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Fibropapillomatosis prevalence and distribution in green turtles *Chelonia mydas* in Texas (USA)

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ABSTRACT: Fibropapillomatosis (FP) is a neoplastic disease that can result in debilitating tumors in sea turtles. Initially identified in Florida, USA in 1937, it has since been detected in green turtle *Chelonia mydas* populations globally. FP was first identified and confirmed in Texas, USA in 2010. No FP tumors were documented in Texas prior to that year, though many green turtles were encountered and examined using standardized procedures since 1980. The present study was undertaken to identify temporal and spatial trends of FP prevalence in Texas since 2010. From 2010 through 2018, 1919 stranded or incidentally captured green turtles were documented with FP in Texas. FP prevalence was significantly correlated with year, hypothermic stunning, geographic region, and turtle size, as determined by logistic regression. FP was documented in <4.0% of the green turtles examined in Texas from 2010 to 2015, increasing to 21.6% in 2016, 27.3% in 2017, and 35.2% in 2018. More than twice as many hypothermic stunned green sea turtles had FP tumors as compared to those that were not hypothermic stunned. In Texas, FP was most prevalent in south Texas, particularly in the Laguna Madre, and associated channels. FP was more prevalent in turtles with straight carapace lengths 40.0–69.9 cm. The impact of this disease on green turtle population recovery in Texas is not yet apparent.

KEY WORDS: Sea turtles · Green sea turtles · *Chelonia mydas* · Fibropapillomatosis · Texas

1. INTRODUCTION

Fibropapillomatosis (FP) is a disease characterized by tumors of the periocular tissues, skin, carapace, and plastron (Herbst 1994, George 1997), although internal tumors have also been found (Herbst 1994, Work et al. 2004, Foley et al. 2005). Initially identified in green sea turtles in the Florida Keys, USA in turtles captured in 1937 (Smith & Coates 1938, Hirama & Ehrhart 2007), FP has since been detected in green sea turtle populations globally (Alfaro-Núñez et al. 2014, Seminoff et al. 2015, Li et al. 2017). FP is considered a threat to some green turtle populations, particularly where there is a high incidence of large tumors that impair vision, movement, or feeding, and thus impact individual survivorship (Ehrhart & Redfoot 1995).

In the USA, large numbers of green turtles have been afflicted with this disease in Florida (Ehrhart & Redfoot 1995, Schmid 1998, Hirama & Ehrhart 1999, 2007, Foley et al. 2005) and Hawaii (Balazs & Pooley 1991, Balazs et al. 1997, 2000, Work et al. 2015). FP was first confirmed in Texas, USA in 2010 (Tristan et al. 2010). Prior to 2010, no green turtles in Texas were documented with tumors or suspect growths consistent with FP, despite thousands of encounters of green turtles by Sea Turtle Stranding and Salvage Network (STSSN) participants and hundreds of encounters by in-water researchers (Manzella et al. 1990, Coyne 1994, Renaud et al. 1995, Arms 1996, Shaver 2000, Metz & Landry 2013, Shaver et al. 2013), confirming that the disease was not missed, but was in fact not present prior to 2010.

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The virus associated with FP, chelonid herpesvirus 5 (ChHV5), is believed to have co-evolved with sea turtles for approximately 1 million yr (Herbst et al. 2004), and has genetic variants between populations (Morrison et al. 2018). ChHV5 is found in FP tumors (Herbst 1994, Herbst & Klein 1995, Quackenbush et al. 1998, Greenblatt et al. 2005, Page-Karjian et al. 2015) and is likely the cause of this disease, though the etiology is not completely understood. There is evidence that turtles acquire the virus after they recruit to a shallow foraging habitat (Ene et al. 2005). Marine leeches, pollution, warm shallow waters, and increasing numbers of green turtles have been implicated in promoting this disease (Greenblatt et al. 2004, Herbst et al. 2004, Foley et al. 2005). Additionally, asymptomatic green turtles can carry the virus, detected as viral DNA in many tissue and fluid types (Page-Karjian et al. 2012, 2015, Alfaro-Núñez et al. 2014, 2016, Chaves et al. 2017).

