A Sea Turtle Population Assessment for Florida's Big Bend, Northeastern Gulf of Mexico

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A Sea Turtle Population Assessment for Florida's Big Bend, Northeastern Gulf of Mexico

Authors
Ryan M. Chabot, Inwater Research Group; Ryan C. Welsh, Inwater Research Group; Cody R. Mott, Inwater Research Group; Jeffrey R. Guertin, Inwater Research Group; Brian M. Shamblin, Warnell School of Forestry and Natural Resources, University of Georgia; and Blair E. Witherington, Inwater Research Group

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Ocean Springs, Mississippi
A SEA TURTLE POPULATION ASSESSMENT FOR FLORIDA’S BIG BEND, NORTHEASTERN GULF OF MEXICO

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ABSTRACT: Coastal waters of Florida’s Big Bend, Gulf of Mexico (GOM) once supported one of the largest sea turtle fisheries in the United States. To fill an information gap in this region on abundance and distribution of sea turtles, we used vessel-based distance sampling and active capture methods to characterize current foraging aggregations near the St. Martins Marsh Aquatic Preserve. Over 10 sampling periods between 2012–2018, we completed 513 km of transects and recorded 819 turtles among 4 species—green turtle (Chelonia mydas, n = 624), Kemp’s ridley (Lepidochelys kempii, n = 147), loggerhead (Caretta caretta, n = 47), and a single hawksbill (Eretmochelys imbricata). Turtle densities in 4 study plots within the 200 km2 study site ranged from 57–221 immature green turtles/km2, 16–56 immature Kemp’s ridleys/km2, and 1–14 juvenile-to–adult loggerheads/km2. Of 200 green turtles captured, 67.5% showed skin tumors consistent with fibropapillomatosis, a frequency similar to that from urbanized estuaries of Florida’s east coast. The largest green turtles (≥ 60 cm straight standard carapace length), abundant in the southern portion of our study area, are of note because this size class is uncommonly recorded within US territorial waters. Analyses of green turtle mtDNA haplotypes found contributions from rookeries in the western GOM, Mexican Caribbean, and Costa Rica. Although Big Bend protected areas were principally designed to conserve marine and coastal habitats, these regulatory zones have also effectively encompassed a hotspot for foraging sea turtles.

KEY WORDS: Marine Protected Area, Distance Sampling, Dip Net Rodeo, Genetic Stocks, Fibropapillomatosis

INTRODUCTION

Population assessments for endangered species assist recovery by revealing hotspots for abundance and potential threats, and by providing data for temporal trends. These assessments are especially helpful in areas that have been historically pressured by harvest (Rebel 1974). In this study, we assessed populations of sea turtles foraging in the Florida Big Bend region of the Gulf of Mexico (GOM). In their comprehensive review of in–water sea turtle assessments, Eaton et al. (2008) identified this area as understudied, yet believed sea turtles are likely to occur here based on habitat, archival harvest records, and reported observations. Since then, Hart et al. (2020) tracked post–nesting loggerhead turtles (Caretta caretta) from around GOM nesting beaches to the Big Bend. Wildermann et al. (2019) also performed opportunistic turtle sightings during vessel surveys and tracked 16 individuals (9 green turtles (Chelonia mydas), 5 Kemp’s ridley turtles (Lepidochelys kempii), and 2 loggerheads) using satellite telemetry to examine movements and habitat use of turtles in the region. Both studies support the assessment made in Eaton et al. (2008) that the area is deserving of more detailed and comprehensive investigation.

Florida’s Big Bend comprises shallow waters off ~350 km of coastline formed by saltmarsh covered, drowned karst limestone (Murali 1982). Marine habitats in the region are characterized by extensive seagrass meadows, oyster beds, and submersine freshwater springs. The region receives low wave energy due to a shallow continental shelf that is over 150 km wide (Murali 1982). Waters of the Big Bend are environmentally diverse and have been described as one of the least polluted coastal regions of the continental United States (Livingston 1980). More recently, Big Bend water quality and seagrass coverage have been impacted by anthropogenic nutrient discharge into regional rivers (Hale et al. 2004). Despite some reduction in historical seagrass coverage, the Big Bend continues to have the second largest contiguous area of seagrass habitat in the eastern GOM (Iverson and Bittaker 1986, Mendelsohn et al. 2017). The importance of this region is recognized through designation of 2 marine protected areas, the Big Bend Seagrasses Aquatic Preserve and the St. Martins Marsh Aquatic Preserve. Protected adjacent terrestrial habitats include the Chassahowitzka National Wildlife Refuge, Cedar Keys National Wildlife Refuge, Lower Suwannee National Wildlife Refuge, and St. Marks National Wildlife Refuge. These, in addition to other conservation lands, protect the majority of Big Bend shoreline from human development (Florida Natural Areas Inventory 2020). We refer to this assemblage of conservation locations here and throughout as Big Bend protected areas.

Results of habitat surveys in Big Bend protected areas and recent satellite tracking and vessel–based survey studies suggest that sea turtles are an important wildlife species benefitting from the region’s ecosystem (Zeiman and Zeiman 1989, Dawes et al. 2004, Wildermann et al. 2019, Hart et al. 2020). However, the ecological contributions of sea turtles in the region were severely diminished by decades of commercial harvest (Caldwell and Carr 1957), which ended in the mid–1970s (Withell 1994, National Research Council 2010). Carr and Caldwell (1956) first described sea turtle demographics within the region using data collected from fishery landings at Cedar Key, located in the central Big Bend. This location hosted...
the last fishery to commercially harvest significant numbers of sea turtles from Florida waters (Caldwell and Carr 1957). The turtle harvest was focused on areas southeast of Cedar Key, near the mouths of the Crystal and Withlacoochee rivers (Caldwell and Carr 1957). Carr and Caldwell (1956) reported that the turtles taken were predominantly immature green turtles (mass range 5—52 kg) and immature Kemp’s ridleys (mass range 2—27 kg), with rare landings of loggerheads and hawksbills (*Eretmochelys imbricata*).

