INTRODUCTION

Fibropapillomatosis (FP) is an infectious, neoplastic disease that affects all sea turtle species, and has been reported in many locations around the world (Aguirre et al. 1994, 1999, Herbst 1994, Williams et al. 1994, Herbst et al. 1995, D’Amato & Moraes-Neto 2000, Hiram & Ehrhart 2007, Chaloupka et al. 2009, Flint et al. 2010, Duarte et al. 2012, Díaz-Delgado et al. 2019). FP tumors are characterized by cutaneous, ocular, and visceral growths that often debilitate affected animals by inhibiting feeding and movement, obscuring vision, and/or leading to organ failure (Balazs 1986, Jacobson et al. 1989). Over the past 3 decades, FP has emerged as an important disease in green turtles Chelonia mydas, which are listed as...
Endangered on the International Union for Conservation of Nature Red List and threatened under the United States Endangered Species Act (Seminoff 2004). In certain green turtle populations of the Caribbean and in Florida and Hawai‘i, USA, FP has reached epizootic proportions (Williams et al. 1994, Hirama & Ehrhart 2007, Chaloupka et al. 2009). Although FP prevalence does appear to be decreasing in some regions such as Hawai‘i, FP prevalence in Florida may be increasing or at least is stable, since both the total number of stranded green turtles with FP have increased since 1995 (Chaloupka et al. 2009, Foley et al. 2015). Additionally, FP is being reported from new localities around the world, including Brazil, West Africa, and the Turks and Caicos Islands, and in more northern latitudes where it has never before been reported (Baptistotte et al. 2005, Loureiro & Matos 2009, Richardson et al. 2009, Duarte et al. 2012, Hargrove et al. 2016).

Stranded sea turtles with FP are often clinically debilitated and/or cachectic and exhibit various abnormalities in clinical pathology data that suggest anemia of chronic disease and chronic antigenic stimulation (Work & Balazs 1999, Aguirre & Balazs 2000, Cray et al. 2001, Hirama et al. 2014, Page-Karjian et al. 2014, Perrault et al. 2017). In sea turtle rehabilitation facilities, FP is a major concern, since extensive quarantine measures are required to accommodate turtles with FP, and because FP tumors can sometimes develop after turtles are admitted for other reasons (e.g. boat strike, fishing gear entanglement) (Page-Karjian et al. 2014). Turtles with severe FP disease often have accompanying co-morbid conditions such as emaciation or poor general health upon admission, buoyancy issues including floating, ileus, boat-strike trauma, and secondary or opportunistic bacterial and fungal infections (Herbst et al. 1995, Work et al. 2004, Page-Karjian et al. 2014, 2015). Furthermore, after FP tumors are surgically removed, there is still a potential for tumor regrowth, since the underlying associated herpesvirus infection (chelonid alphaherpesvirus 5, ChHV5) remains despite tumor removal (Herbst et al. 1995, Lackovich et al. 1999). These clinical factors, along with the infectious and potentially life-threatening nature of FP disease, complicate prognoses and extend rehabilitation times of sea turtles diagnosed with FP.

To objectively understand the various clinical manifestations of FP, it is necessary to apply a standardized method for quantifying and qualifying the extent of the disease. Several different FP scoring systems have been published, which include various considerations for tumor score designation. A simple but effective approach includes classifying the severity of FP disease based on a visual inspection and/or photographs of an individual turtle’s dorsum and ventrum (e.g. Balazs 1991, Hirama & Ehrhart 2007). The first approach for objectively classifying afflicted turtles into 1 of 4 scores (grades 0–3) was based on the maximum size of tumors and had an additional score class (grade 4) for turtles with tumors that physically hampered movement or caused blindness (Wood & Wood 1993). One scoring system developed for green turtles in Hawai‘i classifies tumors into 4 categories according to size, and then uses the number of tumors in each size category to assign an overall tumor score. This scoring system has been widely used; however, this approach was not intended to be universal, and may not be as useful in regions outside of Hawai‘i, particularly since the various physical manifestations of FP disease can be region-specific (Work & Balazs 1999, Work et al. 2004, Chaloupka et al. 2009, Santos et al. 2010, Torezani et al. 2010, Rossi et al. 2016). A more recently published scoring system attempted to improve upon the previous scoring systems by including not only tumor number and size, but additional variables such as tumor location, morphology, and degree of invasiveness, although this method of classification is likely best suited for a clinical setting rather than in the field (Page-Karjian et al. 2014). Researchers in Brazil developed a system that included measuring each individual tumor and then attempting to estimate the percentage of skin covered by tumors in order to provide a quantitative index to represent FP severity (Rossi et al. 2009). Counting and measuring tumors can be very time consuming, may not be conducive to field conditions or animal welfare constraints, and may also lead to large inter-observer differences in measurements translating to variations in tumor score assignments. That scoring system was later improved upon with an updated ‘fibropapillomatosis index’, which is based on a weighted sum model that considers the size category and number of tumors and is more user-friendly under field conditions (Rossi et al. 2016).

