, and between 4 and 7 were caught on 6 sets. Ninety-five percent (95%) of sets showed zero loggerhead turtles recorded. A single loggerhead turtle was captured on 482 different sets, between 2 to 4 were caught on 87 sets, between 5 to 7 were caught on 12 sets, and between 9 to 12 were caught on 5 sets. Mean curved carapace length (CCL, cm) for loggerhead turtles brought on board was 73.3 cm (SD = 27.6).

Descriptive Summary of Pacific Sea Turtle Bycatch Data

Pacific observer records were from 15,472 sets from 460 unique trips during 1994–2014, which included observed capture of 105 leatherback and 222 loggerhead turtles (**Figure 4**). Ninety-nine percent (99%) of sets had zero leatherback turtles recorded. A single leatherback was caught on 103 sets and 2 were caught on 2 sets. Ninety-nine percent (99%) of sets had zero loggerhead turtles recorded. A single loggerhead was caught on 197 sets and 2 or 3 were caught on 25 sets. Mean CCL for all turtles brought on board was 62.8 cm (SD = 11.6), which is smaller than loggerheads measured in the Atlantic (CCL; mean = 73 cm, SD ± 27.8, **Figure 5**). Overall, the range of sizes for loggerhead turtles in the Atlantic was considerably broader than in the Pacific.

Frequency of Capture as a Function of Turtle Size and Sea Surface Temperature

In the Atlantic, the frequency of sets with bycatch in our study was highest within an approximate SST range between 22 to 26°C and 23 to 27°C for loggerheads and leatherbacks, respectively. In the Pacific, the peak range of SST with positive sea turtle

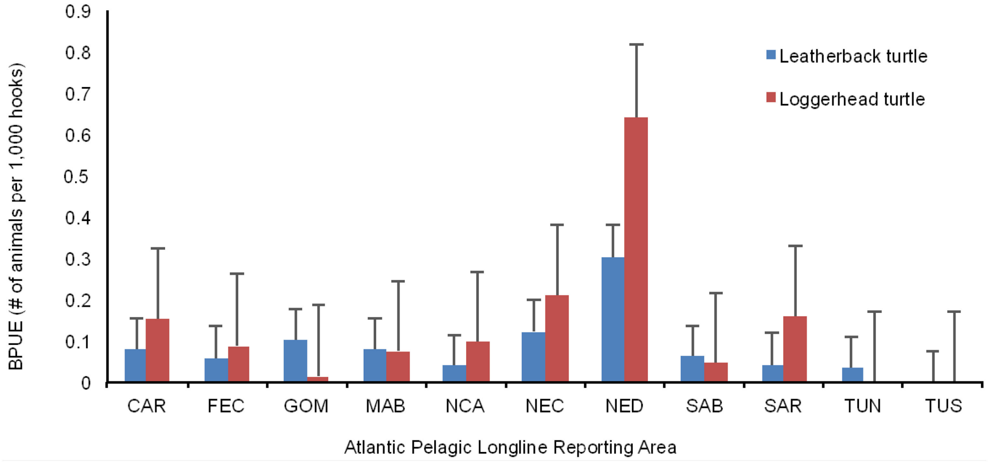

FIGURE 2 | Atlantic data. Mean turtle BPUE (# individuals caugh per 1,000 hooks ±SD) by statistical reporting area. CAR, Caribbean; GOM , Gulf of Mexico; FEC, Florida East Coast; MAB, Mid-Atlantic Bright; NCA , North Central Atlantic; NEC, Northeast Coastal; NED, Northeast Distant; SAB, South Atlantic Bight; SAR Sargasso; TUN, Tuna North and TUS, Tuna South.

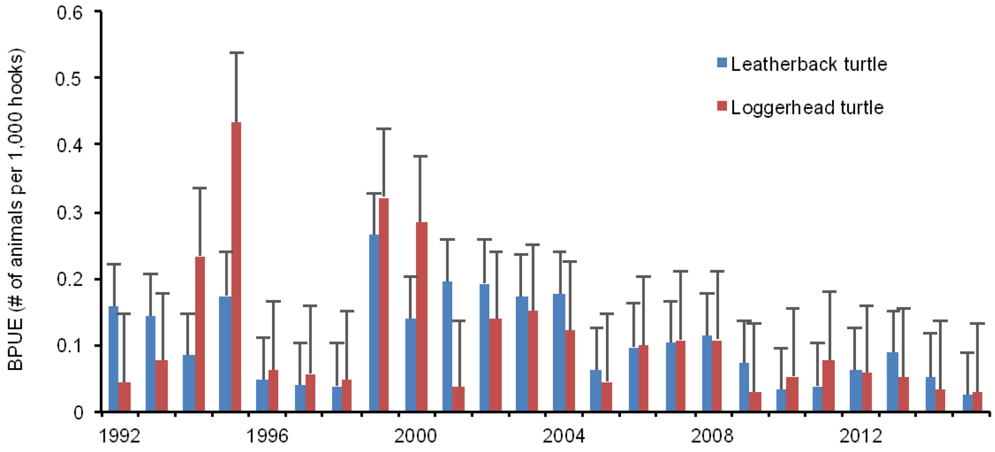

FIGURE 3 | Atlantic data Sea turtle bycatch per unit effort (BPUE, #individuals caught per 1,000 hooks, ±SD) by year.

captures occurred between 17 and 19°C for both loggerheads and leatherbacks (**Figure 5**).

Comparison of Bycatch before and after the Regulations

For Atlantic observer data inclusive of all reporting areas, bycatch rates of leatherback and loggerhead turtles were significantly lower during the post-regulation period, a reduction of 40 and 61%, respectively. Within the NED area alone, which had greater mitigation requirements than the rest of the Atlantic areas, turtle bycatch rates were further reduced during the post-regulation period, by 64 and 55% for leatherback and loggerhead turtles, respectively. For Pacific data, turtle bycatch rates were significantly lower during the post-regulation period, a reduction of 84 and 95% for leatherback and loggerhead turtles, respectively (**Table 3**).

Spatio-Temporal Sea Turtle Bycatch Patterns

Kernel density plots illustrate the distribution of observed longline sets with bycatch of at least one sea turtle by quarter for both the Atlantic and Pacific (**Figure 6**). Spatial and temporal patterns are fishery-dependent and largely driven by the distribution of fishing effort. In the Atlantic, longline sets that captured one or more turtles were most dense in southern latitudes during quarters 1 and 2 and shift northeast to the NED area in quarters 3 and 4. Sets with loggerhead turtle captures in the Gulf of Mexico (GOM) were most prevalent during quarter 2, whereas sets with leatherback turtles were most frequent in the GOM during quarters 1 and 2. Longline sets with leatherback turtles were most dense in the NED and coastal northern U.S. waters during quarters 3 and 4. Sets with loggerhead turtles in the Pacific observer data had less clear patterns, with the exception

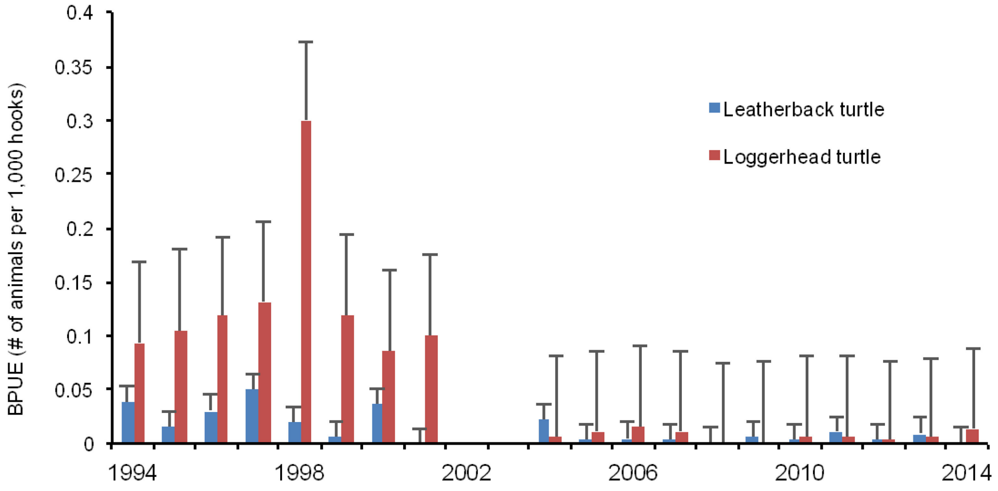

FIGURE 4 | Leatherback and loggerhead BPUE (# individuals caught per 1,000 hooks, ±SD) for Hawaii-permitted shallow set longline fishery by year.

of a high density of sets with loggerhead turtles in quarter 1 northeast of the main Hawaiian Islands. Similarly, sets with leatherback turtle interactions were most dense north of the main Hawaiian Islands during quarters 1 and 2, with density shifted further west during quarter 3.

Model Outputs and Relative Probabilities of Capture

The best-fit models with the final terms for each species and region are summarized in **Appendix**. Turtle bycatch probabilities as a function of significant terms in the model are shown in **Figure 7**. Model estimates of the individual variable effects on bycatch probability for all years of data collections are in **Table 4**. Both absolute and relative probabilities of bycatch for both leatherback and loggerhead turtles in the Atlantic and Pacific were determined using the rescaled GAMM model (**Table 5**). Since probabilities differed among the various statistical reporting areas of the Atlantic, we report on gear comparisons for the area of the NED given the enhanced regulatory requirements in this region and allowing for more valuable comparisons relative to sea turtle bycatch probability as a function of hooks and bait combinations. Bycatch probabilities are also reported for other statistical reporting areas in the rest of the Atlantic for circle hooks with fish bait (**Table 5**).

Probabilities of Turtle Capture

Loggerhead Bycatch in the Atlantic Ocean

The factors found to influence the capture of loggerhead turtles in the Atlantic are number of hooks between floats, ratio of light sticks to hooks, SST, bait type, hook type, and fishing area (**Figures 7A–G**; **Table 1A**). Probability of loggerhead bycatch is expected when the number of hooks between floats is 4 or 5, with light sticks attached at each hook, and when maximum estimated hook depth is ∼22 m or less. The GAMM identified an increased loggerhead catch probability with SST between ∼18 and 24°C. Plots of factors such as bait and hook type indicate loggerhead turtle bycatch probability is lowest when using only fish bait, and significantly increased when using squid bait. The use of J hooks results in significantly elevated bycatch probability as compared to circle hooks for the combined Atlantic statistical areas.

In regards to fishing location in the Atlantic, GAMMs identified the expected probability of catching a loggerhead is highest in the NED and NEC when using circle hooks and fish bait (**Table 5**). The expected probability of catching a loggerhead turtle on a set in the NED area using circle hooks and fish bait is 0.054 as compared to 0.111 if using circle hooks and squid bait, indicating a two-times (2.045) greater catch probability of a loggerhead using squid bait compared to fish bait. There is a reported 1.690 times greater catch probability of catching loggerhead turtles in the NED using J hooks with fish bait compared to using circle hooks with fish bait. In combination, there is a predicted 3.318 times greater catch probability of loggerhead turtles in the NED using the J hooks with squid bait as compared to circle hooks with fish bait (**Table 5**).

Leatherback Bycatch in the Atlantic

Based on the GAMM models, the expected probability of catching a leatherback turtle in the Atlantic Ocean is most influenced by month, number of hooks between floats, SST, bait type, hook type, and statistical reporting area (**Figures 7H–M**, **Table 5**). Bycatch probability is elevated during the months of October through December and within SST in the range of 18–24°C. Leatherback turtle bycatch probability is expected to be lowest when using only fish bait and circle hooks (measured separately) and significantly increases when using squid and J hooks (**Table 1A**). There is a significant elevated bycatch probability of leatherback turtles in the GOM, NEC, and NED regions of the Atlantic statistical reporting area. The probability of catching a leatherback turtle per set in the NED area of the Atlantic while using circle hooks and fish bait is 0.056 as compared to 0.089 if using circle hooks and squid bait, indicating

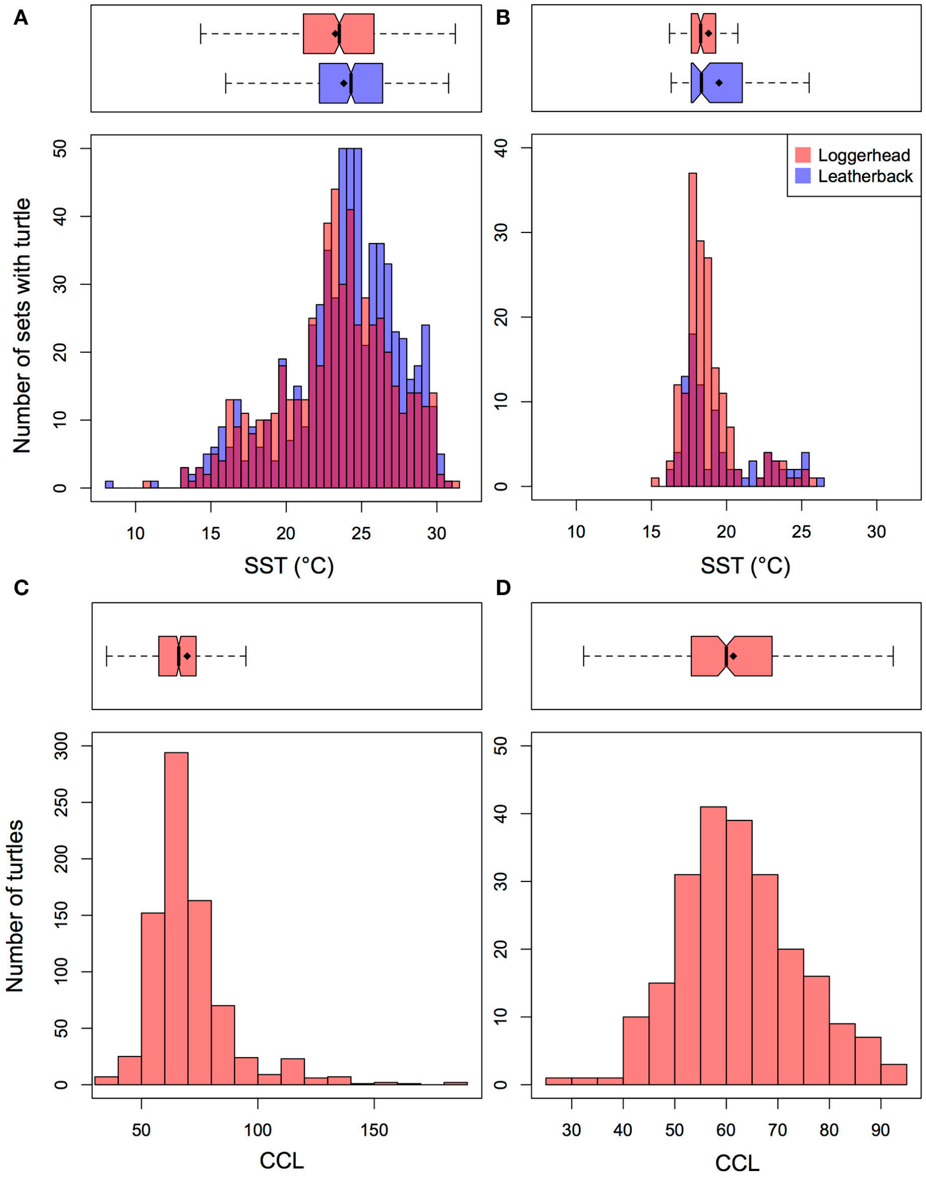

FIGURE 5 | Frequency of sets that caught loggerhead and leatherback turtles by set sea surface temperatures (SST) in the Atlantic **(A)** and Pacific **(B)**. Size frequency of loggerhead turtles captured in longline sets in the Atlantic **(C)** and Pacific **(D)**. Dots in paragraphs indicate mean and lines indicate median. Loggerhead turtle measurements in curved carapace length(CCL, cm).

a 1.589 times greater catch probability of leatherback turtles by using squid (vs. fish) bait. When bait is held constant, there is a 2.284 times greater catch probability of leatherback turtles in the NED using fish bait using J hooks as compared to using circle hooks. There is a 3.475 times greater catch probability of leatherback in the NED using the combination of J hooks with squid bait vs. circle hooks with fish bait (**Table 5**).

Loggerhead Bycatch in the Pacific (Hawaii Shallow Set Fishery)

The key variables influencing the probability of loggerhead bycatch in the Pacific include month, bait type, hook type and location (**Figures 8A–D**, **Table 4**). Loggerhead bycatch is expected to be highest during January and February and in two geographic locations (**Figure 8D**). Loggerhead turtle bycatch probability is lowest with use of fish bait (vs. squid or other) and circle hook (vs. J or other). GAMM results indicate the probability of loggerhead turtle catch in the Pacific is a predicted 2.890 times higher when using circle hooks with squid bait as compared to when using circle hooks with fish bait (0.018 vs. 0.006). There was a predicted 7.313 times greater catch probability of loggerheads using J hooks and fish bait as compared to circle hooks and fish bait. In combination, there is an expected 19.632 times greater catch probability when using the combination of J hooks with squid bait as compared to circle hooks with fish bait (**Table 5**).

Leatherback Bycatch in the Pacific (Hawaii Shallow Set Fishery)

Variables there were expected to influence the probability of leatherback turtle capture in the Pacific include month and hook type (**Figures 8E, F**, **Table 4**). GAMM results indicate a 3.72 times greater catch probability of leatherback turtles on J hooks vs. circle hooks (0.013 vs. 0.004; **Table 5**). Simultaneous gear changes due to regulatory measures limited additional comparisons.

TABLE 3 | Observer sampled nominal mean bycatch per unit effort (BPUE, per thousand hooks) before and after regulations by species by region.

Species	Pre-regulations BPUE (SD)	Post-regulations BPUE (SD)	% Change	Test statistic (Mann Whitney *U*)
ATLANTIC				
Loggerhead	0.17 (0.812)	0.07 (0.444)	−61	$P < 0.001$ (−6.565)
Leatherback	0.13 (0.569)	0.078 (0.378)	−40	$P = 0.002$ (−3.060)
NED				
Loggerhead	0.88 (1.905)	0.39 (1.569)	−55	$P < 0.001$ (−4.516)
Leatherback	0.44 (1.070)	0.16 (0.455)	−64	$P < 0.001$ (−3.866)
HAWAII				
Loggerhead	0.13 (0.468)	0.01 (0.088)	−95	$P < 0.001$ (−25.636)
Leatherback	0.03 (0.209)	0.01 (0.079)	−84	$P < 0.001$ (−8.120)

NED identified in ***Figure 1***.

DISCUSSION

Value of Statistical Models for Bycatch Prediction

In this study, we used statistical models that allow for non-linear relationships (Hastie and Tibshirani, 1990; Guisan et al., 2002) and are thus highly suitable for modeling rare bycatch events, such as sea turtle bycatch in longline fisheries (McCracken, 2004; Coelho et al., 2013). Statistical models, such as GAMs and their extension the GAMMs, have been used extensively in marine fisheries research and management to forecast outcomes such as target species abundance, catch levels, etc. (Walsh and Kleiber, 2001). GAMs can also be used to identify species' associations with environmental variables, such as SST and depth, and therefore be valuable to predict the likelihood that a given species would inhabit or be captured in a particular environment (Forney et al., 2015). Using models to predict the probability of a relatively rare event, such as fisheries bycatch or ship strikes, is challenging due to a high proportion of zero captures resulting in a skewed distribution (Martin et al., 2015).

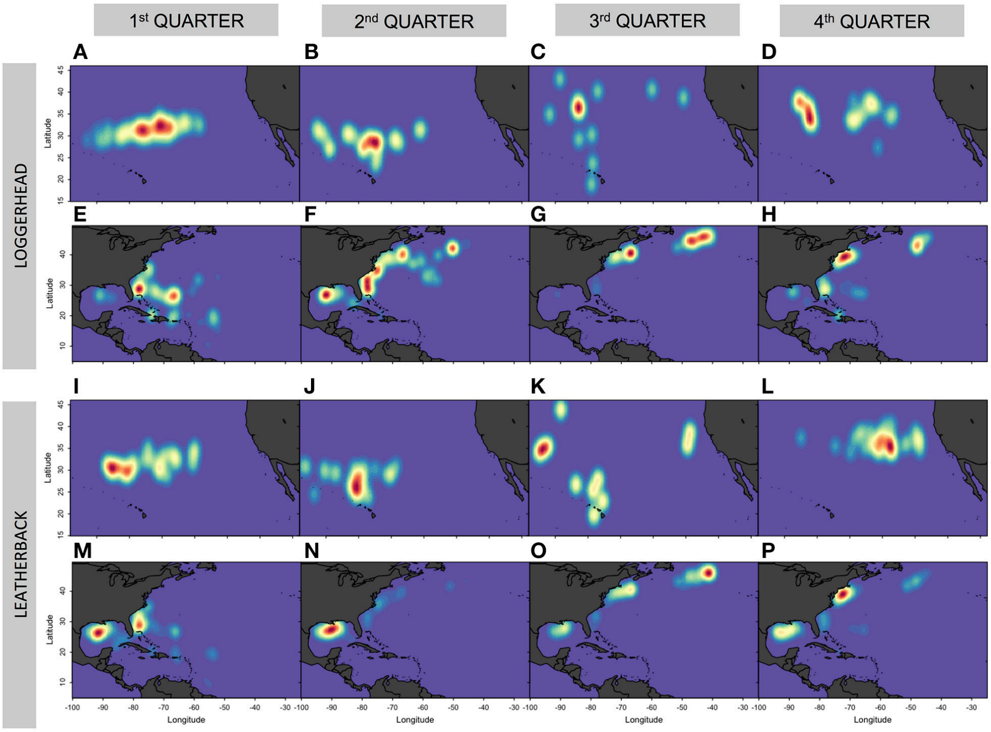

FIGURE 6 | Spatial kernel density plots of loggerhead (**top, upper figure**) and leatherback (**bottom, upper figure**) turtle captures by area and quarter from Atlantic observer data. Spatial kernel density plots of loggerhead (**top, lower figure**) and leatherback (**bottom, lower figure**) turtle captures by quarter from Hawaii observer data. Loggerhead turtle plots **(A–D)** are 1st through 4th quarter in Pacific and **(E–H)** are 1st through 4th quarter in the Atlantic. Leatherback turtle plots **(I–L)** are 1st through 4th quarter in in the Pacific and **(M–P)** are 1st through 4th quarter in the Atlantic.

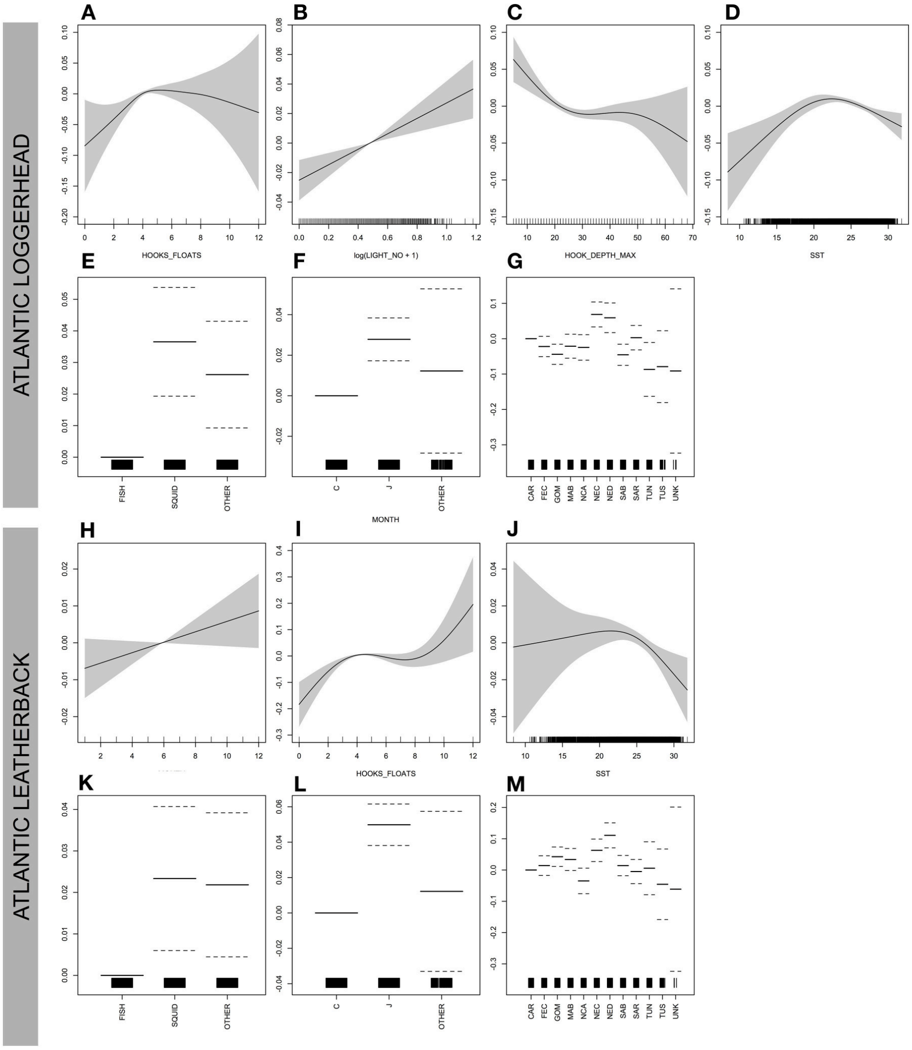

FIGURE 7 | Effect of gear characteristics and weekly averaged satellite derived sea surface temperature (wSST) on catch probability of loggerhead **(A–G)** and leatherbacks in the Atlantic Ocean **(H–M)**. The y-axis is in logit units and represents deviation from the logit mean. The smoothed probability of catch is scaled by its coefficient from the Generalized Additive Mixed Model (GAMM) model. The shaded area represents the upper and lower twice-standard-error curves. For factors (categorical variables), solid lines are the mean and the first factor is the reference group centered at zero, dashed lines represent upper and lower twice-standard errors. Positive (negative) values represent higher (lower) probability of catch from the mean capture probability. Vertical ticks on the x-axis indicate data distribution. For the Atlantic, the probability of loggerhead turtle capture is a function of **(A)** number of hooks between floats,**(B)** ratio of light sticks to hooks (log scale due to improve model fit), **(C)** maximum reported hook depth, **(D)** SST, **(E)** Bait type, **(F)** Hook Type, **(G)** Statistical Reporting, area. For the Atlantic, the probability of leatherback turtle capture is functional of **(H)** Month, **(I)** Number of hooks between floats, **(J)**, SST, **(K)** Bait Type, **(L)** Hook Type, **(M)** Statistical Reporting area.

In spite of the many challenges, recent modeling efforts have provided critical information with direct value to protected species management. Examples are numerous and across taxa, including seabirds (Majluf et al., 2002; Winter et al., 2011; Gilman et al., 2016a), marine mammals (Majluf et al., 2002; Orphanides, 2009; Redfern et al., 2013; Martin et al., 2015), sharks (Walsh and Kleiber, 2001; Minami et al., 2007), and sea turtles (Murray, 2009, 2011).

GAMMs in this study resulted in the highest explanatory power for the probability of bycatch of loggerhead turtles in the Pacific, with the least explanatory ability for leatherback bycatch in the Atlantic. In general, much of the expected bycatch probability for both turtle species was explained in time and space, which is largely a function of the fishery effort and the overlap between target species and sea turtle foraging habitats. Given the fishery-dependent nature of these data, there is no way to isolate the bycatch probability independent from the fishing effort, especially as it relates to space and time. However, these analysis incorporates additional characteristics of the operational components of the fishery including gear specifications such as hook and bait type and approximate hook depth that can provide insights on ways to decrease sea turtle bycatch probability within a specific region during normal fishing operations. As our primary goal was to understand the effects gear differences and spatio-temporal terms have on bycatch probability, we focused on these covariates in the models and their resultant statistical

TABLE 4 | GAMM selection parameters and outputs.

		Models (Species and region)			
		Loggerhead atlantic	**Leatherback atlantic**	**Loggerhead pacific**	**Leatherback pacific**
Parametric terms		**Estimate (sig.)**			
Intercept	**Category**	−3.6308 (***)	−4.47(***)	−5.39 (***)	−5.423 (***)
BAIT (reference: FISH)	SQUID	0.7773 (***)	0.508 (*)	1.074 (*)	(ns)
	OTHER	0.5277 (*)	0.522 (**)	1.01 (*)	(ns)
HOOK (reference: C-HOOK)	J-HOOK	0.5656 (***)	0.906 (***)	2.03 (***)	1.32 (***)
	OTHER	0.009	0.3088	1.21 (***)	0.8313
AREA (reference: CAR)	FEC	−0.5636 (*)	0.1348	–	–
	GOM	−1.6917 (***)	0.754 (*)	–	–
	MAB	−0.5037	0.692 (.)	–	–
	NCA	−0.3759	−0.952 (.)	–	–
	NEC	0.5045	1.045 (**)	–	–
	NED	0.6031	1.481 (***)	–	–
	SAB	−1.1756 (***)	0.1221	–	–
	SAR	−0.2415	−0.2018	–	–
	TUN	−13.5392	−0.0907	—	–
	TUS	−12.7777	−13.0401	–	–
	UNK	−13.7820	−12.1695	–	–
Smooth terms		**EDF (sig.)**			
LAT, LON		–	–	2.91 (***)	(ns)
MONTH		(ns)	1 (.)	1 (***)	1 (***)
SST		2.53 (***)	2.017 (*)	(ns)	(ns)
LIGHTSTICK TO HOOK RATIO		1 (***)	(ns)	(ns)	(ns)
HOOK DEPTH MAX		2.01 (**)	(ns)	–	–
HOOKS PER FLOAT		2.36 (.)	2.334 (*)	(ns)	(ns)
SOAK DURATION		(ns)	(ns)	(ns)	(ns)
Adj. R-sq		5.92%	1.97%	6.15%	0.81%

*Statistical significance (p-values): 0 "***" 0.001 "**" 0.01 "*" 0.05 "." 0.1 "" 1; EDF, Estimated degrees of freedom.*

estimates and significance. Despite the potential importance to turtles' presence in a given location, habitat variables, such as fronts, eddies, and primary productivity, were not included in modeling efforts. We acknowledge, that inclusion of these variables might have improved model fit (adjusted R^2) by explaining the oceanographic context surrounding each longline set.

Interpretation of Findings

Spatial Distribution and SST

Using all Atlantic data, we modeled the probability of turtle bycatch as a function of location (statistical reporting areas) and identified an elevated bycatch risk in the NED and NEC for loggerhead turtles and in the NED for leatherbacks. This finding was expected as the NED is primarily a swordfish-targeting region where hooks are set shallow at night with a high light stick-to-hook ratio that results in a combination of variables associated with an increased probability of catching primarily loggerhead turtles according to our models.

Similar to previous studies, our results identified the influential role of SST regarding the probability of catching loggerhead and leatherback turtles in both the Atlantic and Pacific oceans. In general, there is a broader range of temperatures recorded in the Atlantic (∼10–30°C) vs. Pacific data (∼16–26°C), due to the fact that US fleets operate over a wider latitudinal range in the Atlantic than the Pacific. The frequency of sets with sea turtle bycatch in the Atlantic was highest within approximate SST ranges between 22°C to 26°C and 23°C to 27°C for loggerheads and leatherbacks, respectively. These ranges are nearly identical to those previously reported (Watson et al., 2005; Brazner and McMillan, 2008; Foster et al., 2012; Huang, 2015). The range of SST with positive sea turtle captures was more protracted in the Pacific, with the frequency of sets with sea turtle bycatch highest when SST ranged between ∼17 and 19°C for both loggerheads and leatherbacks. These ranges overlap entirely with the species' at-capture peak SST ranges previously reported in the North Pacific (Howell et al., 2008, 2015; Kobayashi et al., 2008; Abecassis et al., 2013).

TABLE 5 | Capture probabilities of leatherback and loggerhead sea turtles from GAMM models and associated absolute and relative increases in the Atlantic or Pacific Oceans by longline sets for combinations of hook type, bait, and area.

Ocean basin	Area (Atlantic only)	Turtle species	Hook type	Bait type	Capture interaction probability per set	Within hook type comparison (x increase in relative terms)	Between hook type comparison (x increase in relative terms)
Atlantic	FEC	Loggerhead	Circle	Fish	0.018		
Atlantic	FEC	Leatherback	Circle	Fish	0.015		
Atlantic	GOM	Loggerhead	Circle	Fish	0.006		
Atlantic	GOM	Leatherback	Circle	Fish	0.029		
Atlantic	MAB	Loggerhead	Circle	Fish	0.019		
Atlantic	MAB	Leatherback	Circle	Fish	0.026		
Atlantic	NCA	Loggerhead	Circle	Fish	0.021		
Atlantic	NCA	Leatherback	Circle	Fish	0.005		
Atlantic	NEC	Loggerhead	Circle	Fish	0.050		
Atlantic	NEC	Leatherback	Circle	Fish	0.037		
Atlantic	NED	Loggerhead	Circle	Fish	0.054	0.000	0.000
Atlantic	NED	Loggerhead	Circle	Squid	0.111	2.045	2.045
Atlantic	NED	Loggerhead	J	Fish	0.092	0.000	1.690
Atlantic	NED	Loggerhead	J	Squid	0.181	1.963	3.318
Atlantic	NED	Leatherback	Circle	Fish	0.056	0.000	0.000
Atlantic	NED	Leatherback	Circle	Squid	0.089	1.589	1.589
Atlantic	NED	Leatherback	J	Fish	0.128	0.000	2.284
Atlantic	NED	Leatherback	J	Squid	0.195	1.521	3.475
Atlantic	NED	Leatherback	Circle	Fish	0.010		
Atlantic	SAB	Loggerhead	Circle	Fish	0.009		
Atlantic	SAB	Leatherback	Circle	Fish	0.015		
Atlantic	SAR	Loggerhead	Circle	Fish	0.024		
Atlantic	SAR	Leatherback	Circle	Fish	0.011		
Atlantic	TUN	Loggerhead	Circle	Fish	0.000		
Atlantic	TUN	Leatherback	Circle	Fish	0.012		
Atlantic	TUS	Loggerhead	Circle	Fish	0.000		
Atlantic	TUS	Leatherback	Circle	Fish	0.000		
Pacific		Loggerhead	Circle	Fish	0.006	0.000	0.000
Pacific		Loggerhead	Circle	Squid	0.018	2.890	2.890
Pacific		Loggerhead	J	Fish	0.047	0.000	7.313
Pacific		Loggerhead	J	Squid	0.125	2.685	19.632
Pacific		Leatherback	Circle		0.004		0.000
Pacific		Leatherback	J		0.013		3.720

"Within hook type comparison" results represent the percent increase for squid or "other" category baits relative to fish bait within each hook type category (Circle, J, or Other). "Between hook type comparison" results represent the percent increase for sets using J hooks or "other" category hooks with squid or "other" category baits relative to capture probabilities on circle hooks used with fish bait only.

SST has been previously identified as a strong predictor of sea turtle movements (Kobayashi et al., 2008; Mansfield et al., 2009), and thus SST can serve a valuable role as a means to reduce sea turtle bycatch. For pelagic longline fisheries operating in the north Atlantic, Brazner and McMillan (2008) investigated the frequency of loggerhead turtle capture as a function of SST and suggested limiting fishing activity in SST >20°C to minimize loggerhead bycatch. In the North Pacific, Howell et al. (2008, 2015) used extensive satellite tracking data to identify oceanographic features such as SST that could be used to predict the presence of loggerhead and leatherback turtles with the ultimate goal to develop a means to reduce sea turtle captures in longline fisheries. The result is an internet-based product, NOAA TurtleWatch (www.pifsc.noaa.gov/eod/turtlewatch.php), that serves to provide information on preferred sea turtle habitat, specifically SST, that can be used by managers and fishers to make dynamic decisions to reduce the incidental capture of loggerhead and leatherback turtles during longline fishing operations (Howell et al., 2008, 2015). This is particularly valuable in the shallow-set sector of the Hawaii fishery that operates under sea turtle bycatch limits, whereby the fishery is mandated to immediately cease fishing operations until the remainder of the calendar year once a certain number of turtle interactions by species occurs. Howell et al. (2015) proposed a dynamic management concept based upon a SST habitat boundary, whereby fishing effort should be avoided in the

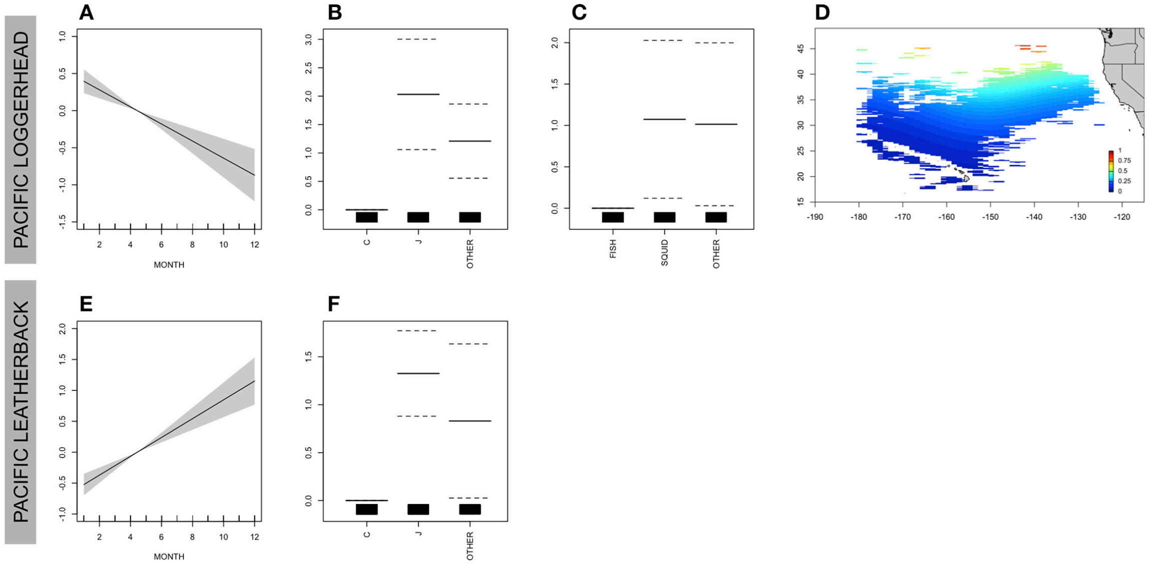

FIGURE 8 | Effect of gear characteristicsand location on the probability of catch of loggerheads **(A–D)** and leatherbacks **(E,F)** in the Pacific. The *y*-axis is in logit units and represents deviation from the logit mean. The smoothed probability of capture is scaled by its coefficient from the Generalized Additive Mixed Model (GAMM) model. The shaded area represents the upper and lower twice-standard-error curves. For factors, solid lines are the mean and the first factor is the reference group centered at zero, dashed lines represent upper and lower twice-standard errors. Positive (negative) values represent higher (lower) probability of catch from the mean capture probability. Vertical ticks on the x-axis indicate data distribution. For the Pacific, the probability of loggerhead turtle capture is a function of **(A)** Month, **(B)** Hook Type, **(C)** Bait Type, **(D)** Location (scaled between 0 and 1 catch probability). For the Pacific, the probability of leatherback turtle capture is a function of **(E)** Month, and **(F)** Hook Type.

SST range of 17.0–18.5°C to minimize interactions with both loggerheads and leatherbacks. In calculating the potential impact of this restriction on the Pacific observer data presented herein, the observed number of turtles captured would have been reduced by 94 (42%) loggerhead turtles and 46 (44%) leatherback turtles.

The Role of Bait in Bycatch

Our findings on the significance of bait type influencing the probability of capturing sea turtles are consistent with other experiments conducted in the Atlantic, whereby it was determined that the largest reduction in (primarily leatherback) turtle bycatch was achieved with use of fish bait, specifically mackerel (Watson et al., 2005; Santos et al., 2012). Based on results of numerous investigations, there is general consensus that replacing squid bait with fish bait will reduce sea turtle bycatch. However, regulations requiring use of fish bait to reduce sea turtle bycatch must be balanced against the potential target species catch loss, a concern that has been previously evaluated (Watson et al., 2005; Yokota et al., 2009; Curran and Bigelow, 2011; Coelho et al., 2012). Further, bait choice can also potentially increase bycatch of certain sharks or other vulnerable species (Foster et al., 2012; Santos et al., 2012; Gilman et al., 2016b). As with other bycatch reduction techniques, success in adopting these measures may be fishery dependent. Among the factors that need to be evaluated include the target species, bait, hook type, and intended hook depth, the species and life-stage of bycaught turtles, fishing area (which is co-related with SST) and season, and other bycatch species potentially affected. In the U.S. Atlantic longline fishery, NED fishers have the choice to use fish or squid bait, yet if they use squid it must be accompanied by a circle hook (18/0) with no offset. It is clear that mandated use of fish bait in all areas would lead to further reductions in sea turtle bycatch.

Ratio of Light-Sticks to Hooks

Unlike leatherback turtles that are primarily externally hooked in the armpit, shoulder or flipper, loggerhead turtles are primarily captured as a result of actively biting and/or swallowing a baited hook (Watson et al., 2005). Based on these observations, Watson et al. (2005) proposed the potential attraction to light sticks, or phototaxis, as an explanation of loggerhead turtle bycatch rates in longline gear. Despite the interest to investigate the role of lights in sea turtle bycatch, such analysis was not possible in the previous NED experiments given that this variable remained constant throughout the time-series. In this study, with data from both swordfish-target and mixed-target sets, variation existed in operational factors such as ratio of lightsticks deployed per hook, whereby lights are generally placed near each hook on swordfish-style sets and likely every other hook in a mixed target set. This overlap allowed for further exploration on the role of lightstick use on the probability of turtle capture. This study identified a positive linear relationship regarding loggerhead turtle bycatch probability and lightstick use. Based on earlier speculations of the role of lightsticks in attracting sea turtles to longline fishing gear, Wang et al. (2007) conducted behavioral experiments with captive loggerhead turtles using an orientation arena to conclude that lightsticks of varying wavelengths significantly attract turtles. These results from the GAMMs, in combination with the laboratory studies (Wang et al., 2007), provide evidence that loggerhead turtles are drawn to the vicinity of longline gear (baited hooks) with increased illumination. Additional bycatch reduction may be gained for loggerhead turtles by reducing or eliminating the use of lightsticks in longline gear.

Hook Type

In all models there was a significant lower probability of bycatch with circle hooks compared to J hooks. The finding of reduced probability of sea turtle bycatch on circle hooks is consistent with a number of different studies and has been thoroughly discussed (Watson et al., 2005; Yokota et al., 2009; Curran and Bigelow, 2011; Santos et al., 2012; Serafy et al., 2012).

Hook Depth

Estimates of theoretical hook depths can be inferred using information on gear characteristics, such as the length of the longline, number of hooks between floats and catenary geometry; however the actual hook depths may be shallower due to shoaling by environmental factors (Bigelow et al., 2006). In this study, two variables serve as a proxy for hook depth: maximum hook depth (sum of the lengths of the gangion, float line, and leader), and number of hooks between floats. The maximum hook depth was reported only in the Atlantic data and does not account for any shoaling, hence the actual hook depth is likely to differ. However, the term was significantly linked to the probability of capturing loggerhead turtles, which is greatly increased when the hook depth is within the top ∼25 m of the sea surface. Additionally, the GAMM indicated an increased probability of capturing a loggerhead when there were 3–5 hooks between floats (**Figure 7A**), indicating a relatively shallow-set longline. These data also indicate a reduced capture risk in sets with fewer than 3 hooks between floats, yet this is likely biased by the relatively few sets in this category (<3% of all sets). The result of an increased bycatch probability for leatherback turtles at greater depth than loggerheads has been previously reported (Watson et al., 2005; Gilman et al., 2006).

