



Fibropapillomatosis dynamics in green sea turtles *Chelonia mydas* over 15 years of monitoring in Akumal Bay, Quintana Roo, Mexico

Fernando A. Muñoz Tenería^{1,*}, Vanessa Labrada-Martagón²,
Roberto Luis Herrera-Pavón³, Thierry M. Work⁴, Erik González-Ballesteros⁵,
Ana Cecilia Negrete-Philippe⁶, Gisela Maldonado-Saldaña⁷

¹Laboratorio de Inmunología, Facultad de Agronomía y Veterinaria, Universidad Autónoma de San Luis Potosí, S.L.P., CP 78399, Mexico

²Laboratorio Ecología de la Salud, Facultad de Ciencias, Universidad Autónoma de San Luis Potosí, S.L.P., CP 78295, Mexico

³Laboratorio de Sistemática, Ecología y Manejo de Recursos Acuáticos, El Colegio de la Frontera Sur, Unidad Chetumal, Chetumal, Quintana Roo, CP 77014, Mexico

⁴US Geological Survey, National Wildlife Health Center, Honolulu Field Station, Honolulu, Hawaii 96850, USA

⁵Campo 1, Facultad de Estudios Superiores Cuautitlán, Universidad Nacional Autónoma de México, CP 54714, Mexico

⁶Jefatura de Fauna Silvestre, Dirección de Conservación, Parque Xcaret, Quintana Roo, CP 77710, Mexico

⁷Kanantik Servicios y Soluciones Ambientales, Cancún, Quintana Roo, CP 77536, Mexico

ABSTRACT: Fibropapillomatosis (FP) is a tumor disease that affects all sea turtle species but is mainly seen in green turtles *Chelonia mydas*. The pathology of FP has been described extensively, but its dynamics in populations over time have been less studied. We analyzed the dynamics of FP in a population of green turtles in Akumal Bay on the central coast of the Mexican Caribbean. A total of 475 green turtles were captured over 15 yr (2004–2018). The highest prevalence of FP was found in the largest turtles, and there was a positive relationship between FP prevalence and size of turtles. FP was first detected in 2008 at a prevalence of 1.6 %, and annual prevalence increased markedly from 17.9 % in 2015 to 54 % by 2018. Likewise, severity of FP increased over time, with most turtles falling into moderately to severely diseased categories (tumor score 2). The average size of turtles with FP was significantly larger than the size of individuals without FP. Regression of tumors was seen in 21 % of turtles, tumor score was higher in smaller individuals, and only tumor score 2 was present in the largest sea turtles. An increase in the prevalence and tumor score of FP coincided with the massive arrival of *Sargassum* in 2015, suggesting that altered environmental conditions may have played a role. The increased prevalence of FP in Akumal Bay prompts the need to explain what might be driving this phenomenon and how widespread it is in the Caribbean.

KEY WORDS: Green turtle · Fibropapillomatosis · Tumor score · *Sargassum*

1. INTRODUCTION

The destruction, degradation, and contamination of habitats contributes to the outbreak and dispersion of diseases, many of which can reach pandemic proportions (Cunningham et al. 2017). One example of

such a disease is fibropapillomatosis (FP), a debilitating chronic tumor disease of sea turtles that affects mainly green turtles *Chelonia mydas*. FP is increasing in prevalence in the southeastern USA and is often associated with eutrophic habitats (Shaver et al. 2019). This disease is characterized by the pro-

*Corresponding author: fernando.munoz@uaslp.mx

gressive development of cutaneous fibropapillomas or papillomas and visceral fibrosarcomas, myxofibromas, or fibromas of indeterminate etiology that are transmissible through filterable tumor extracts (Herbst et al. 1995, Work et al. 2004). Pathology of FP has been described extensively in the literature (Herbst et al. 1999, Work et al. 2004).

A herpesvirus called chelonid alphaherpesvirus 5 (ChHV5) has been consistently associated with tumors, but its role in FP development is uncertain. This is in part because of difficulties in manipulating the virus in the laboratory (Quackenbush et al. 1998, Greenblatt et al. 2005, Alfaro-Núñez et al. 2014, Work et al. 2015). Recent advances in culturing ChHV5 using an *in vitro* culture system that recapitulates the complex 3-dimensional structure of turtle skin may provide a useful tool to understand the pathogenesis of FP in the future (Work et al. 2017).