The present study focuses on quantification of FP tumor cases observed in sea turtles that were documented by the STSSN as stranded (washed ashore or floating, either dead or alive but imperiled) or incidentally captured (entrapped in intake canals of power plants or captured unintentionally while fishing) (Teas 1993) in Texas. Causes of stranding are numerous and include boat strikes, debris entanglement, hypothermic stunning, and disease (Shaver 1998, Shaver et al. 2017). Live stranded turtles are taken to wildlife rehabilitation facilities, while dead turtles are often recovered for investigatory necropsy (Shaver 1998). Our study was undertaken to identify temporal and spatial trends of FP prevalence in Texas since 2010, when the first cases were documented.

2. MATERIALS AND METHODS

2.1. Data collection

STSSN participants in Texas documented sea turtles found stranded or incidentally captured from 1980 through 2018. For each turtle, information was collected on stranding date, location, tag numbers (if identified), visual signs of disease and/or injuries, condition, and final disposition of the animal. Curved carapace length (nuchal notch to posterior tip; CCL) and straight carapace length (nuchal notch to posterior tip; SCL) were measured for most turtles. Turtles documented with visible tumors characteristic of FP were identified; no attempt was made to quantify asymptomatic turtles via viral DNA in tissues or body fluids. Records were categorized as offshore (from shores or waters of the Gulf of Mexico) or inshore (found on shores or waters of passes and bays). Information was recorded on standardized forms sent to state and national STSSN coordinators. Latitudinal bands were used to divide the Texas coast (Fig. 1) into 3 sections: North, which includes coastal areas in Texas from the Louisiana border at latitude 29.684° N through 28.000° N; Central from 27.999° N to 27.000° N; and South from 26.999° N to 25.955° N, for comparison of FP prevalence. These areas of interest were chosen with respect to variation in geographic features and management.

2.2. Permitting and animal welfare

This study was conducted in accordance with the Guide for Care and Use of Laboratory Animals of the National Institutes of Health. Work by National Park Service (NPS) personnel was authorized under US Fish and Wildlife Service Permit TE840727-3, Texas Parks and Wildlife Department Scientific Permit SPR-0190-122, and the NPS Institutional Animal Care & Use Committee permit IMR_PAIS.Shaver.Walker_SeaTurtle.A3.

2.3. Data analysis

The Texas STSSN database was queried for records of green turtles *Chelonia mydas* documented stranded or incidentally captured in Texas from 1980 to 2018. Stranded and incidentally captured turtles were included in all analyses. FP prevalence analyses were restricted to green turtles documented by the STSSN from 2010 to 2018, years FP was observed in Texas. SCL mean, SD, and range were derived. Turtles were categorized into 10 cm SCL size classes to illustrate the frequency distribution of FP among these size classes. For 230 turtles, CCL, but not SCL, measurements were obtained, and these were converted for analysis from CCL to SCL using the formula SCL = 0.294 + (0.937 × CCL) (Teas 1993). Turtles described as skeletal or dry carcass were excluded from analysis due to limitations identifying tumors on carcasses in those conditions (n = 1159; of which only 1 was noted with FP). Repeated measures were also excluded to satisfy model assumptions (n = 56). Means are represented with ±1 SD.

ArcGIS 10.4.1 (ESRI 2016) was used to display the prevalence and distribution of green turtles with FP tumors encountered between 2010 and 2018 in Texas, USA, displayed as prevalence within 10 km hexbins.