The captures of loggerhead turtles in shallow-set gear is consistent with previous studies in the same region (Watson et al., 2005; Brazner and McMillan, 2008; Foster et al., 2012) and can partially explain why rates of turtle bycatch in deep-set longline fishing are an order of magnitude lower than on shallow-set gear (Gilman et al., 2006; Beverly et al., 2009). Elimination of shallow hooks as a means to reduce sea turtle bycatch has been proposed (Polovina et al., 2003; Beverly and Robinson, 2004) and tested. Initial experiments in a deep-set fishery indicated that the method may not be cost-effective due to the reduced catches of economically important epi-pelagic species, such as wahoo (*Acanthocybium solandri*) and the decreased fishing efficiency (Beverly et al., 2009). However, there are ways to set additional weighted lines and to modify fishing vessel speed that can effectively reduce shallow hooks in a deep-set fishery (see Beverly and Robinson, 2004; Gilman et al., 2006). Additional experimentation with modified fishing gear techniques could aid in the identification of fishing methods that optimize the catch of target species while minimizing bycatch. In addition to the effects of other potential gear changes, such as expanded use of circle hooks, more information is required about potential impacts on other species, including other listed species (e.g., sharks, rays) as well as target species (e.g., tuna, swordfish, secondary retained species). The potential economic loss balanced against conservation gains of eliminating shallow hooks is fishery-specific and must be evaluated as such (Beverly et al., 2009; Watson and Bigelow, 2014).

Four Limitations to Analysis

Limitations to Observer Data

The value of observer data is greatly enhanced when efforts are taken to ensure that observations are drawn from a truly representative sample of the fishery at large, both in time and space. In the Atlantic, where observer coverage ranged from 3% to a maximum of 8% of the total fleet effort, the ability to accurately assess the probability of a rare event is limited. This is further hindered by the fact that the 11 statistical reporting areas within the Atlantic are highly heterogeneous, with geographic ranges from the relatively warm waters of the Gulf of Mexico to near frigid waters of the Georges Banks in the North Atlantic. For this and other reasons, annual estimates of both marine mammal and sea turtle bycatch in the U.S. Atlantic pelagic longline fleet are determined specific to each reporting area (Garrison and Stokes, 2014). Additionally, disproportionate sampling in time and space further limits the utility of these data. As a specific example, the potential "hotspot" observed for leatherback turtles captured in the Gulf of Mexico may simply be an artifact of the shift of fisheries observers placed on vessels in this area to ensure adequate coverage after regulations concerning bluefin tuna (*Thunnus thynnus*) quotas. In the near future, we aim to conduct additional analyses specific to individual regions in order to better interpret the bycatch "hotspot" maps given the biases of disproportionate sampling efforts.

Assumptions of Independence of Fishing Sets

Our analyses relies upon an assumption that sets are independent despite the concern that they may not be due to their temporal-spatial similarity to other sets within a single trip (see McCracken, 2004). However, numerous investigations, including Murray (2011) posed that factors affecting estimated bycatch rates were similar between set (haul) and trip and have thus justified its use as the sampling unit.

Changes in Sea Turtle Populations

Our analyses presume that population trends of the bycaught turtles are essentially stable. There are numerous reasons for this decision, including the lack of accurate information regarding the nesting beach origin of the bycaught turtles, which may vary by season of capture; the population trends of each of these nesting populations, as well as the lag time to account for the time between nesting and when they are caught. Therefore, given an inability to calculate population-specific annual trends during this 20+ year period with a high degree of certainty, each model assumes stable trends. A consequence of this limitation is a potential misinterpretation of findings whereby reduced BPUE of turtles post-regulations are erroneously attributed to the effectiveness of mitigation measures when in reality the change is due to a decline in population trends.

An alternative explanation of our findings is that turtle populations are declining, which is certainly the case for leatherback turtles in the Pacific (Tapilatu et al., 2013). The reduced leatherback bycatch rate in the Pacific may reflect population declines. In the Atlantic, however, it has been proposed that adult female (nesting) populations of loggerhead and leatherback turtles are increasing (Ehrhart et al., 2014; Stewart et al., 2016), in which case one might conclude that the fishing restrictions were even more effective than expected. A challenge for future studies will be to incorporate population assessments and life history parameters into ecological models to isolate potential effects of population changes with respect to fisheries effort and sea turtle bycatch rates.

Other Regulatory Changes

Pacific data have unique challenges given the simultaneous nature of regulatory requirements and gear changes in the Hawaii shallow-set fishery that confound data and limit some analysis. The 2004 regulations created an immediate change in use of both bait and hook type, making no allowances for an overlap of different combinations (such as circle hooks with squid bait), rendering it difficult to separate the explanatory effects of bait and hook type. Differences between hook and bait types could only be observed prior to the regulation, yet during this time circle hooks were never used. In this type of scenario, the degree of interrelatedness among hook type, bait type, and year is sufficiently high as to essentially be represented by a single variable. Similarly, location is confounded with SST and thus only location was selected for modeling purposes. Our modeling confirmed these correlations, as regulation, bait, and hook variables all performed similarly as predictors of sea turtle bycatch.

In the Atlantic, the U.S. has multiple regulatory regimes regarding the management of highly migratory fish stocks of tunas, swordfish, billfish, and sharks that may have also influenced fishing effort and observer coverage reported herein. Specifically, year-round closures in the De Soto Canyon of the Gulf of Mexico and the Florida East Coast, as well as seasonal closures in the Charleston Bump and in the Mid-Atlantic (Figure 4.4, NMFS, 2015). Additional regulations involving individual fishing quotas for bluefin tuna and the requirement of weak hooks in the Gulf of Mexico for bluefin tuna bycatch reduction have also been modified during the time period of this analysis (Jan 13, 2011, FR 76, 9). While not intended specifically to protect sea turtles in this region, these closures, as well as changes in gear regulations, may also have affected rates of sea turtle bycatch in Atlantic longline fisheries.

Ecosystem Level Impacts of Findings

This analysis focused on sea turtle bycatch before and after circle hook requirements in the United States. However, there are many other non-target species, such as seabirds, marine mammals, and sharks bycaught in pelagic longline fisheries. Several studies and symposiums have evaluated the effectiveness of circle hooks across and found that reductions are not necessarily achieved for all non-target bycatch species taxa (Kerstetter and Graves, 2006; Serafy et al., 2012; Huang, 2015). In some cases, circle hooks may increase bycatch of sharks (see Gilman et al., 2016b). There is a clear need for further investigation of cross-taxa bycatch solutions in pelagic longline fisheries. Additional research should include evaluating the use of multiple mitigation techniques to reduce the bycatch of several non-target species. For example, testing deep-setting "hook pods" with circle hooks to reduce both seabird and sea turtle bycatch may benefit both taxa. As managers strive to use an ecosystem based fisheries management approach, cross-taxa bycatch reduction studies will become increasingly important. Studies like this will serve as the building blocks for cross-taxa bycatch reduction strategies.

CONCLUSIONS

This study highlights the key variables influencing the probability of sea turtle capture in pelagic U.S. longline fisheries in the Atlantic and Pacific Oceans, as well as how these risks have changed after new regulations. The various analyses have confirmed that in two U.S.-managed longline fisheries, both nominal bycatch and probabilities of bycatch significantly declined, which we attribute to fisheries regulations that mandated changes to traditional longline fishing gear. For combined Atlantic observer data, mean bycatch rates declined by 40 and 61% for leatherback and loggerhead turtles, respectively, for the post-regulation period. Within the NED area alone, where additional restrictions include a relatively larger circle hook (18/0) plus limitations on use of squid bait, sea turtle bycatch rates declined by 64 and 55% for leatherback and loggerhead turtles, respectively, for the post-regulation period. These reductions represent large reductions in sea turtle bycatch despite earlier predictions of even greater conservation gains (Watson et al., 2005). Sea turtle population benefits were even more pronounced for the Hawaii shallow set fishery, where mean bycatch rates declined by 84 and 95% for leatherback and loggerhead turtles, respectively, for the post-regulation period. We consider the existence of numerous confounding factors, as discussed above, in this assessment of the efficacy of the regulation. However, the consistency in observations with results from relevant controlled and comparative experiments (Watson et al., 2005; Gilman et al., 2006) strongly support the inference that the mandated changes in hook and bait were the dominant factors in reducing loggerhead and leatherback bycatch in U.S. commercial longline fisheries during this 20^{+} year investigation period.

In addition to assessing the conservation value of regulatory measures, our work also highlights the value of maintaining a long term (∼22 year) data set of observed target and non-target species caught in U.S. longline fisheries. This information is critical for fisheries managers both in the development of regulatory measures, as well as monitoring and evaluation of their effectiveness. While this research largely relied on data from human observers, in the future, electronic monitoring of vessels when human observation is limited can further assist in assessment of sea turtle (and likely other non-target species) bycatch issues.

The use of statistical models, such as GAMMs, can assist managers in identifying explanatory variables that influence the probability of rare bycatch events, such as sea turtles in longline fishing gear. Information gleaned from these analyses can be applied to management measures that aim to reduce or minimize sea turtle bycatch in longline fisheries. In this analysis, we identified that extending a prohibition of squid bait, eliminating baited hooks at relatively shallow depths ($<\sim 30$ m) and implementing temporary closures specific to SST boundaries could be used in addition to changes in hook requirements to further extend sea turtle protection measures.

Our analyses leads us to conclude that the regulations implemented significantly reduced sea turtle bycatch in U.S. longline fisheries and were effective in achieving management goals. Similar exercises evaluating additional fisheries management actions would be highly valuable, not only for sea turtles but also for additional protected species, such as relative new requirements aimed to minimize bycatch with false killer whales (*Pseudorca crassidens*). Regulatory actions taken by the U.S. can serve as a model for other countries that deploy and manage pelagic longline fishing fleets and organizations, such as regional fisheries management organizations (RFMOs) that manage pelagic longline fisheries. Other nations and RFMOs should evaluate these measures for adoption to significantly reduce sea turtle bycatch in pelagic longline fisheries across the globe.

AUTHOR CONTRIBUTIONS

YS, AG, and BS collectively identified the research questions and pursued refining the questions throughout a year plus writing process. YS and CB worked together on all the statistical analysis. YS did and nearly all of the writing and consulted individuals throughout the process regarding various contributions. KB was useful as a statistical consultant and also assisted with conceptual aspects of the paper as well. KK was instrumental regarding data collection and access to data. KS assisted with data management as well as quantitative aspects of the analysis. KS also assisted with graphing for tables and figures. DF provided expert opinion and commentary throughout the data acquisition, data analysis, and writing of the manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

APPENDIX

TABLE A1 | GAMM best-fit model selection.

Model Terms (Parametric and Smoothed)	**ADJ. R^2**	**AIC**	**Δ AIC**
ATLANTIC LOGGERHEAD			
MONTH + SST + MAXIMUM HOOK DEPTH + SOAK DURATION + HOOKS BTW FLOATS + LIGHTSTICK RATIO + AREA + BAIT TYPE + HOOK TYPE	0.0603	3, 307.168	–
MONTH + SST + MAXIMUM HOOK DEPTH + HOOKS BTW FLOATS + LIGHTSTICK RATIO + AREA + BAIT TYPE + HOOK TYPE	0.0593	3, 304.347	−2.821
SST + MAXIMUM HOOK DEPTH + HOOKS BTW FLOATS + LIGHTSTICK RATIO + AREA + BAIT TYPE + HOOK TYPE	**0.0592**	**3,300.428**	−6.74
ATLANTIC LEATHERBACK			
MONTH + SST + MAXIMUM HOOK DEPTH + SOAK DURATION + HOOKS BTW FLOATS + LIGHTSTICK RATIO + AREA + BAIT TYPE + HOOK TYPE	0.0211	−955.7212	–
MONTH + SST + HOOKS BTW FLOATS + SOAK DURATION + LIGHTSTICK RATIO + AREA + BAIT TYPE + HOOK TYPE	0.0198	−1258.2395	−302.5183
MONTH + SST + HOOKS BTW FLOATS + LIGHTSTICK RATIO + AREA + BAIT TYPE + HOOK TYPE	0.0197	−1270.2896	−314.5684
MONTH + SST + HOOKS BTW FLOATS + AREA + BAIT TYPE + HOOK TYPE	**0.0197**	**−1,278.0982**	−322.377
PACIFIC LOGGERHEAD			
LAT,LON + MONTH + SST + SOAK DURATION + HOOKS BTW FLOATS + LIGHTSTICK RATIO + BAIT TYPE + HOOK TYPE	0.0712	1, 569.829	–
LAT,LON + MONTH + SST + SOAK DURATION + HOOKS BTW FLOATS + BAIT TYPE + HOOK TYPE	0.0712	1, 568.971	−0.858
LAT,LON + MONTH + SST + SOAK DURATION + BAIT TYPE + HOOK TYPE	0.0692	1, 567.155	−2.674
LAT,LON + MONTH + SST + BAIT TYPE + HOOK TYPE	0.0615	1, 550.83	−18.999
LAT,LON + MONTH + BAIT TYPE + HOOK TYPE	**0.0615**	**1,541.656**	**−28.173**
PACIFIC LEATHERBACK			
LAT,LON + MONTH + SST + SOAK DURATION + HOOKS BTW FLOATS + LIGHTSTICK RATIO + BAIT TYPE + HOOK TYPE	0.01	1, 052.135	–
MONTH + SST + SOAK DURATION + HOOKS BTW FLOATS + LIGHTSTICK RATIO + BAIT TYPE + HOOK TYPE	0.00888	1, 049.232	−2.90
MONTH + SST + SOAK DURATION + LIGHTSTICK RATIO + BAIT TYPE + HOOK TYPE	0.00855	1, 048.598	−3.54
MONTH + SST + SOAK DURATION + BAIT TYPE + HOOK TYPE	0.0087	1, 048.598	−3.54
MONTH + SST + BAIT TYPE + HOOK TYPE	0.00827	1, 044.384	−7.75
MONTH + SST + HOOK TYPE	0.0081	1, 040.879	−11.26
MONTH + HOOK TYPE	**0.0081**	**1,036.879**	**−15.26**

GAMMs model catch probability of loggerhead and leatherback turtles on USA pelagic longline fisheries in the Atlantic and Pacific Oceans as a function of gear characteristics, sea surface temperature, and spatio-temporal terms [month, area, latitude (LAT), and longitude (LON)]. Best-fit model indicated by bold text. AIC, Akaike information criteria; DF, Degrees of freedom; ΔAIC, Difference in AIC relative to full model.

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The Role of Taboos in the Protection and Recovery of Sea Turtles

*LoriKim Alexander[1], Andrews Agyekumhene[2] and Phil Allman[1]**

[1] *Department of Biological Sciences, Florida Gulf Coast University, Fort Myers, FL, United States,* [2] *Wildlife Division, Forestry Commission, Winneba, Ghana*

Despite increased efforts from government agencies, scientists, and non-government organizations over the past few decades, anthropogenic sources of sea turtle mortality continue to threaten the survivorship of sea turtle species around the globe. More recent efforts to engage local people with community-based sea turtle conservation programs have been based primarily on economic incentives and less on cultural and social traditions. But there is growing evidence that informal institutions such as, taboos can be extremely effective at promoting wildlife conservation. Ghana is a culturally diverse country where local traditions have shown to improve protection for primates, crocodiles, and many bird species. This study explores the presence of a sea turtle taboo in fishing communities to demonstrate that traditional practices make residents more receptive to sea turtle conservation and more willing to follow government regulations. Fishers in the communities that are aware of the taboo are also more willing to adjust fishing methods to better protect sea turtles. The traditional taboo and national laws appear to be working synergistically to enhance sea turtle conservation in some regions of Ghana. This paper extends the argument that sea turtle conservation strategies succeed when the cultural and social traditions of local communities are integrated with management activities.

Keywords: sea turtles, social taboos, conservation, biodiversity, Ghana, Africa

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United States
Manjula Tiwari,
Southwest Fisheries Science Center
(NOAA), United States

****Correspondence:***
Phil Allman
pallman@fgcu.edu

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INTRODUCTION

Sea turtle conservation efforts around the world have historically incorporated the interests and views of local people. Such community-based conservation (CBC) programs take on a variety of forms, from indirect engagement to the implementation of community-driven conservation goals that prioritize sustainable resource use (Western, 1994). Despite the increasing popularity of CBC approaches in conservation biology, success in achieving recovery outcomes has been mixed (Western and Wright, 1994; Kellert et al., 2000). This is because the effectiveness of CBC is influenced by the program's ability to integrate cultural components, and adjust action plans according to shared values of the community (McShane and Wells, 2004; Peterson et al., 2010).

Taboos and cultural laws are social institutions that govern behavior within communities. The term "taboo" is derived from the Polynesian term "tabu," which means "forbidden." Taboos regulate the way people interact with the world around them by prohibiting the use of items considered sacred. All human societies ascribe to some form of social taboo that is maintained as an informal institution by the cultural standards of its members (Colding and Folke, 2001; Negi, 2010). Unlike judicial law and other types of formal institutions, taboos represent unwritten social rules that regulate behavior in humans that are bound together by common obligations to each person and a shared reverence for the sacred (Freud, 1913). Informal institutions such as, taboos are based on cultural norms that do not require government intervention for proliferation or enforcement (Posner and Rasmusen, 1999).

The role social institutions have in conservation biology has recently attracted more global attention (Colding and Folke, 2001; Riley, 2010), with a growing amount of literature demonstrating taboos improve conservation efforts and resource management (e.g., Uyeda et al., 2014). Tengo et al. (2007) found that adherence to taboos against desecration of sacred forests in Madagascar was influential in maintaining the ecological value of the area. In Indonesia, a taboo held by the To Lindu people affords protection to Tonkean macaques, *Macaca tonkeana*, despite damage to local crops caused by the monkeys (Riley, 2010).

Before the introduction of Western conservation methods to Africa, many communities already had established resource management systems based on complex religious and cultural belief systems that incorporate myths, taboos, totems, and social norms (Ostrom, 1990; Hulme and Murphree, 1999; Roe et al., 2009). These systems originated to protect and promote communal wellbeing, rather than individual interest or that of preserving wildlife (Kideghesho, 2008). In Nigeria for instance, each of the communities surrounding Cross River National Park maintains a sacred forest that protects many species of wildlife, but as Jimoh et al. (2012) reports, the forests are protected for spiritual reasons not directly associated with conservation interests. A sacred grove between Boabeng and Fiema in central Ghana provides a sanctuary for Campbell's guenon, *Cercopithecus campbelli*, and ursine colobus, *Collubus vellerosus* (Wong and Sicotte, 2006; Arhin, 2008). These species are protected from hunting through an oral tradition that describes the monkeys as children of the gods Daworo and Abodwo. Although declining across their range, the increasing population of *C. vellerosus* in this forest is attributed to the taboo that protects them from hunters (Saj et al., 2006). As observed on a global scale, informal institutions in Africa can thus assist conservation efforts in cases where the cultural norms and community behaviors align with the management goals, even in situations when the norms originated for other reasons.

In Ghana, indigenous belief systems have played significant roles in the management of natural resources (Abayie-Boaten, 1998). Ntiamoa-Baidu (1991) identified taboos and totems as the key indigenous methods for conserving biodiversity in Ghana. The West African crocodile, *Crocodylus suchus*, is protected and held sacred by the Tongo-Tengzuk communities of northern Ghana (Arhin, 2008). The crocodiles are protected through a belief that the crocodiles are the incarnation of their important ancestors. This species is therefore abundant in ponds surrounding these communities (Shirley et al., 2009). The Sankana community in northern Ghana serves as a sanctuary for pythons and frogs because community members consider it taboo to consume them (Diawuo and Issifu, 2015). According to oral tradition, the community had to cross a river to escape frequent attacks by slave raiders. A log in the river was used to help cross, but then turned into a python after everyone was safely across. Frogs are also protected because the community believes a frog once led their ancestors to a water source. Moreover, many communities throughout Ghana have sacred groves that serve to protect the area's animal and plant biodiversity (Campbell, 2005).

Informal institutions have historically afforded protection to sea turtles in many cultures around the planet. Pacific islanders throughout Polynesia revere sea turtles as sacred and, therefore, forbid the eating or capture of some species to certain clans or casts (Johannes, 1978; Morgan, 2007), and special rites-of-passage ceremonies (Woodrum, 2010). The fishing community of Buzios Island, Brazil prohibits the consumption of turtle meat as a means of protecting sea turtles. The community restricts captures to only what is necessary for traditional medicine (Begossi, 1992).

Sea turtles are a group of highly migratory species that live primarily in tropical and temperate oceans. They serve keystone functions that collectively maintain healthy and diverse seagrass bed, coral reef, and beach dune habitats (Meylan, 1988; Bouchard and Bjorndal, 2000; Leon and Bjorndal, 2002; Bjorndal and Jackson, 2003). Sea turtles also help maintain a balanced trophic structure that benefits commercially valuable fish species (Houghton et al., 2006; Lynam et al., 2006). Five of the world's seven sea turtle species occur along the western coast of Africa, and at least four species currently nest in Ghana (Allman and Armah, 2008; Allman et al., 2015). The five species that occur in Ghana are the leatherback (*Dermochelys coriacea*), olive ridley (*Lepidochelys olivacea*), green (*Chelonia mydas*), loggerhead (*Caretta caretta*), and hawksbill (*Eretmochelys imbricate;* Allman and Armah, 2008). Each of these species has suffered significant population declines and is currently listed on the IUCN Red List of species threatened with extinction (www.iucnredlist.org). Ghana's Wildlife Conservation Regulation Bill (I.1 685) of 1,971 prohibits the capture or slaughter of sea turtles. Although enforcement is limited, those prosecuted serve time in jail and must pay a fine. Anthropogenic sources of mortality in Ghana include direct harvest of eggs and nesting females, accidental capture in near-shore and offshore fishing gear, and plastic pollution (Agyekumhene et al., 2010).

Ghana's coastal communities consider ocean and marine species to be a significant part of their identity and culture and, as such, there are social norms that influence their daily activities. Many fishing communities throughout Ghana recognize Tuesday to be the day of the sea-God and therefore do not fish (Armah et al., 1998). Furthermore, some community members tell a story that involves a fishing boat becoming lost at sea, but being saved by a sea turtle that directs the boat to shore. Other communities explain how sea turtles saved their ancestors from violent storms or slave ships. One variation explains how sea turtles helped the Dangme people of southeastern Ghana cross the Volta Estuary while retreating from an attacking Ashante army. This taboo originated in southeastern Ghana and has likely decreased the direct harvest of sea turtles since the community elders punish offenders (Armah et al., 1998).

The spiritual world in Ghana serves a significant function in wildlife conservation, and has likely influenced behaviors in Ghana's fishing communities. Fishers in Cape Coast, Ghana accepted a recent government fishing closure of the Fosu Lagoon because traditional practices already reduced fishing efforts in the lagoon. Through a set of taboos that give reverence to Nana Fosu, the God of the lagoon, the fishers understood the need for mangrove restoration to protect the nursery for fish that belong to Nana Fosu (Darkwa and Smardon, 2010).

This study documents a social taboo that serves to support official regulations that protect sea turtles in the water and on the nesting beaches of Ghana. We report interview data from three ethnic groups to explore local knowledge of sea turtles, knowledge of taboos and federal regulations, and their attitudes toward protecting sea turtles.

METHODS

Study Sites

Ghana is located in the western portion of the Gulf of Guinea and contains 535 km of coastline (**Figure 1**). Three ethnic groups populate the coastal region: Nzima in the west, Fante in the central region, and Dangme in the east (Gocking, 2005). For this study, we selected four fishing communities to investigate based on the following criteria: (1) proximity to a known sea turtle nesting beach, (2) minimum of one community within each ethnic group, (3) the presence of an artisanal fishery that utilizes purse seine nets, and (4) varied amounts of Ghana Wildlife Division (GWD) sea turtle conservation efforts in the area.

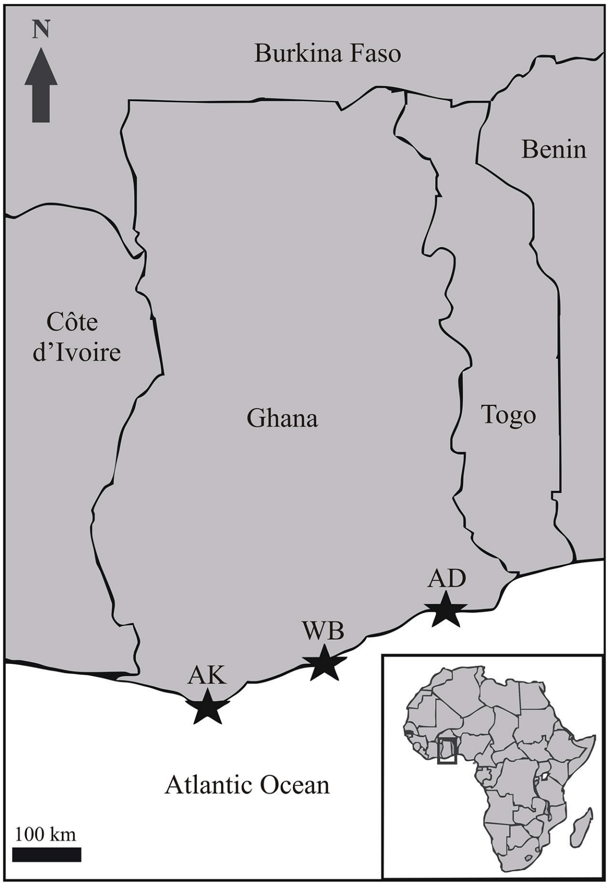

FIGURE 1 | Surveys were conducted in Akwidaa, Winneba, and two villages (Azizenya and Totope) on the Ada peninsula in Ghana, West Africa. AK, Akwidaa; WB, Winneba; AD, Ada.

Community 1

Akwidaa is situated at 4°45′0N and 2°1′0W in the southernmost district of Ahanta West in the Western region. The village is comprised of Nzima people from the original Akan ethnic group that migrated across the Sahara Desert in the eleventh century (Gocking, 2005). Eleven interviews were conducted on the beachfront of Akwidaa Old Town. A non-profit organization briefly conducted sea turtle surveys in this area during the 1990's, but the GWD has not been present. The foreign owner of an eco-lodge near Akwidaa recently attempted to initiate a sea turtle conservation volunteer program, but this has not succeeded thus far.

Community 2

Winneba is located within the Central region at 5°19′60N and 0°37′0W, and is the capital of the Effutu Municipal district. The Fante people of Winneba are also a subgroup of the Akan ethnic group that formed their own state in the fourteenth century (Gocking, 2005). The GWD has a field office here for the primary function of managing the Muni-Pomadze Ramsar site, a 9,461.12 hectares estuarine coastal wetland and lagoon adjacent to Winneba. Since 2011, the local GWD officers have conducted routine sea turtle education, ecotourism, and law enforcement activities in the area. Ten interviews were conducted on the beach in the primary fishing district of Winneba.

Community 3 and 4

Azizenya, at 5°46′0N and 0°39′0E, and Totope, at 5°46′60N and 0°31′60E, are found on the Ada peninsula within the Dangme East district of the Greater Accra region. Azizenya is roughly 8 km east of Totope and 1 km east of the larger township of Ada Foah. The GWD has maintained an office in Ada Foah to protect the Songor Lagoon Ramsar site since 1988. The GWD officers routinely conduct sea turtle education, ecotourism, and law enforcement activities in the area. The Dangme people of this region are known to revere the sea turtle as sacred and consider it taboo to harm or eat them (Armah et al., 1998). Ten interviews were conducted in each village, but these data are presented together due to the proximity and similar characteristics of the two villages.

Data Collection

We conducted interviews in each village between December 2010 and January 2011, with all interviews in a particular community being conducted on the same day. The semi-structured interview design (Creswell, 2013) included questions related to fisher demographics, fishery design, knowledge of sea turtles, and sea turtle conservation. We followed a set order of questions that included open-ended questions and others that allowed participants to choose from a set of possible answers. Open-ended questions gave participants an opportunity to elaborate with examples from their own experiences. We presented photographs of six sea turtle species, a bottlenose dolphin (*Tursiops truncates*), a humpback whale (*Megaptera* *novaengliae*), and the North American gopher tortoise (*Gopherus polyphemus*) to learn which animals have been captured by their nets. Each interview took approximately 30 min to complete. Each interview was conducted with a Ghanaian translator and recorded with a digital voice recorder or video camera.

Fishers were selected for the study using non-probability convenience sampling and snowball sampling. We selected only fishers for this study because they have more direct interactions with sea turtles and are considered a primary stakeholder when promoting sea turtle conservation in the region. When possible, interviews were conducted on Tuesdays when fishers are more available since the taboo forbids fishing on this day. Volunteers and community members fluent in English and Ga, Twi, or Dangme conducted the surveys using local languages. Two individuals then translated each answer to English. Interviews were conducted simultaneously within each village to ensure each respondent fished on a different boat, and therefore acquiring independent samples. Although sample size per village is small, each fishing village utilizes between 12 and 20 boats so we are confident that our sample reflects the activities of the artisanal fishery in each village.

Each respondent was assured of their anonymity in participating in the project, and was asked to sign a consent form to verify their willingness to contribute. In exchange for participation, respondents were given a flag with an original sea turtle logo to fly on their boats. This was presented at the end of the interview so they were not aware of any forthcoming compensation. All activities followed procedures approved by Florida Gulf Coast University's Internal Review Board (IRB#2010-59), and all research participants provided written and informed consent.

track2ptSix key informants from Ada Foah were also interviewed using open-ended questions that further explored the cultural traditions and beliefs surrounding sea turtles in the region. The key informants are community leaders within the traditional authority of the area. Key informants were not given an individual compensation, but school supplies, footballs, and clothes were donated to the chief of Ada and two nearby communities.

Data Analysis

All interviews were translated to English and coded per question. Descriptive statistics are presented to identify trends within, and among, fishing communities and to provide accounts of discrepancies across the research topics. Such approaches have proven useful for identifying trends using similar data structure (Silverman, 2006; Schneller, 2008; Senko et al., 2011; Creswell, 2013).

RESULTS

Ghana's artisanal fishery primarily operates through the use of purse seine nets that are deployed from fishing boats in near-shore waters immediately adjacent to the crew's home fishing village. The crew is comprised of men who are often from the same family, or members of the community who are hired by the boat's owner. The catch is divided among the fishermen in proportions that are negotiated before each fishing trip. Most of the captured fish are sold to women in the community who will then sell the fish in local markets and restaurants. The fishers keep the remaining fish for consumption with their immediate family members.

Fishing is the primary occupation for 92% of all respondents, with only four individuals indicating they have alternative employment when not fishing (**Table 1**). Fishing experience ranged from <1 year to 60 years across all sites. Nearly 93% had 10 or more years of fishing experience and 68% had more than 20 years of experience. Half of the fishers (Akwidaa = 46%; Winneba = 60%; Ada = 47%) own the vessel on which they work, others serve as a member of the crew. All respondents in Winneba and Ada have family members who fish, yet in Akwidaa those with fishers in the family are a minority (9%).

The Fishery

track2ptA large majority of fishers (84% across sites) believe their catch has decreased over the years because of a reduction in fish populations across the region. When asked, many reasons were given for the decline, but the presence of too many boats and the illegal use of lights when night fishing were considered the primary causes (**Figure 2**). Interestingly, 18% (6/34) of fishers attributed the decline to non-anthropogenic causes such as, natural fluctuations, God, and weather conditions. Furthermore 50% of all fishers (Akwidaa = 100%, Winneba = 44%, Ada = 27%) accused sea turtles as a reason for the reduction in fish catch (**Table 3**).

Most individuals (85%) acknowledged they catch sea turtles when fishing, but most were quick to add that it was not intentional (**Table 2**). We could not determine weekly by-catch rates as several fishers noted that catching a sea turtle was rare, with an occurrence rate of 1–3 months, or even 2 years. Fifteen percent across all sites reported catching sea turtles on every fishing trip, but personal experience (Agyekumhene and Allman) indicates it is very unlikely any artisanal fishing vessel would

TABLE 1 | Demography of fishers selected for participation in the study.

Location	Ethnic group	Number surveyed	Fishing is primary occupation	Years of fishing experience	Boat owner	Family members also fish
Akwidaa	Nzima	11	10/11 (91%)	23.5 ± 5.0 (6–50)	5/11 (46%)	1/11 (9%)
Winneba	Fante	10	10/10 (100%)	19 ± 3.2 (0–38)	6/10 (60%)	10/10 (100%)
Ada	Dangme	20	16/19 (84%)	30.5 ± 3.0 (11–0)	9/19 (47%)	19/19 (100%)
Overall		41	36/40 (90%)	30.5 (0–60)	20/40 (50%)	30/40 (75%)

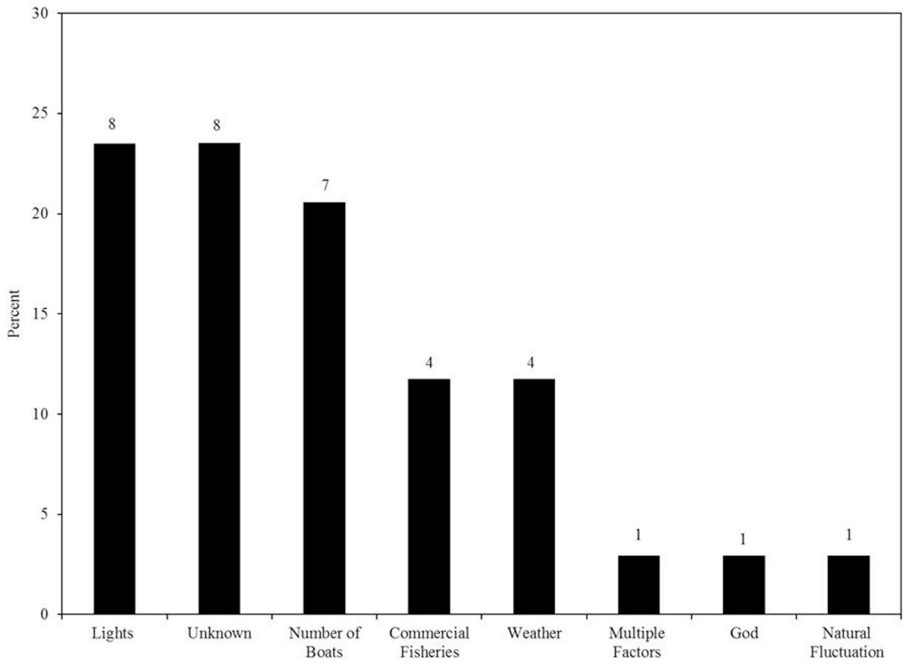

FIGURE 2 | The frequency of responses given by fishers to explain why they have observed a decrease in fish catch over recent years. Data are presented across all sites included in the study.

TABLE 2 | Responses from fishers when asked questions regarding their experiences with sea turtle captures in the artisanal fisheries of three communities in Ghana.

Location	Catch sea turtles	Release captured sea turtles	Captured sea turtles are eaten	Captured sea turtles are sold	Captured sea turtles are eaten and sold	Sea turtles captured all year	Sea turtles reduce fish catch
Akwidaa	9/11 (82%)	7/9 (78%)	2/6 (33%)	2/9 (22%)	1/6 (17%)	1/6 (17%)	8/8 (100%)
Winneba	8/10 (80%)	6/9 (67%)	2/8 (25%)	3/9 (33%)	4/8 (50%)	0/9 (0%)	4/9 (44%)
Ada	18/20 (90%)	15/16 (94%)	1/13 (8%)	0/13 (0%)	0/13 (0%)	1/16 (6%)	4/15 (27%)
Overall	35/41 (85%)	28/34 (82%)	5/27 (19%)	5/31 (16%)	5/27 (19%)	2/31 (7%)	16/32 (50%)

TABLE 3 | Frequency of fishers that reported capturing different species of sea turtles in the artisanal fishery.

Location	Leatherback			Olive Ridley			Green			Loggerhead			Hawksbill		
	DK	No	Yes	DK	No	Yes	DK	No	Yes	DK	No	Yes	DK	No	Yes
Akwidaa	0.00	0.25	0.75	0.00	0.33	0.67	0.00	0.25	0.75	0.00	0.33	0.67	0.00	0.33	0.66
Winneba	0.00	0.37	0.63	0.11	0.11	0.78	0.00	0.22	0.78	0.00	0.22	0.78	0.10	0.45	0.45
Ada	0.00	0.25	0.75	0.00	0.25	0.75	0.00	0.16	0.84	0.11	0.33	0.56	0.12	0.24	0.64

DK, Do Not Know.

catch a turtle on each fishing trip. Interestingly, 29 fishers (93%) indicated sea turtles are not captured year-round, but only during the dry or "Christmas" season.

Only one individual in Ada indicated a captured sea turtle is either eaten or sold (or both), as opposed to 12 fishers that indicated they are not eaten or sold. Fifteen respondents (94%) from Ada indicated they release live sea turtles when captured. Comparing sites, a lower portion of fishers in Akwidaa (78%) and Winneba (67%) return captured turtles to the sea, and a higher percentage eat or sell them (**Table 2**).

Sea Turtle Knowledge, Conservation and Taboo

The fishers were able to identify sea turtles, but they roughly classify leatherbacks as a "large turtle" and all other species as "small turtles". When shown photographs of each species, fishers could identify different species they have encountered. Most fishers reported catching leatherbacks, olive ridleys, and green turtles, with incidence rates of 75–80% across sites (**Table 3**). Over 50% indicated they have captured loggerheads and hawksbills, although these two species do not routinely nest in Ghana. Four individuals indicated they have captured gopher tortoises in their nets (**Table 4**). Ghana's coastal zone is home to three species of hingeback tortoises (*Kinixys* sp.), so it is possible the fishers have encountered a tortoise near the beach. Sixty-five percent of respondents indicate they have captured a dolphin, whereas 55% reported catching a whale (**Table 4**). Dolphins are present in Ghana but rarely seen, and humpback whales migrate through Ghana from September through November each year.

Across all sites, 33 of 34 respondents (97%) asserted that their communities protect sea turtles, but not all relate it to the existence of a taboo (**Table 5**). This protection is manifested by helping hatchlings into the sea, sometimes releasing turtles captured in fishing nets, or alerting authorities about poaching activity. None of the fishers in Akwidda acknowledged a taboo, story, or any other traditional custom that would afford protection to sea turtles, but they were quick to say their community is now protecting turtles because the eco-lodge owner instructed them to. Almost 50% (44%) of the respondents in Winneba are aware of a taboo regarding sea turtles, and therefore, does not harm them. In Ada, 88% of the respondents affirm a history of a taboo against harming sea turtles due to a cultural story that has been passed from generation to generation (**Table 5**). Key informants confirm the existence of an oral tradition and taboo against harming or eating sea turtles in the Ada area, but indicated that the influx of other clans and groups into the area results in more people not knowing the tradition or upholding the taboo. The key informants held that the traditional belief is verbally passed from elders to the younger generation. Further, many stated that the existence of this tradition has led to better receptivity of conservation efforts, namely the federal law.

The majority (86%) of fishers in each village are aware that laws protect sea turtles from harm. Knowledge of the law is highest in Ada (95%) and lowest in Akwidaa (71%; **Table 5**). Thirty percent of the fishers know the penalty for breaking the law is arrest and 24% know that it is the GWD that enforces the law. Only 33% of respondents in Akwidaa stated the law influences their fishing methods. Most fishers in Winneba (60%) and Ada (67%) have made adjustments to their fishing methods because of the legal protection that served to validate the taboo that was already established in these communities.

TABLE 4 | Frequency of fishers that reported capturing a tortoise and marine mammals in the artisanal fishery.

Location	Gopher tortoise			Humpback whale			Bottlenose dolphin		
	DK	No	Yes	DK	No	Yes	DK	No	Yes
Akwidaa	0.00	0.87	0.13	0.00	0.38	0.62	0.00	0.50	0.50
Winneba	0.00	0.87	0.13	0.00	0.67	0.33	0.11	0.22	0.67
Ada	0.00	0.79	0.21	0.00	0.63	0.37	0.00	0.26	0.74

DK, Do Not Know.

TABLE 5 | Frequency of respondents affirming community protection, a taboo, and knowledge of laws that protect sea turtles from harm in Ghana.

Location	Community protect sea turtles	Cultural story regarding sea turtles	Knowledge of law protecting sea turtles	Law influence fishing
Akwidaa	7/7 (100%)	0/8 (0%)	5/7 (71%)	1/3 (33%)
Winneba	7/8 (88%)	4/9 (44%)	7/9 (78%)	3/5 (60%)
Ada	19/19 (100%)	15/17 (88%)	18/19 (95%)	8/12 (67%)
Overall	33/34 (97%)	19/34 (56%)	30/35 (86%)	12/20 (60%)

DISCUSSION

The aim of this study was to explore the presence of a taboo protecting sea turtles, knowledge of federal laws, and willingness to protect sea turtles in Ghana. The interviews conducted among three ethnic groups demonstrate fishers routinely interact with sea turtles and are generally able to recognize different species. In communities where a taboo exists, and the GWD has a presence (Winneba and Ada), fishers are more knowledgeable and receptive to formal regulations that protect sea turtles. Although not directly tested, the protection of sea turtles in Winneba and Ada are likely driven by the taboo, instead of GWD presence, because sea turtle education programs and legal enforcement had not been conducted in Winneba prior to this study. More fishers in these two communities are willing to modify their fishing methods to reduce sea turtle by-catch and mortality. However, individuals in Winneba and Ada express concern about the erosion of cultural heritage due to modernization and immigration of individuals from ethnic groups that do not share the same cultural norms.

Fishing communities in Winneba and Ada observe the taboo protecting turtles, but it is noticeably absent in Akwidaa. The taboo appears to be strongest with the Dangme people of Ada where the story speaks of turtle saving their ancestors during a war with the Ashante. Fishers in Winneba understand the taboo as a cultural norm recognizing sea turtles for saving their fisher ancestors who were lost at sea. Winneba's location in central Ghana makes the town much more ethnically diverse than Ada, with individuals from clans that do not recognize the sea turtle taboo, but also from clans that do (Gocking, 2005). The Nzima fishers of Akwidaa are not aware of a taboo protecting turtles, and therefore are more likely to consume them when captured in nets or observed on the nesting beach.

All communities are aware of the laws protecting sea turtles regardless of whether the taboo is prevalent. Further, each community appears to protect sea turtles, or are at least willing to accept sea turtle conservation efforts. Ada had the highest percentage of respondents who were aware of the legal protection of sea turtles, and the most fishers in the area who adjust their fishing methods in response. Fishers in Ada and Winneba reported that turtles are still sometimes eaten and sold due to lost resources from the damaged net caused by incidentally catching a sea turtle. Therefore, recognition of the taboo does not necessarily result in adherence. Fishers in Ada and Winneba exhibit respect for sea turtles and the taboo, but admit to selling turtle meet to recover the cost of repairing their damaged nets. Some fishers do not consider this an infringement of the taboo since the turtles are captured incidentally.