Other factors such as marine leeches (Greenblatt et al. 2004, Santoro et al. 2007) and environmental cofactors are also associated with FP. The latter seem particularly important, and several authors have suggested that marine pollution, heavy metals, toxins, and eutrophication by human activity play an important role in the pathogenesis of FP (Herbst 1994, dos Santos et al. 2010, Van Houtan et al. 2010, 2014, Keller et al. 2014, da Silva et al. 2016); however, evidence for this is mixed. Although the highest prevalence of FP is found in waters with poor environmental quality, there are also areas with good environmental quality with a lower but significant prevalence of FP (Foley et al. 2005, Perrault et al. 2020).

Host-related factors also play a role in FP. For instance, FP can preferentially affect medium-sized juveniles (Patrício et al. 2016), and host response to the virus varies with geography (Work et al. 2020). Some studies have reported that up to 60% of animals can have tumors that regress in some juvenile green turtles (Bennett et al. 1999, Hirama & Ehrhart 2007, Chaloupka et al. 2009, Rossi et al. 2016). This evidence of tumor regression and the few cases recorded in adult turtles show that FP can resolve spontaneously, suggesting an important role for immune response in pathogenesis of disease. FP is distributed worldwide; its prevalence varies from region to region, but the highest prevalence has been reported in Florida, USA (Hirama & Ehrhart 2007), and more recently, in Brazil (Rodenbusch et al. 2014, Jones et al. 2016, Tagliolatto et al. 2016), with prevalence of FP declining in Hawaii for unknown reasons (Chaloupka et al. 2009).

Akumal Bay in Quintana Roo, Mexico, in the central part of the Mexican Caribbean coast, is a major

area of tourism development, with consolidated areas such as the City of Cancun, as well as areas of incipient growth such as Akumal Bay (Vázquez-Sosa et al. 2015). In recent years, Akumal Bay, an important feeding area for juvenile green turtles, has experienced accelerated growth of tourism due to the attraction of its crystalline waters, coral reefs, sport fishing opportunities and, most importantly, to the possibility of observing and swimming with green turtles that feed in the shallow waters of the bay (Labrada-Martagón et al. 2017). This has resulted in increasing eutrophication of the bay waters with significant adverse effects on marine organisms such as coral reefs (Gil et al. 2015). Akumal Bay was declared a Refuge for the Protection of Marine Life by the Mexican Federal Government on 7 March 2016 (Diario Oficial de la Federación 2016), with the objective of protecting the flora and fauna of the area through sustainable management. A program to monitor the health and ecology of the turtles that inhabit this bay started in 2004 (Labrada-Martagón et al. 2017); it was under this program that the presence of FP was first detected in 2008. The objective of this work was to assess the progression and characteristics of FP based on data collected during 15 yr of monitoring the population of green turtles that inhabit Akumal Bay, and to infer the possible effects of this disease on the green turtle population.

2. MATERIALS AND METHODS

2.1. Study site

The Marine Life Refuge of Akumal Bay (which in the Mayan language means 'place of turtles') is located on the central coast of the State of Quintana Roo ($20^{\circ} 24' 0''$ N, $87^{\circ} 19' 16''$ W). There is a stable population of green turtles of different sizes that feed on pastures of seagrasses such as *Halodule wrightii*, *Thalassia testudinum*, and *Syringodium filiforme* (Molina Hernández & van Tussenbroek 2014). The bay includes a barrier reef about 300 m offshore that forms part of the Mesoamerican Coral Reef (Baker et al. 2013). The depth of the bay ranges from 1.5 to 4 m, with seagrasses distributed mainly in the northern part and in small reef areas.

2.2. Capture of specimens

Turtles were captured manually by snorkelers working off boats every year from 2004 to 2018 during the

summer (July and August), except for 2005 and 2017, using longitudinal mixed capture–recapture sampling (Labrada-Martagón et al. 2017). For all turtles, flexible tape was used to measure maximum curved carapace length (CCL) from the anterior point at the midline (nuchal scute) to the posterior tip of the supra-caudals, and curved carapace width at the widest point. A Monel tag (National Band and Tag Company) was affixed between the first and second trailing axial scales of the right forelimb, after cleaning the application site with povidone iodine and 70% alcohol.

2.3. Documenting tumors

For each turtle with external tumors, the number and largest diameter (cm) of tumors on the skin of the head, eyes, oral cavity, dorsal and ventral surfaces of the flippers, and carapace and plastron were carefully recorded, and based on this, turtles received a tumor score as described by Work & Balazs (1999). Tumor regression (tumor size decreased or absent) or progression (tumor size increased) was recorded for each turtle with 2 or more recapture events. As an indicator of annual abundance (Bjorndal et al. 2000), annual catch per unit of effort (CPUE), was estimated as the number of green turtles captured per year divided by the number of units of effort (h yr^{-1}) (Labrada-Martagón et al. 2010, Bjorndal et al. 2017). Sea turtles captured for the first time with no tag or tag scar were considered neophytes (Richardson et al. 1999). Turtles smaller than 86 cm CCL were considered immature individuals of unknown sex (Labrada-Martagón et al. 2017).