About 2 decades following the end of the sea turtle harvest in Florida waters, Schmid (1998) sampled turtles near Cedar Key between 1985 and 1996, and found that captures were dominated by Kemp’s ridleys. Similarly, Barichivich (2006) also found that Kemp’s ridleys dominated captures from 1996—1999 in Deadman Bay, about 100 km north of Cedar Key. Both studies captured green turtles and loggerheads as well, though species representation may have been influenced by their choice of sampling areas and methods that favored Kemp’s ridley capture (Barichivich et al. 1999).

Although the previous work described above underscores the importance of the Big Bend region for sea turtles, we saw critical gaps in information that would assist management and sea turtle population assessment. With exception to Wildermann et al. (2019), previous work in the region primarily targeted single species and used methods that may have excluded smaller turtles. Additionally, since the assemblages of green turtles in the shallow (< 2 m) coastal waters of the Big Bend are almost exclusively immature animals (Carr and Caldwell 1956, Schmid 1988, Wildermann et al. 2019), they are separated by time and distance from their breeding areas, and their natal regions are unknown. Finally, fibropapillomatosis (FP) is a neoplastic disease evident as lesions on skin, eyes, and internal organs of affected turtles (Herbst 1994), and is especially common in green turtles in Florida, although all species are affected (Jones et al. 2016). The disease prevalence of FP is strongly associated with waters receiving runoff from agriculture, industry, or urban development (Foley et al. 2005, Van Houtan et al. 2010, Jones et al. 2016), and can be used as a way to indicate ecosystem health from a sea turtle perspective (Aguirre and Lutz 2004). To fill these information gaps, our objectives were to: 1) Measure species relative abundance and size distributions using effort—quantified methods expected to represent all species and size classes present within the Big Bend area; 2) Determine green turtle genetic origins and links to regional breeding populations using maternally inherited genetic markers (mitochondrial DNA haplotype frequencies, Allard et al. 1994) to test hypotheses of GOM and Caribbean rookery origins for green turtles; and 3) Assess the frequency and severity of fibropapillomatosis in the relatively pristine Big Bend region.

**Materials and Methods**

**Study Area and Sampling Period**

We represented Big Bend protected areas by sampling nearshore waters surrounding the St. Martins Marsh Aquatic Preserve between the mouths of Crystal River and Homosassa River, FL (Figure 1). Our targeted area totaled about 200 km$^2$ over which water depth was predominantly < 2 m. In this area, sampling was conducted throughout waters accessible to our vessel and where depth would allow turtle sightings and captures (about 0.5—3 m). Benthic habitats in the area consisted predominantly of dense seagrass (primarily *Thalassia testudinum* and *Syringodium filiforme*), but also included sparse seagrass patches, oyster reef, and rocky, submarine, freshwater springs, typical of habitat assemblages found in the region (Murali 1982). We sampled on 51 days over 10 sampling peri-
Sea Turtle Population in Florida's Big Bend

Sea Turtle Sampling Effort

We used vessel-based visual transect surveys to cover the broad study area and generate a spatial data set revealing sea turtle distribution and abundance. Daily surveys comprised multiple Haphazard Unmarked Nonlinear Transects (HUNTs, Bresette et al. 2010). During a HUNT, 2 experienced observers were positioned on a 2 m high elevated tower amidships on a 7 m, flat-bottom skiff. Vessel speed remained close to 9 km/h while observers in the tower searched for turtles. Vessel path during searches and observed/captured turtle locations were recorded using a Garmin GPSMAP 700 Series Chartplotter. HUNT transect locations were distributed throughout the study area, consistent with the ability of the vessel to access shallow water areas ≥ 0.5 m. As we gained additional resources to sample larger regions, we expanded our study area across ~200 km² and divided it latitudinally into 4 equal 50 km² sections, hereafter referred to as sections A – D, where A and D are the northernmost and southernmost sections, respectively (Figure 1, Table S1). Although sections A and B were sampled during each year of the study, section C was not sampled in 2013, and section D was sampled only in 2013 and 2016–2018 (Table S1). We calculated turtle abundance for each section with regard to respective sampling effort. Prior to each HUNT, we recorded conditions that were anticipated to affect detectability and distribution of turtles, i.e., cloud cover and wave height. Turtle sightings by observers were relayed to a data recorder, who noted species, life stage or size class, position in the water column, perpendicular distance of the observation from the transect line, and Global Positioning System (GPS) waypoint. The perpendicular distance from the turtle to the transect line was estimated by observers using length references visible to the observer (e.g., vessel length and beam and capture net length). GPS software (Garmin Mapsource) determined the vessel path distance between HUNT start and end points. GPS waypoints (latitude and longitude position ± 10 m) for turtle locations, and HUNT start and end locations were downloaded at the conclusion of each sampling day. Observers estimated turtle life stage using relative size of the turtle’s carapace (Standard Straight Carapace Length, SSCL) as a guide.