The objective of this study was to conduct a large-scale, retrospective case series analysis of FP in rehabilitating sea turtles in the southeastern USA, in order to (1) assess FP tumor score and regrowth in rehabilitating sea turtles, and (2) provide information on tumor regrowth and survival in turtles with different tumor scores. Evaluating cases of rehabilitating wildlife can be an extremely valuable approach for improving our understanding of pathogen activity in both captive and free-ranging wildlife, and for devel-
Fibropapilloma tumors in rehabilitated green turtles

2. MATERIALS AND METHODS

We retrospectively evaluated medical records of green sea turtles with FP admitted to the following rehabilitation facilities in the USA during 2009–2017: The Turtle Hospital in Marathon, Florida; Clearwater Marine Aquarium in Clearwater, Florida; Sea Turtle Healing Center at the Brevard Zoo in Melbourne, Florida; and Georgia Sea Turtle Center in Jekyll Island, Georgia. Case data evaluated included minimum straight carapace length (SCL$_{\text{min}}$); FP tumor number, size, and location (right/left eye[s], right/left front/hind flipper[s], carapace, plastron, head, neck, inguinal region[s], tail); whether tumor removal surgery was performed, and if so, how many times; whether tumor regrowth occurred, and if so, how many times, how long after removal, and during what month(s); and rehabilitation outcome (i.e. euthanized, died, released, or permanent captive). For the purposes of this study, turtles that were released and those that were transferred into permanent captive care are grouped together as turtles that survived rehabilitation; turtles that were euthanized for humane purposes and turtles that died without euthanasia are grouped together as turtles that did not survive. A tumor regrowth event was defined as one or more tumors arising at the anatomic site(s) of prior surgical removal.

Average (±SD) values were calculated for the following data points: SCL$_{\text{min}}$; number of tumor removal surgeries; number of tumor regrowth events; days between tumor removal and regrowth; number of tumor regrowth events per tumor score category (Page-Karjian et al. 2014 scores only); and tumor scores for each of the 3 FP scoring systems evaluated. Average SCL$_{\text{min}}$ data were compared between turtles that did and did not have tumor regrowth using a Mann-Whitney U-test for non-parametric data (as determined by the Shapiro-Wilk test) with α = 0.05. The numbers of tumor regrowth events per month were analyzed using second-order polynomial regression with α = 0.05. Two-tailed Fisher’s exact tests with α = 0.05 were used to evaluate the relationships between case outcome (survived versus did not survive) and whether tumor removal surgery was performed; and between case outcome and whether tumor regrowth occurred. Binary logistic regression was used to analyze the relationship between total number of tumor removal surgeries and tumor regrowth incidence.

Fibropapilloma tumor scores were assigned to all turtles for which relevant case data were available. Tumor scores were designated by a single experienced reviewer using a combination of tumor measurement data and photographs. Thus, tumor scores (1–3; including [1] mildly, [2] moderately, and [3] severely afflicted) were assigned using 3 different tumor classification systems: one outlined by Work & Balazs (1999); one outlined by Page-Karjian et al. (2014); and one outlined by Rossi et al. (2016). The average tumor scores for each of the 3 scoring systems were compared using a Kruskal-Wallis rank sum test for multiple independent samples with α = 0.05, and post-hoc Dunn’s test with p-values adjusted by the Benjamini-Hochberg false discovery rate method. The total number of turtles that survived or did not survive rehabilitation was calculated for each tumor score category for all 3 scoring systems, and multiple logistic regression was used to evaluate the odds of surviving rehabilitation for the 3 tumor scoring systems evaluated in this study (Work & Balazs 1999, Page-Karjian et al. 2014, Rossi et al. 2016). Since the Page-Karjian et al. (2014) scoring system was developed specifically to evaluate FP score in rehabilitating sea turtles, we used the scores from this scoring system and multiple logistic regression analysis to determine whether tumor score, number of days to first tumor regrowth event, and/or total number of tumor regrowth events were significant predictors of case outcome. These 3 explanatory variables were first assessed for collinearity to ensure that they were independent of each other before inclusion in the model. Because SCL$_{\text{min}}$ data were limited, they were not included in the multiple logistic regression model but were analyzed separately. A Kruskal-Wallis 1-way ANOVA with α = 0.05 was used to compare the average of the following for each of the 3 tumor scores: SCL$_{\text{min}}$ number of regrowth events for turtles that did experience regrowth; and time to first regrowth (in days). Statistical analyses were conducted using SPSS statistical software v.24 (IBM).