2.4. Statistical analysis

Frequency tables and descriptive statistics were evaluated to analyze population structure, number and size of tumors, and prevalence of FP over time. A chi-squared test of goodness of fit was used to evaluate the prevalence of tumors over the years. The assumptions of normality and homoscedasticity were tested for CCL and number of tumors by using Shapiro-Wilk and Bartlett's tests. Differences in the size (CCL) of sea turtles with or without FP were analyzed using a Student's *t*-test. A Kruskal-Wallis test or ANOVA was performed to compare the size of sea turtles between years and tumor score between the years 2012, 2014, 2015, 2016, and 2018 (years with $n > 5$). All analyses were considered significant at an

α of 0.05. Univariate statistical analyses were run in Statistica v.8.

2.5. Modeling of FP

Generalized additive model (GAM) analysis was used to assess the non-linear dynamics of the occurrence of FP (presence/absence) and severity of FP (tumor score) in response to the explanatory variables CPUE, size of individuals (CCL, cm), year, and season of capture. The contribution of season was evaluated as an independent variable (Labrada-Martagón et al. 2013). The variable CPUE was considered an indicator of the relative abundance of green turtles (Bjorndal et al. 2000, Labrada-Martagón et al. 2017). Occurrence and severity of FP were modeled as separate dependent variables; different models were tested to assess the contributions of distinct combinations of covariates. Models were fitted by using cubic regression splines, based in third-order polynomials (Stevenson & Woods 2006, Zuur et al. 2009), to characterize the non-linear relationship between covariates and FP (Patrício et al. 2016) and restricted maximum likelihood (REML) for smoothing parameter estimation. REML is preferred for finite sample sizes in order to avoid overfitting (Stevenson & Woods 2006). A binomial error and a 'logit' link function were used when modeling presence/absence of FP; Poisson error and 'log' link were used for tumor score (Zuur et al. 2009). The contribution of each explanatory variable was evaluated for each independent model ($p < 0.05$), and the overall model fit was assessed by considering Akaike's information criterion, the adjusted R^2 estimates, and by hypothesis testing procedures (chi-squared test) (Stevenson & Woods 2006, Zuur et al. 2009). GAM was performed in R using the 'mgcv' package (Stevenson & Woods 2006) in R v.3.1.3 (R Core Team 2016).

3. RESULTS

Table 1 shows annual mean CCL, prevalence of FP, and CPUE in Akumal Bay, Quintana Roo, Mexico. A total of 475 turtles were captured over a period of 15 yr, with 2 interruptions (2005 and 2017). CCL ranged from 21 to 82.8 cm (Fig. 1A). Turtles captured in 2015 and 2016 were significantly larger ($F_{5,331} = 6.76$, $p < 0.001$). The CPUE was higher in the period 2014–2016 (Table 1). Frequency of neophytes remained constant across years ($\chi^2 = 8.4$, $p = 0.25$), and frequency of recap-

Table 1. Annual mean \pm SD curved carapace length (CCL), number of turtles sampled (n) in each year, number of tumors per year (with prevalence of fibropapillomatosis [FP] in parentheses), tumor score (based on Work & Balazs 1999), and capture per unit effort (CPUE) for green turtles *Chelonia mydas* captured in Akumal Bay, Quintana Roo, Mexico. No turtles were captured during 2005 or 2017

Year	All turtles		n	Prevalence (%)	Turtles with FP		CPUE Annual
	CCL (cm) Mean \pm SD	Range			CCL (cm) Mean \pm SD	Tumor score Mean \pm SD	
2004	57.6 \pm 11.6	32.5–81.0	14	0	—	0	0.9
2006	53.1 \pm 13.8	27.8–77.0	40	0	—	0	3.7
2007	58.8 \pm 12.5	33.0–72.3	17	0	—	0	3.3
2008	59.3 \pm 13.8	32.8–81.3	60	1 (1.6)	65.8	1	2.4
2009	55.4 \pm 15.2	31.0–82.5	42	1 (2.4)	78.3	1	4.7
2010	56.6 \pm 14.1	29.8–81.8	24	2 (8.3)	60 \pm 7.07	1 \pm 0	6.2
2011	60.1 \pm 7.3	50.3–79.3	18	0	—	0	3.3
2012	61.8 \pm 10.2	34.0–80.3	44	3 (6.8)	58.2 \pm 20.9	1.6 \pm 1.1	
2013	60.0 \pm 11.4	21.0–79.0	67	1 (1.5)	64.8	1	6.1
2014	61.1 \pm 10.7	37.2–82.8	70	3 (4.2)	63.5 \pm 2.2	1.3 \pm 0.6	8.9
2015	65.3 \pm 9.2	45.0–78.3	28	5 (17.9)	72.3 \pm 5.1	1 \pm 0	8.5
2016	63.0 \pm 8.9	40.7–80.0	38	10 (26.3)	67.8 \pm 7.4	1.2 \pm 0.6	8.4
2018	61.7 \pm 10.6	47.0–75.1	13	7 (53.8)	69.2 \pm 6.6	1.6 \pm 0.8	5.7