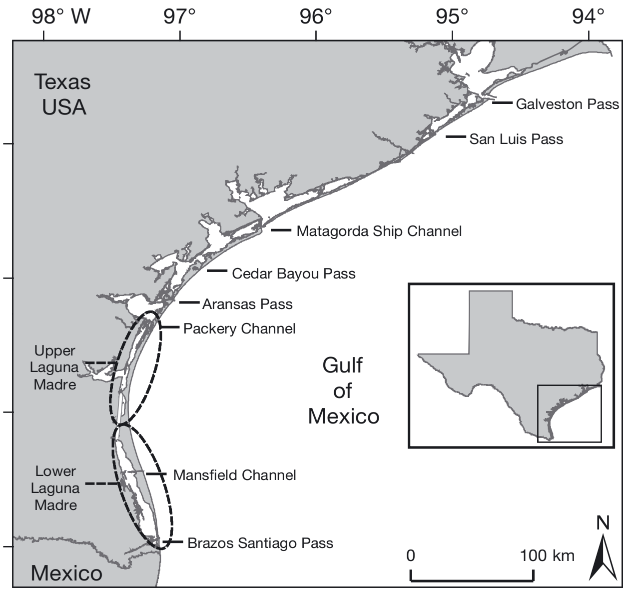

Fig. 1. Texas, USA coast with the locations of major channels and passes as well as the Upper and Lower Laguna Madre (inset: Texas state, with a square around the coastal area)

Table 1. Logistic regression analysis of main effects (Year, Hypothermic stunned, Geographic region, and Size class based on straight carapace length) related to prevalence of fibropapilloma tumors (FP) in *Chelonia mydas* documented in Texas, USA. Estimate: model estimates for log(odds) at intercept and log(odds ratios) for each predictor variable; p-values are for Wald test; *significant ($p < 0.05$)

Predictor variable	Estimate	p
Intercept (Central)	−1064.0	<0.0001*
Region		
North	−0.7508	<0.0001*
South	0.4434	<0.0001*
Inshore/Offshore (Offshore)	−0.0629	0.5870
Hypothermic stunned (True)	0.4912	<0.0001*
Stranded/Incidentally captured (Stranded)	−0.0253	0.9050
Year	0.5250	<0.0001*
Size class	0.0803	<0.0001*

Full model:
FP ~ Region + Inshore/Offshore + Hypothermic stunned (True/False) + Stranded/Incidentally captured + Year + Size class; observations = 11 050

To determine if FP tumor prevalence varied significantly with the factors coastal region, inshore/offshore, hypothermic stunned/not hypothermic stunned, stranded/incidental capture, year, and SCL, generalized linear regression models (glm function) were created using R v.3.5.3 (R Core Team 2018). FP was modeled as a binomial (tumor presence, absence) with log transformation, for all factors (full model). Model reduction, i.e. removing insignificant variables, produced a comparable model that did not relay additional information due to the robustness of the relationships in the full model. This insignificance was confirmed using ANOVA and post hoc chi-squared model residuals. Therefore, only the full model is presented. Collinearity was examined for factors Region and SCL, as described in Section 3.

3. RESULTS

The generalized linear regression model (glm) indicated that the variables coastal region and hypothermic stunned/not hypothermic stunned and the covariates year and SCL were all highly significant effects ($p < 0.0001$) (Table 1). Neither inshore/offshore ($p = 0.5870$) nor incidental capture/stranded ($p = 0.9050$) were significant factors with respect to FP prevalence. FP prevalence increased significantly over the study period (n = 9 years, $p < 0.0001$) (Table 1). A total of 1919 green turtles were detected with FP out of a total of 11 360 stranded or incidentally captured green turtles documented in Texas from 2010 to 2018 (16.9%; Tables 2 & 3). For the state of Texas, the prevalence of FP did not exceed 5.0% during any single year from 2010 through 2015, but increased to 21.6% during 2016, 27.3% in 2017, and 35.2% in 2018 (Table 2). More than twice as many hypothermic stunned green turtles were observed with FP tumors (19.9%) as compared to turtles that were not hypothermic stunned (9.4%) (Table 3).

Table 2. Yearly fibropapillomatosis (FP) prevalence for 3 Texas coastal regions and state totals for *Chelonia mydas* documented with and without FP. n: number of turtles examined; %FP: percentage of turtles documented with FP

Year	North		Central		South		Total	
	n	%FP	n	%FP	n	%FP	n	%FP
2010	168	0.0	250	0.4	293	1.0	711	0.6
2011	108	0.0	802	0.4	996	7.9	1906	4.3
2012	51	0.0	165	0.0	79	2.5	295	0.7
2013	80	0.0	438	0.7	269	13.4	787	5.0
2014	213	1.4	1026	1.3	251	7.6	1490	2.3
2015	105	2.9	613	3.1	156	14.1	874	5.0
2016	67	6.0	225	21.3	116	31.0	408	21.6
2017	120	9.2	598	23.9	484	30.4	1202	27.3
2018	494	26.1	2702	37.4	491	32.2	3687	35.2
Total	1406	10.7	6819	18.2	3135	16.9	11 360	16.9