Capture Efforts

Turtles were occasionally captured following their observation during a HUNT, and in these instances, the turtle sighting location marked the end point of the search transect. We made captures using either hand capture (Rodeo) or a modified version of Rodeo employing dip nets (Dip Net Rodeo). In Dip Net Rodeo a researcher standing on the bow would use a 10 cm mesh nylon net within a 1 m diameter hoop mounted on a long handle to quickly scoop the turtle into the bag of the net (Gorham et al. 2013). Additional crew would ease the netted turtle onto the deck of the vessel. Captures by Rodeo follow typical protocols described by Eckert et al. (1999) and were only used for turtles that were too large or in water too deep for our preferred Dip Net Rodeo capture technique.

Identification, Morphometric Data, and Biological Data Collection

The SSCL was recorded for each captured turtle using forestry calipers as described in Pritchard et al. (1983). Green turtles were considered to be adults if SSCL was > 85 cm (Eaton et al. 2008). We identified captured turtles using both internal and external tags. Inconel #681 tags (Cooperative Marine Turtle Tagging Program, Archie Carr Center for Sea Turtle Research, University of Florida) were applied to the trailing edge of one or both front flippers of turtles with a SSCL ≥ 30 cm. All turtles, regardless of SSCL, were given a passive integrated transponder (PIT) tag (Destron-Fearing) subcutaneously applied proximal to the wrist of the right front flipper. Genetic samples for green turtles were obtained in one of 2 ways, blood collection or skin biopsy. Blood was collected from the dorsal cervical sinus (external jugular veins) using a sterile vacutainer with no additive and a 2.5 cm, 21-gauge sterile needle (Owens and Ruiz 1980). We collected about 4 ml of blood from each turtle and added a few drops to a lysis buffer (100 mm Tris–HCL, pH 8; 100 mm EDTA, pH 8, 10 mm NaCl; 1.0% SDS) in a 1:10 ratio. If we failed to collect blood, a 4 mm biopsy punch was used to acquire a skin sample from the distal edge of one rear flipper. We preserved this tissue in 90% ETOH. Blood or skin were used for mixed-stock analysis to estimate nesting beach contributions to this foraging aggregation.

To avoid resampling, all captured animals were marked on the carapace with a temporary white all-weather Paintstik® live-stock marker. After all measurements and samples were taken, turtles were released in the area where they were captured.

Population Density Estimates

We estimated population densities for green, Kemp’s ridley, and loggerhead sea turtles using Program Distance 7.2 release 1, with both conventional distance sampling and multiple covariates distance sampling. Care was taken in both field and statistical methods to eliminate multiple counting of individuals. Sightings were restricted to within 10 m of the boat for distance analyses to eliminate outliers (a standard practice in Distance Sampling, Buckland et al. 2015) and to reduce double counting of animals on the fringe of sight that may be re-encountered on a different HUNT. Our standard HUNT protocol also restricted the vessel from crossing over a previous track from that same day, in a similar attempt to eliminate the likelihood of the multiple counting of individuals. To satisfy minimum distance sampling sample size requirements (Buckland et al. 2015), detection functions were generated for all turtle sightings by species for Kemp’s ridley and loggerhead turtles regardless of the differing size classes recorded, whereas small and large juvenile classes (smaller and larger than 60 cm SSCL, estimated at sighting) were differentiated for green turtles. Post stratification was then used to gather densities for each field section. We weighted results by total effort of HUNTs in each of the four surveyed sections (Figure 1). Densities (D) were calculated using the transect line formula found in Buckland et al. (2015):

\[ D_j = \frac{n_j f(0)}{2L_j g(0)} \]

Equation 1:
where \( n \) is the total number of turtles observed during surveys, \( f(0) \) is the probability density function evaluated directly on the transect line, \( L \) is the distance surveyed, and \( g(0) \) is the probability of detection on the transect line. Data were truncated to 10 m and binned into 2 m increments for all sections to account for rounding errors during observation. We considered hazard rate and half-normal decay functions as candidate detection functions. For each candidate function, 11 models were compared: one with no covariates, and 10 with each of the temporal and environmental covariates collected (Table 1). Covariates collected included the following: cloud cover (measured categorically in 25% increments; 1 = ≤ 25%, 2 = 26—50%, 3 = 51—75%, 4 = ≥ 75%, recorded at start of each transect); section (section of field site based on the geographic position of observation); size class (estimated categorically as the size class of the animal as determined by the observer during the sighting, used only for loggerhead and Kemp’s ridley models); tide at observation (measured in meters of displacement from mean low water level recorded using Ozello North NOAA station 8727328, based on time of observation); time of day (taken from GPS at time observation was made); transect length (measured in km from GPS track of vessel from start and end points of each individual transect); trip (measured categorically as a number indicating the specific trip to the field site in which the sighting was made); water temperature at observation (taken from transponder on boat during moment of observation, measured in °C); wave height (measured in feet and placed categorically in 4 increments; 0, 1—2, 2—3, and 3—4, recorded at start of each transect); and year (year that observation was made).

We used the Akaike Information Criterion (AIC; Akaike 1973, Burnham et al. 2011) for model selection among the set of candidate models. The model with the lowest AIC value was selected as the most informative among the models tested. Candidate models with ΔAIC values < 2 were considered commensurate, and model selection was then based on the principles of parsimony, as well as visual examination of the probability detection curve, and a \( \chi^2 \) Goodness of Fit test (Δ = 0.05), all of which are provided in the analysis from Program Distance 7.2 release 1.