The overwhelming support for sea turtle conservation in Ada is likely a result of the cultural restriction influencing the fishers to better support the more recent legal protection that is now being enforced by the GWD. Furthermore, the GWD has routinely conducted sea turtle education programs in the Ada region for 20 years before this study. The strong support for sea turtle conservation in Winneba is likely a result of the diverse ethnic groups present in the community, and the constant rotation of fishers among the boats. The GWD office in Winneba only recently began sea turtle education and law enforcement programs. However, the community already had supported sea turtle conservation and had some awareness for the taboo. In contrast, fishers in Akwidaa are not regulated by a taboo or influenced by the GWD and therefore are less willing to adjust their fishing methods in ways that would decrease sea turtle captures. The community affirms an effort to protect sea turtles but this appears to be driven by directives from a British-owned eco-lodge adjacent to the community. The lodge owner requested the community to stop harvesting sea turtles on the beaches visited by the lodge's guests. In the absence of GWD officers in the area, the fishers' interest in sea turtle conservation was limited to requests made by the eco-lodge owners.

Ghana's sea turtle taboo has likely reduced mortality in some areas as sea turtle by-catch was reported by most respondents, but a significant number across all sites indicated that captured turtles are released from the net. The fishers advised that a catch is typically unintentional and not desirable. A captured sea turtle will significantly damage a net by tearing severe holes that are too expensive to repair. Such holes allow some fish to escape and may explain why many fishers remarked that sea turtles are responsible for the observed decrease in fish capture. Our survey questions did not allow us to adequately explore the rate of sea turtle capture, but most fishers indicated that catching a sea turtle is not common. Although a few fishers reported sea turtles are captured year-round, most reported that turtles are captured only during the dry season from November through May. This time period coincides with the primary sea turtle nesting season in Ghana, which suggests fishers may be capturing gravid females that migrate to Ghana for nesting purposes. If fishers are in fact capturing a disproportionally large number of gravid females, then even a small catch rate may have a significant impact to the region's sea turtle populations.

Long term sea turtle recovery will require efforts of the global community to decrease mortality and increase protection of habitats. International laws exist protecting sea turtles worldwide and CBC is now commonplace as a conservation strategy. Co-management is also another increasingly viable option (Campbell et al., 2009). However, keen attention must be paid to the ways in which communities are engaged and efforts must be made to deepen the understanding of officiators with regard to the needs and morays of stakeholders. Access to sea turtles is often regulated by social customs which help define their place in the community (Woodrum, 2010). As such, some CBC efforts around sea turtles and coastal habitat have aimed to include communities by acknowledging the traditions that link them and the turtles (Campbell, 2003). In recent years, traditional knowledge of sea turtles has been one such way to involve community members (Campbell, 2007). But often this is driven by economic incentives while community members' true needs and value are overlooked (Campbell et al., 2007).

Indigenous belief systems commonly incorporate taboos and totems that create environmental ethic behaviors by regulating an individual's interactions with the natural world (Shastri et al., 2002). In this study, we see how informal and judicial law, the sacred and the secular, may work together to aid the protection of wildlife. This relationship is seen throughout Africa, and Ghana in particular, by which traditional sacred sites already delineated by spiritual law afford already judicially protected species within them refuge (Wong and Sicotte, 2006; Diawuo and Issifu, 2015). Indeed, governments often exploit long-established sacred delineations to create parks and reserves since the areas are often the few patches of habitat left intact and are therefore identifiable as in the greatest need for judicial aid (Msuya and Kideghesho, 2009; Jimoh et al., 2012). However, these laws may not afford adequate enough protection, and enforcement may not be without significant complications. For example, the taboos protecting primates do not necessarily afford protection to the animals' habitat or animals beyond the habitat adjacent to the community (Saj et al., 2006). In Madagascar, food taboos regulate wildlife take locally, but state laws are widely known even as they may not be explicitly adhered to (Golden and Comaroff, 2015a). In a separate study, Golden and Comaroff (2015b) point out adherence to taboos is not uniform within cultural groups or even within communities in Madagascar specifically because these taboos are regulators of a very personal narrative. Resource use, therefore, must still be governed by judicial and informal law for conservation to be effective.

The existence of social taboos can ease cultural involvement to make laws more adaptable and enforcement more amenable to community members. Species-specific taboos are generally self-enforcing since penalties of sickness and even death are typically connected to the belief system (Colding and Folke, 1997; Diawuo and Issifu, 2015). In essence, the community has already been policing themselves therefore the judicial law can act as an official acknowledgment of these social restrictions. In Madagascar, Westerman and Gardner (2013) found that local communities around a marine reserve were more compliant with the restriction of use of the area after ceremonies venerating local ancestors were performed to consecrate the reserve. Several taboos which have their origin in an ancestral memory are already in place regarding use of the resources in the area. In Ghana, fishers in Ada and Winneba stated they are likely to release live turtles captured in nets which is in keeping with the judicial law and cultural tradition.

Judicial laws may validate informal institutions but modernization, colonialism, and immigration has been shown to aid in erosion of the social taboos that enable the laws. In terms of conservation, official regulation can aid in filling the cultural gap left by the loss of taboos that would otherwise protect animals and habitat. Key informants advised that Azizenya and Totope are changing in terms of the influx of new community members from other ethnic groups and they believe that this is diluting adherence to the taboo. Historically, colonization and Christianity have also caused cultural degradation in terms of retention of traditional belief structures across the African continent due to the vilification of these beliefs. Taboos against hunting gorillas afford protection to the Cross River gorilla, *Gorilla gorilladiehli*, in Cameroon, but Etiendem et al. (2011) warned this traditional protection is eroding and may disappear. Likewise, Anoliefo et al. (2003) found that urbanization and the adoption of Christianity had adverse effects on the adherence to taboos that protected sacred groves in certain communities in Nigeria. Also in Nigeria, Jimoh et al. (2012) found several social taboos that ultimately lend themselves to biodiversity conservation are under attack from the effects of colonization. Future studies on the sea turtle taboo in Ghana should include an analysis of adherence to it and any pressures that may drive its weakening. Further study of the continuity of the taboo, how those with current knowledge and adherence pass this on to the next generation, is also necessary. Lastly, a look at the ways taboo and education on the judicial law influence each other is needed to tease apart this relationship and minimize confounding factors.

Our connection to sea turtles is complex and inherently culturally driven. Expanding our understanding of these connections can only aid their conservation. While a good amount of literature exists on sea turtle taboos and social customs, further study of these traditions is necessary in locations like Ghana where community engagement in conservation efforts is needed. Interdisciplinary approaches to research like this study, which include the social sciences, can help assess the feasibility of existing conservation initiatives and set a more stable foundation for the development of new ones.

ETHICS STATEMENT

This study was carried out in accordance with the recommendation of the Institutional Review Board (IRB) at Florida Gulf Coast University, with written informed consent from all subjects (IRB #2010-59)

AUTHOR CONTRIBUTIONS

PA conceptualized the research project and directed 20+ students that conducted the interviews. PA also provided oversight to the implementation of the project and then assisted with data analysis and a significant amount of writing and editing. LA finalized the interview questionnaires and helped conduct the study. LA also assisted with data analysis and wrote most of the manuscript draft. AA assisted with the conceptualization of the project and was able to lead the project in the field by coordinating fishermen and translators. AA wrote small portions of the manuscript but provided important review and editorial adjustments.

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frontiers in Marine Science

METHODS
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Comparing Acoustic Tag Attachments Designed for Mobile Tracking of Hatchling Sea Turtles

Aimee L. Hoover[1], *George L. Shillinger*[2,3,4]*, *Jennifer Swiggs*[4] *and Helen Bailey*[1]

[1] Chesapeake Biological Laboratory, University of Maryland Center for Environmental Science, Solomons, MD, United States, *[2] The Leatherback Trust, Monterey, CA, United States,* *[3] The Lost Years – Pelagic Life History Fund, The Ocean Foundation, Washington, DC, United States,* *[4] Indiana University-Purdue University Fort Wayne, Fort Wayne, IN, United States*

The poorly understood movements of sea turtles during the "lost years" of their early life history have been characterized as a "passive drifter" stage. Biologging technology allows us to study patterns of dispersal, but the small body size of young life stages requires particular consideration that such tagging does not significantly impede animal movements. We tested the effect of instrument attachment methods for mobile acoustic tracking of hatchling sea turtles, including a design that would be suitable for leatherback turtles (*Dermochelys coriacea*). We obtained 8-week-old hatchery-reared green sea turtles (*Chelonia mydas*) ($n = 12$ individuals) and examined the effect of attaching Vemco V5 acoustic tags. Each animal's swim speed, swimming depth, and stroke frequency were determined under three scenarios: control, direct Velcro® attachment to the carapace, and harness attachment, to determine if there was a significant difference amongst treatments. Turtle swimming speed was significantly slower during the middle period of the trial for the harness attachment compared with the control. No significant change in swim speed was observed when the tag was attached directly with Velcro®, and no significant change in dive depth was observed for either treatment compared to the control. Stroke frequency was significantly greater compared to the control at the end of the trial for the Velcro® attachment only, although there was no corresponding increase in swimming speed. This information can be used to design effective approaches for actively tracking free-ranging hatchling sea turtles to understand dispersal and survival of these vulnerable marine species.

Keywords: telemetry, sea turtle, behaviour, movement, migration, tracking, dispersal, "lost years"

Edited by:
Sara M. Maxwell,
Old Dominion University, United States

Reviewed by:
Patricia Briones-Fourzan,
National Autonomous University of Mexico, Mexico
Leslie Cornick,
Eastern Washington University, United States

***Correspondence:**
George L. Shillinger
george_shillinger@yahoo.com

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INTRODUCTION

Effective conservation efforts for marine species are hindered by a lack of knowledge regarding movements and habitat utilization (Bowen and Karl, 2007). Highly migratory marine species, such as sea turtles, pose additional complexities for management as they traverse large distances and cross international boundaries throughout their life history (Hays and Scott, 2013). Young life stages, notoriously difficult to track, can even undergo these long migrations (Bolten, 2003; Hazen et al., 2012; Shillinger G. et al., 2012). The "lost years" of sea turtles are an enigmatic period of unknown distribution and developmental habitat after hatchlings leave natal beaches. This oceanic period of sea turtle life history has been increasingly studied in recent years as sea turtle conservation efforts expand beyond terrestrial zones. These investigations of at-sea movements during early life stages have been made possible by advances in biologging technology (e.g., Mansfield et al., 2014; Scott et al., 2014; Thums et al., 2016). Threatened and endangered species with unknown life history patterns, including highly vulnerable sea turtles (Lascelles et al., 2014), have been a research focus to advance conservation and management strategies (Hamann et al., 2010).

Information about sea turtle dispersal and behaviour during the "lost years" has been gained through modelling approaches (Hays et al., 2010; Shillinger G. L. et al., 2012; Putman et al., 2013; Casale and Mariani, 2014), telemetry (Nagelkerken et al., 2003; Witherington et al., 2012; Mansfield et al., 2014; Scott et al., 2014), and other emerging technologies, such as stable isotopes (Bowen and Karl, 2007; Reich et al., 2007; Snover et al., 2010; López-Castro et al., 2014). Due to a lack of information on active dispersal capacity, modelling efforts rely heavily on classifying young turtles as "passive drifters," with little influence on their environment (Hays et al., 2010; Gaspar et al., 2012; Shillinger G. L. et al., 2012; Putman and Mansfield, 2015). Biophysical models can be strengthened and verified by incorporating behavioural data, such as swim speed and orientation (Putman et al., 2012, 2013; Kobayashi et al., 2014; Briscoe et al., 2016), as both swim behaviour and ocean currents control young sea turtles' directionality and influence dispersal outcomes (Gaspar et al., 2012; Putman and Mansfield, 2015; Briscoe et al., 2016). Behavioural data can be collected by deploying instruments to track turtle movements (Putman et al., 2012; Thums et al., 2013; Mansfield et al., 2014; Scott et al., 2014).

Reduction or elimination of tag effects when examining sea turtle early life stages is of high importance in order to maximize field data integrity and minimize negative impacts on tagged individuals (Jones et al., 2013). Acoustic tags are lighter and smaller than satellite tags, but appropriate methods of attaching these tags to turtle hatchlings are still under development and lacking for many species (Hazen et al., 2012; Shillinger G. et al., 2012). Small turtles experience a higher drag ratio compared to larger, more frequently-tracked adult turtles, resulting in higher transport costs of attachments. Impacts on turtle movements and behaviour are presumed to be negligible when below the colloquial 3% tag-to-body-weight threshold. Hatchling sea turtles tagged with miniature acoustic tags generally meet this requirement (Thums et al., 2013, 2016; Scott et al., 2014), but the influence of tag attachments on animal behaviour should still be carefully considered prior to field studies on threatened and endangered species (Vandenabeele et al., 2012). An examination of movement metrics, such as speed and diving depth, should be undertaken prior to deploying transmitters on wild turtles to ensure that the tracking process is unlikely to decrease fitness or survival, whilst providing biologically representative information (Casper, 2009; Mansfield et al., 2012; Jones et al., 2013). A direct attachment method on the plastron has been developed for flatback (*Natator depressus*), green (*Chelonia mydas*), and loggerhead (*Caretta caretta*) hatchlings (Thums et al., 2013, 2016; Scott et al., 2014). However, we sought a design that would be suitable for leatherback turtles (*Dermochelys coriacea*), which have a unique oily skin, and that would detach easily during recovery to ensure the tags were guaranteed to be recovered and removed from a critically endangered sea turtle population in field studies. The direct attachment method would also make it difficult to maintain visual contact with the small, dark bodies of hatchling turtles during mobile active tracking in the open ocean. The existing direct plastron attachment method utilized in other studies (Thums et al., 2013, 2016; Scott et al., 2014) was therefore not suitable and an alternative attachment design was required.

The objectives of this study were to (1) develop a protocol for attaching miniature acoustic transmitters to hatchling sea turtles suitable for mobile tracking of hatchling leatherbacks in the open ocean and (2) quantify the effects of tag and attachment materials on young sea turtle swim speed and dive behaviour. Analysis of the stroke frequency further provided insight into swimming performance. We tested the assumption that attaching Vemco V5 acoustic tags would not affect post-hatchling turtle swim speed, dive depth, and stroke frequency. We sought an attachment design to allow for multiple means of observation to increase the likelihood of maintaining contact in field studies using mobile tracking and detach easily during recovery from these at-risk species. To evaluate potential effects, this study was undertaken with hatchery-reared post-hatchling stage green sea turtles in Grand Cayman serving as a conservative proxy for leatherbacks. Due to a low drag coefficient, combined with their frontal area and carapace length, young green sea turtles may encounter greater drag costs than other sea turtle species (Jones et al., 2013). Therefore, this study will provide a reasonable conservative baseline understanding of potential effects of tag attachment methods for mobile tracking across sea turtle species.

MATERIALS AND METHODS

Tag Attachment Protocol

We conducted experiments with twelve 8-week-old hatchery-reared green sea turtles (*Chelonia mydas)* at the Cayman Turtle Farm on Grand Cayman in December 2014. The sample size ($n = 12$) reflects the number of post-hatchlings available from the hatchery at the time of the study. The turtles remained out of public view prior to experiments. The mean weight of the turtles was 59.9 g (range = 38.3–74.3 g), and the mean straight carapace length notch-to-tip was 73.5 mm (range = 64.0–78.5 mm). All weights were recorded in-air.

We tested two alternative methods for attaching Vemco V5-180 kHz acoustic transmitters (0.65 g) (Vemco Ltd, Halifax, Canada) to hatchling turtles. For both turtle attachment methods, tags were affixed to a tether in a similar arrangement to Gearheart et al. (2011). Attachments had braided monofilament line (1.75 m) suspending two painted floats (4.4 cm by 1.9 cm) behind the turtles (**Figure 1**). The monofilament line was doubled onto itself to mimic the weight of an anticipated longer fieldwork attachment due to restrictions encountered by the size of the tank. The acoustic tag was suspended 0.25 m from the second float, and the combined weight of the line, floats, and tag was 7.5 g. There were two attachment mechanisms tested in this experiment. The line-float-transmitter assembly

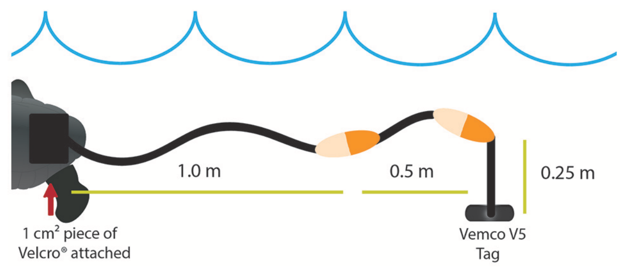

FIGURE 1 | Acoustic transmitter Velcro® attachment method modified from Gearheart et al. (2011). Symbols are courtesy of the Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/symbols/).

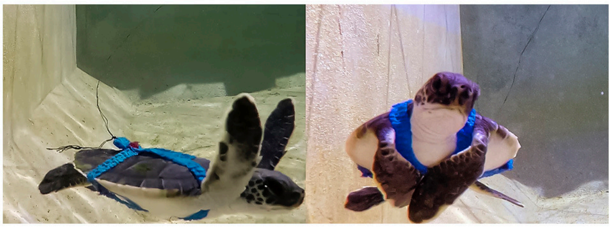

FIGURE 2 | Images of the harness design and application on *Chelonia mydas* hatchlings. Footage from an underwater GoPro camera.

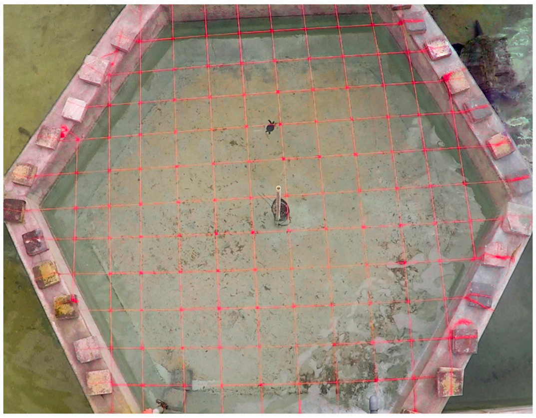

FIGURE 3 | Scale of the tank experiment at the Cayman Turtle Farm. The GoPro camera hoisted 5.1 m above the hexagonal tank filmed each turtle for 25 min per treatment. Each square is 25 cm by 25 cm to serve as a distance reference.

was affixed to the turtles' carapace by one of two methods. For the Velcro® treatment, a 1 cm^2 Velcro® square (1.71 g) was directly bonded to the carapace with several drops of Vetbond™ (Jones et al., 2000; Salmon et al., 2004; Thums et al., 2013; Scott et al., 2014) and linked to a sister piece of Velcro® on the line-float-transmitter assembly. Initial testing of Vetbond™ used for the Velcro® treatment was conducted with naturally deceased hatchlings to ensure the bonding agent would dissolve and separate from hatchlings. The Velcro® attachment could be removed easily with a slight pull within a few days, suggesting the attachment material would be shed easily under natural conditions. For the harness treatment, the line-float-transmitter assembly was linked to a harness (0.47 g) made from 3M™ Coban™, a self-sticking latex/spandex/polyethylene compound. The harness attachment consisted of the same braided monofilament line and float setup, slipped over the head, and wrapped around the widest part of the turtle (**Figure 2**). Trials with the Velcro® treatment, harness treatment, and a control treatment with no attachment were conducted with each turtle in a randomized fashion.

We conducted trials to monitor for behavioural responses of turtles to each treatment in a 12.25 m^2 hexagonal tank filled with seawater to 0.6 m depth with a flow rate of 60 litres per minute (**Figure 3**). A 25 cm by 25 cm grid was placed over the tank to track distance travelled by each turtle for speed calculations. Vertical distance was labelled by a pole with centimetre intervals in the middle of the tank. Every turtle was observed individually for 25 min under each of three treatment scenarios: control, Velcro®, and harness. Turtles were randomly selected for each treatment and given a minimum period of 2 days between treatments over the 2 week study period. Movements were recorded using two GoPro HERO 4 cameras (GoPro, Inc., San Mateo, CA), one placed underwater near a corner of the tank and one hoisted 5.1 m centred overhead.

Our aim was to choose the least intrusive methods of attachment to address potential concerns for animal welfare. There were no evident injuries from the Velcro® or harness attachments. Permission for all procedures was obtained prior to the experiment from the University of Maryland Center for Environmental Science's Institutional Animal Care and Use Committee (Research Protocol No. S-CBL-14-14). The research was conducted under approval of scientific study from the Cayman Islands' Department of Environment.

Horizontal Movement Analysis

Video was compiled with Adobe Creative Premiere Pro CC (Adobe Systems, Inc., San Jose, CA), and turtle movements were analysed using the Tracker Video Analysis and Modeling Tool program, an Open Source Physics Java framework (Brown, 2014). Horizontal swim speed was calculated within the program as a function of movement in the x- and y-directions. Speed was estimated every second and averaged at 10 s intervals for each 25 min trial. This 10 s interval provided a fine-scale measure of the variability in speed without oversampling. Time was then split into 5 min blocks, producing five time periods over each 25 min trial to allow us to investigate changes in the response across a time scale more appropriate to field conditions. Analyses were run in the R statistical software environment (R Core Team, 2016).

A within-subjects repeated measures ANOVA with a block on each post-hatchling was conducted to test differences in speed using the R package "nlme" (Pinheiro et al., 2016). The response variable of speed was square-root transformed based on results of a Box-Cox transformation to meet model assumptions (package "MASS") (Venables and Ripley, 2002). Variation in turtles' speed was investigated using explanatory categorical factors of treatment (control, harness, and Velcro®), time period (5 min blocks), and the interaction of these variables. The best error structure fit with restricted maximum likelihood was a lag 1 autoregressive structure combined with a nested random effects structure of random intercepts among treatments for individual turtles. The autoregressive process of order 1 error structure suggests there is a dependency in the errors between the current value and the previous value, adjusting for correlations among repeated measures. The appropriate fixed effects structure was determined to be the interaction of treatment with time using maximum likelihood. The final model was refit using restricted maximum likelihood. The appropriate ANOVA model was chosen by the Akaike information criterion (AIC) at each step (e.g., "drop1" in R software). The Tukey's honest significant difference test from the package "multcomp" was used in *post-hoc* analysis (Hothorn et al., 2008).

Vertical Movement Analysis

To determine if diving behaviour was affected by transmitter attachments, an underwater camera captured each turtle's movement over time for each treatment. The camera was physically moved side to side as turtles moved throughout the tank to ensure all turtle movements were captured. A depth threshold of 15 cm was set to delineate time spent at the surface vs. time spent diving. For these trials, this resulted in the surface classified as the upper quarter of the water column where swimming was underneath the air-water interface in contrast to definitive diving behaviour. Diving behaviour was measured this way because a true dive depth could not be measured within the available tank. Only a field experiment with a depth recorder could provide this level of estimation without potential tank interference. This classification was a compromise to generically categorize whether having an attachment altered vertical movements through the water column. Water column depth was estimated every second, and these counts of being at the surface or below were compiled every 10 s. This provided a proportion interval similar to the horizontal analysis. Time spent below 15 cm vs. time spent at the surface could then be compared amongst treatments. In a similar manner to speed, data were separated into 5 min blocks across the 25 min recording time. A generalized linear mixed model with a binomial error distribution and logit link function was applied to the response variable of the proportion of time below 15 cm within each 10 s period (package "lme4") (Bates et al., 2015). The categorical explanatory variables were treatment, time in the form of 5 min periods, and their interaction. Using AIC, the best random effects structure was initially found to be a random intercept varying among turtles and among treatments for each turtle (Zuur et al., 2009). To account for model overdispersion, an unstructured random effects term of record number was included in the model. The interaction of treatment and time was significant for the best fixed effects structure. Therefore, the final model was the interaction of treatment and time with 3 random effects: among turtles, among treatments for individuals, and an unstructured error. Model contrasts against the control treatment were completed for each time period to provide a *post-hoc* test for appropriate significance values across these levels and treatments of the linear model.

Stroke Frequency Analysis

The stroke frequency of swimming animals is useful to understanding behavioural changes and swimming performance (e.g., Burgess et al., 2006; Booth, 2014; Sim et al., 2015). Swimming speed is not correlated with stroke frequency in green sea turtles (Booth, 2014), thus making it an independent measurement. We calculated each stroke as the combination of both a down-stroke and up-stroke (Davenport et al., 1984). Strokes were counted during 10 s intervals, and counts were divided by the time spent actively stroking to obtain stroke frequency (Hz) (Ischer et al., 2009). Time spent gliding, dog paddling (Salmon and Wyneken, 1987), resting, or outside the view of the camera was not included in the analysis. As in the previous analyses, time was divided into 5 min blocks, resulting in 5 blocks over the 25 min recording period.

A within-subjects repeated measures ANOVA was run in the same manner as that of speed. A Box Cox transformation indicated the response variable of stroke frequency required a log transformation. Explanatory variables of time (5 min blocks), treatment (control, harness, and Velcro®), mass (g), and the interaction of time and treatment were examined to determine changes in stroke frequency. The best covariance structure allowed for variance to change per treatment combination with the same nested random effects structure used in the prior ANOVA. The best model was chosen at each step through AIC and confirmed with both Bayesian Information Criterion and AICc, which corrects for small sample sizes. *Post-hoc* analysis was completed with Tukey's honest significant difference test.

RESULTS

Horizontal Movement Analysis

There was a statistically significant interaction between treatment and time on turtle speed (**Table 1**; **Figure 4A**). Swim speed was not significantly different for the Velcro® treatment compared to the control for any time periods (Tukey's; α = 0.05). Swim speed was significantly reduced with the harness attachment compared

TABLE 1 | Repeated measures ANOVA results examining square-root transformed speed (cm/s) as a function of the interaction of treatment and time as 5 min periods.

Factors	numDF	denDF	*F*-value	*p*-value
Intercept	1	4,727	512,416	<0.001*
Treatment	2	22	9,440	0.001*
Time	4	4,727	3,185	0.013*
Treatment:Time	8	4,727	5,949	<0.001*

numDF represents the degrees of freedom of the numerator for the F statistic, and denDF is the degrees of freedom of the denominator. Statistical significance is denoted by an asterisk at p < 0.05.

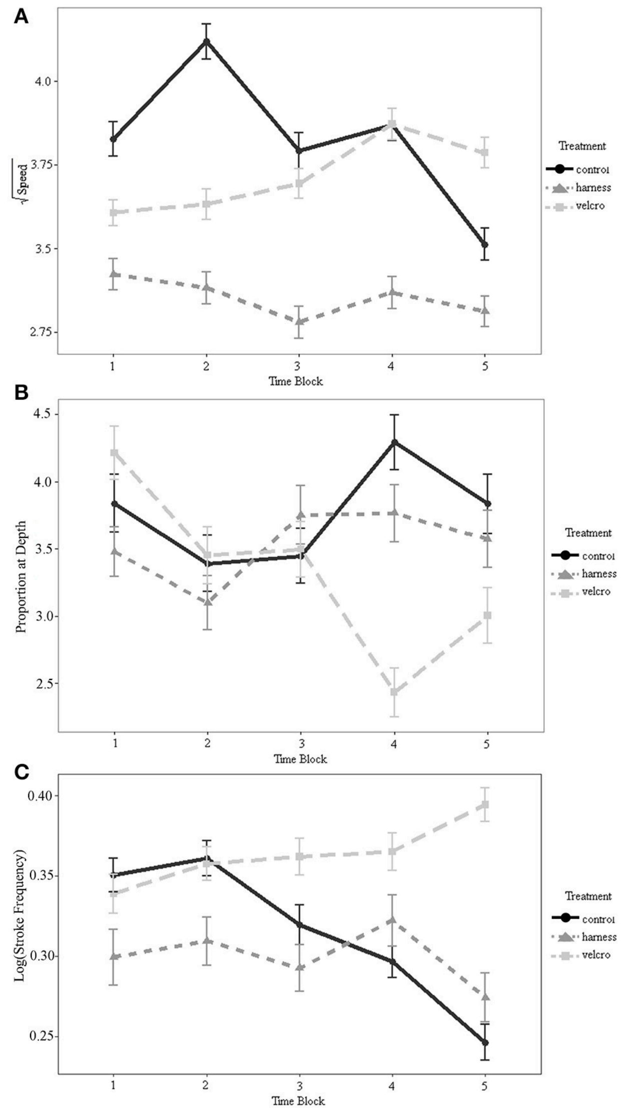

FIGURE 4 | (A) Interaction plot of the square-root transformed speed (cm/s) as a function of time for each treatment. **(B)** Interaction plot of the proportion of time spent below 15 cm as a function of time for each treatment. **(C)** Interaction plot of the log transformed stroke frequency (Hz) as a function of time for each treatment. Minutes comprising the time periods are 1 = (0–5 min), 2 = (5–10 min), 3 = (10–15 min), 4 = (15–20 min), 5 = (20–25 min). Error bars represent standard errors.

to the control during the middle 5-20 min of the trial, time-steps 2–4 (Tukey's; $p < 0.01$; $p = 0.026$; $p < 0.01$, respectively).

Vertical Movement Analysis

Turtles spent 36% of trial time below the surface 15 cm of the tank (**Figures 4B**, **5**). The generalized linear mixed model did not find significant differences in the proportion of time at the surface amongst treatments at each of the 5 min time periods ($\alpha = 0.05$; **Table 2**). There is no evidence to suggest diving behaviour was different between treatments.

Stroke Frequency Analysis

Mass was not statistically significant in the repeated measures ANOVA ($p = 0.941$), but the interaction between treatment and time was significant ($\alpha = 0.05$; **Table 3**; **Figure 4C**). Across all time periods, stroke frequency was not significantly different for the harness treatment compared to the control (Tukey's; $\alpha = 0.05$), and stroke frequency was significantly greater for the Velcro® treatment at the final time-step (at 20–25 min during the trial) compared to the control (Tukey's; $p = 0.024$).

DISCUSSION

We tested miniature acoustic transmitter attachment protocols for efficient tagging of leatherback turtles, using green turtles as a proxy, to minimize impediment of swimming and diving of small sea turtles, while still providing a means of visual contact with diving turtles. Our study suggests outfitting young sea turtles with Vemco V5 acoustic tags will not significantly alter their swim speed or dive behaviour with a Velcro® attachment configuration to the carapace, at least in controlled lab conditions. The Velcro® attachment approach did not result in a significant change in the swim speed or dive behaviour of the turtles at any point during the trials. However, this attachment did result in a faster stroke frequency during the last 5 min block of the trial. This suggests a possible change in the swimming performance or swimming effort at the end of this treatment, which may have resulted in an increase in energetic expenditure. However, the increase in stroke frequency did not correspond with an increase in swimming speed (**Figures 4A,C**). There may have been a compensatory reduction in front flipper amplitude during that time period (Davenport et al., 1984; Booth, 2014). A significant decrease in stroke rate that would be indicative of decreased swimming ability (Burgess et al., 2006) was not observed for either attachment method.

The Velcro® attachment was ultimately determined to be more suitable than the harness attachment, which significantly decreased swim speeds during the middle 15 min of the trial. Our visual observations suggest that the harness disrupted turtle behaviour compared to the control, possibly from constriction of the shoulder girdle, thus reducing swimming speed. We observed that turtles with harness attachments initially spent time at the surface attempting to remove the harness, then conducted a series of rapid dives, whereas the control treatments generally had smooth transitions between the surface and depth separations within the water column. Irritation caused by the harness attachments make this approach less desirable for field experiments and could increase the risk of predation at sea. It could also alter interpretations of past studies that utilized harness methodologies on young sea turtles over short time frames. Based on our results, an experiment utilizing harness attachments should allow for an acclimation period of at least 20 min, while the Velcro® attachment method does not require acclimation. This study can help inform tagging procedures for field studies examining movement of free-ranging hatchling sea turtles.

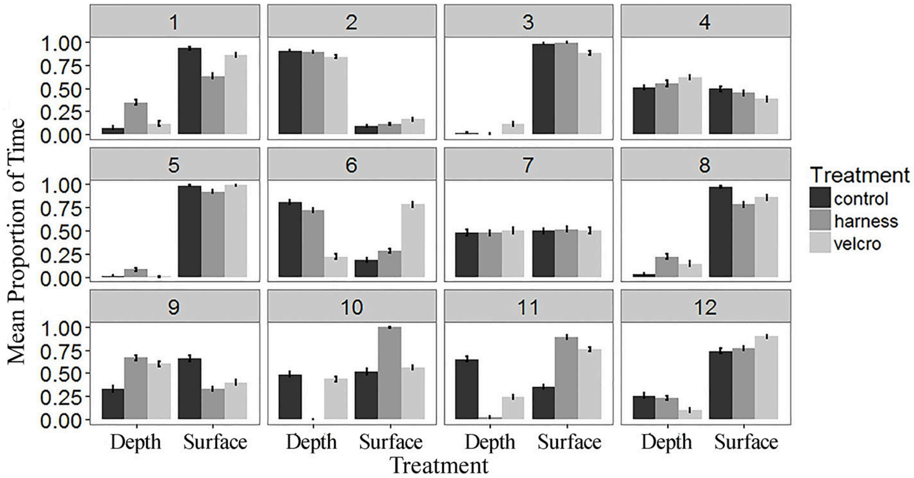

FIGURE 5 | The mean proportion of time spent below 15 cm ("Depth") and at the surface ("Surface") by subject and treatment over the study period. Water column depth was measured every second over the 25 min study period. Each subject is indicated by the number in the grey box. Error bars represent standard errors.

TABLE 2 | The generalized linear mixed model with a binomial error and logit link function results for the proportion of time spent below 15 cm in relation to the interaction of treatment and time.

Factors	Estimate; SE; *p*-value		
	Intercept	**Velcro®**	**Harness**
Time 1 (0–5 min)	−1.82; 1.06; 0.085	0.63; 1.07; 0.56	−0.71; 1.08; 0.51
Time 2 (5–10 min)	−2.57; 1.06; 0.015*	0.41; 1.08; 0.71	−0.82; 1.08; 0.45
Time 3 (10–15 min)	−2.38; 1.06; 0.025*	0.20; 1.08; 0.86	−0.15; 1.08; 0.89
Time 4 (15–20 min)	−2.46; 1.07; 0.17	−1.95; 1.08; 0.072	−2.10; 1.09; 0.31
Time 5 (20–25 min)	−1.97; 1.06; 0.064	−1.01; 1.09; 0.35	−0.73; 1.09; 0.50

Model contrasts were completed at each 5 min period. The estimate, standard error, and p-value are reported. Minutes comprising each period in the stepwise comparison are 1 = (0–5 min), 2 = (5–10 min), 3 = (10–15 min), 4 = (15–20 min), 5 = (20–25 min). Statistical significance is denoted by an asterisk at $p < 0.05$. The control treatment is the reference level.

TABLE 3 | Results of the repeated measures ANOVA examining stroke frequency (Hz) with the interaction of treatment and time (5 min blocks).

Factors	numDF	denDF	*F*-value	*p*-value
Intercept	1	4,546	134,058	<0.001*
Treatment	2	22	2,884	0.077
Time	4	4,546	3,451	0.008*
Treatment:Time	8	4,546	6,553	<0.001*

numDF represents the degrees of freedom of the numerator for the F statistic, and denDF is the degrees of freedom of the denominator. Statistical significance is denoted by an asterisk at $p < 0.05$.

There are limitations to studies such as this because gaining access to endangered species is difficult. The inability to access at-risk sea turtles resulted in a low sample size. Mansfield et al. (2012) utilized an ANOVA framework with smaller sample sizes on sea turtles, and our sample size is within the generally accepted size for this statistical test. While a larger sample size could theoretically increase statistical robustness, this was not feasible given available turtles at the time, and data corrections were applied to meet all model assumptions. Speed is highly variable and individualistic, inconsequential of sample size. Therefore, the sample size may be low, but a larger sample size would not guarantee a more robust statistical test given the high variability inherent in the measured parameter.

Our approach of using a line-float-transmitter attachment was chosen over a direct tag attachment to the plastron at the cost of increased drag because it allows for visual tracking in the water during mobile tracking and should prevent signal dampening or distortion during future field experiments (Thums et al., 2013). This will also help field studies better interpret sources of signal loss at a given location, from events such as predation, tag malfunction, wave interference, or departure from the study site (Thums et al., 2013). Mobile acoustic tracking is very difficult when trying to obtain fine-scale movements through an area when the detection range may extend to 0.25 km. Visual contact with the tracked organism provides the means of fully tracking an organism during a given time period. Although the tag to body weight ratio increases with the Velcro® attachment, there were no significant differences from the control in the swimming speed and diving metrics we measured. Overall, both attachments allow for a safe, full removal from actively tracked turtles, reducing experimental exposure time for wild turtles. Any object placed on an organism adapted to live in its environment may affect its natural behaviours and increase its energetic costs. Consequently, an objective of this methodology was to provide appropriate consideration to the development of tag attachments for leatherback turtles that minimize these negative effects and extend beyond controlled tank environments (Mansfield et al., 2012; Jones et al., 2013). Given that the oily, rubbery skin of leatherbacks could reduce adherence, we wanted to test two attachment techniques in case there were conditions in the field that made a particular method less effective. A vertically attached tag to the plastron, as used by Thums et al. (2016), would not allow for maintained visual contact with deep-diving leatherback hatchlings, as well as provide a very small attachment site on oily skin that has the potential to react differently to Vetbond$^{\text{TM}}$. Therefore, methods that would allow for both visual and acoustic contact to be maintained were considered most effective for actively tracking critically endangered leatherback turtles.

Although there was no significant difference between the control and harness for the proportion of time spent below the surface, this may have resulted from individuals generally spending greater amounts of time at the surface during the control because behavioural reactions to the attachment generally occurred within the surface layer. Any tag attached to an organism should theoretically increase drag, and it is possible the turtles increased power output (e.g., swam harder) to overcome this additional drag, something a longer temporal study might determine (Jones et al., 2013). Stroke frequency was not significantly related to body mass within the limited range of sizes in our study, although such relationships have been found in adult seabirds, pinnipeds, and cetaceans (Sato et al., 2007). Cornick et al. (2006) found harnessed sea lions significantly increased stroke frequency and decreased swimming speed, resulting in overall lower swimming efficiency with reductions in dive durations and foraging times. Further, adult leatherbacks with satellite tags attached to their carapace had faster swimming speeds and longer dive times than harnessed adults, suggesting harnesses were more likely to alter swimming and diving abilities (Fossette et al., 2008). Constraints on the experiment prevented other fine-scale measurements of swimming performance, such as front flipper amplitude, front flipper angle, and proportion of time spent powerstroking, which can aid in understanding the drag costs incurred by individuals (e.g., Davenport et al., 1984; Booth, 2014).

Limitations in both vertical and horizontal movements may have resulted from the experimental tank design. However, visual observation indicated the turtles moved vertically throughout the tank in a similar manner across all treatments, which was supported by the results of the GLM. Edge effects of the tank could alter turtle behaviour through more frequent changes in direction or by seeking shelter, for example, and the depth of the tank may have changed diving patterns. Although the tank was shallower than the length of the attachment, time spent at depth was usually sustained swimming around the circumference of the tank. Therefore, it adequately provides information on whether the attachment changed their vertical movements. We did not provide direct estimates of swimming speed as we recognize that the tank will potentially limit the speed capacity of the turtles, and it would be an inappropriate comparison to other studies of this species. The repeated measures ANOVA appropriately examined changes in speed within individuals, which was the goal of the analysis. Given these turtles generally swam in continuous circles during the study period, we believe any changes in drag which turtles experienced as they moved throughout the tank (e.g., if the line went slack upon changing course) was properly accounted for in our models. A few turtles became entangled in the gear, and untangled themselves. This was an artefact of multiple factors: the size and shape of the tank, as well as the age and behaviour of the turtles. In the open ocean, for which this method was developed, this is not an anticipated concern if turtles are in a frenzied state where swimming will be directed and continuous (Wyneken and Salmon, 1992). Further, the short duration and controlled design did not consider wind drift effects, which have the potential to impact movement during longer studies utilizing these methods (Jones et al., 2013).

Sea turtle early life histories are poorly understood, and lack of knowledge regarding movements and developmental habitat may impede conservation efforts. Advancement of appropriate management strategies requires an understanding of movement and dispersal beyond the adult stage. The "lost years" paradigm begins upon denatant dispersal of hatchlings in a neritic-to-oceanic migration to unknown or unclearly defined nursery habitats. Combining miniaturized tag technology and physical modelling efforts enables much-needed characterization of movement, habitat utilization, behaviour, and life strategies of young sea turtles throughout these cryptic years (Briscoe et al., 2016). As habitats are drastically changed by anthropogenic forces, migrations of many species may shrink or shift (Brower and Malcolm, 1991; Wilcove and Wikelski, 2008). Understanding the mechanisms underlying these movements will improve our ability to describe sea turtle environmental utilization, predict population dynamics, and manage species internationally under changing conditions (Nathan et al., 2008; Bauer et al., 2009). The challenge thus remains to decipher movements among ontogenetic habitats within and across species and understand how to manage these highly migratory species throughout multiple life stages.

AUTHOR CONTRIBUTIONS

GS and HB provided support and assistance with experimental design, data analysis, permits, and manuscript revisions. JS contributed to data collection and experimental design. AH designed, ran, and analysed the experiment, followed by manuscript composition.

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Pre-nesting Movements of Leatherback Sea Turtles, *Dermochelys coriacea,* in the Western Atlantic

Emily P. Bond and Michael C. James*

Population Ecology Division, Fisheries and Oceans Canada, Bedford Institute of Oceanography, Dartmouth, NS, Canada

Understanding high-use areas for highly migratory species and their movements within these areas may provide insight into behaviors such as foraging and mating. In the Western Atlantic, the leatherback sea turtle, *Dermochelys coriacea,* has a broad geographic range extending from nesting beaches at low latitudes to foraging areas off the coast of Eastern Canada. Biotelemetry has revealed much about the movements and habitats of leatherbacks. However, the timing and location of leatherback mating behavior remains unclear. We conducted spatial analyses of the movements of reproductive female leatherbacks prior to their first seasonal nesting events. Using kernel density estimates, high-use areas for seven female turtles originally tagged in Canadian waters were revealed from 50% volume contours depicting pre-nesting movements (120 days prior to confirmed nesting events) and inferred mating behavior (45 days prior to confirmed nesting events). All individuals initially remained offshore within a relatively small range of latitude (10–15° N) before transiting to and residing in coastal waters adjacent to nesting beaches in Colombia ($n = 2$), Trinidad ($n = 3$), Guyana ($n = 1$), and French Guiana ($n = 1$). Comparison of these movement patterns to those of mature male leatherbacks ($n = 12$) revealed similarities. Male and female residency within this offshore high-use area may be indicative of prey exploitation prior to the energetically-costly nesting season. While the offshore residency period of three males and one female extended into the interval in which mating is expected to occur, most males and females transited to coastal waters where they resided throughout this period. High-use areas determined through kernel density analysis support and corroborate previous telemetry work indicating that mature male leatherbacks exhibit seasonal residency adjacent nesting beaches for the early portion of the nesting season, presumably to exploit mating opportunities. Fine-scale analyses of fisheries interactions in both coastal and offshore waters and estimation of accompanying mortality rates is required to evaluate fishery threats to this population during the pre-nesting interval.