Table 3. Fibropapilloma tumor (FP) prevalence in *Chelonia mydas* (n = 11 360) documented in Texas, USA, from 2010 to 2018, categorized by those found hypothermic stunned versus those that were not hypothermic stunned, Texas geographic region, and size class (straight carapace length [SCL], 10 cm bins). Measurements were not obtained for 310 individuals; for 230 individuals, curved carapace length measurements had to be converted to SCL. n: number of turtles examined; %FP: percentage of turtles documented with FP tumors

Factor	n	%FP
Hypothermic stunned		
True	7926	20.1
False	3434	9.5
Region		
North	1406	10.7
Central	6819	18.2
South	3135	16.9
Size class (cm)		
10.0–19.9	85	2.4
20.0–29.9	2385	3.1
30.0–39.9	4773	10.3
40.0–49.9	2334	35.5
50.0–59.9	1069	37.6
60.0–69.9	324	29.0
70.0–79.9	65	10.8
80.0–89.9	13	0.0
90.0–99.9	2	0.0

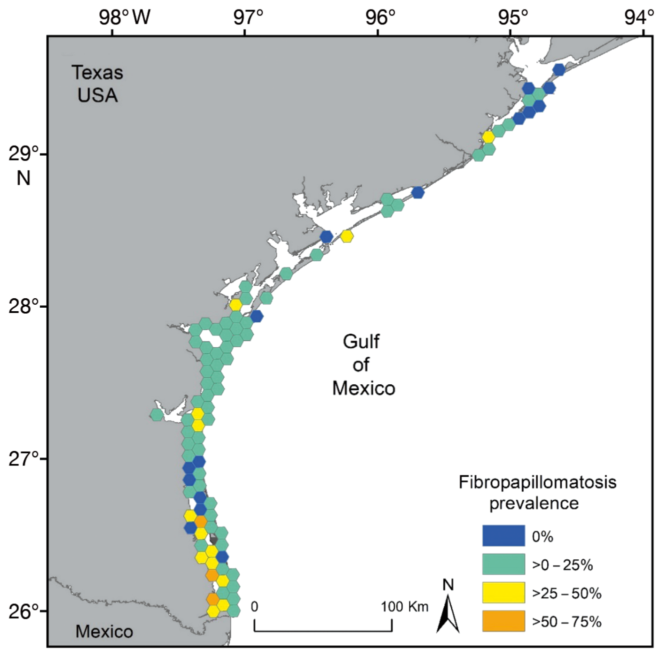

Fig. 2. Distribution and prevalence of green turtles *Chelonia mydas* documented with fibropapillomatosis (FP) in Texas, USA from 2010 to 2018. Data are percentage of green turtles with FP within 10 km hexbins, excluding sections with <10 encounters

Along the Texas coast, significantly more FP-afflicted turtles have been found in the southern portion of the state, south of Cedar Bayou Pass, which crosses between Mustang Island and Matagorda Island at latitude 28° N (Figs. 1 & 2). This section of the Texas coast is >250 km long and has many large bays, including Aransas Bay, Corpus Christi Bay, and the Laguna Madre. For analysis, this section was further divided into a Central region, which includes the Upper Laguna Madre, and South region, which includes the Lower Laguna Madre (Table 2) (see also Section 2.1). FP prevalence differed significantly among these areas (n = 3 regions, $p < 0.0001$), with the highest prevalence found in the Central area (18.2%; Tables 2 & 3). FP prevalence was lower and had a slower rate of increase in the North region, from Cedar Bayou Pass northward to the Louisiana border (Tables 2 & 3). This area of the Texas coast is >350 km long and includes Galveston and Matagorda Bays.