3. RESULTS

Overall, medical records of 756 rehabilitating green sea turtles with FP were analyzed. SCL$_{\text{min}}$ data were available for 329 turtles, and of those, the average (±SD) SCL$_{\text{min}}$ was 37.94 ± 7.45 cm (range = 4.03–63.10 cm; Fig. 1). Of the 756 total cases of green tur-
turtles with FP, 312 (41%) underwent tumor removal surgery. For these 312 turtles, the number of tumor removal surgeries ranged from 1−17 per animal; 215 (69%) turtles had more than one surgery, the average number of surgeries was 4 ± 3 per animal, and the number of regrowth events ranged from 0−9 per turtle (Fig. 2). Tumor regrowth was seen in 155 of 312 (50%) turtles within an average of 46 ± 45 d (range = 2−245 d). There was a total of 332 regrowth events for the 312 turtles that had tumor removal surgery, and multiple regrowth events were observed in 85 of 312 (27%) turtles; for these, the average time to the first tumor regrowth was 50 ± 52 d. The majority of the turtles with tumor regrowth had 1 (70 of 155, 45%) or 2 (47 of 155, 30%) regrowth events during rehabilitative care. The average number of regrowth events was 2 ± 2 per turtle. Of the 756 turtles with FP, 563 (75%) did not survive rehabilitation, including 283 (37%) that were euthanized and 280 (37%) that died without euthanasia; 193 (26%) survived, including 186 (25%) that lived to be released, and 7 (1%) that were placed in permanent captive care. The decision for placement in captive care was made by the attending veterinarians based on a turtle being deemed non-releasable due to its apparent inability to survive in the wild. Common causes for being deemed non-releasable included permanent blindness and unresolved floating (FWC 2016).

A statistically significant relationship was observed between the total number of tumor removal surgeries and whether or not tumor regrowth occurred (odds ratio = 2.56, [90% CI = 2.06−3.18, p < 0.001), suggesting that for each additional surgery performed, the odds of tumor regrowth increase by approximately 2- to 3-fold. Based on the results of a Mann-Whitney U-test, average SCLmin did not significantly differ between turtles that did and did not have tumor regrowth following surgery (p = 0.17). The proportion of total tumor regrowth events by month was found to be: January, 7%; February, 7%; March, 8%; April, 9%; May, 13%; June, 13%; July, 7%; August, 8%; September, 7%; October, 6%; November, 9%; December, 7%. Polynomial logistic regression analysis indicated that there was no statistically significant trend with regards to the total number of tumor regrowth events observed per month (r² = 0.30, p = 0.20).

Of the 756 cases of rehabilitating green turtles with FP, there were 449 cases for which detailed tumor data and photos were available and for which an accurate FP tumor score could be designated. For each of these 449 cases, 3 published FP tumor scoring systems were used to designate 3 FP tumor score values (1−3) per individual turtle. The average (±SD) tumor score was 2.26 ± 0.67 using the Work & Balazs (1999) scoring system, 2.53 ± 0.64 using the Page-Karjian et al. (2014) scoring system, and 1.88 ± 0.78 using the Rossi et al. (2016) scoring system. All of these averages significantly differed from one another, as determined by a Kruskal-Wallis test (H₂ = 69.89, N = 449, p < 0.0001) with post-hoc Dunn’s analysis (all p < 0.004). Based on tumor score categories designated using only the Page-Karjian et al. (2014) scoring system, average SCLmin did not significantly differ between tumor scores 1−3 (H₂ = 4.84, N = 330, p = 0.09). For turtles that had tumor removal surgery, neither the average number of tumor regrowth events (H₂ = 2.48, N = 310, p = 0.29) nor the
average number of days to first regrowth \( (H_2 = 5.38, N = 153, p = 0.07) \) significantly differed between tumor scores 1–3 assigned using the Page-Karjian et al. (2014) scoring system.