Keywords: *Dermochelys coriacea*, habitat, pre-nesting, mating, spatial ecology, satellite telemetry, leatherback sea turtle

Edited by:
Sara M. Maxwell,
Old Dominion University, United States

Reviewed by:
John Roe,
University of North Carolina at Pembroke, United States
Sabrina Fossette,
Department of Parks and Wildlife, Australia

****Correspondence:***
Michael C. James
mike.james@dfo-mpo.gc.ca

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INTRODUCTION

The use of satellite telemetry has provided valuable insight into the distributions and habitat use of many highly migratory species. In the marine realm, satellite telemetry has revealed the distributions and movements of threatened species such as sharks, whales, sea birds, and turtles (Weimerskirch and Robertson, 1994; Godley et al., 2002; Baumgartner and Mate, 2005; Stevens et al., 2010). The ability to remotely observe animals throughout their migrations expands not only our knowledge of their life histories, but may also help identify potential threats that populations face at each phase of their migratory cycle (Hays et al., 2003). In some cases, the use of satellite telemetry has led to the implementation of effective conservation measures such as time-area closures of recreational and industrial fisheries (Domeier, 2006; Jensen et al., 2010). For pelagic species such as most sea turtles, telemetry has provided insight into otherwise enigmatic oceanic movements and residency areas. Advancing telemetry methods have aided in the investigation of mating behavior for populations of loggerhead (Henwood, 1987; Hays et al., 2010; Schofield et al., 2010), green (Balazs and Ellis, 2000), and Kemp's ridley turtles (Shaver et al., 2005).

The leatherback sea turtle, *Dermochelys coriacea*, is the most widely distributed of all sea turtles, with mature individuals in the North Atlantic making annual migrations from their nesting beaches in the Caribbean and South America to foraging grounds in the Northeast (Witt et al., 2007; Fossette et al., 2010) and Northwest Atlantic (James et al., 2007). The species is classified as endangered in Canada (SARA (Species at Risk Act), 2002). In order to effectively protect this species, their full range of habitats and high-use areas must be understood. Advancements in telemetry have led to discoveries such as leatherback migratory routes (James et al., 2005a,b; Dodge et al., 2014) and northern foraging habitats (James et al., 2006; Jonsen et al., 2007). However, knowledge gaps in the life history of this species remain, including understanding of female pre-nesting and mating behavior. Consistent paternity documented across successive nests suggests that sperm storage occurs from mating event(s) prior to the nesting season (Crim et al., 2002; Stewart and Dutton, 2011; Figgener et al., 2016) and that mating during inter-nesting periods may be rare. Therefore, as mature male and female turtles presumably aggregate to breed in specific areas, identifying where pre-nesting season mating opportunities occur may offer conservation value to the Atlantic leatherback population.

The first published hypothesis regarding timing and location of mating activity in North Atlantic leatherbacks suggested that mating occurred in close proximity temporally and spatially to a female's first oviposition on the nesting beach (Lazell, 1980). An alternate hypothesis was published shortly thereafter, asserting that since there were no reported first-hand observations of mating near nesting beaches, such activity must occur in distant offshore waters (Pritchard, 1982). This topic was revisited again in 1988, when Eckert and Eckert inferred pre-reproductive movements of females through the study of epibionts that colonize females once they arrive in warm, tropical waters. The results of this work suggested that females do not arrive in tropical waters early enough to allow for localized mating near nesting areas, but instead must mate prior to their arrival in tropical waters (Eckert and Eckert, 1988). The advancement of satellite telemetry allowed for these hypotheses to be re-visited in 2005, when James et al. reported that mature male leatherbacks tagged at high-latitude foraging areas migrated to waters adjacent nesting beaches, supporting the earlier hypothesis that mating likely occurs in these areas. However, these findings have yet to be corroborated with mature female movement data.

Inter-nesting and post-nesting movements of mature female leatherbacks have been well documented (Reina et al., 2005; Eckert, 2006; Eckert et al., 2006, 2009; Hays et al., 2006); however, movements of female leatherbacks prior to their first seasonal nesting event have not yet been described. This arises from challenges associated with tracking turtles to their nesting beaches. To investigate behavior prior to the deposition of a female leatherback's first clutch of the season, satellite tags deployed on nesting females must be retained and remain operational for 2–3 years (until their next nesting season), a feat which has not yet been achieved with current technology and tag attachment methods (Hays et al., 2007). Alternatively, transmitters may be deployed on females in northern foraging areas prior to migration to nesting areas. While the required duration of tag retention and operation is considerably shorter in these cases, loss of tags and or transmissions before turtles reach nesting areas is unfortunately the norm, resulting from incidental mortality, biofouling, tag (including battery) failure, and/or tag attachment failure (Hays et al., 2007). These logistic challenges mean that documentation of pre-nesting behavior is exceedingly rare: of 57 mature females satellite tagged off the coast of Atlantic Canada (2000-2016), only six (10.5%) have retained their transmitter through to a confirmed nesting event.

Here we present the first analysis of the movements of female leatherbacks upon their arrival in tropical waters prior to their first confirmed nesting event, and compare their movement patterns to those of mature male turtles. By outlining areas of high-use habitat for both mature male and female turtles prior to the onset of nesting season, we can evaluate mating hypotheses using empirical data.

METHODS

Field Sampling

Seven female leatherback sea turtles were equipped with satellite transmitters while foraging in shelf waters off mainland Nova Scotia, Canada (∼44°N, 64°W) and Cape Breton Island (∼47°N, 60°W) (**Table 1**). Monel flipper tags (style no. 49, National Band and Tag Company, Newport, KY, USA) were applied to both rear flippers and passive integrated transponders (Avid, Calgary, AB, Canada; Biomark, Boise, ID, USA; Trovan, Douglas, UK) were implanted in the right shoulder. Individuals were equipped with satellite-linked transmitters [Wildlife Computers, Inc., Redmond, WA, USA; models SSC3 ($n = 1$), MK10-A ($n = 1$), MK10-AF ($n = 2$), SPOT5 ($n = 2$) and SPLASH10 ($n = 1$)]. All turtles were released immediately after tag attachment. Research and associated protocols were reviewed and approved

TABLE 1 | Summary data for seven mature female leatherback turtles equipped with satellite transmitters off the coast of Nova Scotia, Canada prior to confirmed nesting events.

Turtle ID	Curved carapace length (cm)	Transmitter deployment date	Date of first nest	120 Days prior to first nest	Nesting location
A	155	21-Jul-03	21-Apr-2005	22-Dec-2004	Shell Beach, Guyana
B	174.5	24-Jul-08	22-Mar-2009	22-Nov-2008	Awala Yalimapo, French Guiana
C	152.4	17-Jul-08	25-Apr-2009	26-Dec-2008	La Playona, Colombia
D	147.4	22-Jun-12	20-Apr-2013	21-Dec-2012	Bobalito, Colombia
E	151.9	25-Aug-13	22-Mar-2014	22-Nov-2013	Grande Riviere, Trinidad
F	159.2	5-Aug-14	2-May-2015	2-Jan-2015	Matura, Trinidad
G*	152	12-Jul-16	N/A	22-Jan-2017*	Trinidad-Guiana Shield

**Note that Turtle G was confirmed entangled in coastal fishing gear in Venezuela only days prior to her predicted first nesting event. Her pre-nesting interval was, therefore, estimated based on coastal residency behavior in nearshore waters off Trinidad and Venezuela.*

by Dalhousie University Committee on Laboratory Animals or the Fisheries and Oceans Canada Maritimes Animal Care Committee or the Fisheries and Oceans Canada Maritimes Animal Care Committee to meet standards established by the Canadian Council on Animal Care. Research was conducted under scientific license from Fisheries and Oceans Canada and Species At Risk Act (SARA) Section 73 permits.

Spatial Analysis

Location data were acquired via the Argos satellite network[1]. Locations classified as LC3, LC2, LC1, or LC0 are defined as within 150 m, 150–350 m, 350–1,000 m and >1,000 m of the true location, respectively[1]. Location class A (LCA), for which Argos does not provide an estimated range of positional accuracy, has been shown to be as accurate, if not more so, than LC0 transmissions (Vincent et al., 2002). Therefore, location classes 3, 2, 1, 0, and A were analyzed in this study. Transmitters deployed in support of several research projects across multiple years had varying programming parameters, including different user-defined transmission intervals. To address this, prior to spatially analyzing these tracks, a daily median position was calculated for each individual, and tracks were linearly interpolated using packages "plyr" (Wickham, 2011) and "zoo" (Zeileis and Grothendieck, 2005) in R 3.0.2 (R Core Team, 2013) (**Figure 1A**). Linear interpretations assumed constant speed and direction for days in which locations were not generated. The pre-nesting interval was defined as the 120 days prior to each individual's first seasonal nesting event; this interval allowed for discerning marked changes from migratory to residency behavior.

To infer potential areas of high use during the pre-nesting interval, interpolated tracks were spatially analyzed in ArcGIS 10.2.2 software (ESRI) using the kernel density tool within the Home Range Tools toolbox (MacLeod, 2013) (**Figure 1B**). Smoothing parameters for this analysis were calculated using the *ad hoc* approach in order to minimize fragmentation of potential high-use areas (Kie, 2013; Schuler et al., 2014). From kernel density results, percent volume contours were generated, outlining areas in which 50 and 95% of each individual's locations have the probability of being detected within the 120-day pre-nesting interval (**Figure 1C**). The 50% volume contours were used to infer high-use areas, while 95% contours showed the range of each individual throughout the pre-nesting interval.

To corroborate leatherback high-use areas derived from female pre-nesting telemetry data, we also considered tracking data from 12 mature male leatherbacks, including data from seven turtles previously analyzed by James et al. (2005a) (Table S1). Male tracking data were analyzed for all dates spanning 120 days prior to the earliest recorded nest (March 22; Female B) through to the onset of each male's northward migration (Figure S2). Male and female high-use areas defined by their 50% volume contours were overlaid to determine areas of overlap using the Clip tool within the Spatial Analysis toolbox (ESRI).

Inference of Mating

Unlike other sea turtles, the timespan between mating and nesting events has not been directly observed in leatherbacks. Therefore, we inferred this period from known follicular development intervals of other sea turtles. Studies of captive green turtles indicate the first oviposition typically occurs ∼34–45 days after observed copulation (Simon et al., 1975; Wood and Wood, 1980); however, this interval may reach up to 60 days (Wood and Wood, 1980). The mating period for loggerhead sea turtles has been documented to last up to 42 days (Miller et al., 2003). Leatherbacks are predicted to have a similar timespan between mating and nesting to that recorded for loggerhead and green turtles, as egg incubation and inter-nesting intervals are similar across these species (Hirth, 1980). This interval is also supported by the findings of Eckert and Eckert (1988), who estimated an interval of ∼30 days between mating and egg production based on the colonization of tropical epibionts on nesting leatherback females. To remain conservative in our estimation of follicular development time, we assumed the period between mating and first nesting spans 45 days.

To identify potential areas of mating activity, individual female movement datasets were truncated to the inferred mating period (45 days prior to first seasonal nesting events), and spatial analyses applied to the pre-nesting interval (see above) were repeated (Figure S3). Male movement data were analyzed for all dates spanning 45 days prior to the earliest recorded nest (March 22; Female B) through to the onset of each male's northward migration. From kernel density estimates, 50% volume contours were generated for both females (**Figure 4**) and males (**Figure 5**). Movement data for turtle F were not analyzed within the mating

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[1] www.argos-system.org

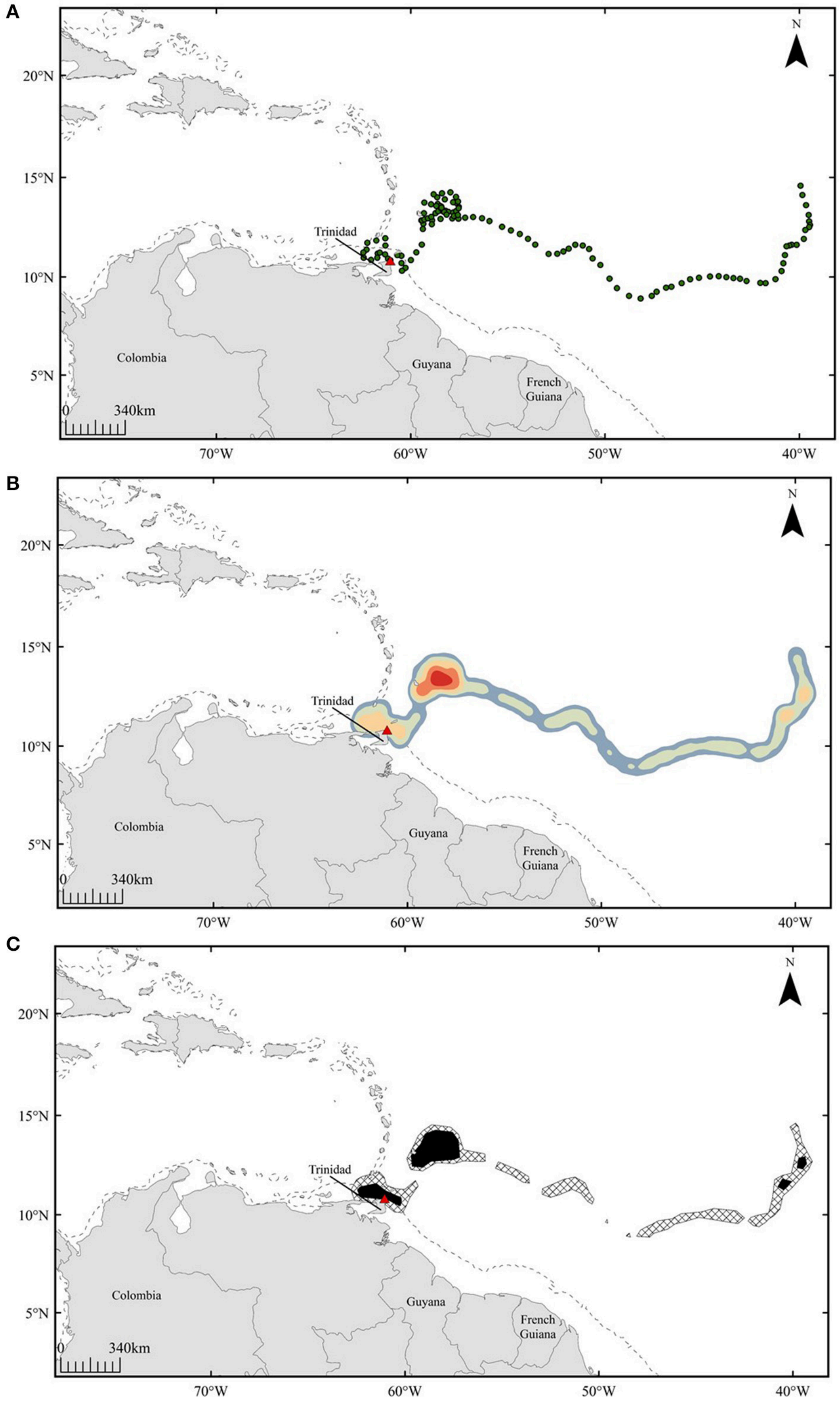

FIGURE 1 | Representative example of spatial analyses conducted for pre-nesting leatherbacks: turtle E. Panels show interpolated positions for 120 days prior to nesting **(A)**, heatmap displaying areas of high density (red) **(B)**, and 50% (black) and 95% (hatched) volume contours generated from kernel density results **(C)**. Dashed line represents 200 m isobath.

period (45 days prior to nesting), as transmissions temporarily ceased during this time interval. Nesting events were confirmed through observations of high-quality coastal Argos locations derived from continuous tag transmissions during satellite passes and/or extended surface/dry time logged by tag depth sensors, consistent with turtles coming ashore at known nesting areas ($n = 1$), or through encounters of tagged animals by collaborating beach monitoring organizations ($n = 4$). For one female (turtle G), the pre-nesting and mating intervals were estimated based on coastal residency behavior in nearshore waters off Trinidad and Venezuela prior to her fatal entanglement just days before her first predicted nesting event.

RESULTS

Female Movements within the Pre-nesting Interval

All mature female leatherbacks equipped with satellite transmitters off Nova Scotia exhibited seasonal residency in Atlantic Canadian waters from the time of tagging through to late September ($n = 2$), October ($n = 4$), or November ($n = 1$) of their respective deployment years before assuming southward migration. Females traveled southward within a narrow longitudinal range (∼35°–50°W). Upon reaching southern waters corresponding roughly to the North Equatorial current (∼10–15°N), seven females (turtles A-G) traveled westward toward beaches in Colombia ($n = 2$), Trinidad ($n = 3$), Guyana ($n = 1$), and French Guiana ($n = 1$) (Figure S1).

Combining the 50% volume contours produced from kernel density estimates for all seven female leatherbacks revealed patterns in female behavior during the 120-day pre-nesting interval (**Figure 2**). While offshore high-use areas spanned a wide longitudinal range (∼40°–60°W), ∼75% of activity in these areas (across all corresponding pre-nesting seasons) occurred within a narrow range of latitude (∼5°; 10–15°N). After initially departing offshore high-use areas, tracking data from six females (turtles A-E, G) revealed secondary residency areas in coastal waters proximate to their respective nesting beaches, used immediately prior to their respective first nesting events.

Male Movements within the Pre-nesting Interval

Male satellite tracking data ($n = 12$) also revealed offshore high-use areas within the pre-nesting interval (**Figure 3**). Similar to patterns observed in female tracking data, 76.4% of total male residency fell within the 10–15°N latitudinal range. Overlap between mature male and female leatherback high-use areas (revealed from 50% volume contours) occurred in offshore areas spanning latitudes of 40–60°W, as well as in coastal waters off Trinidad and French Guiana (**Figure 3**). Within the 10–15°N latitudinal range, each female exhibited at least one high-use 50% volume contour that corresponded to male residency areas, representing a strong affinity for both males and females for this area over the course of multiple breeding seasons ($n = 13$).

Female and Male Movements within the Mating Period

Throughout the inferred mating period, six females (turtles A-E, G; turtle F not included due to transmission gaps) resided in coastal waters proximate to nesting beaches in Trinidad ($n = 2$), French Guiana ($n = 1$), Guyana ($n = 1$), and Colombia ($n = 2$) (**Figure 4**). Offshore residency throughout the mating period was observed in one female, turtle E, which occupied waters beyond the 200 m isobath prior to transiting and residing in coastal waters proximate to Trinidad. Each female was present in continental shelf waters (inshore of the 200 m isobath) in the days immediately preceding confirmed nesting events. Mature male tracking data were analyzed to corroborate probable mating areas. Three males exhibited offshore residency throughout the mating period (**Figure 5**, Figure S4); however, the majority of

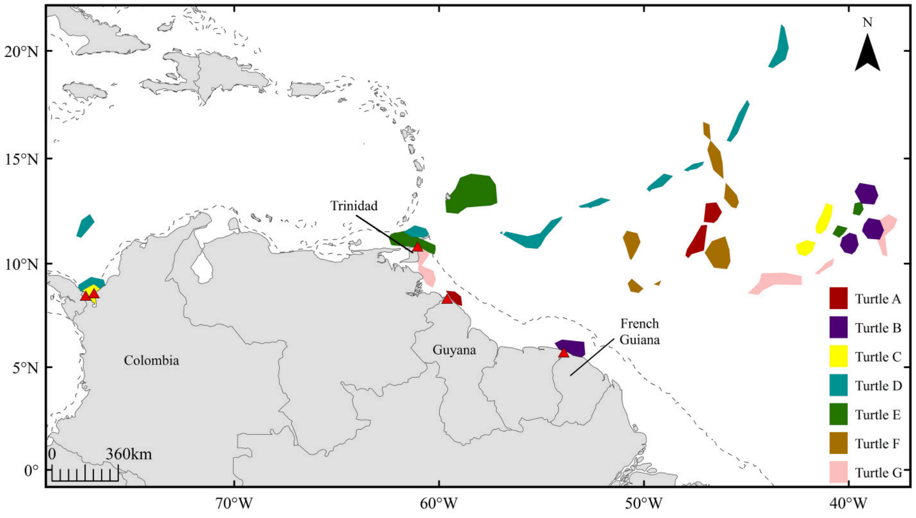

FIGURE 2 | High-use areas defined by 50% volume contours for seven female leatherbacks (turtles A-G) for 120 days prior to first seasonal nesting events; red triangles indicate corresponding nesting sites in Guyana, French Guiana, Trinidad, and Colombia. Dashed line represents 200 m isobath.

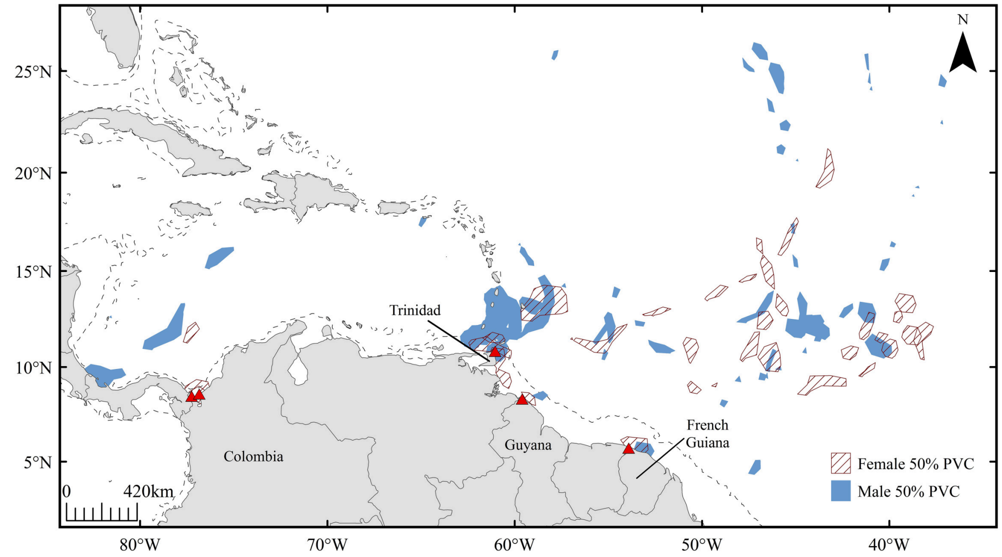

FIGURE 3 | High-use areas defined by 50% volume contours for mature male ($n = 12$, blue) and female ($n = 7$, red hatched) leatherbacks throughout the 120-day pre-nesting interval. Dashed line represents 200 m isobath.

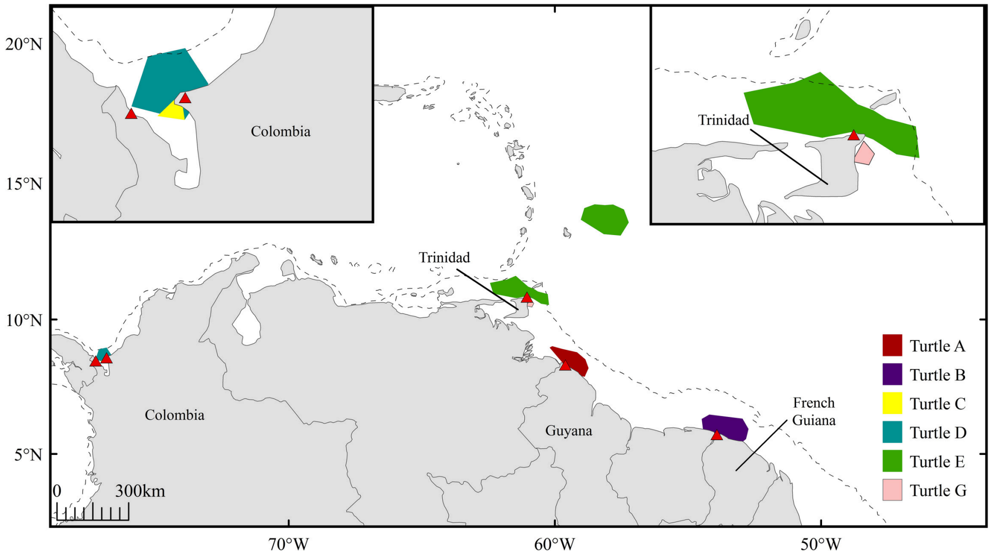

FIGURE 4 | High-use areas defined by 50% volume contours for 6 female leatherbacks (turtles A-E, G) during inferred mating period 45 days prior to confirmed nesting events; red triangles indicate corresponding nesting sites in Guyana ($n = 1$), French Guiana ($n = 1$), Trinidad ($n = 1$), and Colombia ($n = 2$). Dashed line represents 200 m isobath.

male high-use areas corresponded to coastal waters directly adjacent nesting beaches in Trinidad, French Guiana, St. Lucia, St. Vincent, Grenada, and Panama prior to the onset of their northward migration.

DISCUSSION

Offshore High-Use Areas

Satellite telemetry data from mature leatherbacks tagged in Canadian foraging habitat can be used to identify probable mating areas and provide the first insights into the pre-nesting behavior of this species. Consistent with James et al. (2005a), results of the present spatial analyses showed that mature males spend extended time proximate to nesting beaches within the inferred mating interval. However, this study highlights an additional high-use area for both mature males and females prior to their arrival in coastal waters proximate to nesting beaches (**Figure 3**). All seven female leatherbacks and seven of 12 males we tracked frequented this offshore area prior to assuming directed movement toward nesting beaches. Residency

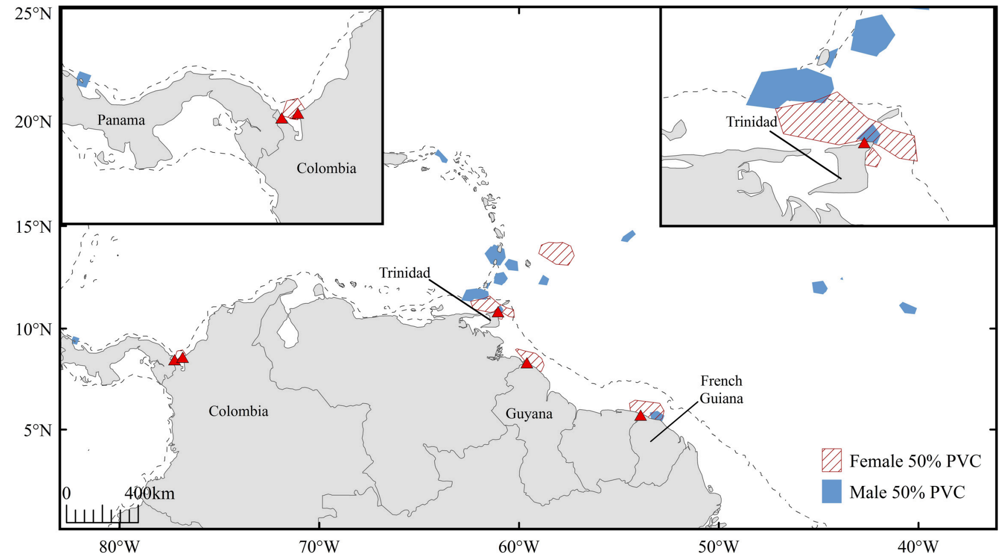

FIGURE 5 | High-use areas defined by 50% volume contours for mature male (*n* = 12, blue) and female (*n* = 6, red hatched) leatherbacks throughout the 45-day inferred mating period. Dashed line represents 200 m isobath.

of reproductively-active females and males in this offshore area over multiple breeding seasons (*n* = 13) underscores the importance of these waters to the Northwest Atlantic leatherback turtle population.

The fundamental goals of long-distance migrations are often resource driven linked to exploitation of spatially limited food, mates, or shelter, all of which may result in temporary aggregation (Dingle and Drake, 2007). The foraging grounds of Atlantic Canada have been identified as critical habitat for mature leatherbacks (James et al., 2006), and both morphometric and physiological indicators have been used to determine that leatherbacks are capital breeders (James et al., 2005b; Davenport et al., 2011; Plot et al., 2013). Throughout the nesting season, leatherbacks rely on energy stores and become anorexic (Plot et al., 2013). The sub-Equatorial region spanning 5–15°N has previously been posited as an area of foraging success for post-nesting leatherbacks, corroborated by low leatherback travel rates and modeled annual zooplankton biomass (Fossette et al., 2010). It is possible that this offshore high-use area provides foraging opportunities for pre-nesting leatherbacks as well, enabling acquisition of valuable energy reserves prior to the start of the energetically-costly nesting season.

Inferred Mating Behavior

After exhibiting seasonal residency in offshore waters, presumably to exploit available prey, reproductively active male and female leatherbacks transited to coastal waters where they resided throughout the inferred mating period preceding first seasonal nesting events (**Figure 5**). While three males remained in offshore waters throughout this time interval, six females and nine males exhibited coastal residency during the inferred mating period. Our results corroborate the findings of James et al. (2005a), suggesting that mature leatherbacks frequent coastal waters adjacent to nesting beaches in order to exploit mating opportunities prior to the nesting season.

Coastal mating areas have also been identified for various populations of other sea turtle species. Mature male and female green sea turtles have been documented in coastal waters of Hawaii (Dizon and Balazs, 1982), Costa Rica (Carr et al., 1978), and Australia (Booth and Peters, 1972) prior to the onset of seasonal nesting. Satellite tracking data of male green sea turtles near Ascension Island have revealed temporary residency in the coastal waters of high-density nesting beaches (Hays et al., 2001). Male green turtles have also been observed transiting through coastal waters of multiple high-density nesting sites prior to and during the nesting season, potentially mating in coastal waters of multiple rookeries in Cyprus and Turkey (Wright et al., 2012). Mating pairs of olive ridley sea turtles have been documented offshore from nesting beaches just prior to high-density arribada nesting events (Kalb et al., 1995; Jensen et al., 2006). Similarly, coastal mating has been confirmed in the Laganas Bay population of loggerhead sea turtles, where mating pairs have been directly observed in close proximity to rookeries over the course of multiple breeding seasons (Schofield et al., 2006). Our results suggest that leatherbacks behave similarly to other sea turtle species, with mature males and females residing in coastal waters prior to the onset of seasonal nesting.

Genetic analyses have previously identified reproductively isolated leatherback nesting assemblages within the Northwest Atlantic. Stewart et al. (2013) combined the use of passive identification tagging, satellite telemetry, and mitochondrial DNA analyses to identify the natal origins of 288 leatherbacks sampled off the coast of Atlantic Canada. Results indicated that individuals from the Guiana Shield population (encompassing nesting populations from Trinidad, Guyana and French Guiana) were genetically distinguishable from individuals originating from Costa Rica, Panama, and Colombia. The present telemetry results, also representing turtles sampled in Canadian waters, support the findings of Stewart et al. (2013), as both Colombian-nesting females (turtles C and D) spent their presumed mating period in coastal waters of Colombia. In contrast, females nesting in Trinidad, French Guiana and Guyana (turtles A, B, and E) exhibited longer residency in the North Equatorial Current offshore area throughout their pre-nesting interval, followed by secondary residency periods in waters proximate to their respective nesting beaches throughout the mating period (**Figure 4**).

A metric of reproductive success among oviparous organisms is the quantity of eggs successfully fertilized (Parker, 1984). As such, reproductively successful males will have morphological or behavioral adaptations that increase their likelihood of fertilizing as many eggs as possible. For male leatherbacks, these adaptations may include the areas they select to intercept females prior to the nesting season. While all species of sea turtle exhibit polyandry (Kichler et al., 1999; Ireland et al., 2003; Jensen et al., 2006; Zbinden et al., 2007; Theissinger et al., 2009; Joseph and Shaw, 2011), multiple paternity in Atlantic leatherback clutches has been observed in low proportions (10–41.7%; Crim et al., 2002; Stewart and Dutton, 2011, 2014; Figgener et al., 2016). Few instances of inter-nesting mating have been identified in leatherbacks (Figgener et al., 2016), and successive nests laid by most females reveal consistent paternities throughout the nesting season, indicative of sperm storage from mating event(s) occurring prior to the nesting season (Crim et al., 2002; Stewart and Dutton, 2011; Figgener et al., 2016). Therefore, male leatherbacks must intercept reproductive females upon arrival in low latitude waters prior to the onset of nesting, highlighting the importance of leatherback high-use areas prior to the nesting season.

Confirmation of leatherback mating interactions during the inter-nesting interval in nearshore waters off Pacific Costa Rica has been achieved through deployments of animal-borne video recorders on nesting turtles (Reina et al., 2005). However, logistical challenges have so far precluded application of this technology on turtles during the pre-nesting period; thus direct confirmation of the times, areas, and behaviors associated with mating remains elusive. With the rapid evolution of animal-borne imaging systems, visual confirmation of pre-nesting mating behavior may eventually be possible.

Fishing Interactions and Significance for Conservation

Identification of high-use areas for leatherbacks is critical to evaluating where this species may be vulnerable to fisheries interactions (James et al., 2005c; Fossette et al., 2014). Artisanal gill net fisheries have been identified as a serious threat to nesting leatherbacks in Trinidad and Tobago (Lee Lum, 2006) and Grenada (Georges et al., 2007), as well as in French Guiana and Suriname (Chevalier et al., 1999; Georges et al., 2007). One female leatherback in this study (turtle G) exhibited pre-nesting behavior consistent with other females (turtles A-F), however, she was confirmed dead, entangled in coastal fishing gear, just prior to the date of her first predicted nesting event. This case highlights the threat coastal fishing gear presents to mature leatherbacks in waters proximate to nesting beaches prior to and during the nesting season.

While artisanal fisheries adjacent to many nesting beaches are an important source of mortality for the Northwest Atlantic leatherback population, this species may also be vulnerable in the additional high-use offshore area identified here, where mature turtles aggregate prior to their arrival in nearshore coastal waters.

Atlantic basin-wide analyses have identified areas where leatherbacks may be vulnerable to interactions with high seas fisheries (Hays et al., 2004; Lewison et al., 2004; Wallace et al., 2010; Fossette et al., 2014). However, where satellite telemetry data has been incorporated into such analyses, mainly post-nesting movements have been considered, potentially missing key areas of reproductive leatherback aggregation including the pre-nesting movements described here. A detailed bycatch analysis quantifying leatherback-fishery interactions and subsequent mortality rates in offshore high-use areas is required to better evaluate the potential impact on the Northwest Atlantic population at this stage of their migratory cycle.

AUTHOR CONTRIBUTIONS

EPB led the analysis and writing of the manuscript. MCJ conceived the study, conducted fieldwork, collected data, and contributed to the analysis and writing of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fmars.2017.00223/full#supplementary-material

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METHODS
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Novel Bio-Logging Tool for Studying Fine-Scale Behaviors of Marine Turtles in Response to Sound

Reny B. Tyson[1]*, Wendy E. D. Piniak[2], Camila Domit[3,4], David Mann[5], Michael Hall[5], Douglas P. Nowacek[6] and Mariana M. P. B. Fuentes[7]

[1] Sarasota Dolphin Research Program, Chicago Zoological Society, Sarasota, FL, United States, [2] Department of Environmental Studies, Gettysburg College, Gettysburg, PA, United States, [3] Laboratório de Ecologia e Conservação, Centro de Estudos do Mar, Universidade Federal do Paraná, Paraná, Brazil, [4] Associação MarBrasil, Paraná, Brazil, [5] Loggerhead Instruments, Inc., Sarasota, FL, United States, [6] Nicholas School of the Environment & Pratt School of Engineering, Duke University Marine Laboratory, Beaufort, NC, United States, [7] Department of Earth, Ocean, and Atmospheric Science, Florida State University, Tallahassee, FL, United States

Increases in the spatial scale and intensity of activities that produce marine anthropogenic sound highlight the importance of understanding the impacts and effects of sound on threatened species such as marine turtles. Marine turtles detect and behaviorally respond to low-frequency sounds, however few studies have directly examined their behavioral responses to specific types or intensities of anthropogenic or natural sounds. Recent advances in the development of bio-logging tools, which combine acoustic and fine-scale movement measurements, have allowed for evaluations of animal responses to sound. Here, we describe these tools and present a case study demonstrating the potential application of a newly developed technology (ROTAG, Loggerhead Instruments, Inc.) to examine behavioral responses of freely swimming marine turtles to sound. The ROTAG incorporates a three-axis accelerometer, gyroscope, and magnetometer to record the turtle's pitch, roll, and heading; a pressure sensor to record turtle depth; a hydrophone to record the turtle's received underwater acoustic sound field; a temperature gauge; and two VHF radio telemetry transmitters and antennas for tag and turtle tracking. Tags can be programmed to automatically release via a timed corrodible link several hours or days after deployment. We describe an example of the data collected with these tags and present a case study of a successful ROTAG deployment on a juvenile green turtle (*Chelonia mydas*) in the Paranaguá Estuary Complex, Brazil. The tag was deployed for 221 min, during which several vessels passed closely (<2 km) by the turtle. The concurrent movement and acoustic data collected by the ROTAG were examined during these times to determine if the turtle responded to these anthropogenic sound sources. While fine-scale behavioral responses were not apparent (second-by-second), the turtle did appear to perform dives during which it remained still on or near the sea floor during several of the vessel passes. This case study provides proof of concept that ROTAGs can successfully be applied to free-ranging marine turtles to examine their behavioral response to sound. Finally, we discuss the broad applications that these tools have to study the fine-scale behaviors of marine turtles and highlight their use to aid in marine turtle conservation and management.

Keywords: bio-logging, tagging, marine turtle, anthropogenic sound, behavioral response

Edited by:
Ellen Hines,
San Francisco State University,
United States

Reviewed by:
Nathan D. Merchant,
Centre for Environment, Fisheries and Aquaculture Science, United Kingdom
John A. Cigliano,
Cedar Crest College, United States

***Correspondence:**
Reny B. Tyson
rtyson@mote.org

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INTRODUCTION

Increases in the spatial scale and intensity of activities that produce anthropogenic sound in the marine environment have led to a rapid growth in the number and scope of scientific studies that assess the potential physiological and behavioral impacts of anthropogenic sound on marine organisms (Richardson et al., 1995; National Research Council, 2000, 2003, 2005; Nowacek et al., 2007; Popper and Hastings, 2009). Sounds produced by anthropogenic activities such as shipping, seismic surveys, dredging, pile driving, low-frequency sonar, and wind turbines have been shown to induce stress in organisms such as marine mammals and fish, which may contribute to suppressed growth, reproduction, and immune system functioning (Romano et al., 2004; Wright et al., 2007; Rolland et al., 2012), to elicit behavioral responses (e.g., Richardson et al., 1995; Nowacek et al., 2007; Popper and Hastings, 2009), and to cause temporary and permanent threshold shifts in hearing (reviwed by Finneran, 2015). Anthropogenic sound may also mask important acoustic cues that marine species rely on for communication, navigation, foraging, or reproduction (Richardson et al., 1995; Halpern et al., 2008). Most of the research to date on effects of anthropogenic sounds on marine species has focused on marine mammals and fishes (reviewed by Nowacek et al., 2007; Slabbekoorn et al., 2010), with substantial knowledge gaps remaining in relation to the responses of marine turtles to sound (Popper et al., 2014). While marine turtles are known to detect and respond to low-frequency acoustic stimuli (i.e., 50–2,000 Hz) (Ridgway et al., 1969; O'hara and Wilcox, 1990; Bartol et al., 1999; McCauley et al., 2000; Bartol and Ketten, 2006; DeRuiter and Doukara, 2012; Martin et al., 2012; Piniak, 2012; Lavender et al., 2014; Piniak et al., 2016); such as those commonly produced by anthropogenic activities (Hildebrand, 2009), further research is needed to examine the extent that such activities may affect the behavior and physiology of marine turtles (Popper et al., 2014; Nelms et al., 2016).

The few behavioral studies conducted with marine turtles in response to sound have primarily been conducted in enclosed or semi-enclosed settings, and in response to high-intensity seismic air guns, limiting the ability to assess behaviors of freely swimming turtles to specific types, levels, and frequencies of anthropogenic sound. For example, McCauley et al. (2000) exposed a green (*Chelonia mydas*) and a loggerhead turtle (*Caretta caretta*) to an approaching-departing single air gun in a cage and found that the turtles increased their swimming activity when the air gun sound levels were equivalent to 166 dB re 1 μPa, and that they demonstrated more erratic behavior at sound levels greater than 175 dB re 1 μPa, possibly indicating an agitated state. Alternatively, O'hara and Wilcox (1990) found that loggerhead turtles generally remained near or moved toward the sound source when exposed to air guns in a 300 × 45 enclosure in a 10 m deep canal, but they did not report sound levels. Finally, Moein et al. (1994) reported that repeated exposure of loggerhead turtles to air guns in an 18 m × 61 m enclosure in a 3.6 m river exhibited avoidance behavior upon the first presentation of the stimulus, but that repeated exposure did not elicit significant behavioral responses, suggesting that the turtles had habituated to the sound or had temporary shifts in hearing capabilities (sound source ranges were not reported). Physiological measurements showed increases in stress levels, and pre- and post-hearing threshold measurements showed a temporary decrease in hearing sensitivity in some turtles after seismic air gun exposures. To our knowledge the only studies conducted on free-swimming marine turtles in an open water environment were conducted by DeRuiter and Doukara (2012) and Weir (2007), whom visually tracked loggerhead turtles during seismic air gun surveys. The two studies report contrasting results with DeRuiter and Doukara (2012) documenting that loggerhead turtles dove immediately following an air gun shot (estimated received level of 191 dB re 1 μPa-peak at 130 m and 175 dB re 1 μPa-peak at 830 m), possibly eliciting a startle response, and Weir (2007) reporting that 80% of olive ridley turtles (*Lepidochelys olivacea*) remained at the surface during air gun activity. The differences between the two studies could be related to variations in behavioral responses by different species, variations in air gun source levels, frequencies, and/or distances, or some other unknown environmental or ecological parameter. These differences and the lack of additional studies available examining marine turtle responses to anthropogenic sound, particularly in open water environments, highlight the urgent need to develop tools and techniques that enable us to fill this important research gap.

Studying the movements and behaviors of free-swimming marine animals, especially in relation to sound sources, has many logistical challenges, but the advent and use of bio-logging tools, such as radio, acoustic, or satellite-telemetry tags and data archival tags has greatly expanded the capacity to conduct such analyses. Bio-logging tools can provide insights into the behaviors and movements of marine species over long and broad temporal and spatial scales as well as at depths where these animals are not visible. Therefore, they are an increasingly popular method for examining the movements, behaviors, physiology, and/or environment of free-ranging marine megafauna, including marine turtles (Payne et al., 2014). For example, satellite tags have been used to document the broad-scale movements of marine turtles, as well as to infer their home range, migratory corridors, suitable habitats, and exposure to threats (e.g., reviewed by Godley et al., 2008). In addition, passive and active acoustic telemetry tags (e.g., Taquet et al., 2006; Lamont et al., 2015), time-depth recorders (TDRs; e.g., Eckert et al., 1989; Hill et al., 2016), and animal-borne video tags (e.g., Seminoff et al., 2006; Arthur et al., 2007) have been used to infer fine-scale movements and behaviors of animals, particularly in relation to foraging ecology and diving behavior. Bio-logging tools that combine acoustic and fine-scale movement measurements (e.g., digital acoustic recording tags, Dtags, Johnson and Tyack, 2003; Acousonde, Burgess, 2009) are a particularly useful tool for quantifying behaviors of marine organisms in response to sound; however, to our knowledge have not yet been applied with marine turtles. These tools are commonly used in behavioral response studies (BRS) and controlled exposure experiments (CEEs) of marine mammals whereby a specific stimulus [e.g., sonar, predatory acoustic cues, an acoustic deterrent device (ADD)] is played to an individual that is fitted with a tag, which measures the animal's response as well as the received sounds, thus providing a combined record of stimulus and response (e.g., Johnson et al., 2009; Tyack, 2009; Tyack et al., 2011; Southall et al., 2012; DeRuiter et al., 2013; Goldbogen et al., 2013; Stimpert et al., 2014; Harris et al., 2016; Southall et al., 2016). These tools can also be used passively to examine an animal's behavior in relation to sounds in their environment, such as sounds produced by the tagged individual (e.g., Tyson et al., 2007; Stimpert et al., 2012; Sayigh et al., 2013), calls from conspecifics (e.g., Jensen et al., 2011) or predators (e.g., Curé et al., 2013; Bowers, 2015), and anthropogenic sound sources such as noise produced by shipping and small vessels (e.g., Aguilar Soto et al., 2006; Jensen et al., 2009; Parks et al., 2011).