SCL of green turtles documented in Texas from 2010 to 2018 ranged from 10.5 to 94.0 cm (mean: 37.7 ± 10.4 cm, n = 11 050); those recorded with FP ranged from 12.5 to 78.3 cm SCL (mean: 44.7 cm ± 9.0 cm, n = 1901). FP was present in turtles of all 10 cm size classes except 80.0–89.9 and 90.0–99.9 cm, with the 40.0–49.9 and 50.0–59.9 cm size classes having the highest FP prevalence (Table 3).

A pairwise *t*-test indicated that turtles in the South region were significantly larger (mean SCL: 39.6 ± 11.2 cm; n = 3016) than the other 2 regions (both comparisons, $p < 0.0001$; North [mean SCL: 37.1 ± 10.6 cm, n = 1372] and Central [mean SCL: 36.9 ± 9.8 cm, n = 6612], which were not significantly different from each other, $p = 0.45$), indicating it was prudent to investigate possible collinearity between the relationship for SCL:Region with FP prevalence by plotting these factors against each other. Collinearity was not observed and was rejected.

4. DISCUSSION

The numbers of green turtles documented with FP tumors in Texas has increased at an alarming rate starting in 2015, with higher prevalence in south Texas than north Texas. This trend of increasing tumor prevalence over time was documented in Florida and Hawaii starting in the late 1980s (Foley et al. 2005, Chaloupka et al. 2009). Prevalence of FP varies greatly among other studied areas, though some examined numbers of turtles were captured by netting or other methods. Our inclusion of stranded turtles could cause a sampling bias because debilitated turtles, such as those with advanced FP, may be more likely to be found stranded (Foley et al. 2005, Chaloupka et al. 2008, Page-Karjian et al. 2014). However, bias is also possible in netting studies since debilitated turtles may also be more likely to be captured by netting (Limpus et al. 1994). We examined FP prevalence across time and space in Texas, with the source of study animals (stranded and incidentally captured turtles) held constant, limiting bias. Additionally, since 69.8% of green turtles examined for the present study were hypothermic stunned, capture bias is likely reduced because that stranding cause is likely more indiscriminate, supported by the fact that many otherwise healthy animals are found (Shaver et al. 2017).

High prevalence of FP has been found in other regions of the USA. In fact, high prevalence of FP occurred concurrently during the late 1980s through 1990s in areas of Hawaii and Florida (Balazs et al. 2000, Hirama & Ehrhart 2007, Chaloupka et al. 2009). However, tumor regression has been noted in many individual green sea turtles in Hawaii, and FP prevalence declined there from >50% in the mid-1990s to 9.4% in 2007, which was similar to levels documented before 1990 (Chaloupka et al. 2009). Prevalence of FP ranged from 4.5 to 7.8% among different areas for turtles netted in the Key West National Wildlife Refuge (Florida, USA) from 2003 to 2012 (Herren et al. 2018), though it remains as high as 70% for some lagoons on Florida's Atlantic coast (Ehrhart et al. 2016). FP was present in 13.4% of netted green turtles in the Turks and Caicos Islands, West Indies (Stringell et al. 2015). Additionally, prevalence of FP fluctuated from 0 to 75% in netted green turtles at 2 foraging sites in Puerto Rico from 1997 to 2014, with complete regression of visible tumors in individuals occurring within 2.7 yr on average (Patrício et al. 2016). FP tumor presence (31%, 2008–2010) and regression has been documented in Brazil (Guimarães et al. 2013). In Australia, 7.9% of green turtles captured in Moreton Bay (1990–1992) had FP tumors (Limpus et al. 1994).