Using 2-tailed Fisher’s exact tests, turtles that had tumor removal surgery were significantly more likely to survive rehabilitation than turtles that did not (\( p < 0.001 \)), but tumor regrowth following surgery was not significantly associated with survivorship (\( p = 0.57 \)). Case outcomes (survived versus did not survive) for each tumor score for the 3 scoring systems are detailed in Table A1 in the Appendix. In total, 57–67% of turtles with tumor score 1, 68–80% of turtles with tumor score 2, and 76–82% of turtles with tumor score 3 did not survive rehabilitation efforts. When the 3 tumor scoring systems (Work & Balazs 1999, Page-Karjian et al. 2014, Rossi et al. 2016) were compared using multiple logistic regression, the odds of survivorship for the Page-Karjian et al. (2014) system were approximately half of the other 2 (Table 1). Tumor score (Page-Karjian et al. 2014 scoring system only), number of days to first tumor regrowth, and total number of tumor regrowth episodes did not significantly affect survivorship, as shown by multiple linear regression (\( p > 0.05 \)).

**4. DISCUSSION**

The results of this study may be used by caretakers and veterinarians to help guide clinical decision making and determine prognoses for rehabilitating sea turtles with FP. Tumor regrowth may be expected in about 50% of cases involving surgical removal within an average of 46 d, and ~27% of turtles may be expected to undergo multiple rounds of surgery and regrowth. Most (75%) of the turtles that had tumor regrowth in this study had only 1 or 2 regrowth events. The regrowth rates reported here differ from 2 previously reported FP regrowth rates of 0 and 39%, likely due to differences in sample size and triage/treatment protocols between different rehabilitation facilities (Tristan et al. 2010, Page-Karjian et al. 2014). Fibropapilloma tumor regrowth following surgery likely occurs due to the continued presence of presumptive inciting cause(s) (e.g. ChHV5 infection, immunosuppression), even though the clinical signs of disease (FP tumors) are surgically removed. Data from this study indicate that with each additional surgery performed, the odds of tumor regrowth increased by approximately 2- to 3-fold. Triage-based, *a priori* case selection for surgery candidates helps explain the paradoxical finding that although tumor removal surgery was significantly associated with survivorship, tumor regrowth following surgery was not. Specifically, these results may be explained by the fact that the ‘sickest’ turtles were usually not considered good candidates for tumor resection and were instead elected for euthanasia; and tumor regrowth following surgery was often not considered grounds for euthanasia by the attending veterinarian(s), but rather was considered as an indicator for another round of surgical tumor removal. Tumor score was not a significant predictor for the event or extent of FP tumor regrowth. Consistent with previous reports that FP is of greatest concern in juvenile sea turtles in nearshore habitats, all of the green turtles with FP in this study were classified as juveniles (<65 cm SCLmin; Aguirre et al. 1994, 1998, Herbst et al. 1999, Ene et al. 2005, Hirama & Ehrhart 2007, Bresette et al. 2010, Page-Karjian et al. 2014).

The majority (75%) of the turtles with FP in this study did not survive following admission into a rehabilitation facility, irrespective of whether or not tumor regrowth occurred following surgery. Wildlife survivorship in rehabilitation facilities is usually heavily dependent upon triage protocols, and triage procedures at sea turtle rehabilitation facilities often include outright euthanasia for cases of internal tumors or severe FP that irreversibly diminishes an animal’s well-being and ability to survive. Successful triage strategies for neoplastic diseases are often accompanied by tumor scoring systems, and FP is no exception. Various tumor scoring systems have been published and used to categorize FP tumors based on tumor number, size, location, and

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient (β)</th>
<th>SE</th>
<th>Wald</th>
<th>p-value</th>
<th>Odds ratio</th>
<th>95% CI for odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.60</td>
<td>0.42</td>
<td>2.03</td>
<td>0.15</td>
<td>1.81</td>
<td>0.80–4.10</td>
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<tr>
<td>B/W tumor score</td>
<td>0.06</td>
<td>0.27</td>
<td>0.05</td>
<td>0.27</td>
<td>1.06</td>
<td>0.62–1.82</td>
</tr>
<tr>
<td>P-K tumor score</td>
<td>-0.65</td>
<td>0.26</td>
<td>6.26</td>
<td>0.01*</td>
<td>0.52</td>
<td>0.31–0.87</td>
</tr>
<tr>
<td>Rossi tumor score</td>
<td>-0.15</td>
<td>0.19</td>
<td>0.60</td>
<td>0.44</td>
<td>0.86</td>
<td>0.59–1.26</td>
</tr>
</tbody>
</table>