In this paper, we introduce the application of the Remora-OpenTag (ROTAG), a fine-scale digital acoustic and movement tag custom designed by Loggerhead Instruments, Inc. for use with free-ranging marine turtles. We include a description of the ROTAG and demonstrate proof-of-concept via a case study of a successful deployment on a juvenile green turtle in the Paranaguá Estuarine Complex (PEC) off Paraná state in southern Brazil. Because the habitat use of marine turtles overlaps extensively with sound produced by anthropogenic activities such as fishing, shipping, and coastal and marine development (Van Dolah and Maier, 1993; Wallace et al., 2010; Lewison et al., 2014; Fuentes et al., 2016), the application of the ROTAG will enable researchers to gain important insights regarding how and to what extent such activities may affect marine turtles. This information is critically needed and can be used to aid in marine turtle conservation and management.

ROTAG DESCRIPTION

The ROTAG is a fine-scale digital acoustic and movement tag designed and developed by Loggerhead Instruments, Inc. specifically for use with marine turtles. The system combines an Arduino-compatible open-source inertial measurement unit (IMU) for recording high speed motion sensor data (OpenTag motion datalogger, Loggerhead Instruments Inc.) with a state-of-the-art acoustic tag for measuring sound in the marine environment (Remora-ST, Loggerhead Instruments, Inc.) and a rechargeable lithium polymer battery encapsulated with epoxy (Epotek 301) in a 3D printed ABS plastic housing pressure tolerant to 300 m depth (**Figure 1**). The tag is designed to sit upon an ABS plastic plate (4.45 × 4.06 cm, 0.64 in thickness) that is affixed to a turtle's carapace with epoxy (e.g., Sonic Weld). A 0.22 mm stainless steel wire is used to attach the ROTAG to this plate, and can be programmed to corrode at a specified time to release the ROTAG from the plate and the turtle. A positively buoyant syntactic foam float is attached to the anterior portion of the tag to allow for the tag to float upon release from the turtle. Two VHF radio telemetry transmitters and antennas are positioned within the ROTAG to allow for tag and turtle tracking as well as retrieval of the tag: one antenna (218–220 MHz) is positioned to transmit signals when the turtle surfaces to breath, while the other antenna (149–150 MHz) is positioned to transmit signals when the tag is floating after release. The tag is powered by three 850 mA rechargeable lithium polymer batteries, and the total power draw is ∼25 mA. The dimensions of the ROTAG body and float are 25 × 5 × 2.8 cm and the in-air the tag weighs 220 g. The shape was chosen to minimize the cross-sectional area exposed to flow in order to minimize drag.

The movement tag within the ROTAG includes a three-axis accelerometer, gyroscope, and magnetometer (sampling rate up to 100 Hz), which can be used to calculate a turtle's pitch, roll, and heading; a pressure sensor (1 Hz sampling rate) to record turtle depth; and a temperature sensor (1 Hz sampling rate) to record ambient water temperature. Data recorded by this tag is stored on a micro SD memory card that can be removed from the device and saved to a computer after a successful deployment. The acoustic tag within the ROTAG can be programed to sample sound at 24, 48, 72, 96, 144, or 288 kHz with memory capabilities of 128 GB with X3 compression. The tag can be connected to a computer via a USB cable to download acoustic files after a deployment. The hydrophone of the acoustic tag has a sensitivity of −211 dBV/µPa and can be set to have either low (21 dB gain ∼190 dB re 1 µPA clip level) or high gain (33 dB gain ∼178 dB re 1 µPa clip level).

The ROTAG can be customized in many ways depending on the user's needs. For example, the movement tag can stream all internal sensors (accelerometer, magnetometer, and gyroscope) at 100 Hz each. However, a user can choose to only record data from a specific sensor (e.g., accelerometer) at a higher sample rate without data loss (e.g., 200 Hz). The ROTAG can also be set to record all data streams continuously or with a specified duty cycle. This includes an option to turn off the VHF transmitter at specified depths (e.g., greater than 1 m), which may be beneficial as the frequencies emitted by the VHF transmitter can be received by the acoustic tag possibly hindering acoustic measurements. In addition specific sensors could be set to turn on upon motion detection (i.e., data will be recorded for a specified duration when a defined threshold is exceeded on the accelerometer). The deployment duration of the ROTAG depends on these user-specified settings, but ranges from ∼3–7 days.

CASE STUDY: ROTAG DEPLOYMENT ON A JUVENILE GREEN TURTLE

On 26 May 2016 we deployed a ROTAG on a juvenile green turtle during a pilot study conducted in the PEC (**Figure 2**). The results of the pilot study are reported elsewhere (see Tyson et al. unpublished report 2016), but data collected during this deployment are described here to showcase how ROTAGs can be used to examine marine turtle behavior and concurrent anthropogenic sound.

A local fisherman captured the turtle using a bottom-set gillnet on the morning of 26 May 2016. The turtle was transferred by boat to our research team ∼1 h after capture. A veterinarian assessed the turtle's health (e.g., body condition, behavior, presence of fibropapilloma or other external pathologies and injuries), which was deemed to be good, and we collected standard morphometric measurements: the turtle weighed 12.28 kg and its curved carapace length was 38.50 cm

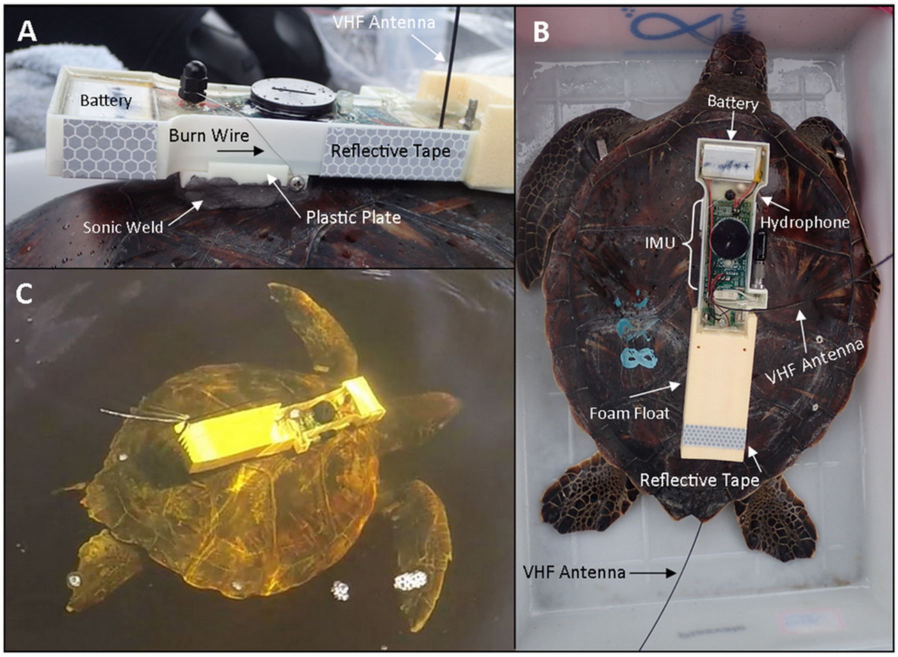

FIGURE 1 | A ROTAG deployed on a juvenile green turtle. **(A)** A side view of the ROTAG affixed to the turtle's carapace. A 0.22 mm stainless steel wire attaches the ROTAG to a plastic plate that is affixed to the turtle's carapace with epoxy. Reflective tape is included to assist with tag retrieval at night. **(B)** An overhead view of the ROTAG affixed to a turtle, indicating the main components of the tag. **(C)** A freely-swimming juvenile green turtle with a ROTAG. Once the burn is complete the tag will release from the plate and float to the surface. The small plastic plate will remain affixed to the turtle until it is worn off.

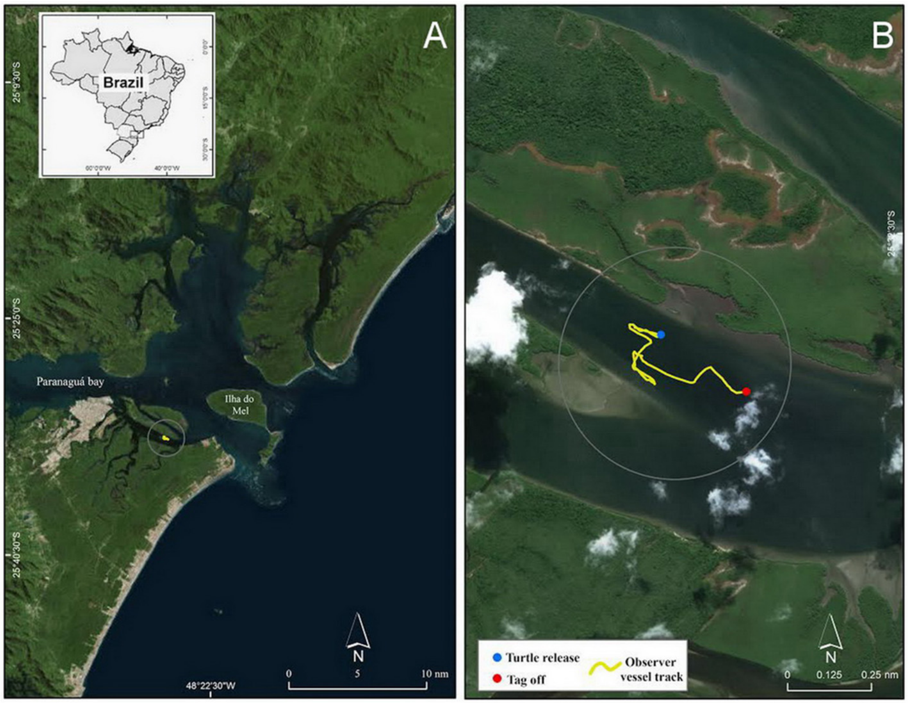

FIGURE 2 | **(A)** Map of Paranaguá Estuarine Complex located off the northern coast of Paraná state, Brazil, and **(B)** an inset showing the turtle release and tag off locations as well as the observer vessel track in Canal da Cotinga.

and curved carapace width was 36.90 cm. Prior to tag attachment, we cleaned the anterior portion of the turtle's carapace of barnacles and other epibionts, sanded it with coarse sandpaper (e.g., 60-grit), and washed it with distilled water, 91% isopropyl, and acetone to increase bonding strength. We applied a steel-reinforced epoxy (Sonic-Weld) to the base of the plastic plate to which the ROTAG was attached and placed the plate on the 2nd vertebral scute for (**Figure 1**). Following SEFSC (2008) protocols, the total weight of the tag plus epoxy used for attachment was less than 5% of the turtle's body weight, minimizing the effects from increased drag and mass. Preliminary analyses by Tyson et al. (2016) using ROTAGs deployed in this manner report that respirations of a turtle before and during a deployment were statistically similar, suggesting minimal behavioral interference from the tags.

We waited ~10 min for the epoxy to dry then released the turtle at 13:23:36 local time (−3 GMT) in Canal da Cotinga, an estuarine area surrounded by mangroves (**Figures 1C**, **2**). We programmed the ROTAG to sample the accelerometer, magnetometer, and gyroscope at 100 Hz and the pressure and temperature sensors at 1 Hz. We recorded sound at 96 kHz with a low gain. We also programmed the ROTAG to turn off the VHF signal when the turtle was ≥10 m and to activate the wire attaching the ROTAG to the fixed plastic plate to start burning 250 min after the tag start time (i.e., just before dusk) to initiate tag release. During the deployment we tracked the turtle via the integrated radio telemetry tags and a VHF receiver and antenna from an observation vessel (26 foot center console vessel with two 150 Hp engines) positioned > 200 m from the perceived turtle's location. As we rarely visually spotted the turtle, these observations consisted primarily of recordings of the time points for which we heard the VHF signal, which was indicative of the turtle being at the surface to breathe. We also recorded other events of interest, such as the presence of any vessels near the perceived turtle's location (i.e., within ~2 km). As scheduled, at 16:50:00 the wire burn was initiated and at 17:04:50 the ROTAG released from the turtle and floated to the surface. Upon retrieval, we downloaded the ROTAG data onto a computer for analysis.

We extracted and calibrated the movement data using custom written code in the R Statistical Program (R Core Team, 2016). We used the pressure and temperature sensor data to estimate depth and temperature, respectively (**Figures 3A,B**). The turtle dove 30 times during the deployment (dives were considered to be any submergence greater than 0.2 meters deep and longer than 5 s). Maximum dive depths (mean (median) ± SD) were 6.22 (6.78) ± 3.01 m, dive durations were 408.53 (256.00) ± 399.04 s, and post-dive surface durations were 22.80 (13.50) ± 23.04 s. Temperature was 21.17 (21.09) ± 0.21°C. The large decrease in temperature seen at the beginning of the deployment record and during surfacings (**Figure 3B**) corresponds to the ROTAG transitioning from air to water temperatures. We subsampled the accelerometer and magnetometer data to 5 Hz and used them to calculate the pitch, roll, and heading of the tagged turtle as well as the vector of the dynamic body acceleration (VeDBA), a proxy for energy expenditure (Qasem et al., 2012; **Figures 3C–F**, respectively). These parameters demonstrated variations in movements while the turtle was diving and minimal movement when the turtle was resting at or near the sea floor.

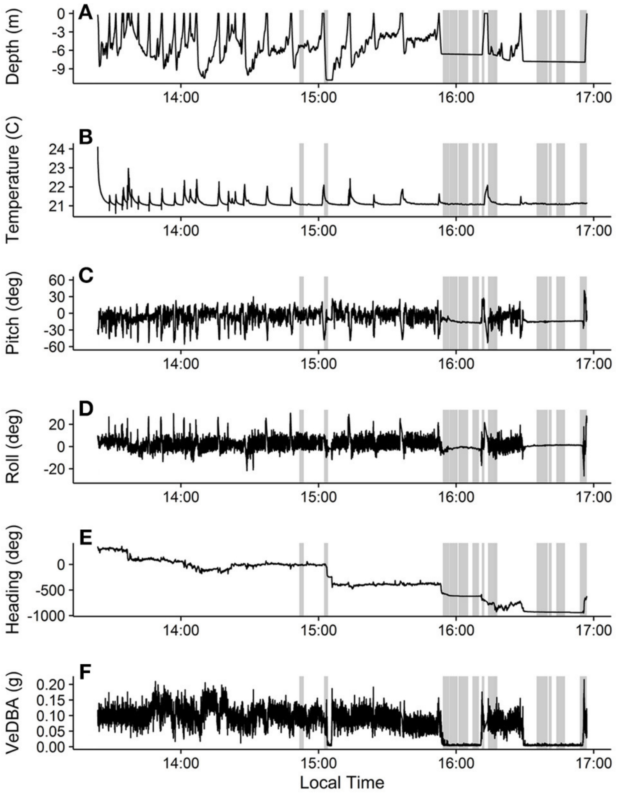

FIGURE 3 | Movement and temperature data collected during the ROTAG deployment on a juvenile green turtle. Depth **(A)** was estimated from the pressure sensor, temperature **(B)** was measured by the temperature sensor, and pitch **(C)**, roll **(D)**, heading **(E)**, and VeDBA **(F)** were estimated from the accelerometer and magnetometer data. Heading data were unwrapped to deal with issues arising from circular data (i.e., crossing 0 and 360) using the unwrap function in the signal package for R (Signal Developers, 2013; R Core Team, 2016). Gray boxes represent approximate times when vessels passing near the turtle were detected on the hydrophone.

The acoustic data from the ROTAG was examined using Adobe Audition® (V1 Build 3211.2) and analyzed using the Fast Fourier Transformation (FFT) with 4,096 points FFT order, Hanning windows, and a 50% overlapping of successive windows. From this examination, we were able to pinpoint the start and end time points of all instances when vessels passed closely by the turtle (estimated distance is <2 km given the location of the observation vessel and surrounding land masses) (**Figure 3**). During many of these instances, the turtle appears to remain still on or near the sea floor. **Figure 4** showcases the movements of the turtle and the corresponding spectrogram of sounds for the dive that occurred from 15:52:36 to 16:12:24. While there is no clear behavioral response between individual boat passes and turtle behavior (despite the slight change in pitch, roll, heading at VeDBA at ~15:56:33), this schematic showcases how useful the information recorded on these tags can be in examining turtle behaviors in response to anthropogenic sound. Spectrum levels of

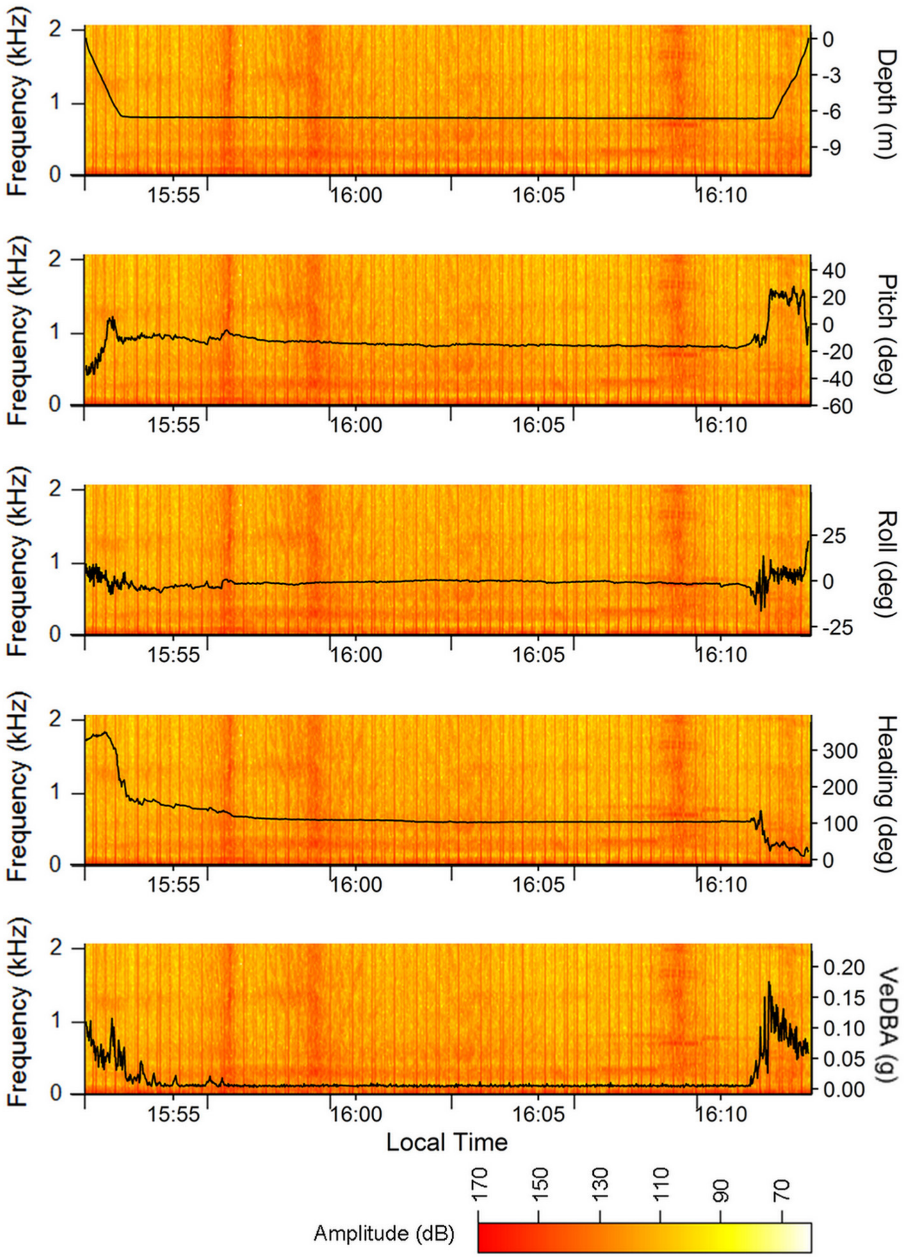

FIGURE 4 | Depth, pitch, roll, heading, and VeDBA of the juvenile green turtle during a dive with multiple vessel passes overlaid onto the simultaneous acoustic record (i.e., spectrogram). The vessel passes are the broadband signals with varying amplitudes and durations found at ∼15:56:28, 15:58:50, 16:03:05, 16:08:44, and 16:10:36. VHF transmitter signals picked up by the audio board are apparent as regularly spaced broadband clicks of consistent sound pressure levels. Spectrograms were created in R using the spectro function in the seewave package for R (Sueur et al., 2008; R Core Team, 2016) with 4,096 points FFT order, Hanning windows, and a 50% overlapping of successive windows.

the vessels (**Figure 4**) were measured and compared to an average audiogram of a green turtle in **Figure 5**.

Spectra for noise level measurements were calculated by isolating 32,768 points in between the VHF pings. A Hanning window was applied to each segment and a FFT calculated (frequency resolution = 2.93 Hz) using MATLAB. Spectrum level was calculated by adjusting for the hydrophone sensitivity, gain, Hanning window (+6 dB), and subtracting 10(log(frequency resolution)). Ten sequential spectra were averaged to calculate an average spectrum level that covered about 5 s of recording time from the original recording (**Figure 5**). While these vessels produce generally broadband signals (**Figure 4**), the turtle may have detected their presence as the spectrum levels of all vessels were above their hearing thresholds, specifically in the 200–400

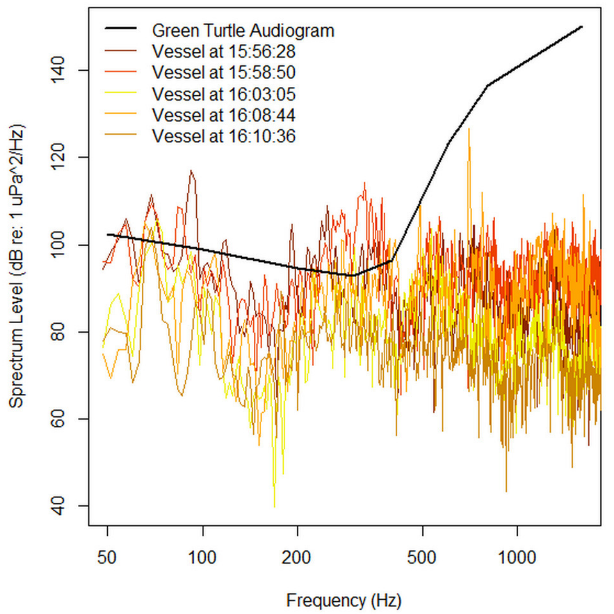

FIGURE 5 | Audiogram of a juvenile green sea turtle (adapted from Piniak et al., 2016 and measured in dB re 1 uPa) and spectrum levels of vessels that passed within ~2 km of the tagged turtle (true distances between vessel and turtle are unknown) between 15:52:36 and 16:12:24 (**Figure 4**). Spectrum levels were measured at the times when the vessels were estimated to be at their closest approach of the turtle.

Hz range. Even if detected; however, the turtle may not have responded because the measured spectrum levels were much lower than those previously shown to illicit behavioral responses in turtles (**Figure 5**; e.g., ≥166 dB re 1 μPa, McCauley et al., 2000). In addition, during the 221 min of the deployment, 13 vessels passed closely by the turtle. Therefore, it is likely that this turtle is either habituated to the sounds produced by these vessels or that they have experienced some level of hearing loss due to their constant presence. More records of vessels near tagged turtles, their approximate distances, and estimates of their spectrum levels are needed to determine if turtles, such as the one presented here, responded to the sounds produced by vessels such as this and if so what this response may be (e.g., remain still on sea floor, surface, swim away).

RO-TAG APPLICATIONS

Marine turtle populations have experienced severe declines globally due to direct harvest, incidental capture in fisheries, and the destruction of foraging and nesting habitats (Lutcavage et al., 1996; Wallace et al., 2011a,b). Because the habitat use of marine turtles overlaps extensively with anthropogenic activities that produce sounds such as fishing, shipping, and coastal and marine development (Van Dolah and Maier, 1993; Wallace et al., 2010; Lewison et al., 2014; Fuentes et al., 2016) it is important to develop tools and techniques that enable us to examine how such activities affect the behavior and physiology of these threatened species. The case study presented here showcasing a successful deployment of a ROTAG on a juvenile green turtle provides proof of concept for using these tags to examine marine turtles in response to sound and demonstrates their utility in examining the general soundscapes of marine turtles. These advancements will increase our understanding of the acoustic threats marine turtles face and can be used to aid in their successful conservation and management.

Marine species such as fish and marine mammals have been observed to respond to anthropogenic sounds produced at construction and demolition sites (e.g., Popper and Hastings, 2009; Buckstaff et al., 2013), by vessels (e.g., Aguilar Soto et al., 2006; Lemon et al., 2006; Jensen et al., 2009; Parks et al., 2011), and from low-frequency sonar (DeRuiter et al., 2013; Goldbogen et al., 2013). At this time, it is unknown whether free-ranging marine turtles respond to such sounds and to what extent their responses may be if present. Given that the sounds produced by many of these activities dominate the frequencies within the range of best hearing for marine turtles (50–500 Hz; Hildebrand, 2009) turtles are particularly susceptible to their presence. The ability to tag a turtle with a tool such as a ROTAG would enable researchers to examine if turtles respond to these types of anthropogenic activities, and if so the type and potential fitness consequences of these responses. For example, Goldbogen et al. (2013) and DeRuiter et al. (2013) used DTAGs, a bio-logging tool from which the ROTAG is based, to examine whether blue whales (*Balaenoptera musculus*) or Cuvier's beaked whales (*Ziphius cavirostris*) responded to mid-frequency (1–10 kHz) active (MFA) sonar. Both species exhibited responses to the MFA sonar: blue whales ceased deep activities, increased their swimming speed, and directed travel away from the sound source (Goldbogen et al., 2013). Cuvier's beaked whales (*Ziphius cavirostris*) responded by ceasing normal fluking and echolocation, and swimming rapidly and silently away from the sound source extending both their dive duration and subsequent non-foraging interval (DeRuiter et al., 2013). Similar insights in behavioral responses of marine turtles to sound can be obtained from the tags presented here (e.g., significant changes in parameters such as dive depth, heading, VeDBA, or dive duration) and could be used to help managers and policymakers to develop appropriate mitigation strategies, enact restrictions or legislation to limit such activities, and guide monitoring programs in regions where marine turtles may be present and vulnerable.

Sound has also been used to warn or repel animals from dangerous areas or activities. For example, one such anthropogenic activity of particular concern to marine turtles is dredging to sustain the demand for sediment (e.g., for beach nourishment projects), and maritime development (e.g., ports) to maintain shipping channels (Dickerson et al., 2004; Sundin, 2007; Goldberg et al., 2015). Dredging is often conducted with hopper dredges, which remove sediment through suction (Banks and Alexander, 1994; Goldberg et al., 2015). The hopper dredge dragheads are slow-moving and nearly silent and can cause physical harm (e.g., injuries, fractures, and hemorrhage) and mortality to marine turtles and impacts to their habitat (Dickerson et al., 1991, 2004; Banks and

Alexander, 1994; Goldberg et al., 2015). Strategies to mitigate impacts of dredging on marine turtles and their habitat include: relocating marine turtles away from dredging sites (Dickerson et al., 2007; Sundin, 2007), modifications to hopper dredges drag heads (Banks and Alexander, 1994), and temporal restrictions to reduce the likelihood of turtle and hopper dredger interactions (Dickerson et al., 2004). Another potential strategy for minimizing interactions of marine turtles with anthropogenic activities, including dredging, is to warn or repel marine turtles from areas where potentially harmful activities are or will take place. Given that marine turtles can detect and respond to low-frequency acoustic stimuli, acoustic harassment devices (AHDs) and acoustic deterrent devices (ADDs) could be used as a strategy to successfully repel marine turtles from various threats, including from areas that are being dredged or potentially high interaction fishing areas (Van Der Meij et al., 2015). The ROTAG could be used to test and determine the effectiveness of various ADDs or AHDs providing insights into further development of these devices and their suitability as mitigation strategies to protect marine turtles.

While little evidence exists that sea turtles use sound for communication, sea turtles may use sound for navigation, locating prey, predator avoidance and environmental awareness (Piniak et al., 2016). It is likely that they, like other vertebrates, use ambient soundscapes to create an auditory scene that can provide them with critical information about their environment and their position (Bregman, 1990; Slabbekoorn and Bouton, 2008). For instance, the sound of waves crashing on a beach could indicate that they were close to shore, or near an appropriate nesting beach. The sounds of chorusing fish or snapping shrimp could provide a signpost that they were near an appropriate foraging area like an estuary or coral reef. Anthropogenic sounds have the potential to mask some of these important soundscape auditory cues. The biological significance of sound for marine turtles is not well understood, and the use of a ROTAG to describe the overall soundscape of turtles could provide interesting and important information regarding their ecology and biology that to date have been hard to obtain.

SUMMARY AND CONCLUSIONS

The case study presented here provides proof of concept that ROTAGS can successfully be applied to free-ranging marine turtles. The information available from these tools can be used in a variety of ways for marine turtle conservation and ecology in particular to determine the response of marine turtles to anthropogenic sound. While these tags are useful for many applications, several limitations still exist at this time. Being high-resolution recording tags, ROTAGs can only be deployed on turtles for a short period of time (i.e., ∼3–7 days depending on user settings). However, the richness and breadth of the data collected by ROTAGs cannot currently be obtained with longer-term bio-logging tags, such as satellite tags or other time-depth recorders, and therefore can be used to enhance our understanding of fine-scale marine turtle behavior, specifically in relation to sound. Another limitation common to all capture/release and tagging studies, the possibility of inducing a capture and/or tagging response by the animal under study, is something that needs to be considered. For example, Thomson and Heithaus (2014) and Hazel et al. (2009) found that some green turtles fitted with animal borne video cameras and TDRs, respectively, were influenced by capture stress for several hours (0–6) after release. In contrast Seminoff et al. (2006) reported that turtles tagged with a Crittercam^{TM} resumed normal activities within minutes of being released. The ROTAG will create drag for the turtle, which may result in modified swimming behaviors. For example, van der Hoop et al. (2014) found that bottlenose dolphins carrying DTAGs swam more slowly than dolphins without DTAGs possibly in an effort to reduce the metabolic cost of drag created from the tags. Drag may also be induced by the plastic plate left affixed to the turtle after the ROTAG is released (Jones et al., 2013), but given the low profile of the plate we anticipate this drag to be minimal. More deployments are needed to further investigate whether a capture or tagging response is elicited with ROTAGS and if so how they can be minimized. Finally, the presence of flow noise recorded by the tags, which increases with decreasing frequency (Burgess et al., 1998; Merchant et al., 2015), may mask sounds of interest within the range of marine turtles best hearing (i.e., <2,000 Hz). In the case study presented here; however, flow noise was typically at frequencies less than 70 Hz (e.g., **Figure 4**) and therefore was minimal.

Given the threatened status of many species and populations of marine turtle, mitigation measures are needed, and often required by laws and regulations, to reduce marine turtle injuries and mortalities associated with these activities (Wallace et al., 2013; Fuentes et al., 2015). Until the threat of anthropogenic sound on marine turtles can be assessed, such mitigation measures may be unproductive in conserving these species. The application of the ROTAG or similar acoustic-movement tags will enable researchers to start addressing this potential threat and to determine in what ways and to what extent anthropogenic sound may impact marine turtles. This information can then be used to more appropriately guide laws and regulations devised for conserving these threatened species. In addition, the broad range of applications the ROTAG presents make it a unique tool for expanding our understanding of the fine-scale movements and behaviors of marine turtles, which may provide new insights into their ecology, such as their foraging behavior, physiology, and predator response.

AUTHOR CONTRIBUTIONS

RT, WP, CD, DN, and MF contributed to the acquisition, analysis, and interpretation of the data as well as by drafting and revising the manuscript. DM and MH designed and built the RO-TAG used in this study and contributed to the analysis and interpretation of the data, as well as by revising intellectual content.

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Use of Particle Tracking to Determine Optimal Release Dates and Locations for Rehabilitated Neonate Sea Turtles

Natalie A. Robson[1]*, Yasha Hetzel[2], Scott Whiting[3], Sarath Wijeratne[2], Charitha B. Pattiaratchi[2], Philip Withers[1] and Michele Thums[4]

[1] School of Animal Biology, University of Western Australia, Crawley, WA, Australia, [2] School of Civil, Environmental, and Mining Engineering, UWA Oceans Institute, University of Western Australia, Crawley, WA, Australia, [3] Marine Science Program, Department of Parks and Wildlife, Kensington, WA, Australia, [4] Australian Institute of Marine Science, Indian Ocean Marine Research Centre, University of Western Australia, Crawley, WA, Australia

Sea turtles found stranded on beaches are often rehabilitated before being released back into the wild. The location and date of release is largely selected on an informal basis, which may not maximize the chance of survival. As oceanic conditions have a large influence on the movements of neonate sea turtles, this study aimed to identify the best locations and months to release rehabilitated sea turtles that would assist in their transport by ocean currents to the habitat and thermal conditions required for their survival. A particle tracking model, forced by ocean surface velocity fields, was used to simulate the dispersal pathways of millions of passively drifting particles released from different locations in Western Australia. The particles represented rehabilitated, neonate turtles requiring oceanic habitats [green (*Chelonia mydas*), hawksbill (*Eretmochelys imbricata*) and loggerheads (*Caretta caretta*)] and flatback turtles (*Natator depressus*) which require neritic habitats. The results clearly identified regions and months where ocean currents were more favorable for transport to suitable habitats. Tantabiddi, near Exmouth on the north-west coast, was consistently the best location for release for the oceanic species, with dominant offshore-directed currents and a very narrow continental shelf reducing the time taken for particles to be transported into deep water. In contrast, release locations with more enclosed geography, wide continental shelves, and/or proximity to cooler ocean temperatures were less successful. Our results produced a decision support system for the release of neonate marine turtles in Western Australia and our particle tracking approach has global transferability.

Keywords: decision support, Leeuwin Current, Western Australia, lost years

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Mariana M. P. B. Fuentes,
Florida State University, United States

Reviewed by:
Nathan Freeman Putman,
University of Miami, United States
Erin Seney,
University of Central Florida, United States
Graeme Clive Hays,
Deakin University, Australia

***Correspondence:**
Natalie A. Robson
nrobson_92@hotmail.com

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INTRODUCTION

Commonly, sick or injured sea turtles are taken into care and where possible are rehabilitated and released back into the wild (Louisiana Department of Wildlife and Fisheries and Audubon Aquarium, 2013; Craige, 2014). For most rehabilitation centers or management authorities, the decision process around the selection of release sites is not well documented. Others use a best available knowledge approach, such as releasing turtles at the location where they were found or where that species and size class are known to occur (Mandelc et al., 2002;

Wallace, 2012; Louisiana Department of Wildlife and Fisheries and Audubon Aquarium, 2013; The Turtle Hospital, 2017). This best available knowledge approach is currently used in Western Australia, but given that all sea turtle species are threatened and the significant level of community labor and expense involved in rehabilitation, a more considered approach using multiple lines of evidence might increase chances of survival for these individuals (Caillouet et al., 2016). Quantitative data that incorporate knowledge of the species, size class and preferred physical environment would help guide effective decisions on release locations and dates.

Post-hatchling and neonate green (*Chelonia mydas*), hawksbill (*Eretmochelys imbricata*), loggerhead (*Caretta caretta*) and flatback sea (*Natator depressus*) turtles commonly strand in Western Australia (Department of Parks and Wildlife unpublished data). They are all listed as either vulnerable or endangered under the Australian Government's Environment Protection and Biodiversity Conservation Act 1999 (Environment, 2014) and as endangered or critically endangered (except the flatback which is data deficient) by the IUCN (IUCN Red List, 2014).

Sea turtles inhabit a range of ecosystems from terrestrial nesting grounds to developmental and foraging habitats in both coastal and oceanic water (Bolten, 2003; Putman et al., 2010; Shillinger et al., 2012). Loggerhead, green and hawksbill turtles follow the same oceanic-neritic developmental pattern (Collard and Ogren, 1990; Putman et al., 2012; Ascani et al., 2016). Early development occurs offshore until reaching a certain size range; 35–40 cm for hawksbill and green turtles (Moon et al., 1997; Hochscheid et al., 2007) and 65–90 cm for loggerhead turtles (Limpus and Limpus, 2003; Ascani et al., 2016). Later development then occurs on the continental shelf (Zug and Glor, 1998; Bolten, 2003; Scales et al., 2011). The Australian flatback turtle has a completely neritic developmental pattern, with no oceanic phase (Walker and Parmenter, 1990; Salmon et al., 2010).

Water temperature is critical to the survival and health of sea turtles with distributions usually limited to a minimum between 15 and 20°C (Coles and Musick, 2000; McMahon and Hays, 2006) but with variation between species. Sea turtles have been shown to experience a reduction in swimming ability in colder water and can cease feeding when they move into water below their minimum temperature range (Moon et al., 1997). Average sea surface temperatures along the Western Australian coast can range between 15 and 30°C, with the lower temperatures occurring between 32 and 36°S (**Figure 1**; NOAA 2015).

Whilst directional swimming is clearly a component of post-hatchling and neonate turtle's migratory paths (Hamann et al., 2011; Putman and Mansfield, 2015; Christiansen et al., 2016), ocean currents strongly influence their movement (Carr, 1987; Polovina et al., 2000; Gaspar et al., 2006; Bentivegna et al., 2007; Okuyama et al., 2009). The proximity of nesting beaches to favorable ocean currents highlights the importance of ocean currents to sea turtles, with higher nest densities found close to currents that promote hatchling dispersal to suitable habitats (Putman et al., 2010; Shillinger et al., 2012; Ascani et al., 2016). Ocean circulation along the WA coast is dominated by the Leeuwin Current system that consists of the southward flowing Leeuwin Current at the surface, underlying Leeuwin Undercurrent, and variable northward wind-driven coastal currents including the Capes and Ningaloo Currents (**Figure 1**). The Leeuwin Current flows contrary to other eastern boundary currents, transporting warm tropical water poleward along the continental shelf break (Cresswell and Golding, 1980; Pattiaratchi and Woo, 2009). This causes the favorable thermal range for turtles to extend to approximately 32°S, further poleward than for most other west coasts globally (Smith et al., 1991; Feng et al., 2003; NOAA, 2015). However, the Leeuwin Current system is highly variable, characterized by meanders and eddies and seasonal and inter-annual changes (Feng et al., 2003; Rennie et al., 2007). As a result, entrainment of rehabilitated juvenile turtles by the Leeuwin Current has the potential to transport the turtles far from their optimal habitat and, when they are expelled from the warm current, they may become stranded in sub-optimal water temperatures. As the strength and direction of currents (including local tidal and wind-driven currents) varies both spatially and temporally (Pearce and Phillips, 1988; Hanson et al., 2005), dispersal patterns of rehabilitated sea turtles should vary with release location and time of year, resulting in different end points and therefore survival rates.

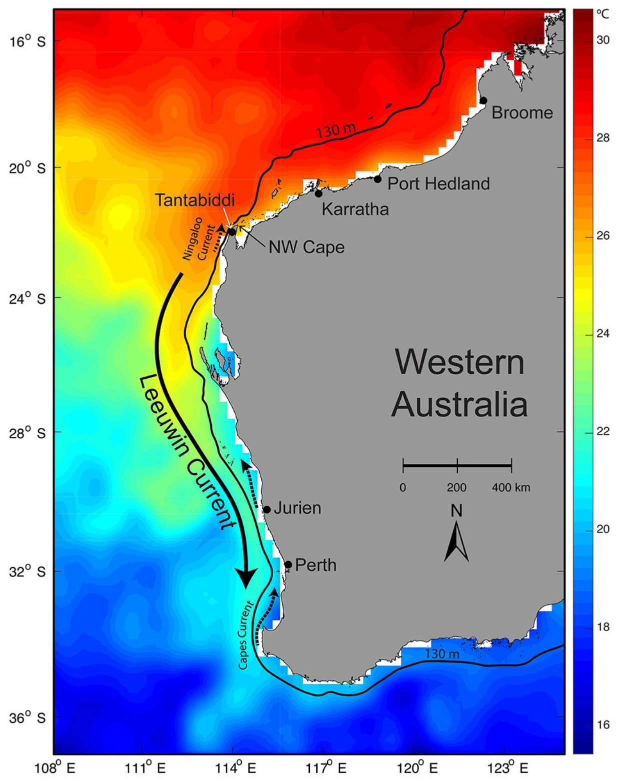

FIGURE 1 | Map of Western Australia showing particle release sites/potential release sites of rehabilitated turtles along the Western Australian coast. Dominant coastal currents are indicated with arrows overlaying NOAA OIv2 sea surface temperature for 17 March, 2010 and 130 m depth contour is shown to indicate the continental shelf edge.

Particle tracking models, driven by hydrodynamic models, are commonly used to determine potential dispersal/drift pathways of marine organisms, pollutants or other objects that can be transported by ocean currents. In a particle tracking model, virtual drifters representing marine organisms or other suspended matter are advected by ocean currents predicted by hydrodynamic models forced by atmospheric hindcasts or forecasts and predicted tides. Some particle tracking models ascribe behavioral attributes to the particles (e.g., swimming, vertical movements, directional cues) whilst others treat the virtual drifters as purely passive objects that are advected by the underlying ocean currents in order to reduce uncertainties, as much behavior is difficult to quantify and validate (Condie and Andrewartha, 2008; Condie et al., 2011; Lynch et al., 2014). For example, turtle movements globally have been extensively studied using particle tracking with ocean circulation models (Okuyama et al., 2011; Putman and He, 2013; Putman and Naro-Maciel, 2013; Proietti et al., 2014). Many of the studies on neonate and juvenile sea turtles use passive drifter models, assuming a minimal swimming influence (Okuyama et al., 2011; Proietti et al., 2014).

In this paper, we used a passive particle tracking model forced by predicted ocean currents and temperature, with particles released at varying times of the year and at multiple locations along the WA coastline (shoreline release of rehabilitated turtles is most common) to assess the influence of ocean circulation on particle trajectories and the potential movements of rehabilitated neonate sea turtles. We assessed each particle as successful or not based on the optimal habitat and thermal conditions required for their species and size-class and modeled success as a function of site, month and year for each species. Our objective was to provide a quantitative and objective approach for the selection the release locations and seasons where the probability of transport by ocean currents to favorable environments would be highest. The results will directly inform management agencies in their timing of release and selection of release sites and the methodology is transferrable to other regions.

METHODS

We used a stepped approach to the problem of maximizing survivorship of rehabilitated turtles which involved:

1. Identifying species and size classes.
2. Identifying potential release sites.
3. Determining depth and temperature boundaries identified for each species and size class.
4. Developing a particle tracking model with an appropriate hydrodynamic model.
5. Developing criteria for particle/turtle success.