### Genetic Stock Analyses for Green Turtles

Control region sequences were generated from 177 individuals using PCR primers LCM15382 and H950 and sequencing primers LCM15382 and CM1820 as described by Shamblin et al. (2015). Sequences were assigned haplotypes based on standardized nomenclature (Archie Carr Center for Sea Turtle Research website; http://accstr.ufl.edu/resources/mtdna—sequences/). Expanded haplotypes retained their number designations based on the inclusive 490 base pair original haplotypes, with suffixes indicating variation outside this fragment. In addition to the control region, individuals carrying haplotype CM—A1.1 were sequenced at the geographically informative mtSNP position 12958 using PCR primers CM12751F and CM13064R and sequenced with CM12781 as previously described (Shamblin et al. 2017). All CM—A5.1 individuals were sequenced at the regionally informative mtSNPs 10745 and 14726 as described by Shamblin et al. (2012). Haplotype frequencies of the Big Bend protected areas foraging aggregation were compared with others in the GOM: the neritic juvenile foraging aggregation in Texas (TX, Shamblin et al. 2017), neritic foraging aggregation in the Dry Tortugas and Everglades (SGoM, Naro—Maciel et al. 2017), and surface—pelagic juveniles from the northern GOM (NGoM, Shamblin et al. 2018). Rookery contributions to the Big Bend aggregation were estimated based on a Bayes-

<table>
<thead>
<tr>
<th>Covariates</th>
<th>Method of Collection</th>
<th>Included in Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cloud Cover</td>
<td>Measured categorically in 25% increments. 1 = ≤ 25%, 2 = 26—50%, 3 = 51—75%, 4 = ≥ 75%, recorded at start of each transect.</td>
<td>Cc, Lk, SCm, LCM</td>
</tr>
<tr>
<td>Section</td>
<td>Section of field site based on the geographic position of observation.</td>
<td>Cc, Lk, SCm, LCM</td>
</tr>
<tr>
<td>Size Class</td>
<td>Measured categorically as the size class of the animal as determined by the observer during the sighting.</td>
<td>Cc, Lk</td>
</tr>
<tr>
<td>Tide at Observation</td>
<td>Measured in meters of displacement from Mean Low Water level recorded using Ozello North NOAA station 8727328 (28.86333, -82.66667) based on time of observation.</td>
<td>Cc, Lk, SCm, LCM</td>
</tr>
<tr>
<td>Time of Day</td>
<td>Taken from Global Position Unit at time observation was made.</td>
<td>Cc, Lk, SCm, LCM</td>
</tr>
<tr>
<td>Transect Length</td>
<td>Measured in kilometers from Global Positioning Unit mapped track of vessel from start and end points of each individual transect.</td>
<td>Cc, Lk, SCm, LCM</td>
</tr>
<tr>
<td>Trip</td>
<td>Measured categorically as a number indicating the specific trip to the field site in which the sighting was made.</td>
<td>Cc, Lk, SCm, LCM</td>
</tr>
<tr>
<td>Water Temperature at Observation</td>
<td>Taken from transponder on boat during moment of observation and measured in °C.</td>
<td>Cc, Lk, SCm, LCM</td>
</tr>
<tr>
<td>Wave Height</td>
<td>Measured in feet and recorded at start of each transect. Placed categorically in 4 increments. 0, 1—2, 2—3, and 3—4.</td>
<td>Cc, Lk, SCm, LCM</td>
</tr>
<tr>
<td>Year</td>
<td>Year that observation was made</td>
<td>Cc, Lk, SCm, LCM</td>
</tr>
</tbody>
</table>

TABLE 1. List of covariates used in distance sampling analysis. Method of Data Collection explains how and when each covariate was collected during field sampling from 2012-2018. Included in analysis indicates for which species/size classes each covariate was included in distance sampling analyses. Cc = loggerhead, Lk = Kemp’s ridley, SCm = small juvenile green turtle, and LCm = large juvenile green turtle.
Fibropapilloma (FP) Tumor Score

Fibropapilloma (FP) tumors associated with FP were measured and recorded on a standardized tumor score sheet. A tumor score (the number of tumors found on an individual) was calculated for each turtle that exhibited external evidence of FP. Applying the technique of Balazs (1991), we used the number and size of all tumors present to assign individual turtles to FP severity categories (i.e., Balazs score). We used the program R (R Core Team 2017) to conduct a Pearson’s $X^2$ test with $\alpha = 0.05$ to search for patterns of tumor abundance among different sizes of green turtles (the species most commonly afflicted with FP) using crossed counts of size class and tumor number. For these analyses, green turtles were divided into 4 SSCL size bins: 20–29.9 cm; 30–39.9 cm; 40–49.9 cm; and 50.0–82.0 cm. Because few turtles > 50 cm SSCL were captured, animals above this threshold were combined into one category to provide an adequate sample size. Tumor number was also binned; no tumors; 1–10 tumors; 11–20 tumors; 21–30 tumors; 31–40 tumors; and > 40 tumors.

**Results**

During our HUNT surveys in Big Bend waters from 2012–2018, we observed 819 sea turtles, of which 386 turtles were captured. Green turtle sightings were most frequent (n = 624), followed by Kemp’s ridleys (n = 147), loggerheads (n = 47), and a single hawksbill.

**Size Class Distributions**

Capture attempts were made on 587 turtles, where the overall capture success rate was 66% (386/587). By species, capture rates were 75% (15/20) for loggerheads, 52% (200/381) for green turtles, 92% for Kemps ridleys (170/185) and 100% for hawksbills (1/1). The mean ± sd SSCL of the captured animals were: loggerheads (75.9 ± 17.8 cm, range = 33.9–97.0 cm, n = 15); green turtles (38.7 ± 9.7 cm, range = 23.9–81.3 cm, n = 200); Kemp’s ridleys (44.2 ± 6.5 cm, range = 20.0–56.5 cm, n = 170); and one hawksbill (40.5 cm). Size—class distributions of captured individuals varied among species (Figure 3). Loggerhead captures were sparse relative to green turtles and...
Kemp’s ridleys, and sizes ranged between small juveniles and adults. Captured green turtles were most frequently between 25—45 cm SSCL, except those captured in section D, where the 19 green turtles captured were between 33—82 cm SSCL (57.9 ± 15.3 cm). This contrasts with green turtles captured in sections A, B, and C, which overall were smaller, with a mean SSCL of 36.7 ± 10.3 cm (range = 23.9—67.6 cm). Size frequency of Kemp’s ridleys peaked near 50 cm SSCL, with only 5% of captured individuals larger than this value (Figure 3).