Strong evidence suggests that juvenile green sea turtles are infected by ChHV5 once they reach the inshore neritic foraging habitat (Ene et al. 2005). All individuals detected with FP in our study were juveniles (12.5–78.3 cm SCL) in and near neritic foraging habitat. No nesting green turtles encountered in Texas, to date, have been found with FP tumors, and all have been over 84.0 cm SCL. Though 18.7% of green turtles found inshore (n = 9138 examined) had FP tumors, compared to 9.6% (n = 2222 examined) of those found offshore, this difference was not significant. Those found offshore are likely moving there from the inshore areas temporarily, to avoid cold inshore water temperatures during the winter or to migrate to other areas. All Texas bays and estuaries are connected by both oceanic routes and inshore waterways, with the exception of Galveston Bay, which is not connected via the inshore waterway southwest of the 29° N latitudinal line, limiting some inshore movement in that area. The lack of intracoastal waterway connection to Galveston Bay may have reduced movement of diseased animals to this area and may be the reason we see a significantly lower prevalence of green turtles with FP north of 29° N latitude (North region). Another possible reason that prevalence of FP was lower in the northern portion of Texas is that warmer waters are associated with FP tumor presence and development (Page-Karjian et al. 2014), and water temperatures are on average cooler in the northern part of the state. In Florida, FP prevalence is also more common in the southern portion of the state and very few cases north of 29° N latitude have been reported (Schmid 1998, Foley et al. 2005, 2007), strikingly similar to what we report here for Texas.

In Texas, FP tumors were observed most commonly on the ventral surfaces of flippers and on other body surfaces except the carapace. During the study period, no cases of oral FP tumors were recorded in green turtles in Texas. Although oral tumors are rare in Florida (Balazs et al. 1997, 2000), 40–61% of FP-afflicted turtles documented in Hawaii had oral tumors (Balazs et al. 1997, 2000). It is unknown why this disparity exists, but differences in infection route or genetic variants of ChHV5 are implicated (Balazs et al. 1997, 2000). Additionally, internal tumors have been reported in some severe FP cases in the USA (Herbst 1994, Work et al. 2004, Foley et al. 2005), but only 5 cases of internal tumors have been documented to date in green turtles in Texas.

Hypothermic stunning was a significant factor influencing the prevalence of FP tumors documented on turtles in this study. In fact, more than twice as many hypothermic stunned green turtles were observed with FP tumors as compared to turtles that were not hypothermic stunned. The cause of this disparity is not apparent. Further study of the prevalence of FP in green sea turtles in Texas waters is warranted, using a standardized capture method, such as netting.

The fact that FP tumors were first detected in green turtles at neritic foraging areas in southern Florida in 1937 (Hirama & Ehrhart 2007) and in the Hawaiian

Islands in 1958 (Balazs & Pooley 1991), but not in Texas until nearly half a century later, is compelling evidence and could provide clues regarding how the disease spreads to new areas. The Texas neritic foraging juvenile green turtle population is genetically distinct, indicating isolation of these western Gulf of Mexico juvenile green turtles (Shamblin et al. 2017). Thus, the late appearance and increasing prevalence of FP in Texas green turtles could be due to novel incursion of the virus into an immunologically naive population. Increasing FP prevalence is also likely due in part to the concurrent increasing population of Texas juvenile green turtles such that this provides high host density for this infectious disease. It has also been associated with near-shore waters with low water turnover such as lagoons, that contain pollutants, agricultural runoff, or marine biotoxins, and near-shore waters that are adjacent to large human populations (Limpus et al. 1994, Herbst & Klein 1995, George 1997, Aguirre & Balazs 2000, Foley et al. 2005, Arthur et al. 2008, dos Santos et al. 2010, Van Houtan et al. 2010, 2014, Patrício et al. 2016). Additionally, if FP prevalence is a reliable method to monitor ecosystem health in near-shore marine habitats as suggested by Aguirre & Lutz (2004), the health of Texas waters could be in decline.

The green turtle population in Texas, which was decimated by the late 1800s due to over-harvest and hypothermic stunning (Hildebrand 1983), has been increasing since 2007 (Metz & Landry 2013, Shaver et al. 2017), just prior to the appearance of FP. FP can cause significant debilitation and even death of individuals. However, it is unknown whether increasing prevalence of FP will slow green turtle population recovery in Texas. FP does not appear to be affecting population demographics for green turtles elsewhere (Foley et al. 2005, Chaloupka et al. 2009, Patrício et al. 2016). It will be vital to monitor FP prevalence over time to identify long-term trends, signs of regression in affected individuals, and possible impacts to the rapidly increasing green turtle population and the health of critical developmental habitat in Texas.

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