Species and Size Class

The size at which turtles utilize the open ocean varies between species (Bolten, 2003; Putman et al., 2010; Shillinger et al., 2012). Success criteria considered whether particles remained in water depths and temperatures that were favorable for each species survival (Coles and Musick, 2000; McMahon and Hays, 2006). Green and hawksbill turtles utilize the open ocean during neonate stages and recruit to inshore neritic habitats at approximately 40 and 30 cm curved carapace length (CCL) respectively (Moon et al., 1997; Hochscheid et al., 2007). Loggerhead turtles spend longer in the open ocean and recruit to neritic habitats across a large size range with the smallest at approximately 65 cm CCL (Limpus and Limpus, 2003; Ascani et al., 2016). Flatback turtles do not have an oceanic stage and remain on the continental shelf at all stages (Walker and Parmenter, 1990; Salmon et al., 2010). Therefore, these models will assist the return of green, hawksbill and loggerhead turtles of small sizes (oceanic life stages) to the open ocean and for the release of flatback turtles of all sizes to remain on the continental shelf.

Potential Release Locations

Turtles strand anywhere along the WA coast, with current rehabilitation centers located in the Perth Region (Bunbury, Rockingham, Hillarys) (Dolphin Discover Centre, 2015; Rockingham Regional Environment Centre Naragebup, 2015; AQWA, 2017), Shark Bay on the mid-west coast (Ocean Park, 2017), and in the northwest at Broome (Chelonia, 2017; **Figure 1**). Turtles are transported to rehabilitation centers by road and air. It is not always appropriate to release turtles at the site of stranding as many have been cold stunned or are far from their preferred conditions. Modeled release locations were selected based on proximity to rehabilitation centers and access for transport of turtles, with remote and unfeasible sites not considered. Although releasing rehabilitated turtles at sites near to rehabilitation centers reduces transport time to the release site, releasing turtles near to the Perth region centers is not recommended as these are located south of 32°S and outside the preferred water temperature of most species and size classes. Previously, the Department of Parks and Wildlife has released rehabilitated turtles at Exmouth, Karratha, and Broome based on logistics and local knowledge of species (**Figure 1**). To test release scenarios, we selected seven proposed release sites (including those previously used) across six regions covering a representative range of habitats (from north to south): Broome, Port Hedland, Karratha, Ningaloo (North West Cape and Tantabiddi), Jurien Bay, and Perth (**Figure 1**). Shark Bay sites were not considered due to limitations of the hydrodynamic model (Hetzel et al., 2013). Please see the Supplementary Material for a detailed description of the dominant physical processes in Western Australian waters that influence these sites.

Ocean Circulation Model

The particle tracking model was forced by surface velocity fields extracted from a hindcast application of the Regional Ocean Modeling System ROMS (http://www.myroms.org/) (Haidvogel et al., 2008). The ROMS hindcast was run without data assimilation for the years 2000–2016 and termed OzROMS (Wijeratne et al., in review). OzROMS is a fully three-dimensional (3D) high resolution circulation model, configured to include the entire Australian continental shelf, slope and the adjacent deep ocean using ROMS. The main advantage of the OzROMS model compared with other coarser resolution hindcasts such as the Bluelink ReANalysis (BRAN) (Oke et al., 2013) or HYCOM (Chassignet et al., 2007) is the inclusion of tides and higher resolution near the coast that includes processes not resolved in coarser models (Putman and He, 2013). Further details and validation of the OzROMS model are given in Wijeratne et al. (in review). Daily mean outputs of current velocity from OzROMS were used to drive the particle tracking model. Please see the Supplementary Material for a detailed description of the ocean circulation model.

Particle Transport Model

We used a particle tracking modeling framework, a commonly applied approach that uses current velocity fields from ocean circulation models, to force a Lagrangian drift model that calculates the trajectories of virtual "drifters" released in the model domain. For this, we used a freely available java tool, ICHTHYOP-3.2 (Previmer, 2010), which was designed to study the effects of physical factors on ichthyoplankton dynamics (Lett et al., 2008). This tool has been used successfully to model dispersal patterns of sea turtles (Proietti et al., 2014; Putman et al., 2016) as well as a range of other marine organisms (e.g., pelagic fish eggs and larvae, Pagán, 2003; Condie et al., 2011).

Simulated surface velocity fields from a high resolution 3-D ocean circulation model for Australia—OzROMS (Wijeratne et al., in review) were used to drive the ICHTHYOP-3.2 particle tracking model (see details below). Particles were "released" at the seven locations along the WA coastline corresponding to potential rehabilitated turtle release sites (**Figure 1**) in different months over a 2-year period (2010–2011). The particles were treated as passive drifters (no swimming behavior assigned), as the aim was to identify where and when ocean currents could act in the turtle's favor, not to investigate actual turtle dispersal patterns as turtle swimming behavior cannot be easily or realistically parameterized in these models, and attempting to do so would introduce unknown errors.

Based on a preliminary analysis of inter-annual variability over the 16 year OzROMS archive and a review of regional oceanography, two representative years were selected for particle tracking simulations. The model was run for 2010, representing conditions similar to a "normal" year, and 2011 to look at the effects of La Niña (Boening et al., 2012). These 2 years represent two contrasting extremes: 2010 was cooler than normal but with an average strength Leeuwin Current, whilst 2011 experienced above average temperatures and a strong Leeuwin Current (Boening et al., 2012). Conditions for other years are expected to be represented within the range of conditions experienced during 2010–2011. Spatial patterns common to both years would have a higher probability of occurring in any given year.

We used the Runge Kutta numerical advection scheme with a time step of 180 s to simulate the transport of particles forced by daily averaged surface velocity fields from OzROMS. The relatively small internal timestep was selected to ensure the particle model did not become unstable when high velocities and small grid cells were encountered. This internal 180 s time-step was paired with a record frequency of 240 s in ICHTHYOP 3.2, so that particle positions were recorded every 12 h. To account for horizontal dispersion caused by turbulent processes not resolved by ocean models, the particle tracking model included a random-walk component parameterized by the horizontal dispersion rate that was set to 1×10^{-9} m^2 s^{-1} following Peliz et al. (2007).

The daily averaged OzROMS surface velocity fields meant that simulated particle trajectories included the residual (i.e., net) effect of tides but did not resolve movements related to individual flood or ebb tidal cycles. This only created limitations near shore in the far north of the region where extreme tides occur and this was taken into consideration when interpreting results.

Please see the Supplementary Material for a detailed description of the main user inputs for the particle tracking model.

Optimal Depth and Temperature Boundaries Identified for Each Species

Depth criteria were based on a literature review of the life cycles of each of the four species. The depth and temperature criteria used in this study may differ to similar studies, as here we used optimal conditions for a release and not mortality (Putman et al., 2012). Particles representing turtles requiring pelagic habitat (all except flatback turtles) were required to leave the continental shelf (>130 m) within 7 days of the release due to the high predation risk on the continental shelf (Bolten, 2003; Bornatowski et al., 2012; Putman et al., 2012). Successful particles representing flatback turtles (shallow water species) were required to stay on the shelf for at least 30 days (<130 m). Sensitivity tests showed that results were insensitive to the number of days chosen for the depth threshold at most sites, with the exception of the NW Cape site where probability of success increased by up to ∼80% for all species when more lenient thresholds were chosen, indicating that results for NW Cape are a lower bound of success estimates.

Water temperature thresholds were set at 20°C for green (Moon et al., 1997; Hochscheid et al., 2007) and flatback turtles (Moon et al., 1997; Coles and Musick, 2000; Mandelc et al., 2002; McMahon and Hays, 2006), 18°C for loggerheads (Lutz et al., 1989; Polovina et al., 2004), and 15°C for hawksbills (Moon et al., 1997; NSW National Parks and Wildlife Service, 2002; McMahon and Hays, 2006). While turtles are able to survive temperatures below the criteria outlined above, and have been known to hibernate at extreme temperatures (Carr et al., 1980; Hochscheid et al., 2007), the temperature criteria outlined are considered optimum for success. Corresponding monthly averaged satellite sea surface temperature data from 1/4° resolution NOAA Optimum Interpolation OIv2 dataset (Reynolds et al., 2002) were used to quantify the temperatures experienced by each particle at each time-step. The monthly averaged temperature data were chosen to remove cloud effects and lessen the chances of a particle being deemed "unsuccessful" if it encountered a small patch of cold water not representative of the surrounding environment. By utilizing satellite SST instead of OzROMS temperatures we eliminated bias that may have been present in the model.

Criteria for Particle Success

Post processing of particle trajectories to determine the "success" of each particle considered that each particle represented a turtle of each of the four species that commonly wash ashore in Western Australia.

The associated temperature and water depth for each particle along the predicted pathways were extracted for each time step and each particle was assessed as passing the criteria or not. Particles were classed as unsuccessful if any of the following occurred:

1. Particles came back on shore (beached).
2. Particles did not leave the shelf (<130 m) within 7 days (green, hawksbill and loggerhead turtles) or particles drifted into deep water (>130 m) within 30 days (flatback turtles).
3. Particles experienced water temperatures below species-specific thresholds.

Data Analysis and Interpretation

In order to determine which sites and months were best, we calculated the proportion of successful particles for each month, site, year, and species, then a suite of generalized additive models (GAM) were constructed for each species using the MGCV (Wood, 2011) library in R (R Core Team, 2014), including all combinations of the individual variables, the two-way interactions and the three-way interaction. Success of particles for each species was the response variable where prior weights were used to give the number of trials (total number of particles in our case) and site, year and month were predictor variables. Month was modeled as a continuous variable whereas site and year were categorical variables. We compared and ranked models using weights of Akaike's information criterion (AIC). AIC weight varies from 0 for no support to 1 for complete support (Burnham, 2002), relative to all models in the set. We also calculated the percent deviance explained as a measure of goodness of fit.

For plotting purposes, we also calculated seasonal means (of all criteria) for the core austral season months [i.e., summer (Jan-Feb), autumn (Apr-May), winter (Jun-Jul), spring (Sep-Oct)]. Particle trajectories were interpolated onto a 0.1° grid and the percent of particles passing through each grid cell and the mean drift time to each cell were determined for monthly and seasonal means of all particle releases.

Based on the analysis of the relative success of the particles, we developed a decision support system for what constitutes a successful release site and time for each turtle species. The sites and months were categorized based on the probability of success. These categories were classified as "Very High" (70–100%), "High" (50–70%), "Medium" (30–50%) and "Low" (1–30%). For neritic species (flatback turtles) the category "Indeterminate" (1–30%) was added instead of the "Low" category, as the model results were considered less reliable in places where many particles beached and the total sample size was small. By definition, neritic species remain near shore, and any oceanic features that act to retain turtles (particles) at the coast would in fact be beneficial to their survival. For example, consistently strong onshore winds during the wet season along the north coast caused large numbers of particles to become "beached" in the model simulations, while in reality the likelihood of "beaching" is much less due to the turtles' ability to swim. Irregardless of swimming ability particles are very unlikely to be transported into deep and/or cold water under these conditions. Therefore, even if quantification of probability of success of neritic species is not possible with this model configuration, qualitative conclusions about regions and times more favorable for neritic species can still be made and results are still useful for management purposes. The lower categories should only be considered if no higher options are available and the turtle needs to be released.

RESULTS

Statistical Analysis

The majority of release sites were not successful, with only Tantabiddi, NW Cape and Jurien Bay showing success in some months for the green, hawksbill and loggerheads, and Broome, Port Hedland, Karratha and NW Cape for flatbacks, so the statistical model therefore only included these sites. There was high variability in success between months, sites and years (**Figure 2**), with the additive model including the three way interaction between site, year and month having majority support for all three species ($wAIC = 1$) and the proportion of deviance explained ranging from 0.61 to 0.66. Out of the three individual factors, site accounted for largest proportion of the deviance explained (0.43 for green turtles, 0.33 for hawksbill turtles and 0.35 for loggerhead turtles). The three way interaction accounted for an additional proportion of 0.24 for green turtles and 0.28 for hawksbill and loggerhead turtles.

Tantabiddi was the most successful site (5.90–99.1%), across all months and years for greens, hawksbills, and loggerheads (**Figures 2m,n,mm,nn**). Seasonal trends were also clear, with a higher probability of success in late summer (January and February) and early spring (September and October) compared to autumn and winter in 2010 (**Figures 2m,n**). The particles released in 2011 showed different seasonal trends for these species, with a higher probability of success in the cooler months, including autumn, winter and early spring (**Figure 2**). For flatback turtles, Broome was the most successful site with a higher probability of success in the cooler months, including autumn, winter (**Figures 2c,cc**) and only minor differences between years. Although the probability of success was higher during the cooler months due to retention of particles on the shelf in warm water in the northern region, a more qualitative interpretation where particles retained at the shoreline is beneficial eliminates the seasonal dependence for success across the north where monsoon winds reverse seasonally. The Perth region (the region supporting most of the rehabilitation centers) had the lowest success as a release site as many particles either beached or were transported into cold water.

Particle Trajectories and Environmental Variables

The differences in particle dispersal pathways (and thus relative success as a turtle release site) between sites and months are explained by local bathymetry, surface current regimes, and winds (**Figures 3**, **4**). The most important factor determining particle success was advection into unsuitable depths (due to beaching and width of the continental shelf) whilst exposure to cold temperatures was secondary (**Figure 5**). This was in part because drift times to reach cold water were often greater than

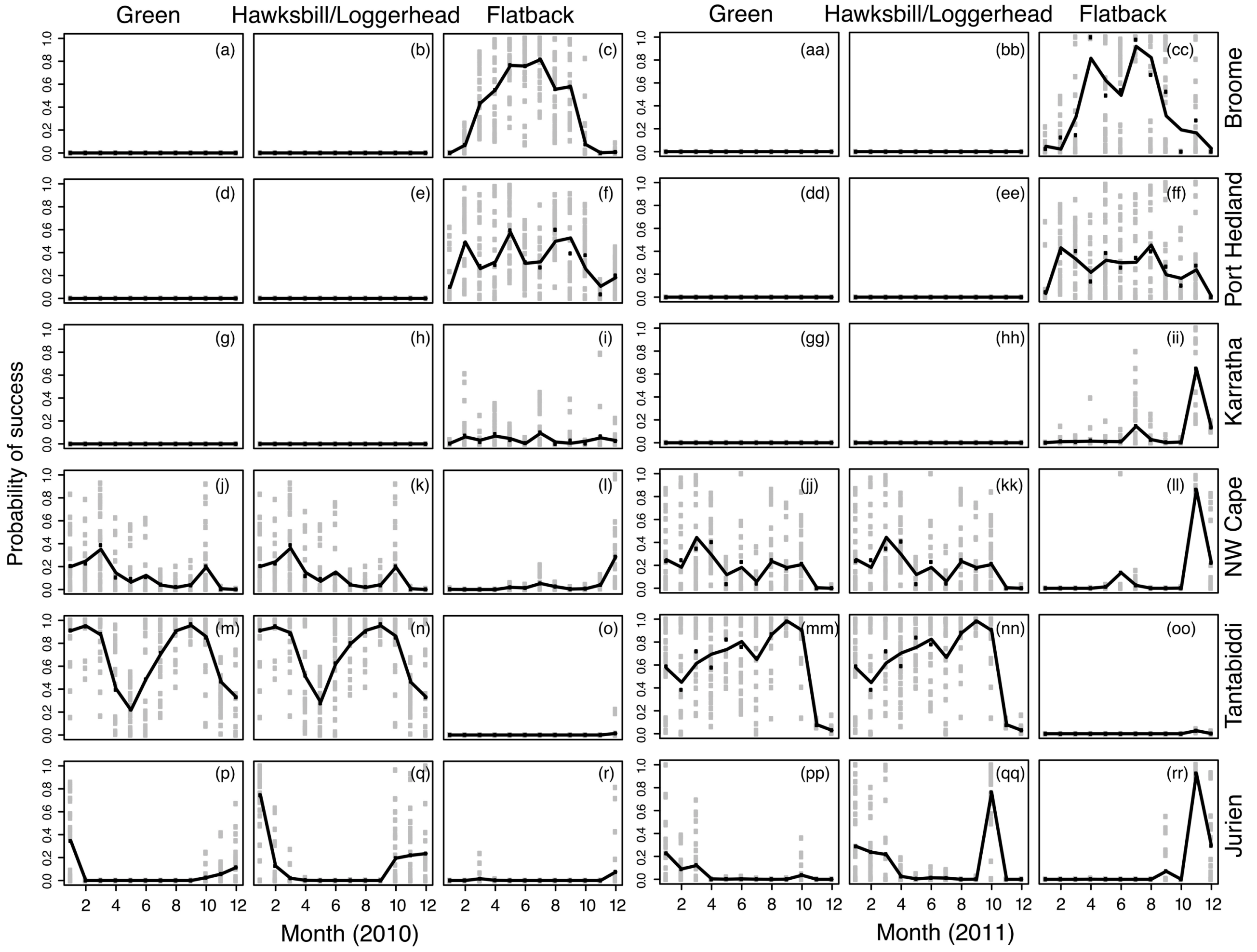

FIGURE 2 | Predicted probability of success (success of each particle is determined by the criteria: temperature, distance offshore, and beaching) from the model used to explain the relationship between particle success and month, site and year for each turtle species in 2010 (a-r) and 2011 (aa-rr). Plots for hawksbills and loggerheads are combined as they had alike results due the similar criteria used for success. Shown in black is the fitted line and gray points are average daily success and black points are mean monthly success.

the 60 day drift duration prescribed to the particles (**Figure 4**). Seasonality was more important at some sites than others, with inter-annual differences less important than site and season. Across all sites, there was a strong link between "success" and the width of the continental shelf—where the shelf was narrow, particles were more likely to end up in deep water and vice versa (**Figures 1**, **4**). In the north (Broome to Karratha), particles generally remained on the shelf due to the wide shelf and weaker residual currents. Along the west coast from NW Cape to Perth, the strong Leeuwin Current and eddies dispersed particles over a much broader area, and further south along the coast (**Figure 3**).

Inter-Site Variability

The strongest determinant of success rates was variability in the time for particles to drift into deep water and beaching. Along the north coast (Karratha-Broome), the mean time to reach deep water was 20–30 days; for Ningaloo sites it was ~10 days; and for the SW sites 10–20 days (**Figures 4**, **5**). Greater than 80% of particles were still in shallow water after 7 days at all sites except Tantabiddi (**Figures 5g,h**), explaining the poor success for oceanic species at most sites. Only at NW Cape, where particles took closer to 14+ days to reach deep water, were success rates increased substantially if the threshold was longer. Correspondingly, near zero counts of particles drifted into deep water within 30 days along the north coast (explaining higher flatback success at sites on the north coast, **Figures 2c,f,i,l**); >50% at Ningaloo sites, and variable rates up to 50% at SW sites (**Figures 5c,d**). Rates of beaching were more variable among sites, but followed similar patterns and ranged from >90% at Karratha and Broome to <5% at Tantabiddi. More than half of all particles beached at all sites except Tantabiddi (**Figures 5g,h**), particularly those sites with enclosed geography such as Perth and Karratha.

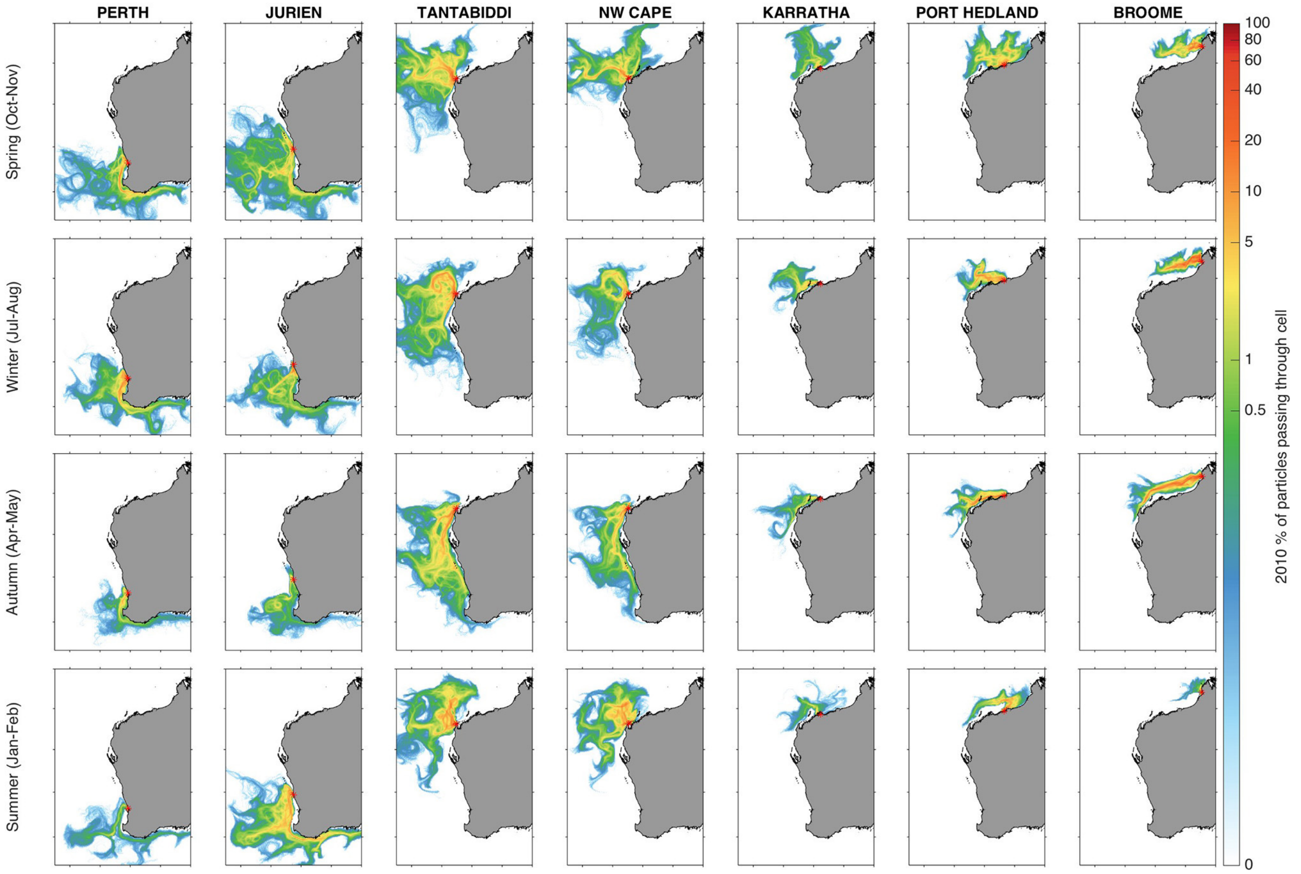

FIGURE 3 | 2010 Seasonal averaged drift trajectories for all seven sites indicating percent of particles passing through each grid cell (seasonal averages of particles released for one month and drifting for 2 months). Red asterisk indicates release location and the color bar is scaled logarithmically to show relevant gradients.

Temperature effects explained less of the success rates than the depth criteria but did show high variability between release sites. Particles released at the southernmost sites were more likely to breach the temperature criteria. At the Perth site the particles were transported south with the Leeuwin Current in all months (**Figure 3**), carrying <5% of the particles into water temperatures <15°C and 20–30% into water below 18°C. Jurien particles experienced similar counts into 18°C water (20–30%), mostly during winter (**Figures 5i–l**). Whilst the particles generally still moved southward with the Leeuwin Current across all months, they also moved further offshore in some months, and occasionally drifted north with the Capes Current during summer and spring (**Figure 3**). Fewer particles experienced water below 20°C at the more northerly sites, reducing from 100% at Perth to <5% at NW Cape. Along the north coast no particles experienced water below 20°C (**Figures 5m,n**), resulting in these sites being best suited for flatbacks requiring neritic habitat and water temperatures >20°C.

Particles released at Tantabiddi, the most successful site for oceanic life stages, were transported in more northerly and offshore directions, with the particles generally advected quickly offshore and remaining offshore for the duration of the drift phase in warm water (**Figure 4**).

Seasonal and Inter-Annual Variability

Along the west coast, drift pathways varied with season, with the particles moving southward and closer to shore in autumn and at the start of the winter, whilst moving northwards and offshore in summer and spring (**Figure 3**). At all sites except Tantabiddi that experienced little seasonality, there was a 5–10 day change in the mean-time to drift into deep water between seasons (**Figures 5a,b**). The pathways from NW Cape followed the same seasonal patterns observed at Tantabiddi (**Figure 3**). However, the particles released at NW Cape took longer to get offshore, with more seasonality, and less direct pathways to deep water, thus influencing their overall success as a turtle release site. Stronger southerly winds that occurred during summer and spring (more so in 2010 than 2011) reduced transport time to deep water (**Figures 5a,b**), improving success for oceanic life stages (**Figures 2j–n**).

Along the north coast of WA at Karratha, Port Hedland, and Broome, the majority of particles took longer to leave the wide continental shelf and were more likely to beach. These pathways were different across seasons, with the particles advected west and south in the autumn and winter months (dry season), while moving north and east in summer and spring (wet season) (**Figure 3**). This region is influenced by SE trade winds during the

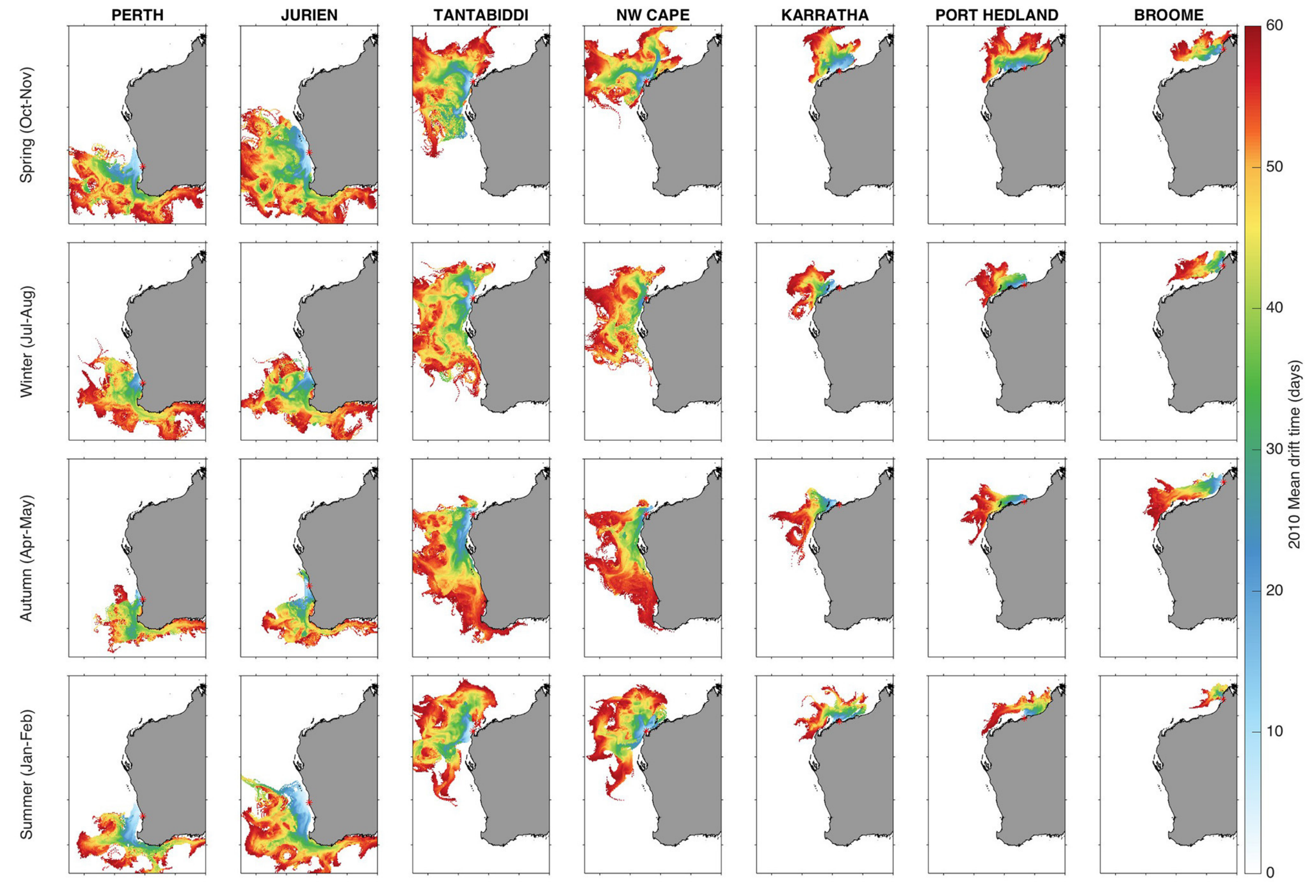

FIGURE 4 | Mean seasonal drift time in days for particles released at seven sites along the Western Australia coast during 2010. Each map shows the mean of ~60,000 individual particle tracks.

dry season and NW monsoon winds during the wet season (See Supplementary Material for more detail). At Broome almost all particles were beached during the wet season, and <20% beached during the dry season when offshore winds advected particles away from the coast (**Figures 5i,j**). Despite the high rates of beaching (and inability to quantify success during the wet season at Broome), a qualitative interpretation that considers beached particles as successful for flatback species, would indicate that flatback success may not be limited to a certain time of the year.

Similarly, in the SW (Perth, Jurien), the highest beaching rates occurred due to onshore currents/winds, except in this case those winds were associated with winter cold fronts approaching from the South Indian Ocean. Generally, cold fronts (and onshore winds) do not extend as far north as Ningaloo and so those sites experienced less seasonal variability and less beaching.

The Leeuwin Current was stronger and water temperature was warmer in 2011 compared with 2010. This warmer water was advected relatively further south and as a result the pathways in 2011 (**Figure S1**) were slightly different compared to 2010 (**Figure 3**). The seasonal patterns were still very similar to those observed in 2010, with the particles moving southward in the autumn and winter months, and moving northwards in summer and spring across most sites (**Figure 3**; **Figure S1**). The only sites that differed substantially between years were Jurien and Tantabiddi. The particles released from Jurien in summer 2011 initially moved further north in the Capes current (**Figure S2**), before being transported south in the Leeuwin Current. During autumn of 2011 at Jurien, particles were ~10 days faster to move off of the shelf (**Figures 5a,b**), and were also less likely to experience water <18°C (**Figures 5m,n**). Likewise, fewer particles released from Tantabiddi in autumn and winter 2011 moved into colder waters (**Figure S1**), compared with 2010 (**Figures 5m,n**). Particles released in summer of 2011 took a longer time to get offshore than in 2010 presumably due to weaker wind conditions during this period (**Figures 5a,b**).

Decision Support System

Based on the results of the particle transport model, a decision support system for when and where to release rehabilitated sea turtles was developed for each species under both 2010 (weak El Niño/ neutral ENSO) and 2011 (strong La Niña) conditions. This system, in the format of a flow diagram (**Figure 6**), was designed to be easily applied by conservation managers. For oceanic species (green turtles less than 40 cm CCL, hawksbill turtles less

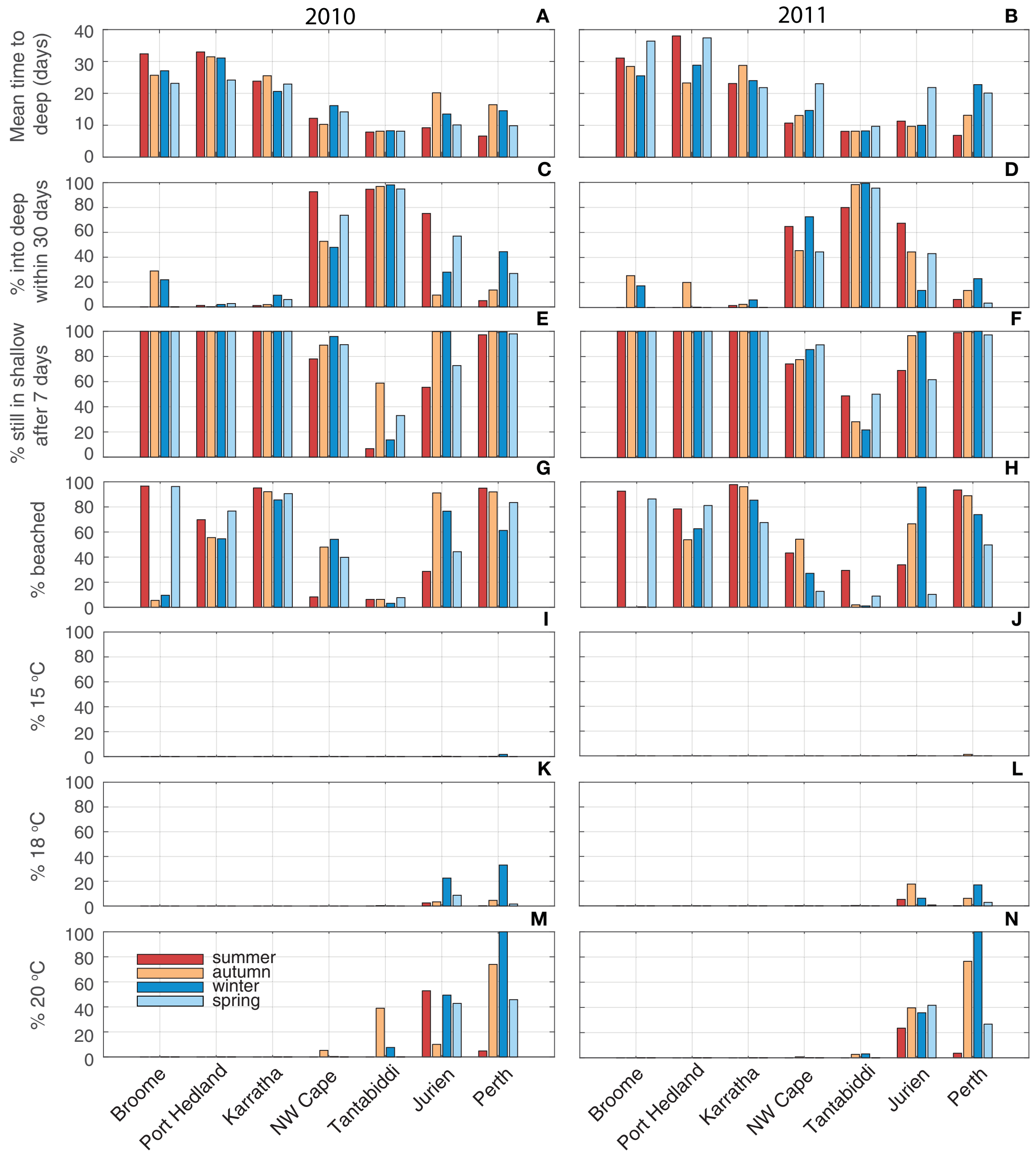

FIGURE 5 | Proportions of particles fulfilling each criteria used to determine success, including 2010 and 2011 seasonal averages for seven sites of **(a,b)** mean time in days for particles to reach the edge of the continental shelf (>130 m depth); **(c,d)** proportion of particles that drifted into deep water (>130 m) within 30 days—if so, flatback species were unsuccessful; **(e,f)** proportion of particles that were still in shallow water (<130 m) after 7 days—if so, deep water species were unsuccessful; **(g,h)** proportion of particles that beached resulting in failure for all species; **(i–n)** proportion of particles experiencing temperature thresholds of 15, 18, and 20°C relevant to each corresponding species.

than 30 cm CCL and loggerhead turtles less than 65 cm CCL) the best release site, under "normal" conditions, is Tantabiddi in summer and winter, and under La Niña conditions the optimal release is from Tantabiddi in autumn, winter and spring (**Figure 6**). Tantabiddi also provides the benefit of providing inshore habitat if turtles in the larger range recruited early to the nearshore habit. For neritic species (flatback turtles of all sizes) the best release site, under "normal conditions" is Broome in winter, followed by the average release sites; Port Hedland in summer, autumn and winter, and Broome in autumn (**Figure 6**). Under La Niña conditions the optimal release site for neritic species is Broome in autumn and winter, followed by the average release sites; Port Hedland in winter and Karratha and NW Cape, in spring (**Figure 6**). A qualitative interpretation that considers

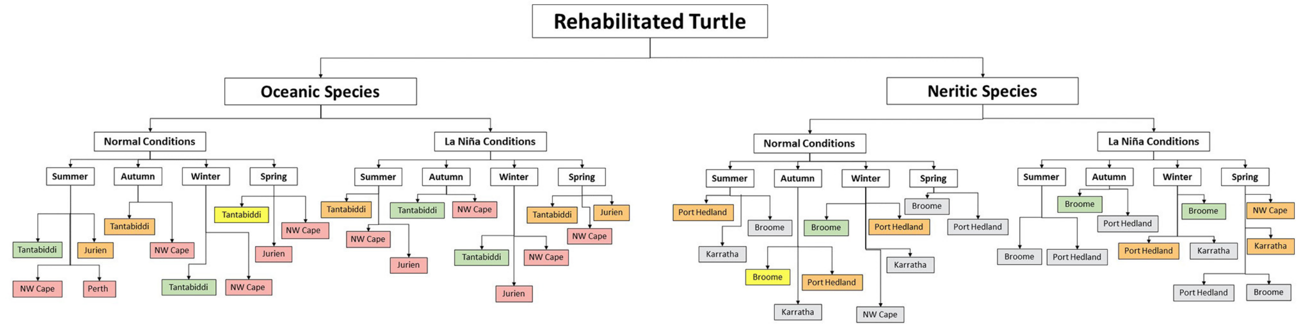

FIGURE 6 | A decision support system to inform the release of rehabilitated sea turtles, with both oceanic and neritic life histories, in Western Australia based on 2010 (normal) and 2011 (La Niña) conditions. The colors indicate the suitability of a site in relation to the percentage of successful particles; green is "Very High" (70–100%), yellow is "High" (50–70%), Orange is "Medium" (30–50%), red is "Low" (<30%) and gray is "indeterminate" (<30%).

beached particles as successful for flatback species, would indicate that flatback success may potentially be higher at many protected sites, particularly along the entire north coast, and may not be seasonally limited. Although Jurien and Perth were found suitable by the model, they were excluded because flatbacks are not commonly found at these locations.

DISCUSSION

Turtle migration and transportation by ocean currents has been widely studied using particle tracking with ocean circulation models, for both adult and neonate turtles, but this study was to our knowledge the first to attempt to use it to provide a quantitative approach to the selection of release sites and timing for rehabilitated sea turtles. We found that release site, year, and month were important parameters in explaining the success of released particles and therefore these factors will all be important in influencing the success of released, rehabilitated sea turtles to oceanic habitat. Within Western Australia, Tantabiddi, near Exmouth, is the optimal site to release oceanic life-stage green, hawksbill and loggerhead turtles. Whilst turtles are not passive particles and have the ability to influence their own pathway and orientation, we have identified the release sites and seasons that will result in the most favorable oceanic conditions for rehabilitated turtles to be released at, with the highest probability of being transported to the correct habitat and thermal conditions required for them to survive.

The higher success of particles released in 2010 in the summer and spring months, compared to the autumn and winter months, could be due in part to the changing strength of the Leeuwin Current. The current flows weakly against maximum southerly winds in October to March, and is stronger in April to September due to the absence of dominant southerly winds and other factors (Godfrey and Ridgway, 1985; Woo et al., 2006; Ridgway and Godfrey, 2015). This caused more particles to be transported south in autumn and winter into cooler waters resulting in lower success. Along the Western Australian coast, southerly winds are prevalent in spring and summer; stormy conditions with no prevailing wind direction in winter; and, generally weaker winds during autumn (Verspecht and Pattiaratchi, 2010). The winter storms usually last 1–2 days and are related to the passage of frontal systems, when the region is subject to strong winds from the north-west, which rapidly changes direction to the west then south-west over 12–16 h, gradually weakening over the subsequent 2–3 days. These wind patterns likely affected the success of particles. The generally weaker winds in autumn and winter storms caused more particles to be blown onshore or become beached or remain on the shelf for a longer period of time, whilst the southerlies in summer and spring could prevent the beaching of particles. The sea breeze system is superimposed on the larger scale atmospheric circulation in the region during summer months and tends to dominate water movements near shore (Pattiaratchi et al., 1997). In this system, differential heating of the land and ocean causes a diurnal cycle of offshore directed winds at night and during the morning and strong onshore directed flow during the afternoon when land heats up. Although the particle tracking model provides an estimate of the net movement on the daily time scale, improved success could be achieved if turtles were released during favorable weather conditions when local winds blow offshore and assist their migration offshore.

Particles released in 2011 had higher success in the autumn and winter months than those released in 2010, particularly at the Ningaloo sites. The differences between the years could be attributed to the effects of La Niña (Boening et al., 2012). The 2010/11 La Niña resulted in a stronger Leeuwin Current that intensified earlier than normal in February (Pearce and Feng, 2013). This had the largest impact on the success of particles in the autumn and winter months when the Leeuwin Current is already seasonally strong (Godfrey and Ridgway, 1985; Woo et al., 2006). The particles released in summer in 2010 had a greater percentage of survival than those released in summer 2011. In summer and spring southerly and easterly winds are more prevalent (Bureau of Meteorology, 2014), assisting the transport of particles offshore. However, in 2011 lighter winds were experienced (Boening et al., 2012), which could have resulted in the lower success in summer 2011. The stronger than normal LC during summer in 2011 could have also contributed to this, but it is difficult to determine the exact cause owing to the high number of factors involved.

This inter-annual variability in ocean current velocity and particle dispersal patterns has previously been shown in other particle tracking studies (Hays et al., 2010). This variability between dispersal patterns indicates the importance of particle tracking, for turtle releases and other ocean migration and connectivity studies. These studies can be done using past data from years with similar oceanographic and weather conditions or by using real-time forecasts of ocean currents and the same particle tracking methodology applied here. Comparing those results to what we presented here would be the best approach. In addition to this inter-annual variability, seasonal current bifurcation was observed at most sites. In general, northward coastal currents are restricted to summer and spring seasons with dominant southward flow in autumn and winter. These seasonal drift patterns have been shown to influence hatchling dispersal and subsequent adult migration routes (Scott et al., 2014). This current divergence could explain the different migratory paths taken by nesting female sea turtles (Hays et al., 2010; Whittock et al., 2014). Particle tracking can therefore be used to assist in the prediction of migration patterns of adult turtles, based on the year and season that they hatched, as well as hatchling dispersal patterns.

One of the main criteria for the success of the three oceanic species was being transported off the continental shelf within 7 days and a very high percentage of particles released at Tantabiddi achieved this. This could be attributed to the narrow continental shelf in this area which is the narrowest on the entire Australian mainland (Woo et al., 2006), meaning that particles were likely to be transported off the continental shelf faster at Tantabiddi due to a shorter distance compared to the other sites. Previously the majority of turtles rehabilitated in Western Australia have been released from NW Cape, with very little success. Our study indicated that particles released at NW Cape took longer to move offshore into deep water resulting in lower success (below 40% across all seasons in both years). Over 50% of particles released from NW Cape took more than 7 days to leave the continental shelf across all months and years. Sensitivity tests indicated that increasing the number of days allowed for particles to move into deep water could dramatically improve success at NW Cape, indicating that our results were somewhat sensitive to the exact time threshold chosen. Previous studies have found a strong correlation between nest density and the distance to favorable currents (Putman et al., 2010). Shorter distances offshore will increase the likelihood of hatchlings reaching favorable currents and suitable habitats, as well as decreasing the risk of predation (Putman et al., 2010). This supports our results and favors Tantabiddi over the NW Cape as the optimal release site for rehabilitated neonate turtles (**Figure 4**). Targeting release dates to favorable local conditions such as strong southerly or easterly winds and an outgoing tide to assist turtles offshore could improve success compared to the non-targeted approach assessed here. However, given the proximity of the Tantabiddi site, it would make more sense to release turtles there, where they have higher chances of success. Whilst our results showed that many of the release sites tested here resulted in zero success, it is important to remember that we did not account for turtle swimming ability in our particle tracking model, so that actual success of real rehabilitated turtles could be higher. Success at some sites could be increased slightly by releasing further offshore and subsequently reducing the likelihood that the particles (turtles) would beach. However, releasing further offshore would not change the general advection patterns and further distances may not be feasible for rehabilitation centers.