We estimated the size of green turtles sighted but not captured (n = 424), assigning them to 2 simple size class categories — small (< 60 cm SSCL) and large (≥ 60 cm SSCL) immatures; no green turtles of adult size were observed or captured during the course of this study. All measurements of captured green turtles were within the estimated size category assigned at sighting. We attribute this high level of accuracy to the use of experienced observers familiar with estimating green turtles by size in multiple habitats, a low number of green turtles documented between 55–65 cm SSCL (Figure 3), and possibly bias where observers knew size class frequencies from previous sample periods.

**Population Density Estimates**

We completed 513.25 km of HUNTs over 10 sampling periods, during which we observed 819 turtles among 4 species. Of the 4 survey sections evaluated within our study area (Figure 1), section B was surveyed the most (243.04 km), followed by sections A (149.12 km), C (80.05 km) and D (44.04 km). To test hypotheses on size—dependent distribution patterns, we divided green turtles between 2 categories of size estimated at sighting: smaller turtles (about < 60 cm SSCL, n = 565) and larger turtles (about 60–85 cm SSCL, n = 59).

The best—fit detection model for small juvenile green turtles used a Hazard Rate function, with tide at observation as a covariate, while the best—fit detection model for Kemp’s ridleys also included a Hazard Rate function with no temporal or environmental covariates (Table 2). The best—fit detection model for large juvenile green turtles and loggerheads was a Half Normal function, with length of transect included as a covariate for large juvenile green turtles, while the loggerhead model contained no temporal or environmental covariates (Table 2).

Turtle population density estimates varied among study area sections and species/size classes (Figure 4). Density esti-

**TABLE 2.** Model selection table displaying top 3 ranked Distance Sampling models generated using Program Distance 7.2 release 1 for loggerhead, Kemp’s ridley, small, and large juvenile green turtles, based on data collected from Florida’s Big Bend 2012–2018. Model covariates in bold are the selected models using selection based on the principles of parsimony, as well as visual examination of the probability detection curve, and a χ² Goodness of Fit test (α = 0.05).

**Hazard Rate Function**

<table>
<thead>
<tr>
<th>Model Covariates</th>
<th>Parameters</th>
<th>AIC Score</th>
<th>ΔAIC</th>
<th>Model Covariates</th>
<th>Parameters</th>
<th>AIC Score</th>
<th>ΔAIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Covariates</td>
<td>2</td>
<td>368.03</td>
<td>0.00</td>
<td>Observation</td>
<td>3</td>
<td>369.60</td>
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**Half Normal Function**

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**FIGURE 4.** Density estimates by study area section in the Florida Big Bend region derived from distance sampling analyses 2012–2018, separated by species/size class groupings. Small juvenile green turtles are < 60 cm SSCL and large juvenile green turtles are >60 cm SSCL. See Figure 1 for location of sections.
mates for small juvenile green turtles were the highest overall among the species/size classes, with 220.8 turtles/km² (range, 157.5–307.8) in section A, 158.0 turtles/km² (range, 122.2–202.9) in section B, 69.8 turtles/km² (range, 32.5–142.8) in section C, and 56.8 turtles/km² (range, 23.9–110.1) in section D. Loggerheads had the lowest density estimates overall, with 0.9 turtles/km² (range, 0.3–2.5) in section A, 4.9 turtles/km² (range, 2.1–13.9) in section B, 14.0 turtles/km² (range, 4.6–35.9) in section C, and 14.4 turtles/km² (range, 5.2–27.8) in section D. Kemp’s ridleys had density estimates of 29.8 turtles/km² (range, 13.6–51.7) in section A, 56.4 turtles/km² (range, 33.2–93.2) in section B, 26.8 turtles/km² (range, 11.3–55.4) in section C, and 56.8 turtles/km² (range, 23.9–110.1) in section D. Large juvenile green turtle sightings were restricted to section D, with density estimates of 110.2 turtles/km² (range, 44.0–212.5).

Green Turtle Genetic Stocks

The large number of green turtle captures presented us with the greatest potential for insightful mixed-stock analysis among the 4 sea turtle species. Polymorphic sites in the mitochondrial control region resolved 15 different haplotypes in the green turtle aggregation we sampled (Figures 2, 5, Table S2). One individual carried an orphan haplotype of unknown origin (CM—A29.1). Haplotype frequencies indicated similar stock compositions between Big Bend juveniles and those in SGoM, but strong differentiation of these from the TX aggregation and the nearby NGoM surface–pelagic aggregation (Figure 2, Table S2). Mixed-stock analyses estimated that the majority of Big Bend juveniles originated from Mexican genetic stocks, particularly from WBCMX (Tamaulipas/Veracruz) and Quintana Roo (QRMX), with additional important contributions from Tortuguero, Costa Rica (TORT) and minimal contributions from other locations (Figure 5, Table S2).