Table 1. Multiple logistic regression analysis to ascertain odds of surviving rehabilitation for green turtles assigned with tumor scores 1–3 using the 3 fibropapillomatosis scoring systems including that of Work & Balazs (1999) (B/W tumor score), Page-Karjian et al. (2014) (P-K tumor score), and Rossi et al. (2016) (Rossi tumor score). Note that odds of survivalship using the P-K system were approximately half of the other 2 scoring systems. (*) denotes statistically significant difference (\( p \leq 0.05 \)).
degree of invasiveness (Wood & Wood 1993, Work & Balazs 1999, Rossi et al. 2009, 2016, Page-Karjian et al. 2014). The percentage of turtles that survived was inversely related to FP tumor severity (i.e. tumor score), since summarized data from all 3 scoring systems indicate that rehabilitation survivorship for turtles with FP was only 32–43% for turtles with tumor score 1; 20–32% for turtles with tumor score 2; and 18–24% for turtles with tumor score 3. This finding is in alignment with data from another retrospective case series analysis of green turtles in Florida, which showed that of turtles with FP admitted to Florida facilities during 2006–2016, only 21% of turtles with tumor score 2 and 6% of turtles with tumor score 3 survived rehabilitation (Stacy et al. 2018). Therefore, particularly in situations of limited resources, and taking into account any co-morbid conditions, focusing rehabilitation efforts on turtles with lower tumor scores (i.e. 1–2) will help further streamline admission and triage of turtles with FP in rehabilitation facilities, and lead to higher rehabilitation success rates.

The averages of the 3 scoring systems differed significantly. Furthermore, the differences observed in survivorship depending on which scoring system is used underline the fact that FP scoring systems are usually developed based on site-specific data and/or for specific purposes and function best when applied to situations for which they were intended. For example, the Page-Karjian et al. (2014) FP scoring system incorporates tumor size, number, location, morphology, and whether internal tumors are diagnosed or not, and was developed specifically for use with rehabilitating sea turtles to help provide a prognostic indicator (tumor score) based on these factors. Application of this scoring system coupled with rigorous triage and admission criteria for stranded turtles with FP can effectively help reduce facilities’ burden in terms of rehabilitating fewer turtles with poor prognoses. More research is needed to define the effects of prolonged captivity and repeated cycles of FP tumor removal and regrowth on FP detection, prognosis, and general turtle health.

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LITERATURE CITED


www.helpingseaturtles.org.
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**Table A1. Rehabilitation survivorship for green turtles with fibropapillomatosis (FP) tumor scores 1–3**

<table>
<thead>
<tr>
<th>Tumor score</th>
<th>Work &amp; Balazs (1999) FP scoring system</th>
<th>Page-Karjian et al. (2014) FP scoring system</th>
<th>Rossi et al. (2016) FP scoring system</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of turtles that survived rehabilitation (%)</td>
<td>No. of turtles that did not survive rehabilitation (%)</td>
<td>Total</td>
</tr>
<tr>
<td>Tumor score 1</td>
<td>23/58 (40%)</td>
<td>35/58 (60%)</td>
<td>58/449 (13%)</td>
</tr>
<tr>
<td>Tumor score 2</td>
<td>54/217 (25%)</td>
<td>163/217 (75%)</td>
<td>217/449 (48%)</td>
</tr>
<tr>
<td>Tumor score 3</td>
<td>31/174 (18%)</td>
<td>143/174 (82%)</td>
<td>174/449 (39%)</td>
</tr>
<tr>
<td>Tumor score 1</td>
<td>16/37 (43%)</td>
<td>21/37 (57%)</td>
<td>37/449 (8%)</td>
</tr>
<tr>
<td>Tumor score 2</td>
<td>44/136 (32%)</td>
<td>92/136 (68%)</td>
<td>136/449 (30%)</td>
</tr>
<tr>
<td>Tumor score 3</td>
<td>48/276 (17%)</td>
<td>228/276 (83%)</td>
<td>276/449 (62%)</td>
</tr>
<tr>
<td>Tumor score 1</td>
<td>54/165 (33%)</td>
<td>111/165 (67%)</td>
<td>165/449 (37%)</td>
</tr>
<tr>
<td>Tumor score 2</td>
<td>34/172 (20%)</td>
<td>138/172 (80%)</td>
<td>172/449 (38%)</td>
</tr>
<tr>
<td>Tumor score 3</td>
<td>20/112 (18%)</td>
<td>92/112 (82%)</td>
<td>112/449 (25%)</td>
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<tr>
<td>Total</td>
<td>108/449 (24%)</td>
<td>341/449 (76%)</td>
<td>449</td>
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