The particles released from Jurien in 2010 were more successful in summer. This could be due to the weaker Leeuwin Current at this time of the year and/or the existence of a northward flowing Capes Current (Godfrey and Ridgway, 1985; Woo et al., 2006). With a weaker Leeuwin Current, less particles were transported south into cooler water. However, in 2011 the Leeuwin Current flowed stronger in summer and transported more particles south (Feng et al., 2003; Boening et al., 2012). This indicates that during a "normal" year, Jurien could be considered for release of turtles in summer, although the probability for success is much lower than at Tantabiddi.

Flatback turtles need to remain on the continental shelf and in warm water, above 20°C (NSW National Parks and Wildlife Service, 2002; McMahon and Hays, 2006). Theoretically, Broome is an ideal release site as there is a wide continental shelf and warm water. However, becoming beached was a major cause of failure for flatback turtles at the Broome site, particularly in summer (wet season) when winds were generally from the west, so the highest rates of success would likely be achieved during the dry season. In reality, success would likely be higher across all sites and seasons than the models predict, as turtle's swimming ability would decrease the likelihood of beaching (Carr, 1987; Polovina et al., 2000; Bentivegna et al., 2007; Okuyama et al., 2009). While the model does not accurately give the probabilities for success for flatback turtles in some regions due to high beaching rates, the results do indicate where currents act to retain particles nearshore, which is ideal for this species. High levels of beaching can therefore be interpreted as favorable for flatback success.

As our aim was to identify where and when ocean currents could act in favor of rehabilitated, released turtles, we purposefully did not account for turtle swimming ability so as to not introduce unknown errors into our modeled dispersal pathways. As we have suggested, the low probability of success that we predicted for flatback turtles due to particle beaching and the zero probability of success for the oceanic species at some sites, might not be realistic in relation to real turtles given that their swimming behavior would clearly influence the modeled pathways. This would be especially true for the larger size classes and our modeled pathways might be more representative of small neonates around <20 cm CCL and for species requiring oceanic habitat (Bolten, 2003). We acknowledge this limitation but argue that our approach has met the stated objective of informing management agencies in the selection of the *best* times and sites for release and our approach would be equally successful in other areas around the world needing decision support for the release of rehabilitated turtles. Another important factor influencing these results is the large tidal influence in north

Western Australia. The OzROMS model output used to drive the particle model consisted of daily velocity fields rather than hourly velocity fields. This is not an issue when the particles are released further offshore, as the path the particles follow is very similar. However, when the particles are released closer to shore, the trajectories resulting from hourly velocity fields are much more variable at short time scales than those computed from the averaged currents. Resolving instantaneous tidal velocities in the particle tracking model, however, would further compound issues related to beaching and would require more complex particle behavior that introduces yet more uncertainty in the results. The results from this study can therefore be considered conservative estimates for Broome, Karratha, and Port Hedland, as these are sites with larger tidal ranges and more enclosed topography. However, since the particles were released offshore and the analysis identified robust general patterns across sites and seasons, we are still confident of the recommendations we provide here. Due to the strong tidal currents at these northern sites, it is important to release flatback turtles at high tide and at a greater distance from shore to prevent beaching or becoming unnecessarily fatigued.

Turtle rehabilitation is often community driven with dedicated groups and individuals spending their own time and money to care for sick and injured individuals. The value of rehabilitation is not only through the release of a healthy individual into the population, but extends to community education and capacity building, scientific information on threats through a compilation of stranding events and through general community support to sea turtle conservation. The final step in the rehabilitation process is the release of the individual into suitable habitat (Caillouet et al., 2016). This study provides a quantitative process to assist conservation decision makers to select release sites and dates to provide these rehabilitated turtles the best survival chance post-release. For each release, logistical factors must also be considered which include a consideration of the transport options available (e.g., air or road), minimizing overall travel time between rehabilitation center and release site and staff support at both ends. For individuals requiring oceanic habitat, releasing the turtles offshore using a vessel should always be considered.

CONCLUSIONS

Analysis of more than 3,000,000 simulated particle trajectories for seven sites over 2 years indicated that Tantabiddi, near Exmouth on the Pilbara Coast is the optimal site to release rehabilitated oceanic life stage green, hawksbill and loggerhead turtles in Western Australia. The best time of year for a release depends on the weather conditions, including ENSO variability. To increase a turtle's chance of survival, it is recommended that they are released at high tide, and preferably with easterly winds to assist their transport offshore. Our study is, to the best of our knowledge, the first to have used particle tracking models to determine the optimal release time and location for rehabilitated turtles. This method is preferable to the ad hoc approach used currently, as it allows for a quantitative approach for selecting release sites and times based on the oceanography—one of the main drivers of their movement. Importantly, this project provides objective information to guide conservation management decisions and protocols within Western Australia and provides a useful approach to assist with release decisions for rehabilitated turtles around the world.

ETHICS STATEMENT

Animal ethics approval was not required for this study, no animals were used during any process of this research.

AUTHOR CONTRIBUTIONS

NR originally wrote this manuscript as an honors thesis. She did all of the particle tracking modeling and all of the writing for the manuscript, as well as contributing to the experimental design, graphs and statistics. YH contribution to this manuscript was the analysis of the particle tracking data, as well as providing edits and advise throughout the writing of the manuscript. SWh presented the original research idea and experimental design, he also advised and edited throughout the research and writing process. SWi provided the ozROMS data for the ocean current model, he also assisted with final manuscript edits. CP assisted with obtaining the ozROMS ocean circulation model; and also contributed to the experimental design. PW was a supervisor for the original honors thesis. He provided edits and advise throughout the research and writing process. MT contributed to the experimental design and conducted the statistical analysis. She also provided edits throughout the writing process.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fmars.2017.00173/full#supplementary-material

Figure S1 | 2011 Seasonal averages of drift trajectories for all seven sites indicating percent of particles passing through each grid cell. Red asterisk indicates release location and colorbar is scaled logarithmically to show relevant gradients.

Figure S2 | Mean seasonal drift time in days for particles released at seven sites along WA coast during 2011. Each map consists of the mean of 60,000 individual particle tracks.

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frontiers in Marine Science

REVIEW
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Advances in the Application of Genetics in Marine Turtle Biology and Conservation

Lisa M. Komoroske[1], Michael P. Jensen[1], Kelly R. Stewart[1, 2], Brian M. Shamblin[3] and Peter H. Dutton[1]*

[1] Marine Mammal and Turtle Division, Southwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, La Jolla, CA, United States, [2] The Ocean Foundation, Washington, DC, United States, [3] Daniel B. Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA, United States

Marine turtles migrate across long distances, exhibit complex life histories, and occupy habitats that are difficult to observe. These factors present substantial challenges to understanding fundamental aspects of their biology or assessing human impacts, many of which are important for the effective conservation of these threatened and endangered species. The early development and application of genetic tools made important contributions to understanding marine turtle population and evolutionary biology, such as providing evidence of regional natal homing by breeding adults, establishing connectivity between rookeries and foraging habitats, and determining phylogeography and broad scale stock structure for most marine turtle species. Recent innovations in molecular technologies, statistical methods, and creative application of genetic tools have significantly built upon this knowledge to address key questions in marine turtle biology and conservation management. Here, we evaluate the latest major advances and potential of marine turtle genetic applications, including improved resolution and large-scale syntheses of population structure, connectivity and phylogeography, estimation of key demographic rates such as age to maturity and operational or breeding sex ratios, insight into reproductive strategies and behavior, and assessment of differential human impacts among populations. We then discuss remaining challenges and emerging capabilities, such as rapid, multiplexed genotyping, and investigation of the genomic underpinnings of adaptive variation afforded by high-throughput sequencing technologies.

Keywords: sea turtle, population genetics, wildlife genomics, management units, conservation management

Edited by:
E. Christien Michael Parsons,
George Mason University,
United States

Reviewed by:
Gail Schofield,
Deakin University, Australia
Antonios D. Mazaris,
Aristotle University of Thessaloniki,
Greece

***Correspondence:**
Lisa M. Komoroske
lisa.komoroske@noaa.gov

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INTRODUCTION

Complex behaviors and life histories have long made it challenging to assess key biological parameters and human impacts in marine turtle species. Long distance migrations between breeding and foraging grounds, differential use of neritic, oceanic, and terrestrial (beach) habitats across life stages, and divergent patterns among populations require researchers to creatively develop a wide diversity of approaches to answer even seemingly straightforward questions for these threatened and endangered species (Wyneken et al., 2013). After decades of multidisciplinary, collaborative research, a striking body of knowledge has been synthesized in marine turtle biology, yet some key questions remain unresolved (reviewed in Hamann et al., 2010; Rees et al., 2016). In parallel with innovations in other fields (e.g., animal telemetry and population modeling), advances in molecular genetics continue to play a central role in furthering our understanding of marine turtle biology.

Early studies using genetic tools in marine turtle research provided key evidence of regional natal homing by breeding adults, established connectivity between rookeries (i.e., nesting colonies) and foraging habitats, and defined phylogeography and broad scale stock structure for most species (reviewed in Jensen et al., 2013a). Recent innovations in molecular technologies and statistical methods, improved sampling efforts, and the creative application of genetic tools (**Box 1**) have significantly built upon this knowledge. Importantly, advances in marine turtle genetics include not only studies investigating population genetics or phylogeography directly, but also those leveraging genetic tools to quantify other biological parameters that are challenging to measure, such as age to reproductive maturity. Here we evaluate the latest major advances in marine turtle genetics (and now genomics) to address key remaining gaps in knowledge, identify remaining challenges, and highlight emerging developments with future applications. Rather than provide an exhaustive review of all genetic studies conducted, we focus on key and forthcoming advances as well as practical guidance, generating a valuable resource for marine turtle biologists that conveys how genetic tools may be used to address a wide diversity of evolutionary, ecological, and conservation management questions.

EVOLUTIONARY HISTORY AND PHYLOGEOGRAPHY

The taxonomy of marine turtles is now well-established based on both nuclear and mitochondrial genes (Naro-Maciel et al., 2008; Duchêne et al., 2012), yet population genetic and phylogeographic studies continue to reveal complex population structuring within each species. Such studies inform both broad scale contemporary patterns of geographic variation, as well as inferring historic patterns that led to the current distribution of genetic variation. Perhaps the most important advances to this field have been made through expanded sample collections that provide key insights informing designation of units to conserve on a global scale, such as Evolutionary Significant Units (ESUs), Distinct Population Segments (DPSs), and Regional Management Units (RMUs; see **Box 2**, **Table 1** for definitions and applications of conservation units). As sample coverage continues to improve for all species in parallel with better analytical tools, marine turtle studies continue to explore questions of past glacial refuges (e.g., Hamabata et al., 2013; Naro-Maciel et al., 2014a), colonization routes (Dutton et al., 2014a; Shamblin et al., 2014; Gaos et al., 2016), and multiple colonization events that create such complexity in marine turtle phylogeographic patterns (e.g., Dethmers et al., 2006; Vargas et al., 2016). The field of marine turtle phylogeography will undoubtedly continue to advance in coming years with the development of genomic approaches and novel analytical tools. By offering insight into evolutionary history and patterns of biodiversity over geologic timescales, phylogeographic studies provide important context for effective conservation management. However, in accordance with the aims of this review, below we principally focus on describing the contemporary distribution of genetic variation within species that is directly used in marine turtle management and conservation frameworks.

DEFINING POPULATION BOUNDARIES AND CONNECTIVITY

How Are Rookeries Connected to Each Other and Linked to Foraging Grounds?

Defining biologically relevant population units for monitoring and management is an important first step in conservation because different populations may have distinct habitat and ecological needs, be subject to differential threats, and be under different geopolitical and management jurisdictions. Given the complex life cycle of marine turtles, it's also essential to understand the spatial ecology of individuals representing each of these populations, or stocks (see **Box 2**, **Table 2**), as they disperse and migrate through oceanic and neritic foraging habitats. Because it is difficult to make direct observations to define population boundaries and migratory connectivity of marine turtles, genetic studies have played a pivotal role in achieving these goals.

Two key criteria for accurate assessment of population structure are: (1) sufficient power of the molecular markers to resolve differentiation present among populations, and (2) comprehensive and representative sampling of individuals and rookeries in each region to facilitate precise estimates of the fine-scale genetic differentiation among rookeries. Ideally, the latter includes genetic sampling of males as well as females at nesting grounds because nuclear gene flow via males represents the upper geographical scale of a population (NRC, 2010). These criteria are also important prerequisites for accurately assigning foraging turtles to their respective nesting populations to quantify connectivity. Thus, while early research delineated numerous major population boundaries, the recent development of high resolution markers and the expansion of worldwide sampling efforts have significantly improved our capacity to assess fine-scale population structure and connectivity (see **Box 1**). To illustrate these advances, here we focus on recent progress in green turtles, where these approaches have been particularly fruitful in resolving global stock structure and foraging ground connectivity (see Jensen et al., 2013a for a recent comprehensive review of stock structure for each species).

Global Green Turtle Stock Structure: What Have We Learned from Increased Sampling Efforts?

Nearly three decades ago, mitochondrial DNA (mtDNA) restriction site analysis of western Atlantic green turtles provided the first genetic evidence supporting the hypothesis of natal

TABLE 1 | Examples highlighting how genetic tools have been used to address key research questions in marine turtle biology and conservation.

Research question	Approaches/genetic tools	Examples
POPULATION BOUNDARIES AND CONNECTIVITY		
• How isolated or connected are rookeries from one another through the exchange of nesting females?	Population structure analyses of nesting females across regions of interest (mtDNA haplotypes and/or fine-scale nuclear markers[a])	Examples since comprehensive review in Jensen et al. (2013a) include: Dutton et al. (2014b), Shamblin et al. (2014), Vargas et al. (2016), Gaos et al. (2016), and Matsuzawa et al. (2016)
• How does male-mediated gene flow influence population structure among rookeries?	Population structure analyses, various methods including: sampling nesting females, hatchlings and/or males in-water at breeding grounds (fine-scale nuclear markers; mtDNA haplotypes under specific circumstances[b])	Bagda et al., 2012; Dutton et al., 2013; Roden et al., 2013
• Which nesting stocks use which foraging grounds?	Mixed stock analysis (mtDNA haplotypes) and/or assignment testing (fine-scale nuclear markers)	Stewart et al., 2013; Read et al., 2015; Jensen et al., 2016b; Shamblin et al., 2017
• How do environmental factors influence population connectivity?	Combining genetic analyses with oceanographic modeling of key abiotic factors (e.g., ocean currents or temperature)	Putman and Naro-Maciel, 2013; Naro-Maciel et al., 2014a, 2017
EVOLUTIONARY HISTORY AND PHYLOGEOGRAPHY		
• How are marine turtles species related?	Phylogenetic analyses across marine turtle species (mtDNA and/or nuclear markers)	Bowen et al., 1993; Dutton et al., 1996; Naro-Maciel et al., 2008; Duchêne et al., 2012
• What historical patterns have led to current patterns of diversity and population structure?	Phylogeographic analyses within marine turtle species (mtDNA and/or nuclear markers)	Dethmers et al., 2006; Reis et al., 2010; LeRoux et al., 2012; Dutton et al., 2014a; Naro-Maciel et al., 2014b; Vargas et al., 2016
LIFE HISTORY		
• How old are turtles when they start to reproduce?	Genetic CMR and relatedness analyses (fine-scale nuclear markers)	Dutton et al., 2005; Dutton and Stewart, 2013
• How can we assess the male component of the population?	Paternal genotype reconstruction to assess OSRs/BSRs (fine-scale nuclear markers)	Wright et al., 2012b; Phillips et al., 2013; Stewart and Dutton, 2014; Tedeschi et al., 2015
• What reproductive strategies and behaviors do turtles use?	Maternal genetic ID; Multiple paternity analysis (fine-scale nuclear markers)	FitzSimmons, 1998; Shamblin et al., 2011; Stewart and Dutton, 2011; Frey et al., 2014; Phillips et al., 2014; Sari et al., 2017
HUMAN THREATS AND IMPACTS		
• How are populations impacted by threats away from nesting grounds?	Mixed stock analysis (mtDNA) and assignment testing (fine-scale nuclear markers)	LaCasella et al., 2013; Clusa et al., 2016; Stewart et al., 2016
• How do recent population declines affect resilience?	Analyses to detect population bottlenecks/diversity loss (mtDNA haplotypes and/or nuclear markers)	Carreras et al., 2007; Rodríguez-Zárate et al., 2013; Gonzalez-Garza et al., 2015
• How are marine turtles affected by habitat alterations?	Physiological responses to abiotic conditions (mRNA/transcriptomic assays); Tracking M:F foraging ground rookery origins over time (mtDNA haplotypes and/or nuclear markers)	Gomez-Picos et al., 2014; Tedeschi et al., 2016; Bentley et al., 2017

We focus primarily on examples from recently published articles, and note that there are many ongoing studies that will soon add to our knowledge for the questions listed. Additionally, there are numerous other applications that have been used in other wildlife taxa not yet realized for marine turtles that may be helpful to guide future research (see Remaining Challenges and Future Directions Section).

[a] *E.g., panels of informative microsatellite and/or SNP markers.*

[b] *mtDNA haplotypes may be informative in specific cases, such as sampling in-water males in breeding areas within regions where there is clear differentiation between rookeries based on nesting female haplotypes.*

TABLE 2 | Summary of the current status of different conservation unit designations for each marine turtle species.

Species	Region	RMU	DPS	MU	IUCN sub-population	Notes and references
Green	Pacific	7*	6**	29	1a****	Sampling coverage: Atlantic is considered good; Pacific has recently improved but some gaps remain; major gaps remain in the Indian Ocean. DPS information from Seminoff et al. (2015)
	Atlantic	5	2	20		
	Mediterranean	1	1	6		
	Indian	4	3**	14		
Loggerhead	Pacific	2	2	4	2	Sampling coverage: Atlantic and Pacific are considered good; gaps remain in the Indian Ocean. Conant et al. (2009), USFWS NOAA (2011), Shamblin et al. (2014), Casale and Tucker (2015) and Matsuzawa et al. (2016)
	Atlantic	3	3	17	3	
	Mediterranean	1	1	10	1	
	Indian	4 (1 is putative)	3	3	4	
Leatherback	Pacific	2	tbd	3	2	Sampling coverage: Atlantic and Pacific are considered good; coverage of nesting in the Indian Ocean is limited. Dutton et al. (2013) and Wallace et al. (2013)
	Atlantic	3	tbd	7***	3	
	Indian	2	tbd		2	
Hawksbill	Pacific	6 (4 are putative)	tbd	9	tbd	Sampling coverage: Atlantic is limited; Pacific and Indian Oceans have recently improved but major gaps remain. LeRoux et al. (2012), Trujillo-Arias et al. (2014), Vargas et al. (2016), and Gaos et al. (2016)
	Atlantic	3	tbd	12	tbd	
	Indian	(4 are putative)	tbd	3	tbd	
Kemp's Ridley	Atlantic	1	Not designated	Not designated	n/a	Sampling coverage: very good but genetic analysis currently pending for MU designations
Olive Ridley	Pacific	3	tbd	2*****	tbd	Sampling Coverage: Atlantic is limited; major nesting sites sampled in the Pacific and Indian Oceans, but many remaining gaps for smaller rookeries. López-Castro and Rocha-Olivares (2005), Hahn et al. (2012), Shanker et al. (2004), and Jensen et al. (2013b)
	Atlantic	2	tbd	1	tbd	
	Indian	3 (1 is putative)	tbd	4	tbd	
Flatback	Pacific	1	Not designated	5	n/a	Sampling Coverage: good geographical coverage however, several smaller populations remain unsampled. Pittard (2010)
	Indian	1	Not designated	2	n/a	

RMU information across all species is from Wallace et al. (2010b); references for information specific to each species is listed within the table. Unless otherwise denoted, groups east of Torres Strait and North of Java were counted as part of the Pacific Ocean region, while locations with the reverse criteria were counted in the Indian Ocean region. Summaries reflect designations at time of publication of this article, however ongoing studies will continue to change totals (particularly in for MUs, which will likely continue to increase as regions that are currently data deficient are adequately sampled).

(#) = putative RMUs of the total number listed.

**Four adjacent RMUs in coral triangle overlap.*

***The 'East Indian/West Pacific' DPS is included in both the Pacific and Indian counts (i.e., there are 11 green turtle DPSs total globally).*

****9 DIPs -see **Box 2** text.*

*****Proper IUCN global reassessment hasn't been completed, but Hawaiian population has been designated as a subpopulation.*

******Peninsular vs. continental MUs suggested based on significantly lower genetic diversity (López-Castro and Rocha-Olivares, 2005).*

homing by nesting females (Bowen et al., 1989; Meylan et al., 1990). Since that time, considerable progress has been made in refining the global population structure for this species through improved sampling efforts and development of genetic tools with increased power to detect population structure (**Figure 1**, **Box 1**). First, sequencing of the mtDNA control region (490 bp) from nine Atlantic and Mediterranean rookeries detected 6-fold greater genetic diversity compared to previous RFLP data (Encalada et al., 1996), significantly improving stock structure resolution and identifying at least six management units (MUs; see **Box 2** for additional discussion of conservation units). Further progress was then made by increasing sampling sizes and coverage in the Florida nesting aggregation, clearly demonstrating that Florida is genetically distinct from the Quintana Roo, Mexico rookery and identifying at least two MUs within Florida, separated by the St. Lucie Inlet (Shamblin et al., 2015a). Additional studies added novel rookeries and increased sample sizes regionally in the Atlantic, resulting in the recognition of a total of 12–13 MUs using mtDNA control region haplotypes (490 or 817 bp, see **Box 1**; Bjorndal et al., 2006; Formia et al., 2006; Ruiz-Urquiola et al., 2010; Shamblin et al., 2015b).

In contrast to the Atlantic, progress on delineating population boundaries in the Indian and Pacific Oceans has been slowed by challenges to carrying out comprehensive rookery sampling. With globally significant rookeries scattered throughout the Indian and Pacific Oceans that are often located at remote islands and atolls, sampling is difficult and requires long-term international collaborations (see also *Social Dimensions* section). However, more than two decades of focused efforts to advance the genetic sampling of green turtle rookeries across this region has started to bear fruit. The number of rookeries sampled has increased from 14 across six countries at the beginning of the

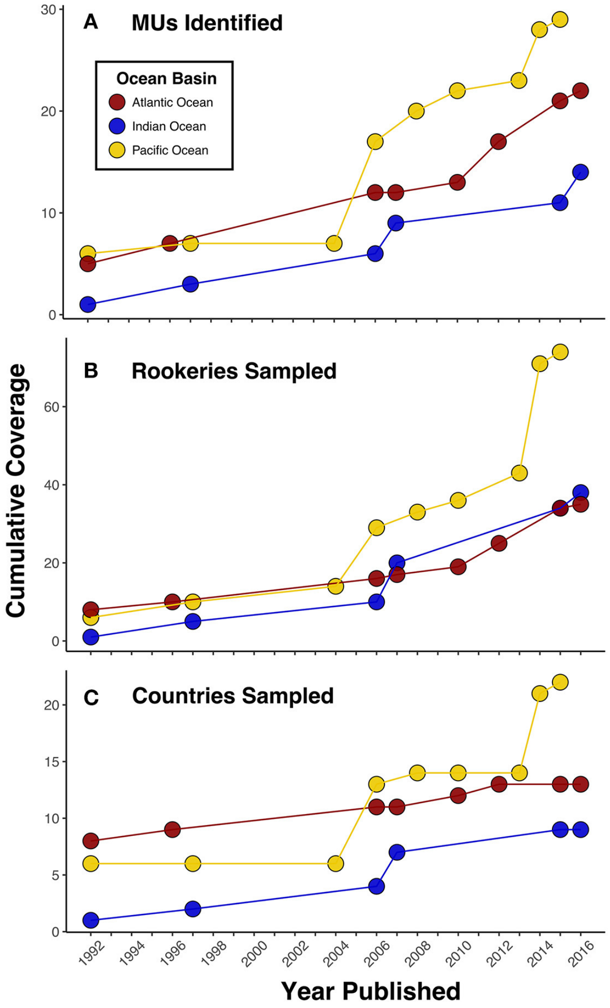

FIGURE 1 | Progress in (A) management unit (MU) identification and genetic sampling by **(B)** rookeries and **(C)** countries for green turtles over the past 25 years in each ocean basin as measured by published studies. While trends among panels generally depict the same pattern, in some instances the number of MUs increases without corresponding new sampling locations. This highlights that increases in MU identification have resulted from both expanded sampling efforts and improvements in genetic marker resolution. These data also depict how MU delineation in the Atlantic was the first to advance, but there has been recent progress in the Indian and Pacific Oceans as well.

millennium (Bowen et al., 1992; FitzSimmons et al., 1997) to more than 80 rookeries across 23 countries by 2016 (**Figure 1**), now covering the majority of the green turtle distribution across the regions (e.g., Hamabata et al., 2013; Dutton et al., 2014a,b; Jensen et al., 2016a). As a result, our ability to define population boundaries for Pacific green turtles has improved significantly. Dethmers et al. (2006) first pioneered large-scale Indo-Pacific green turtle population structure analyses, covering an impressive 27 rookeries identifying 17 MUs using mtDNA control region haplotypes (386 bp). Since then, studies including additional beaches in Taiwan (Cheng et al., 2008) and Japan (Nishizawa et al., 2011; Hamabata et al., 2013) have shown population differentiation between rookeries separated by no more than 150 km, identifying six new MUs in the Northwest Pacific. Recent regional analyses of Southwest and South-central Pacific green turtle rookeries also filled a significant gap across a large portion of the western Pacific and identified seven MUs (Dutton et al., 2014b; Read et al., 2015). In contrast, in the central and eastern Pacific only five MUs have been identified, with no genetic differentiation detected between neighboring rookeries in the Hawaiian archipelago (>500 km), the Revillagigedo Islands (∼400 km), or between rookeries in the Galapagos and mainland Ecuador (>1,000 km) (Dutton et al., 2014a; Chaves et al., 2017).

Population Boundaries: When and How do Higher Resolution Markers Help Us?

Despite impressive progress in filling sampling gaps, questions about the scale of natal homing and demographic isolation of rookeries have persisted. Using data on mtDNA control region haplotypes, no population differentiation was detected between several green turtle rookery pairs despite being separated by several hundred kilometers. Examples included Aves Island and Suriname in the Northwest Atlantic (Encalada et al., 1996), the Brazilian archipelago rookeries of Fernando de Noronha and Atol das Rocas (Bjorndal et al., 2006), and the individual rookeries comprising the central and eastern Pacific stocks discussed above. Such observations provoke the important question: *Is this apparent lack of population structure between rookeries within a region due to recent shared common ancestry, ongoing gene flow via female exchange across rookeries, or due to lack of resolution in the genetic markers employed?* Haplotypes may be shared regionally or even across entire ocean basins, and may potentially obscure population boundaries (e.g., Formia et al., 2006). Further, even when rookeries are considered demographically isolated through haplotype frequency differences, haplotype sharing can lead to uncertainty around stock contributions in mixed stock analyses (MSA; see **Box 1**). These ambiguities can hinder defining appropriate population boundaries for management frameworks (see further discussion of conservation units in **Box 2**). In these circumstances, the use of additional genetic markers beyond traditional mtDNA control region sequences can provide insight, and in several cases have already been instrumental in the resolution of marine turtle population boundaries. Multiple marker types may reveal previously obscured population structure, and the 'best' marker depends on the biological context and evolutionary histories of the particular rookeries being investigated. To demonstrate this, we discuss examples of improvements in green turtle population structure resolution using a variety of genetic markers, several of which show further utility for other populations and species (see **Box 1** for additional details on marker development).

Mitogenomic Sequencing and Mitochondrial Short Tandem Repeats

In green turtle rookeries of the western Atlantic, extensive sharing of four common mtDNA 490 bp control region haplotypes hampered assessments of population boundaries. In particular, within the eastern Caribbean, the large rookeries of Suriname and Aves Island are nearly fixed for haplotype

CM-A5 (Encalada et al., 1996), which is also detected at low frequency in the Tortuguero, Costa Rica rookery (Bjorndal et al., 2005). These patterns made it unclear if there is mixing among females nesting at these rookeries, or if there is population structure undetected by this particular marker. To resolve this question, Shamblin et al. (2012a) sequenced nearly the entire 16,000 bp mitogenome from 20 individuals from these rookeries as well as Buck Island, US Virgin Islands (USVI) to detect informative variable positions outside of the standard control region sequence. They found four variants in other regions of the mitogenome that divided CM-A5 into three haplotypes that were regionally structured. Statistical analysis of the new haplotypes indicated that all four rookeries were demographically isolated from each other, and qualified as distinct MUs. It is not yet known if such informative mitogenomic variants are common for marine turtles, or if their presence is unique to these eastern Caribbean green turtles. Efforts are underway for green turtles in regions of the Pacific where haplotype sharing similarly confounds population structure assessment (A. Frey, pers. comm.). Such exploration could also be valuable in other species where refinement of methods to detect population differentiation is needed to meet management goals, such as leatherbacks that can share haplotypes across entire ocean basins (Dutton et al., 2007), and eastern Pacific olive ridleys in Costa Rican and Mexican rookeries that are undifferentiated based on traditional mtDNA control region sequences (López-Castro and Rocha-Olivares, 2005).

Much like the problem in the eastern Caribbean green turtles, one common mtDNA control region haplotype dominates Mediterranean rookery profiles (CM-A13), with only the Cyprus rookery being distinct from the others (Bagda et al., 2012). However, using a mitochondrial short tandem repeat (mtSTR) marker downstream of the standard control region sequence used for haplotype designation, Tikochinski et al. (2012) determined that this common haplotype could be subdivided into 33 variants. Examining differences in the frequencies of these mtSTR variants from the Israeli rookery compared to stranding's along the Israeli coast suggested that population structure is present among Mediterranean green turtle rookeries. In another example, Shamblin et al. (2015c) combined mtSTR markers with sequencing of the extended control region from two northern Brazil island rookeries that were previously undifferentiated (Fernando de Noronha and Atol das Rocas; Bjorndal et al., 2006). This allowed them to discern cryptic diversity and clearly demonstrate that the two rookeries were demographically distinct populations, and also provide novel population markers for future MSA studies. Though consistent sequencing of mtSTRs can be challenging due to technical methodological issues, further investigation may also prove useful for identification of fine-scale patterns in other populations and species where shared control region haplotypes are common.

Nuclear Markers

The application of maternally inherited mtDNA markers for defining population structure and connectivity is useful given that the scale of female natal homing is a key element in determining population boundaries. However, mtDNA markers only clarify the female portion of the story and can lack resolving power due to the slow rate of mitochondrial evolution inferred for turtle species. In contrast, nuclear markers incorporate male connectivity that is essential to delineate populations with greater accuracy, and can have increased statistical power, e.g., microsatellites and single nucleotide polymorphisms (SNPs; see Glossary and **Box 1** for further details). Early studies found congruent or weaker patterns of population structure with nuclear markers relative to mtDNA, suggesting the presence of weak male natal homing behavior and/or mating during periods when individuals from different populations overlap at foraging sites (FitzSimmons et al., 1997; Roberts et al., 2004). More recent research employing a higher number of nuclear markers detected significant population differences across key green turtle rookeries in the Pacific, providing evidence that male-mediated gene flow between regional nesting stocks may be more limited than previously believed and facilitating clearer stock delineation (Roden et al., 2013). Several rookeries in Turkey also displayed different microsatellite allele frequencies (Bagda et al., 2012), suggesting there was underlying population structure not detected by mtDNA. These results correspond with recent findings in other species such as leatherbacks, where using mtDNA alone distinguished seven populations in the Atlantic, but using microsatellites it was clear that there were nine populations (Dutton et al., 2013). However, in western Atlantic green turtles, microsatellites discerned population structure that was congruent with but weaker than that identified by mtDNA control region sequences (Naro-Maciel et al., 2014a). Thus, while it is clear that combining mtDNA and nuclear markers can provide insight into male-mediated gene flow and population boundaries, patterns may differ between regions and species, and additional studies are needed to clarify their generality and context dependencies. As technological advances continue to improve researchers' abilities to generate robust nuclear data that are comparable across laboratories, nuclear markers are poised to complement mtDNA in further advancing our understanding of female and male natal homing and fine-scale population structure.

Green Turtle Habitat Connectivity: Which Nesting Stocks Use Which Foraging Grounds?

Along with other approaches such as flipper tagging, satellite telemetry, and stable isotope analysis, genetics have been instrumental in quantifying connectivity between rookeries and foraging grounds. Early work in the Atlantic recognized the importance of both ocean current patterns and natal homing behavior in shaping the distribution of juvenile green turtles at foraging sites (Luke et al., 2004; Bass et al., 2006). Recently, improved sampling efforts have coincided with advances in statistical analyses such as MSA to clarify how populations are linked to foraging habitats (see **Box 1**). This provides researchers and practitioners working in foraging grounds with knowledge about where the turtles are coming from, which is informative for public outreach engagement, identifying regional and international management partners, and integrating threats at nesting beaches and foraging grounds into risk assessments. This information also enables estimation of proportional contributions of each source nesting stock to the foraging population.

With the advancements defining rookery population structure across the Pacific, an increasing number of foraging grounds have now been analyzed using MSA across northern Australia (Dethmers et al., 2010), Southeast Asia (Jensen et al., 2016b; Joseph and Nishizawa, 2016), Southwest Pacific Ocean (Read et al., 2015; Jensen et al., 2016a), Northwest Pacific Ocean (Nishizawa et al., 2013), Central Pacific (Dutton et al., 2008; Naro-Maciel et al., 2014b), and the East Pacific (Amorocho et al., 2012; Chaves et al., 2017). These studies have shown considerable variation in results, with some foraging ground aggregations being composed mostly of turtles from the nearest stock (see Glossary) such as Hawaii, Aru, Gulf of Carpentaria, and the northern and southern Great Barrier Reef (Dutton et al., 2008; Dethmers et al., 2010; Jensen et al., 2016a), while other aggregations include significant numbers of turtles from distant stocks over 2,000 km away such as Palmyra, New Caledonia, Colombia and Japan (Amorocho et al., 2012; Nishizawa et al., 2013; Naro-Maciel et al., 2014b; Read et al., 2015). A similar pattern is evident in the Atlantic basin where transatlantic dispersal has been identified from Suriname to the West coast of Africa and from rookeries in western Africa to the Brazilian coast (Monzón-Argüello et al., 2010). While these studies have expanded our understanding of connectivity between rookeries and foraging areas, they do not provide information on migration routes or the factors that influence the dispersal of turtles. In recent years, multidisciplinary approaches have combined MSA and high-resolution ocean circulation modeling to further our understanding of marine turtle movement (e.g., Putman and Naro-Maciel, 2013; Naro-Maciel et al., 2017). These studies have revealed that while ocean currents play a vital role in the spatial distribution of turtles they do not always correlate with MSA results, suggesting that other factors such as swimming behavior play important roles in the distribution of turtles (Putman and He, 2013; Hays et al., 2014a; Naro-Maciel et al., 2014b, 2017; Christiansen et al., 2016).

Finally, MSA can also help identify how threats and conservation efforts in foraging areas may affect nesting populations and vice versa, and could allow scientists and managers to study changes in population composition over time (also see *Human Impacts and Threat Assessment* section). For example, a recent study of green turtles at six major feeding grounds along the Great Barrier Reef used MSA to show that fewer juvenile turtles (61, 52, and 47% at the three most northern foraging grounds, respectively) originated from the northern Great Barrier Reef nesting beaches when compared to adult turtles (91, 69, and 69%), supporting a hypothesis that hatching success at northern Great Barrier Reef rookeries has been declining since the mid-1990s (Jensen et al., 2016a).

Cautionary Tales: The Importance of Understanding MSA Limitations

While considerable progress has been made, it is important to recognize that knowledge gaps still exist, and efforts to sample new rookeries and employ higher resolution markers need to continue to enable accurate MSA. This is highlighted by the fact that orphan haplotypes (see Glossary) are still common, and several recent cases illustrate how misinterpretation of MSA results may lead to incorrect conclusions under such scenarios. In one instance, incomplete baseline sampling led to the potential misinterpretation of MSA results for foraging juveniles. CM-A13 is the dominant Mediterranean haplotype, and was detected among foraging juveniles in the Greater Caribbean region. This introduced the possibility of dispersal from the Mediterranean into the western Atlantic (Bjorndal and Bolten, 2008; Anderson et al., 2013). However, this haplotype was later found to occur at low frequency in the Florida nesting aggregation (Shamblin et al., 2015a). Analysis of the mtSTR for CM-A13 turtles nesting and foraging in Florida yielded a shared repeat haplotype that was not among the 33 described thus far from the Mediterranean (Tikochinski et al., 2012), revealing that the foraging turtles most likely originated locally within the Greater Caribbean region (Shamblin et al., 2015b). In a second example, in the northern Greater Caribbean region, CM-A1 and CM-A3 are the dominant haplotypes shared among the rookeries of Quintana Roo, Mexico; Guanahacabibes Peninsula, Cuba; and Florida rookeries (Encalada et al., 1996; Ruiz-Urquiola et al., 2010; Shamblin et al., 2015a). An MSA of juvenile green turtles in Texas foraging grounds suggested a large Florida rookery contribution based on the presence of these haplotypes at high frequencies (Anderson et al., 2013). Yet later analysis of the green turtle rookery at Rancho Nuevo, Tamaulipas, Mexico found CM-A1 and CM-A3 in the same frequencies as the central Florida MU, so the source of the majority of juveniles foraging in Texas remained unresolved using standard markers (Shamblin et al., 2015a, 2017). However, mitogenomic sequencing of the CM-A1 females nesting in Rancho Nuevo and Florida identified a single diagnostic mtDNA SNP between the two rookeries. This marker demonstrated that turtles nesting in Florida and Tamaulipas represented distinct populations. Subsequent MSA including the mtDNA SNP excluded Florida as a source of Texas foraging juveniles, and established the strong connectivity between this foraging aggregation and the newly proposed western Gulf of Mexico MU (Shamblin et al., 2017).

Delineating Population Boundaries: Best Practices and Remaining Challenges

The case studies discussed above for green turtles highlight several important insights for using genetics to inform our understanding of population boundaries and connectivity. First, the presence of multiple MUs along the Florida and Mexican coasts demonstrate the importance of sampling the complete geographic extent of a nesting aggregation to test for structure, rather than assuming none is present. Second, the CM-A13 story from the western Atlantic reinforces the importance of adequate rookery sampling to capture rare haplotypes that are present at low frequencies. Third, the Texas foraging aggregation example highlights that a low frequency of orphan haplotypes from a foraging aggregation does not necessarily imply that all potential source rookeries have been adequately sampled. As haplotypes are subdivided into ever-larger numbers of distinct genetic markers, there is also a greater need to increase sampling depth at rookeries and foraging aggregations to reduce sampling error. This includes sampling of males at breeding grounds, which is a frequently overlooked component necessary to estimate male-mediated gene flow and define population boundaries. Finally, taken collectively these examples clearly illustrate that there is not a "one size fits all" solution to potential genetic resolution issues. Efforts that have proven useful in some situations have failed to detect any additional structure in others, and finding the "best" approach for a specific biogeographical scenario may involve some trial and error.

In addition to the examples discussed above, many new genetic studies of nesting and foraging grounds in green turtles and other species are already underway, guaranteeing improvement of our understanding of marine turtle population boundaries and connectivity in the future. However, despite these advances, challenges in the interpretation of genetic data also remain. Turtle mitochondrial DNA evolves slowly relative to that of many other vertebrate species (Avise et al., 1992), so demographic isolation can sometimes outpace the rates of mutation and drift. This may diminish the ability of these genetic markers to detect such demographic changes, and consequently, apparent lack of population structure may reflect a lack of power of the markers employed rather than true panmixia, particularly at demographic levels. Additionally, genetic differentiation in marine turtles is typically not well correlated with geographic distance, so there is no universal benchmark that can predict the scale of structure across populations. In many cases, rookeries several hundreds of kilometers away are not genetically differentiated using traditional markers, but may not have significant contemporary demographic connectivity. Therefore, best practices entail understanding the strengths and limitations of each genetic dataset, and evaluating it along with data from complementary sources (e.g., flipper tag returns, telemetry, stable isotopes, oceanographic modeling, the relative population differentiation observed elsewhere for a species, etc.) to make inferences about population boundaries and connectivity. Integrating this information helps ensure that the best available science is used to inform management decisions. While genetic data are key components of these evaluations, in some cases there may be valid reasons to consider treating rookeries as demographically distinct for management, even in the absence of genetic evidence that they are isolated.

LIFE HISTORY

How Old Are Female Turtles When They Start to Reproduce?

Age to maturity (ATM) is one of the key parameters required for estimating how long recovery could take for depleted populations because it is needed to calculate generation time. Determining ATM is difficult in marine turtles due to challenges related to both longevity and life history, and both empirical and indirect approaches have been pursued. For example, coded wire tags were injected into juvenile Kemp's ridley turtles (*Lepidochelys kempii*), and recovered via dead stranded animals years later to estimate a minimum ATM of 10–14 years (Shaver and Caillouet, 1998; Caillouet et al., 2011). This was similar to an ATM using capture-mark-recapture (CMR) and skeletochronology in headstarted Kemp's (10–17 years; Snover et al., 2007), but longer than an estimate from captive animals (5–12 years; Bjorndal et al., 2014), suggesting that growth and maturity may differ in the wild. Skeletochronology has also shed light on ATM in green, loggerhead, and leatherback turtles (Avens et al., 2009; Turner Tomaszewicz et al., 2015), as have a small number of direct observational studies from headstarted turtles (e.g., Florida and Caribbean green turtles, Keske et al. (2016) and Bell et al. (2005), respectively), CMR via carapace punches in South African loggerheads (Tucek et al., 2014) or growth rates (Casale et al., 2009). The general consensus emerging from these studies, is that each species and perhaps each population may have different ATM ranges.

Techniques such as genetic CMR offer exciting new opportunities to directly measure ATM, however the process may require significant cost and long-term commitment of several decades, depending on the species. For example, Dutton and Stewart (2013) began a long-term genetic CMR effort in 2009 with leatherback hatchlings at Sandy Point National Wildlife Refuge (US Virgin Islands) with the goal of live-sampling every hatchling leaving the nesting beach. Genetic fingerprints developed through the use of microsatellites (Stewart and Dutton, 2011) were applied to DNA from the hatchling samples to identify each one individually, effectively serving as a lifelong genetic "tag." Concurrently, they sampled and genetically fingerprinted each new nesting female at Sandy Point with the same microsatellite markers to identify when the former hatchlings return as nesting adults (Stewart and Dutton, 2011, 2014). The project continues annually, with new nesting females being compared with hatchling turtles that left the beach years earlier. To date, there have been no matches (K. Stewart and P. Dutton, unpublished data), but continual investigation using microsatellites in combination with new SNP markers should yield results that are informative for leatherback ATM estimation in the near future (albeit only for the female portion of the population). This genetic fingerprinting technique also has potential for estimating other parameters essential to accurate population models for conservation management, such as survivorship from hatchling stage to adulthood.