Size-Specific Fibropapilloma (FP) Tumor Score

None of the Kemp’s ridleys nor the single hawksbill captured during this study presented with FP tumors. One loggerhead had one FP tumor (Balazs score = 1). Tumor prevalence was documented for 199 green turtles, in which 64 (32.2%) did not present with FP tumors, 67 (33.7%) had between one and 10 tumors, 25 (12.6%) had between 11 and 20 tumors, 18 (9.0%) had between 21 and 30 tumors, 14 (7.0%) had between 31 and 40 tumors, and 11 (5.5%) had > 40 tumors. Sixty-four (32.2%) of the green turtles had a Balazs score of 0 (no FP tumors), 52 (26.1%) had a Balazs score of 1 (mild), 77 (38.7%) had a Balazs score of 2 (moderate), and 6 (3.0%) had a Balazs score of 3 (severe). Results of the Pearson’s χ² test indicated a significant association between the size class of green turtles and their number of FP tumors (χ² = 40.003, p < 0.001). Evaluating standardized residuals of the chi-squared test (Figure 6) revealed that fewer green turtles in the smallest size bin (20–29.9 cm) presented with FP tumors than was expected by
the model. Observed presence of FP tumors in the 2 middle—size bins was higher than expected by the model, with individuals in the 30—39.9 cm size bin having fewer tumors, and those in the 40—49.9 cm size bin having more. Turtles in the largest size bin (50—82 cm) had fewer tumors than expected, although less markedly so than turtles in the smallest size bin.

**Discussion**

**Size Class Distributions and Suggestions of Species Recovery**

Green turtles were the most frequently sighted species and had the highest density estimates based on our distance sampling analyses. Comparing these results to historical data from the region highlights possible demographic shifts. Green turtle capture efforts by Schmid (1998) from 1986—1995 at a location just north of our study site resulted in only 10 green turtles, all > 40 cm SSCL, with the majority > 60 cm SSCL. In contrast, about 92% of green turtles in our study were < 50 cm SSCL. We are cautious in drawing conclusions about this apparent shift toward smaller (younger) green turtles because the large—mesh tangle netting method used by Schmid (1998) favored capture of larger turtles. Our observations are that the hand—capture method we used was equally successful for all turtle sizes. With caveats that our methods are minimally comparable to historical efforts and habitats sampled, we hypothesize that the high frequency of small green turtles we observed reveals high recruitment to this region. This high recruitment, combined with positive trends in nest numbers across north Atlantic rookeries (Chaloupka et al. 2008, Seminoff et al. 2015), offer an encouraging assessment of conservation efforts over the past few decades. These efforts have included reduction of harvest of green turtles and eggs as food throughout the region (Seminoff et al. 2015).

The number of Kemp’s ridley captures we made was in keeping with those from the mid—1980s and mid—1990s (Schmid and Witzell 1997, Schmid 1998, Barichivich et al. 1999), but was high in comparison to capture numbers from Crystal River, FL during the 1950s (summarized in Barichivich et al. 1999). These previous studies used mixed capture methods including strike netting, tangle netting, and rodeo, so a direct comparison of catch—per—effort is not possible. However, to the extent that size—class distributions among these projects (including ours) are comparable, there is evidence of an increase in capture frequencies of Kemp’s ridleys < 50 cm SSCL. Our size distribution of Kemp’s ridleys from the Big Bend region is similar to that of the recent sampling by Lamont and Johnson (2020) in the nearby Florida Panhandle. We hypothesize that this apparent size—distribution shift represents increased recruitment of young Kemp’s ridleys.

Our size distribution of Kemp’s ridleys, with few adult turtles (SSCL ≥ 60 cm, Eaton et al. 2008), was different from sites in the northern GOM west of the Mississippi Delta (Shaver and Rubio 2008, Seney and Landry 2008, 2011). This difference is in keeping with telemetry data, which show that a small proportion of adult Kemp’s ridleys tracked from nesting beaches forage in the Big Bend region, with most occupying the northern GOM westward (Shaver et al. 2013, Shaver et al. 2016). Because about one—third of our sampling took place following the post—nesting migration season of Kemp’s ridleys (May—August, Shaver et al. 2016), we do not believe that our capture period excluded the detection of adult female ridleys. Loggerhead captures in our study were sparse relative to green turtles and Kemp’s ridleys, with a size—class distribution similar to the one observed by Schmid (1998) for loggerheads in waters surrounding Cedar Key, FL to the north of our study site.

**Turtle Distributions and Density Estimates**

One concern when conducting haphazard versus traditional transects is the potential for multiple counts of the same individual during the same sampling period, particularly when working with highly vagile species like sea turtles. As described in the methods, we took care to not conduct HUNTS over areas previously traversed during the same sampling day, and we marked all captured turtles with a highly visible temporary number on their carapace for easy identification during sampling weeks. During the course of this study, only one previously sampled turtle was re—sighted on the same day it was captured, suggesting a low (1/386 = 0.26%) rate of double counting. Additionally, there were only 3 instances in which turtles were re—sighted within the same multi—day sampling period, which supports our assumption that double counting individuals was a low likelihood event.

We recorded fewer loggerhead sightings and estimated lower loggerhead population densities than those of green turtles and Kemp’s ridleys. Loggerhead density estimates were highest in sections C and D of our study area, which was similar to regional loggerhead sightings by Wildermann et al. (2019). Although there are no extensive surveys for benthic prey items preferred by loggerheads in our shallow coastal study area, we hypothesize that the lack of large hardbottom structure within the area makes this habitat less preferred. The relatively dense foraging aggregations of immature and adult loggerheads in the shallow waters of Florida Bay occur within a mosaic of seagrass and hardbottom habitats (Bjorndal et al. 2013). Similarly, nesting female loggerheads tracked by Girard et al. (2009) and Tucker et al. (2014) from southwestern FL, by Hart et al. (2020) from various GOM beaches, and by Hardy et al. (2014) from GOM and Atlantic beaches, dispersed across the northeastern GOM in deeper waters of the West Florida Shelf where there is considerable hardbottom habitat (Schroeder et al. 1988, Broadbent et al. 2019).

The highest densities of small juvenile green turtles were found in sections A and B of our study area, whereas the highest density of Kemp’s ridleys occurred in section B. These results are consistent with sightings of these species recorded by Wildermann et al. (2019) in a similar study area to ours. These 2 studies provide evidence that Florida’s Big Bend protected areas provide important foraging habitat for juvenile green turtles and Kemp’s ridleys. Distributions of these species may be influenced by concentrations of high value prey items, although no fine scale data are currently available regarding distributions of benthic invertebrate prey items preferred by
Sea Turtle Populations in Florida's Big Bend

Kemp’s ridleys (Witzell and Schmid 2005, Servis et al. 2015) in the study site. The study area contains extensive seagrasses and algal species (Florida Department of Natural Resources 2017), representing the predominant food items consumed by green turtles in the northwest Atlantic (Bjorndal 1997).

Green turtles are hypothesized to occupy successive developmental habitats, with spatial shifts that are correlated with body size (Meylan et al. 2011). In keeping with this hypothesis, we observed smaller immature green turtles that were spatially separated from larger immatures. Although we recorded only one large juvenile green turtle (> 60 cm SSCL) in study area sections A, B, or C, density estimates for large juveniles in section D were equivalent in scale to the densities of small juvenile green turtles in the other sections. Large juvenile green turtles are uncommonly sampled in U.S. Atlantic waters and are principally known from foraging grounds in the Florida Keys (Bresette et al. 2010, Fujisaki et al. 2016). Large juvenile green turtles are also uncommon in the nearshore waters off the west coast of Florida, where animals of this size comprise between 1–10% of green turtles recovered during mass stranding events (Foley et al. 2007, McMichael et al. 2008, Avens et al. 2012). The same pattern is observed along the coast of Texas where large juvenile green turtles comprised < 10% of in-water captures in the Laguna Madre (Metz and Landry 2013) and about 3% of green turtles recovered during mass cold stun events in the same region (Shaver et al. 2019).

Because the vast majority of our large green turtle sightings occurred near a 3 m deep dredged channel in section D, we hypothesize that bathymetry is a principal environmental variable influencing foraging site selection by larger green turtles. In a review of predator effects on adult sea turtles, Heithaus et al. (2008) found that adult green turtles in Shark Bay, Australia predominantly occupied areas with access to deep water, which they surmised provided escape from predators like tiger sharks (Galeocerdo cuvier). Because tiger sharks and bull sharks (Carcharhinus leucas, another large predator) are known to frequent our study area (Peterson et al. 2020), we propose that the largest green turtles in our study would benefit from foraging locations close to deeper water with more three-dimensional space to maneuver and avoid predation.

We did not collect representative habitat data (depth, bottom type, salinity, etc.) that could inform a spatial model for sea turtle distribution. However, some general observations help form hypotheses that could be tested in a subsequent study. The hypothesized relationship between water depth and turtle size is one example, as is the influence of salinity varying by river mouth location. Because the bottom type we observed was predominantly dense seagrass, within what has been described as the second most expansive seagrass habitat in the eastern GOM (Hale et al. 2004), there may be limited opportunity to discern influence from this habitat variable. Relative to observations of large immature green turtles near the dredged channel in Section D, we did not observe any obvious increase in seagrass within the channel.

Some spatial and temporal limitations of how well our data represent the study area include a lack of winter sampling due to poor visibility and turtle sighting conditions during these months. However, for the effort spent between late spring and late summer, we detected little spatial or temporal bias in the haphazard effort. Nevertheless, some bias may have resulted from sampling only during spring and summer, only in daylight, and limiting sampling to waters between 0.5 and 3.0 m.

**Green Turtle Genetic Stocks**

We found strong genetic connectivity between the green turtles sampled in our study area and females nesting on beaches in the southern GOM and Mexican Caribbean. These linkages highlight the importance of management and conservation efforts across international boundaries. Comparisons among green turtle foraging aggregations in the GOM showed similarity between benthic/neritic groups in Florida's Big Bend (this study) and southern GoM (SGoM), but strong differentiation from neritic green turtles in TX and from young, surface–pelagic green turtles in the northern GOM (NGoM). These TX and NGoM foraging aggregations primarily comprised individuals from WBCMX rookeries, with minimal contributions from Quintana Roo (Shamblin et al. 2017, 2018). The larger Caribbean (Quintana Roo, Mexico and Costa Rica) contributions detected in the eastern GOM are likely influenced by the Loop Current and surface eddy patterns in the region (Shamblin et al. 2018). The orphan haplotype of unknown origin (CM–A29.1) carried by one individual in this study was previously detected in the SGoM foraging aggregation (Naro–Mael et al. 2017). Although nearby southern and central eastern Florida rookeries account for the vast majority of green turtle nesting in the southeastern U.S. (Florida Fish and Wildlife Conservation Commission, Fish and Wildlife Research Institute 2020), these “downstream” rookeries provided a minimal genetic contribution to turtles at our Big Bend study site.