In addition, much else may be learned from knowing the genetic identities of thousands of individuals. For example, leatherback tissue samples from stranded animals, in-water captures and bycatch may all be genetically identified and compared to known individuals (through comparison to data from the larger stocks or from hatchlings sampled at Sandy Point). However, reliably detecting matches requires profiling a high proportion of the population, and for some species the turtles are too numerous or accessing all potential nesting habitat is not feasible. Given these constraints as well as the costs and required time investment, this approach is currently best applied under certain contexts, such as in smaller populations where turtles have high site fidelity, long-term project investment is feasible (including the capacity to store and track DNA samples for years to decades), and where there are clear research questions (**Table 3**). However, rapidly evolving high-throughput technologies with the capacity to analyze thousands of samples concurrently will make it more feasible to conduct mass-tagging experiments in the future, provided that infrastructural support is available.

How can Genetics Be Used to Assess the Male Component of the Population?

Genetic fingerprinting may also yield important information about a component of the adult marine turtle population that is rarely assessed, the males. Questions related to the sex ratios of breeding adults, mating patterns such as levels of multiple paternity, and male reproductive site fidelity may all be answered through intensive studies of nesting females and their hatchlings. By comparing maternal and hatchling leatherback genetic identities at Sandy Point, Stewart and Dutton (2011, 2014) were able to reconstruct the genetic identities of individual males contributing to each nest laid during several nesting seasons. Using this approach, males may be identified without being sampled directly (Wright et al., 2012a; Tedeschi et al., 2015; Sari et al., 2017). Then by comparing all male genetic identities within a nesting season, the number of successful breeding males may be determined, providing an annual population census for all males and females. However, it is important to note that these estimates represent the minimum number of breeding males, since all males may not successfully sire offspring due to mating and sperm competition. This work also requires consistent monitoring of females and nests directly because the maternal identity of each nest must be known to assess females and hatchlings and then by inference, the male identities.

In addition, the levels of multiple paternity at breeding sites are able to be determined with this approach targeting females and hatchlings. Multiple paternity has been detected in hawksbills (Phillips et al., 2013), loggerheads (Sari et al., 2017), leatherbacks (Stewart and Dutton, 2011), olive ridleys (Jensen et al., 2006), and green turtles (FitzSimmons, 1998), and the number of studies in this area is increasing. By tracking male identities over several nesting seasons, male remigration/breeding intervals may also be determined by recording how often a known male is detected in hatchling genetic signatures. However, to be successful, this approach requires consistent and comprehensive monitoring over time, and male turtles without any or fewer reproductive successes due to competition and other factors can be missed.

Nonetheless, this application of genetic fingerprinting has the potential to advance our understanding of how males contribute to nesting populations. Genetic studies on males may be undertaken in conjunction with other methods, such as satellite tracking (Hays et al., 2010, 2014b) to improve estimates of breeding sex ratios across populations and to answer questions about site fidelity and how rookeries are connected to form populations. By identifying males that have made reproductive contributions to each clutch, we can also assign individual hatchlings to fathers and assess the relative contributions by different fathers (for clutches with multiple paternity), and therefore gain insight on reproductive strategies and success for the males as well as the females (Stewart and Dutton, 2011). However, complementary studies sampling in-water males at breeding grounds are needed to assess the number of males in the population with no reproductive success (e.g., due to competition).

Operational sex ratios (OSRs) or breeding sex ratios (BSRs) are important to understand and monitor over time, particularly given growing concern that climate change will affect sand temperatures where marine turtle nests incubate and alter hatchling sex ratios. As clutches of hatchlings are generally female-biased (Wright et al., 2012a; Hays et al., 2014b), understanding how this translates to adult sex ratios is important for tracking effects over time and understanding climate change risks to nesting populations. To date, from the studies that looked at this ratio specifically, there does not appear to be a reduction in the proportion of males in breeding populations, despite there being female biases in the hatchling sex ratios. For example, Wright et al. (2012b) found 1.4 males for every female in a green turtle population, Stewart and Dutton (2011, 2014) found 1.4 males and 1 male (respectively) for every female in leatherback turtles, and Phillips et al. (2013) found 1.1 males for every female in a hawksbill population. In all of these studies, there were more males than females detected within the breeding population within a single year. Developing baselines for populations for OSRs or BSRs will be important for monitoring risk from climate change to populations over time.

What Reproductive Strategies and Behaviors Do Turtles Use?

Robust estimates of reproductive vital rates such as clutch frequency, the number of clutches a female lays in a given nesting season, and remigration interval (the number of years a female skips between nesting seasons) are important for monitoring and modeling population recovery. However, some females may disperse their nests beyond the limits of the areas of beach monitoring for tagging or observation, or there may be insufficient resources to conduct consistent monitoring, leading to missed turtles, sparse recapture data, and biased estimates of these key parameters. Shamblin et al. (2011) developed a technique to address this limitation by extracting maternal genomic DNA from freshly laid loggerhead eggs, permitting individual identification of females without the need to physically intercept them during the nesting process. This type of sampling allows genetic CMR on spatial scales that would be logistically impossible to replicate through traditional tagging approaches. A subpopulation-scale genetic CMR project has been underway since 2010 for loggerhead turtles nesting in the United States north of Florida to refine nesting female abundance estimates, assess reproductive parameters, determine the level of nest site fidelity, and calculate annual survival rates (www.seaturtle.org/nestdb/genetics). Nest sampling may also supplement traditional tagging approaches thus improving annual censuses of nesting populations where nesting females cannot be consistently observed. For example, Frey et al. (2014) used parentage analysis and genetic fingerprinting for a limited number of known Kemp's ridley mothers along with hatchlings from unidentified nests to assign mothers for every nest. They

TABLE 3 | Generalized summary of marine turtle genetic/genomic research to inform future project development.

Research stage	Main components	Potential considerations
Study design	Define research goal(s) Determine appropriate sampling schema and analysis approaches* Create project plan *(includes attaining necessary permits for access, sampling and/or transport)*	• Can research questions be addressed with a single sampling effort, or do they require longer-term sampling and possible integration into ongoing biological monitoring? • How will we organize and track samples, genetic data and corresponding metadata? • What resources do we have to accomplish our desired project plan *(includes expertise, financial and infrastructure resources)*? • How can we scale our project to match our resources or attain what we lack (e.g., via funding and/or partnerships)?
Field sampling and storage	Prepare sampling materials Personnel training Collect samples and metadata Store/archive samples	• How will genetic sampling be conducted to capitalize on but not interfere with other on-going studies? • Will we need to catalog and store samples for only short periods, or do we need an organizational system and appropriate equipment for longer-term archiving?
Molecular laboratory analyses	DNA (or RNA) extraction and quantification PCR amplification and/or library preparation Visualization and/or sequencing	• What expertise and facilities do we need for our selected approaches, and do we have access to it (independently, or via partnerships or contracting)? • Does our project design require continuous access to molecular facilities, or can we partner with off-site laboratories to process samples in discrete timeframes (e.g., off-season)? • At what scale will we need to store extractions, PCR products or libraries?
Data analyses and storage	Quality assessment and control of data Archive raw and processed (e.g., genotypes) data linked to metadata Statistical Analyses *(widely vary depending on research question)* Document/store analyses procedures (e.g., scripts) and results for reproducibility	• What will be our standard operating procedures for data QA/QC, and how will this be documented so results are consistent over time and comparable across laboratories? • At what scales will we need to archive &/or share data, and what resources do we need to accomplish this over the entire course of the project? • How will we archive analytical procedures and results and make accessible for reproducibility?

While not comprehensive (because key components and considerations can vary widely across study types and contexts), these ideas offer general guidance for incorporating genetics into research and monitoring programs. Although budget is also a critical component of project design, we do not include cost estimates because these are highly dependent upon a large number of factors (e.g., infrastructure and equipment availability), and continue to rapidly change with on-going technological developments. Thus, it is best for interested programs to confer with experienced researchers or contracting molecular analysis companies when designing studies to develop realistic budgets to meet project goals.

**This step includes robust understanding of genetic, physiological, &/or ecological principles to guide study design, such as selection of individuals (e.g., nesting females, breeding males, foraging juveniles, hatchlings), sample sizes, timescales, and study sites, as well as appropriate data analyses and interpretations.*

BOX 1 | Technological and analytical improvements.

Key methodological advances in the past decade underpin the growing capacity to tackle a diversity of questions in wildlife biology and conservation. Broadly, these developments offer improved resolution relative to early molecular markers and analyses, facilitate larger-scale spatial and temporal syntheses, and generate capability for novel applications. Here we provide a general overview within the context of recent and emerging marine turtle applications, emphasizing that there is not one universal "best" approach, but rather the most appropriate tools for particular research questions and conservation needs, budget, and biological contexts.

Expansion of molecular markers

The majority of marine turtle genetic studies to date have used mitochondrial DNA (mtDNA) control region (d-loop) as a molecular marker of choice because it is (1) generally fast and easy to sequence, (2) the principal non-coding region of mtDNA and often highly polymorphic, and (3) mtDNA is maternally inherited. These factors have made it a useful tool for many studies, including assessment of phylogenetic relationships, establishing broad population stock structure, and species identification of unknown individuals. However, this marker can have limited resolution, and some research questions require nuclear markers (i.e., inherited both maternally and paternally, such as studies of male-mediated gene flow). In the past decade, researchers have tackled these limitations via development of both (1) mtDNA markers complementary to the control region, and (2) informative nuclear markers.

For mtDNA, this began with the development of primers that target an extended control region sequence (i.e., 766 vs. 490 bp; Shamblin et al., 2012b), and recently extended to identification of diagnostic variants in other mtDNA regions (Shamblin et al., 2012a; Tikochinski et al., 2012) and whole mitogenome sequencing (Duchêne et al., 2012). In cases where there is not extensive haplotype sharing among rookeries, the extended control region sequence can effectively identify natal origin of foraging turtles (LeRoux et al., 2012). However, when overlaps in control region haplotypes among rookeries are prevalent, the addition of other mtDNA markers or complete mitogenomic sequencing can help resolve uncertainties (Shamblin et al., 2012a). Informative nuclear markers have also now been developed for all marine turtle species, offering finer-scale resolution in some biological contexts, and applicability to research questions about the male component of populations. This has included informative microsatellites (see Glossary; e.g., Aggarwal et al., 2004; Jensen et al., 2006; Shamblin et al., 2007, 2009, 2012c; Alstad et al., 2011; Roden and Dutton, 2011) and more recently, single nucleotide polymorphisms (SNPs; Roden et al., 2009; Hancock-Hanser et al., 2013; Hurtado et al., 2016). With many alleles at each locus, nuclear microsatellites can be employed for applications such as individual and familial genotyping (Selkoe and Toonen, 2006). However, laboratory analyses of microsatellites can be time and labor intensive, and although technical advances (e.g., multiplexing) have helped, scaling up in projects requiring analyses across thousands of samples can be challenging. Additionally, generating microsatellite data that is comparable across laboratories is problematic, which can impede large-scale stock structure and MSA syntheses. In contrast, SNPs typically have lower power per locus relative to microsatellites, but hundreds to thousands of SNP loci can now be rapidly and reliably quantified across large numbers of individuals (Campbell et al., 2015; Ali et al., 2016; Andrews et al., 2016). Additionally, SNPs in coding regions can be used to understand genotype and phenotype linkages and identify loci under selection if adequate genomic and budget resources are available (Hoban et al., 2016; Todd et al., 2016).

Transcriptomic and epigenetic markers have also recently been developed to understand biological processes in marine turtles. Measuring changes in gene expression may provide insight into the key genes and cellular pathways involved in physiological responses to environmental conditions or development cues, for example during sex determination in embryonic development or exposure to pollutants and environmental stressors (Sifuentes-Romero et al., 2013; Gomez-Picos et al., 2014; Díaz-Hernández et al., 2015; Tedeschi et al., 2016; Bentley et al., 2017). To date, most published studies in marine turtles have developed and applied functional genetic markers using quantitative PCR, targeting specific genes based on *a priori* knowledge from other taxa (e.g., Sox9, CIRBP, and aromatase). However, projects quantifying global gene expression (i.e., measuring all genes being expressed in a given sample) are underway, which can be used to address a diversity of research questions from comparative functional genomics of development to the physiological impacts of contaminants. Additionally, markers quantifying DNA methylation have been developed to investigate the role of epigenetics in these processes (Venegas et al., 2016). It is important to realize that these techniques provide the strongest biological insight when accompanied by companionate phenotypic information, and may require further validation for different species and environmental contexts to ensure accurate data interpretation (particularly *in situ*). Nonetheless, along with other emerging approaches (e.g., environmental DNA and DNA meta-barcoding), these techniques are rapidly becoming more common across wildlife taxa, and are well-poised to further our understanding of the genomic and cellular processes underlying physiological responses and adaptations in marine turtles.

High-throughput sequencing

High-throughput sequencing (HTS; also referred to as next generation sequencing, massively parallel sequencing or second-generation sequencing) is a category of approaches that employ massively parallel processing for DNA and RNA sequencing. These technologies are rapidly advancing, and detailed evaluation of these methodologies is available elsewhere (Morey et al., 2013; Reuter et al., 2015). However, there are a few key elements of HTS that are important to understand within the context of marine turtle genetic and genomic advances. First, in contrast to Sanger sequencing (which has been the most widely used method for almost four decades; Sanger et al., 1977) that produces each DNA sequence individually, HTS approaches rapidly generate millions of sequences simultaneously by employing massively parallel processing. Sanger sequencing is still useful for small-scale DNA sequencing projects, but HTS allows rapid sequencing across hundreds to thousands of loci and individuals. Secondly, HTS approaches are versatile. For example, in addition to DNA sequencing HTS techniques, RNA sequencing (RNA-Seq) can be employed to quantify gene expression (in lieu of lower throughput techniques such as quantitative PCR) and conduct functional genomics studies. Although to date HTS has been more widely used in other wildlife taxa (e.g., mammals and fish; Shafer et al., 2015; Cammen et al., 2016), in marine turtles HTS approaches have been used to construct the complete green turtle reference genome (Wang et al., 2013), generate mitogenomes of all seven marine turtle species for phylogenetic analyses (Duchêne et al., 2012), and discover SNPs for green, hawksbill and loggerhead turtles (Hancock-Hanser et al., 2013; Komoroske et al., 2016). While HTS approaches effectively eliminate bottlenecks in data production, challenges in data quality and interpretation can remain, particularly for wildlife species with limited genomic resources. However, continued decline of HTS costs coupled with development of complementary tools (e.g., targeted DNA/RNA library techniques and bioinformatic resources-see below) is greatly expanding accessibility for cost-effective use of HTS approaches in marine turtle research, and will likely become more common in the near future.

Quantitative analyses

Bayesian mixed stock analysis

Mixed stock analysis (MSA) has become a key tool for marine turtle research aiming to connect turtles sampled away from nesting beaches (e.g., foraging areas, migratory corridors, fisheries bycatch, or strandings) to their natal rookeries. Software packages like BAYES (Pella and Masuda, 2001) and the "mixed stock" R package (Bolker et al., 2007; Bolker, 2008) uses the frequencies of genetic markers for estimating the most likely proportions of "source populations" (rookeries) sampled in a "mixed population." Robust MSA depends on three key factors: (1) a comprehensive sampling of potential source populations (rookeries), (2) suitable sample size of the mixed population and (3) strong genetic structure to differentiate between source populations. Common problems limiting MSA accuracy in

(Continued)

BOX 1 | Continued

marine turtles have been lack of detectable population structure and/or limited sampling among source rookeries, resulting in imprecise estimates surrounded by large levels of uncertainty. In such cases, it can be advantageous to use Bayesian approaches that allow the use of informed priors to influence the distribution of the relative baseline frequencies. Prior information based on biological knowledge guides the output in situations where genetic structure is weak between the baseline samples, such as shared haplotypes between rookeries. For marine turtles, the most commonly used priors are the relative size of source populations (assuming that larger rookeries are more likely to contribute) or the geographical distance between rookeries and foraging grounds (assuming that closer rookeries are more likely to contribute). This can greatly assist MSA, but there is an inherent danger with using priors because if these underlying assumptions are not true, the results may be erroneous. Additionally, MSA methods can differ in their underlying algorithms, so it is advisable for researchers to compare multiple methods as they can produce different results (e.g., see Jensen et al., 2016a). Researchers using MSA need to understand the strengths and limitations of their dataset and method, and make good arguments for the use of specific priors. Nonetheless, MSA is an effective tool for providing vital information on the geographical boundaries of marine turtle populations that is crucial to the conservation and management of these animals.

Assignment testing

Assignment testing methods can be used for a variety of wildlife applications by assigning individuals by probability to their source (see Manel et al., 2005 for method overview). Numerous programs have been developed and are best applied under differing biological and data conditions. One program, ONCOR (originally used for stock assignment of Pacific salmon), that analyzes genetic data and conducts simulations to make assignments (Kalinowski et al., 2007) was recently adapted and validated in leatherback turtles (Stewart et al., 2013). This approach was then adapted in combination with MSA to assign bycaught turtles to source nesting populations with high precision (Stewart et al., 2016). ONCOR is open source software that is user friendly and has good documentation, and has strong potential for future applications in other marine turtle populations and species. However, like MSA, an important caveat is that accurate assignment requires availability of robust baseline data for all possible source populations, and that the markers used can differentiate among them.

Open source software and reproducibility initiatives

There is an increasing diversity of open source software for genetic and genomic analyses, but programs are often developed by different research groups, resulting in discontinuity (e.g., dissimilar required data formats and variable levels of documentation) and making it time-consuming and frustrating for researchers to easily find and correctly implement them. Recent efforts to make tools more accessible via user-friendly, well-documented workflows have made significant strides in tackling these issues. For example, the National Evolutionary Synthesis Center sponsored a recent Hackathon that produced a suite of R packages for population genomics analyses, each of which are now publicly available for download and have corresponding documentation in a special issue of *Molecular Ecology Resources* (see Paradis et al., 2016 and original articles within this special issue). Similar barriers can hamper HTS analyses, often compounded by data scale issues (i.e., simple tasks that can be manually completed in small data sets require automation with "big data"). Well-documented and user-friendly bioinformatics tools and pipelines are becoming increasingly available, greatly assisted by open access online repositories such as GitHub and Dryad. These resources allow researchers to share both code and data, complimenting public genomic databases such as NCBI, the UCSC Genome Browser and Ensembl. For example, marine turtle mitogenome analysis, SNP discovery and genotyping scripts, as well as the complete green turtle reference genome, mitogenomes for all species, and targeted capture array probe sequences are now publically available (see data accessibility links in Duchêne et al., 2012; Hancock-Hanser et al., 2013; Wang et al., 2013).

BOX 2 | Marine turtle conservation units.

Conservation units are often defined at multiple hierarchical levels in globally distributed species, and it can be challenging to understand how each is used in management frameworks and decisions. Evolutionary Significant Units (ESU) and Distinct Population Segments (DPS) are below the species level and are shaped by processes over evolutionary timescales that result in global partitioning of genetic variation that are relatively easy to detect with genetic markers such as the mtDNA control region (Taylor et al., 2010). Status assessments for marine turtles under the US Endangered Species Act now seek to identify DPSs that would represent a significant loss of genetic diversity for the species were they to go extinct (USFWS NOAA, 1996). Regional management units (RMUs) are analogous to DPSs and also recognize broad global conservation units below the species level for marine turtles (Wallace et al., 2010b). The IUCN has also recognized the need to identify broad population units below the species level for conservation, and has listed these as "subpopulations" in a recent global Red List assessment for leatherback (Wallace et al., 2013) and loggerhead turtles (Casale and Tucker, 2015). These large-scale units are helpful for assessing conservation priorities from a global perspective, but in most cases, each ESU, DPS, or RMU comprises multiple demographically independent nesting populations, known as management units (MUs). MUs are defined on the basis of significant differences in mitochondrial haplotype and/or nuclear allele frequencies (Moritz, 1994), reflecting regional natal homing to nesting sites by females. As such, MUs represent discrete populations over ecological time frames and reflect the appropriate scale for monitoring population dynamics. These MUs may also be referred to as genetic stocks, and the level of connectivity between MUs characterizes the degree of population structure and vulnerability to threats, and the ease with which demographic structure can be detected with genetic markers. Demographically independent populations (DIPs) are characterized by a low level of differentiation that is harder to detect with mtDNA markers (Dutton et al., 2013), and failure to detect demographic independence when it exists may lead to inappropriate management policy (Taylor and Dizon, 1999). In marine turtles, there are recently published genetic studies that have not yet been incorporated into conservation unit delineations (e.g., Gaos et al., 2016). As these are integrated, further sampling gaps are resolved, and the application of finer-resolution markers increases, the number of MUs and DIPs will likely continue to increase in coming years for several species. Established broader scale conservation units (e.g., RMUs) are likely to only change modestly, mostly via additional sampling that reduces current uncertainty of turtle population distributions across foraging areas, breeding grounds, and migratory pathways. Nonetheless, in combination with other forthcoming studies (e.g., assessing connectivity via telemetry), new fine-scale genetic information will help refine population boundaries for management frameworks, such as designating of DPSs for several species that have not yet been conducted (**Table 2**). Additionally, these advances are likely to be particularly valuable for linking threats with demographically independent nesting population abundance trends (e.g., Stewart et al., 2016).

were able to reconstruct genotypes for the mothers that were not observed and match unknown nests to mothers that had been sampled. They found that the number of nesting females in Texas was likely to have been underestimated based solely on nest counts or on the number of known mothers.

HUMAN IMPACTS AND THREAT ASSESSMENTS

Though it is well-established that many different human threats impact marine turtles, it is challenging to link human activities to population level effects, which is often key information for conservation and management action (Wallace et al., 2011). For example, to understand how human-caused mortality in foraging grounds may influence population abundance declines, we must distinguish the impacts among nesting stocks. Tackling these problems is unquestionably multi-faceted, and the role of genetics in unraveling these complexities has continued to expand in recent years. Genetic tools have been used to quantify impacts over both short and long-term timescales, and are well-suited for many recently identified and emerging threats such as climate change.

How Are Populations Impacted by Threats Away from Nesting Grounds?

For threats occurring in foraging grounds or during migration transit, identification of natal origins is crucial to assess and compare impacts within and across populations. Advances in genetic marker resolution and analytical tools (see **Box 1**) have allowed recent studies to make substantial headway in accomplishing this goal. For example, LaCasella et al. (2013) used MSA on loggerhead turtles bycaught in the US pelagic longline fishery in the North Atlantic Northeast Distant (NED) region to estimate that over 99% of juveniles using NED habitat belong to the Northwest Atlantic DPS. Researchers have used comparable approaches to identify population sources of harvested green turtles in Malaysia (Joseph et al., 2014) and ghost fishing net mortalities of olive ridley turtles in Australia (Jensen et al., 2013b), however these studies were constrained by small sample sizes and shared or orphan haplotypes.

Employing finer resolution nuclear markers, Stewart et al. (2013) used an assignment testing method combined with mtDNA-based MSA to determine source populations of individual leatherbacks bycaught in the US western North Atlantic pelagic longline fishery (Stewart et al., 2016). This quantified differential natal stock origins of leatherbacks impacted across 11 geographical regions, shining a light on the disproportionate impact on Costa Rican leatherbacks—one of the only North Atlantic populations not experiencing significant nesting increases (Troëng et al., 2007). Similarly, Clusa et al. (2016) coupled genetic assignment with stable isotope analyses to determine that bycatch risk to foraging loggerhead turtles in the Mediterranean Sea is dependent on the geographical region of a fishery, rather than differential fishing gear types. Although MSA alone will continue to contribute to threat assessments (particularly when only mtDNA data are available), these examples highlight the increased power of combining MSA with assignment testing to understand the relative risks away from nesting beaches at finer scales. However, there are some location and species-specific limitations in the applicability of these approaches because in some cases microsatellites have not provided any added resolution relative to mtDNA markers (e.g., Northwest Atlantic loggerheads; Bowen et al., 2005).

While bycatch is one of the principal threats to marine turtle populations globally (Wallace et al., 2010a), genetic assignment has potential to inform assessments of other human impacts, such as boat strikes, poaching or marine macro-debris (e.g., ghost fishing nets), as well as additional conservation contexts such as identification of differential foraging habitat use among populations (e.g., for critical habitat assessments). These applications will be most biologically informative when comprehensive genetic characterization of all potential natal origin stocks has been conducted (see *Social Dimensions Section below*).

How Do Recent Population Declines Affect Marine Turtle Long-Term Resilience?

Many marine turtle populations have suffered large declines due to anthropogenic activities, and there is evidence that human-caused reductions began several centuries ago (Bjorndal and Jackson, 2003; McClenachan et al., 2006). Some populations have shown encouraging signs of recovery due to conservation actions in recent years, while others continue to remain low or further decrease (NRC, 2010). Marine turtles of today may be relics of historically larger and possibly biologically different populations, but it is unclear if or how such declines might impact population recovery and resiliency. One way that species declines (natural or human caused) can impact population resiliency is through the loss of genetic diversity underlying phenotypic variation, which may reduce adaptive potential and increase inbreeding impacts (Willi et al., 2006; Allendorf et al., 2013). For example, population bottlenecks have been shown to have strong negative impacts on hatching success in endangered birds (Heber and Briskie, 2010), and low major histocompatibility complex (MHC) genetic variation in Tasmanian devils *(Sarcophilus harrisii)* contributes to a high susceptibility to deadly transmissible cancers (Jones et al., 2004; Siddle et al., 2010). Longevity and other life history traits of marine turtles provide buffers from diversity loss relative to other taxa (e.g., if depletion does not persist over several generations, some populations can recover quickly without necessarily losing genetic diversity; Dutton et al., 1999), but it may also make it challenging to detect. Early research suggested that contemporary genetic bottlenecks in small nesting populations of Mediterranean loggerheads could be mitigated by male-mediated gene flow (Carreras et al., 2007). However, a recent study in olive ridley turtles in Mexico discerned a human-induced contemporary bottleneck across an entire metapopulation where strong impacts of a commercial fishery from 1960 to 1990 significantly reduced allelic diversity (Rodríguez-Zárate et al., 2013) as well as phenotypic variation of reproductive modes (i.e., solitary nesters vs. arribadas; Abreu-Grobois and Plotkin, 2008). Additionally, lower levels of genetic variation in younger vs. older turtles were recently reported in Yucatan peninsula hawksbills (Gonzalez-Garza et al., 2015), which is the largest nesting population in the Atlantic and critical for long-term hawksbill persistence (Mortimer and Donnelly, 2008). This work highlights that although marine turtles have buffers to maintain genetic diversity in the face of human-driven declines, they are not completely immune, and once it is lost it would likely take a long time to regenerate (i.e., low diversity may persist even as abundances of depleted populations recover).

These studies are an important first step in advancing our understanding of diversity loss and maintenance in marine turtles, but it is not yet known if or how such changes negatively impact marine turtle population resiliency, particularly in the face of other stressors such as disease and climate change. To our knowledge, only one study to date has investigated connections between genetic variation and phenotypic traits related to fitness in marine turtles, and there were no significant relationships between measures of reproductive success (i.e., clutch size, hatching success, and frequency of infertile eggs) and neutral nuclear loci in hawksbill turtles (Gonzalez-Garza et al., 2015). However, examination of functional genomic regions (MHC loci) in loggerhead turtles suggested that locally adapted pools of MHC alleles at the margins of the population distribution combined with male-mediated gene flow may be key to sustaining adaptive potential across the entire rookery (Stiebens et al., 2013a,b). As diversity is increasingly characterized across more marine turtle species and geographical regions (e.g., Ng et al., 2014; Vargas et al., 2016) and capacity to assess both neutral and adaptive genomic variation expands, our understanding of how human activities might impact marine turtle genomic diversity and ultimately long-term resiliency will continue to advance.

Emerging Applications for Threat Assessment: How Are Marine Turtles Affected by Habitat Alterations?

Habitat loss and degradation created by coastal development, pollution, climate change and other human activities increasingly threaten marine turtles at nesting and foraging grounds. In addition to direct mortalities, these may impact populations indirectly in ways that are much more difficult to quantify (such as altering population connectivity, demographic structure, or imparting sub-lethal impacts). Techniques such as MSA and assignment testing, passive maternal CMR via eggshell sampling, and gene expression assays have good potential to understand indirect consequences of human-caused habitat alteration. For instance, research is currently underway combining MSA and sex determination via hormone assays in foraging juvenile turtles to assess shifting sex ratios due to increasing sand temperatures at rookeries (M. Jensen and C. Allen, unpublished data). Tezak et al. (2017) also recently validated an immunohistochemical method to quantify protein expression of cold-induced RNA-binding protein (CIRBP) that reliably identifies hatchling sex, which could have potential for adaptation into a minimally invasive gene expression assay for hatchling sex determination. If possible, this may support rapid, direct monitoring of sex ratios over larger spatial and temporal scales, facilitating robust estimates of climate change impacts on this important demographic parameter. Functional genomics may also be useful in understanding underlying genomic and physiological processes and investigating sub-lethal impacts. For example, gene expression assays have recently been used to begin studying the effects of exposure to endocrine-disrupting pollutants in hatchling development (Gomez-Picos et al., 2014) and heritability of physiological stress responses to increased incubation temperatures under climate change (Tedeschi et al., 2016). Recently identified genes underlying thermal stress responses in marine turtle embryos also may serve as candidates to examine the adaptive capacity of different populations and species to cope with increasing nest incubation temperatures (Bentley et al., 2017).

Finally, human activities such sea wall construction and beach nourishment projects that restrict or remove access to key marine turtle reproductive habitats continue to increase as humans react to these threats themselves. These are occurring in concert with changes in habitat suitability due to warming and sea level rise (Pike, 2013), though it currently remains unclear how much plasticity or local adaptation exists in climatic niches across marine turtle populations (Mazaris et al., 2015). Evolutionary history tells us that extant marine turtles have found ways to persist in the face of large-scale climatic and habitat changes across millennia, and these strategies will likely help buffer impacts of ongoing environmental changes on marine turtle population viability. However, evidence of recent radiation and colonization events along with existence of many extinct marine turtle lineages also reminds us that evolutionary processes are dynamic, and that persistence is not guaranteed (Pritchard, 1997). We must also recognize that such habitat alterations are co-occurring with other human-caused stresses on marine turtle populations. Future research using approaches such as genetic CMR, assignment testing and functional genomics could be leveraged to track and understand changes due to these habitat changes over space and time at individual, rookery and population levels, possibly addressing questions such as: *Under what contexts will turtles relocate to another rookery or lay eggs in sub-optimal or unviable conditions? How does this affect population connectivity and demographic structure? Do species exhibit local adaptation or broad plasticity in nesting climatic niches?*

SOCIAL DIMENSIONS

Collaborative Synthesis

It's common for researchers to conduct genetic studies of nesting populations with a local or regional focus. However, extensive dispersal and migrations of marine turtles across large geographical distances necessitates large-scale analyses and international collaboration to resolve connectivity and phylogeography questions. This is particularly important for identifying population "units to conserve" (see **Box 2**) and for conducting meaningful MSA and individual assignments that require a comprehensive rookery baseline dataset of potential natal stock sources. Lack of this information can significantly limit MSA and assignment inferences, and in some cases can lead to inaccurate interpretation of results (Shamblin et al., 2017). In the case of oceanic juvenile loggerheads foraging in the North Atlantic, baseline 817 bp haplotype data were initially lacking for several potentially contributing rookeries, preventing robust MSA. In 2009, NOAA-NMFS and the Archie Carr Center for Sea Turtle Research at the University of Florida convened an Atlantic-Mediterranean Loggerhead Genetics Working Group (LGWG) to bring together researchers and assess the state of knowledge of loggerhead turtle genetics in the Atlantic and Mediterranean basins. While each group was working on regional studies of loggerhead turtle stock structure, they did not individually have access to complete datasets for the whole Atlantic that is required for meaningful MSA. The LGWG provided a formal structure for individual sample and data holders to safely share data prior to publication of regional datasets to address data gaps and develop large-scale, synthetic stock structure analyses and facilitate robust MSA. The LGWG also recognized that the baseline would require continual updating as additional rookery data become available to maintain relevance for MSA in the future, and established a website to provide a forum to obtain updated results (Shamblin et al., 2014). These cooperative efforts represent significant advances in marine turtle biology and conservation, and directly enabled the assignments of animals bycaught in fisheries. Although such syntheses require substantial time commitments and large-scale international cooperation, continued efforts in other species and regions will be instrumental in facilitating comprehensive threat assessments and mitigation efforts. The reluctance or lack of capacity in many countries to deal with the bureaucratic burden of CITES has been an impediment to interchange of samples for larger-scale, comparable analyses, and is likely to continue without substantial investment in infrastructural support. However, opportunities may exist for establishing or expanding collaborations like the LGWG and for building regional capacity or a network of in-country laboratories to generate and share genetic data within existing frameworks such as the IUCN Marine Turtle Specialist Group, The State of the World's Sea Turtles (SWOT) working group, or in some cases bilateral collaborations (see Matsuzawa et al., 2016).

Public Engagement and Outreach

In addition to collaborative efforts within the scientific community, another advance has come from sharing results with stakeholders and the public in accessible platforms to promote citizen engagement to achieve goals in large-scale synthetic genetic projects. For example, the Northern Recovery Unit Loggerhead DNA Project is using genetic CMR that spans nearly all beach monitoring programs from the Florida-Georgia border through North Carolina. Representatives from these projects enter nesting data for each recorded clutch in an online nesting database maintained by seaturtle.org. Following individual assignment of each genetic sample, a turtle ID unique to each nesting female is uploaded into the database. This provides volunteers collecting samples for the genetics project real time feedback on female identity as samples are processed. Participants from each beach project have access to the nesting history for any female that has ever laid a clutch on their beach. This feedback mechanism has strongly contributed to volunteer engagement and buy-in, particularly when the project was in its infancy. There is also a summary webpage on the project available to the general public that highlights some basic demographic data at various spatial scales and includes some example nesting histories. A project of this scale simply would not be possible without the support and cooperation of the marine turtle management programs in the respective state agencies and the many organizations and volunteers that comprise the marine turtle nest monitoring networks in each state. Integrating collaborative initiatives with easy to use, standardized methods enables consistent data collection and maintenance across the subpopulation and facilitates large-scale analyses. As these online resources and social media tools for citizen science become more accessible, we anticipate increasing opportunities to use this approach across a variety of marine turtle genetics research applications.

REMAINING CHALLENGES AND FUTURE DIRECTIONS

Lessons Learned and Best Practices

Several key themes emerge from the diverse examples discussed that are useful in guiding future projects using genetic tools for marine turtle biology and conservation research. First, there is not one best approach. Rather, it is most important to match the right tools to the research question and biological context, and for researchers employing genetics to understand the underlying theory to ensure appropriate inferences from their data (Karl et al., 2012). Particularly in conservation contexts, budget constraints often need to be considered, making it even more important to prioritize research and management goals to make sure they are in line with research study designs. Some conservation questions may be adequately addressed using traditional markers or without sampling every individual or location. However, it is also essential to recognize and pursue synergistic opportunities that can build capacity for future research and progress our state of knowledge. Many of the advances discussed here were made by combining banked specimens with new samples and/or re-analyzing samples with new genetic markers, demonstrating the importance of long-term biological collections. Insights of future studies using new technologies and/or evaluating spatiotemporal patterns will undoubtedly similarly rely on such resources, so while it is not always feasible to collect and store samples without a specific purpose, it is important to support these efforts when possible. Additionally, it can be difficult to justify using limited funds to develop resources that do not immediately address management questions (such as genome assemblies and annotation, new techniques or markers, pedigrees, and genetic linkage maps), but these resources open the door for a tremendous diversity of future studies highly relevant to conservation (e.g., well-annotated genomes and other molecular resources facilitate cost-effective studies of environmental adaptation and disease impacts in salmon and mammals; Miller et al., 2011; Epstein et al., 2016). By reaching out across disciplines, marine turtle biologists may likely find opportunities to partner with scientists in other academic fields as well as the biotechnology industry with expertise, interest and resources to develop these tools to build future capacity for marine turtle conservation genomics.

Secondly, undertaking large-scale or long-term sampling and monitoring programs such as the genetic fingerprinting projects in St. Croix and the Southeast US require substantial forethought of logistical coordination, standardized sample collection and storage, and data management. For programs embarking on incorporating genetic sampling into monitoring plans for the first time, learning from the challenges and best practices that have emerged from current genetic fingerprinting projects and long-term tagging databases, and investing time in developing infrastructure, training and data organization strategies can greatly facilitate project success (**Table 3**). Many of the examples detailed in this review also demonstrate the importance of working groups and international collaborations in determining global marine turtle population boundaries, life history strategies, and threat assessments. As we strive to put together the remaining pieces of these puzzles and address outstanding big questions in marine turtle biology and conservation, working together across boundaries will continue to be paramount to success.

Finally, we recognize that for many conservation programs, despite continued cost reductions and increasing technological accessibility (**Box 1**), it may still not be feasible to independently integrate genetic sampling and analyses into biological monitoring due to financial, expertise and infrastructure barriers. However, interested organizations may be able build partnerships and scale projects to capitalize on available resources (**Table 3**). For example, some programs may have capacity to collect and store samples, but lack funding or infrastructure to conduct analyses. These groups can develop sampling schemes best suited for their resources and biological questions by conferring with experienced researchers and using validated methods for collection and storage (e.g., Dutton and Stewart, 2013) in preparation for future analysis and funding opportunities. Organizations may also be able to build partnerships with other wildlife genetics researchers that have existing infrastructure and expertise to make costs feasible with existing resources or work collaboratively to seek funding together. While it certainly does not make sense for every program to conduct extensive genetic sampling, employing these and other creative strategies can help make these approaches and the knowledge they generate accessible to the broader marine turtle conservation research community.

Emerging Applications

As we look toward the future, what are the key remaining challenges, and how can we use genetics and genomics to address major unresolved questions in marine turtle biology, as well as emerging issues such as climate change? New studies creatively adapting traditional approaches will continue to play important roles, and there are also many exciting new techniques with great potential to expand our knowledge. Here, we highlight several promising avenues on the horizon, recognizing that there are many more possibilities that will likely emerge. First, integrating genetic tools with complementary data types (such as stable isotopes, skeletochronology, hormones, telemetry, unmanned aerial vehicles, and oceanographic modeling) has recently provided novel insight into marine turtle biology (Stewart et al., 2013; Naro-Maciel et al., 2014a). Many of these fields are undergoing revolutionary technological advances akin to those occurring in genomics, so the potential for combined novel applications will likely continue to grow into the future. For example, "landscape genomics," a discipline integrating genetic and environmental data to understand how environmental factors shape population connectivity and adaptation, is almost two decades old and has contributed to a wide-range of conservation management applications (Manel and Holderegger, 2013). However, high-resolution environmental data resources in the oceans have been more limited compared to terrestrial ecosystems, so analogous studies in the marine environment have lagged behind. But recent advances have facilitated the rapid expansion of seascape genomics studies that have diverse applications in conservation and resource management contexts (Benestan et al., 2016; Selkoe et al., 2016). These integrative approaches have good potential in marine turtle studies for tackling emerging threats such as monitoring foraging grounds to detect early signs of recruitment decline, or tracking possible phenological and range shifts due to habitat alteration and climate change. Minimally invasive techniques that have been validated and are currently being employed in other marine wildlife may also prove to be useful in marine turtles, such as environmental DNA (eDNA) sampling to estimate presence of a particular species (Kelly et al., 2014), and gene expression profiling via blood sampling to evaluate sublethal impacts of environmental stressors such pollutants or capture stress (Morey et al., 2016). High-throughput sequencing (HTS; see **Box 1**) also holds promise for expanding our understanding of fundamental marine turtle ecology and evolution. The generation of genome-wide datasets open the door to phylogeographic and comparative genomic analyses that have yielded remarkable insight into evolutionary histories in other taxa (Cammen et al., 2016), but have not been possible in marine turtles due to data and genomic resource limitations. But beyond this, the versatility of HTS offers potential for a broad diversity of applications, such as genome-wide association studies (GWAS) to identify the genomic basis of key phenotypic traits (Korte and Farlow, 2013), rapid genotyping of individuals tracked over larger spatio-temporal scales (e.g., to identify phenotypic plasticity in key behaviors, such as nesting site fidelity or breeding intervals), or evaluating genomic signatures of introgression (Reis et al., 2009; Vilaça et al., 2012) and monitoring future hybridization events under changing demographic conditions (e.g., altered sex ratios under climate change).

CONCLUSIONS

Over the past several decades, genetics have helped answer an increasing diversity of research questions in marine turtle biology and conservation. Rapidly expanding genetic and genomic toolboxes will undoubtedly continue to expand our knowledge in coming years. By collaborating and integrating these innovations with those in complementary disciplines, marine turtle conservation biologists can leverage these tools to tackle the remaining and emerging challenges in marine turtle ecology, evolution and conservation management.

AUTHOR CONTRIBUTIONS

LK, MJ, KS, BS, and PD developed the ideas, performed the literature searches, and wrote the manuscript.

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GLOSSARY

Genetic bottleneck: A loss of genetic diversity due to a severe reduction of population size.

Microsatellite: Tandem repeats of short DNA sequences, usually in pairs, triplets, or quadruplets that are used as neutral markers for identifying individuals, inferring parentage, or assigning individuals to specific populations.

Nuclear vs. mitochondrial DNA: In the marine turtle context, nuclear DNA is inherited from both parents while mitochondrial DNA is transmitted from mother to offspring. The divergent modes of inheritance as well as rates and mechanisms of mutation make it important to consider which type of marker is appropriate for the question being addressed.

Orphan haplotype: A mitochondrial DNA variant described from a foraging area but with no known nesting population of origin.

Panmixia: Random mating within a breeding population; no genetic structure. In a marine turtle context, it can also mean sufficient female movement among rookeries such that the rookeries would behave as part of the same population.

Phylogeography: The study of the geographical distribution of genetic lineages, generally attempting to explain contemporary patterns through inferring historical processes.

Polymorphic: Variable, often used in the context of nuclear markers where informative allelic variation is present.

Rookery: Marine turtle nesting beach colony. Definition and usage in the literature varies and can create confusion; can refer to one isolated nesting beach or several adjacent nesting beaches. These are often delimited *a priori* based on physical proximity, not based on data quantifying demographic or population connectivity. One or more rookeries typically make up a genetic stock or management unit (MU), based on genetic and complementary data, and several stocks/MUs make up larger units like DPS or RMU. (see *further discussion of conservation units in* ***Box 2***).

SNP: Single Nucleotide Polymorphism (pronounced "snip"), sequence variation at a specific position in a DNA sequence.

Stock: Demographically independent nesting populations (DIPs), defined by genetic and complementary data. While this term can be used differently in the literature among taxa and disciplines (e.g., fisheries science), in the context of marine turtles it is considered synonymous with management unit (MU). To avoid confusion, in this review we primarily refer to "MUs," but use "stock" where necessary (e.g., in discussions of marine turtle applications of Mixed Stock Analysis (MSA), which was originally developed for fisheries applications).

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