**Green Turtle Fibropapilloma (FP) Tumors**

Although the causative agent of green turtle FP is a Chelonid alphaherpesvirus (Herbst et al. 1995, McGeoch and Gatherer 2005), environmental factors are commonly implicated as contributors to tumor expression (Dos Santos et al. 2010, Van Houtan et al. 2010). Biotoxins and anthropogenic contaminants are suspected to act during a tumor promoting phase of FP within turtles that already have the virus (Arthur et al. 2008).

We found a size–class frequency of green turtle FP fits the profile of gradual disease expression following recruitment of green turtles into coastal waters, a pattern seen in other green turtle assemblages (Ehrhart 1991, Jones et al. 2016). Namely, we found FP tumors to be most frequent and most severe in the middle size classes of immature individuals. This is in keeping with the hypothesis that green turtles recruit from oceanic/pelagic habitat without the disease, acquire the disease after they arrive in coastal habitats and begin benthic foraging, and either die or undergo tumor regression with acquired immunity that minimizes tumor expression as they mature (Foley et al. 2005). Our success rate in capturing green turtles was about 52% (200/381 green turtle capture attempts). We have
no evidence that FP was over—represented in captured turtles; FP presented in varied degrees of severity, predominantly with low tumor scores that do not correlate with encumbered swimming ability (Li and Chang 2020, McNally et al. 2020).

The high prevalence of FP among green turtles in our study (67.5%), from a relatively pristine area of Florida, is contrary to the hypothesized correlation of high green turtle FP rates with adjacent urbanized areas (Jones et al. 2016). Green turtle FP prevalence at our study site is comparable to rates recorded from the Indian River and Lake Worth Lagoons on Florida’s densely populated east coast. These east coast lagoons are poorly circulated with turbid waters that receive large discharges of agricultural and urban runoff (Hirama and Ehrhart 2007, Gorham et al. 2016). There is a lack of data regarding toxin and pollutant concentrations important to FP tumor expression in green turtles (Jones et al. 2016). However, to the extent that pollutants and toxicants are indeed factors in green turtle FP presence and severity, there are a number of contributing sources in the Big Bend region. Mouths of 3 river systems (Crystal, Homosassa, and Chassahowitzka Rivers) empty into neritic waters directly adjacent to our study site and could carry pollutants from terrestrial sources. Other rivers also empty into regional marine waters, including the Fenholloway River, which is about 150 km to the north of our study area. This river receives discharge from a cellulose (paper) mill and is thought to contribute to marine eutrophication, algal blooms, and elevated sulfates (Mattson 2000). These and other potential FP cofactors could be transported from other regions within the GOM or wider Caribbean by the Loop Current and associated eddies. We recommend that future research in Florida’s Big Bend protected areas assess toxin loads in sea turtles and their habitats, their effects on health and immune function, and potential sources of these pollutants.

Multi—Population Sea Turtle Assemblage as a Critical Resource within Big Bend Protected Areas

Systematic planning for protected areas requires spatially and demographically explicit data for local species in need of protection. Few Marine Protected Areas (MPAs) have these data. Where MPAs are surveyed for foraging sea turtles, information is most often limited to relative distributions of turtles based on a sample of telemetered animals (Maxwell et al. 2011, Schofield et al. 2013, Hays et al. 2014, Dawson et al. 2017, Selby et al. 2019). Only rarely are abundance estimates and detailed genetic stock assessments presented for these areas (Herren et al. 2018, Fuentes et al. 2019). Although these genetic assessments and abundance estimates are labor intensive to collect, they are critical to conservation planning approaches that seek to balance economic and conservation values (Ban et al. 2009).

The demographic specific sea turtle abundance and distribution data reported here showcase the value of Big Bend protected areas to western Atlantic sea turtle populations. This value applies to at least 4 species, with genetic representation in one of these species, the green turtle, spanning 5 more populations with nesting beaches in the GOM and Caribbean.

Although Big Bend protected areas were principally designed to conserve marine and coastal habitats, these regulatory zones have effectively encompassed a diverse hotspot for foraging sea turtles. Patchy distributions of juvenile sea turtles are hypothesized to stem from patterns in drift within ocean currents and in turtle swimming patterns that vary between regions (Christiansen et al. 2016). We have no data to describe the Big Bend region as a special recruitment area for any sea turtle species, but the size classes of green turtles and Kemp’s ridleys we recorded suggest that the region encompasses more new recruits than older foraging juveniles. We propose that the productive seagrass and hardbottom habitats in the region explain the abundance of sea turtles, especially green turtles and Kemp’s ridleys.

Because sea turtles are migratory between multiple life stages (Bolten 2003), individual MPAs are unlikely to contain entire populations. However, strategically planned regulations in multiple areas can approach full protection. Where sea turtles have benefitted from extensive protection like this, populations have shown impressive recovery. In the south Atlantic, green turtles migrate from breeding beaches at Ascension Island to foraging grounds along the Brazilian coast (Luschi et al. 1998). At both locations, green turtles have received efficient protection for the past few decades (Broderick et al. 2006), protections that are credited with the steep increase seen in females migrating to nest at Ascension Island beaches (Weber et al. 2014). Population wide protections are also a likely explanation for the recovery of green turtles in Florida (Florida Fish and Wildlife Conservation Commission 2020). Sea turtle nesting beaches in Florida and throughout the southeastern U.S. are protected from direct take of individuals, as are U.S. waters (Butler 1998). However, there are many threats to sea turtles incidental to lawful human action, which occur in many habitats where sea turtles live. It is for this reason that MPAs, with restrictions on human activities harmful to sea turtles and their habitats, are beneficial to sea turtle population recovery. We feel that protected areas of the Big Bend region contribute to a key combination of protections across sea turtle ranges.

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Chabot et al.


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