tures during 2012–2018 was twice that of sea turtles recaptured during the period 2004–2011 ($\chi^2 = 34.4$, $p < 0.001$) (Fig. 2). Of 487 turtles, 33 had FP (6.8%); the highest prevalence of FP was found in the largest turtles, mainly those in the subadult class (range 60.4–80.5 cm of CCL, 5.95%, $n = 29$ turtles) (Fig. 1A). The first case of FP in the Akumal Bay was detected in 2008; the annual prevalence remained below 10% until 2014. From 2015, the annual prevalence increased steadily until reaching 54% in 2018 (Table 1, Fig. 2). The average (\pm SD) size of turtles with FP (77.67 ± 15.2 cm CCL) was

significantly larger than the size of individuals without FP (65.3 ± 18.1 cm CCL; $t = -4.86$, $p < 0.001$) (Fig. 1B).

Of the turtles with FP, 58% were categorized as tumor score 1, 38.5%, as tumor score 2, and 4% as tumor score 3. All tumors were found on soft tissues and mainly in the cranial half of the body (63.6%), including the neck ($n = 9$ tumors), axillary region ($n = 4$), and forelimbs ($n = 54$), as well as on the eyelids and conjunctiva of the eye (31.4%, $n = 36$). A much smaller proportion (5.1%) of tumors were on the hindlimbs ($n = 4$), inguinal region or tail ($n = 2$). No

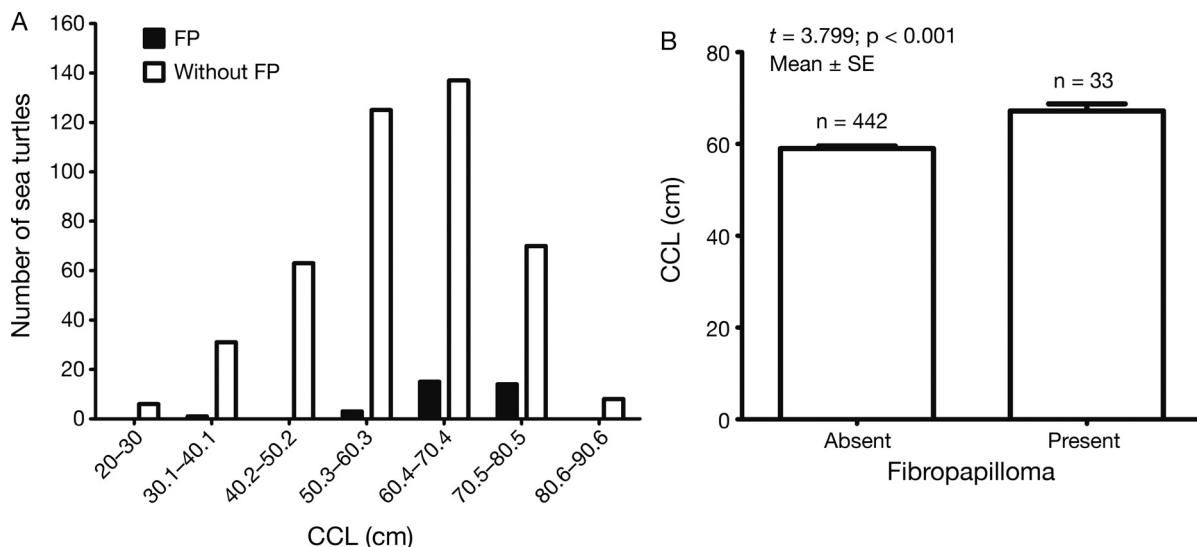


Fig. 1. (A) Frequency distribution of curved carapace length (CCL) and (B) size differences of green sea turtles *Chelonia mydas* with and without fibropapillomatosis (FP) from Akumal Bay, Quintana Roo, Mexico, captured over a 15 yr period (2004–2018)

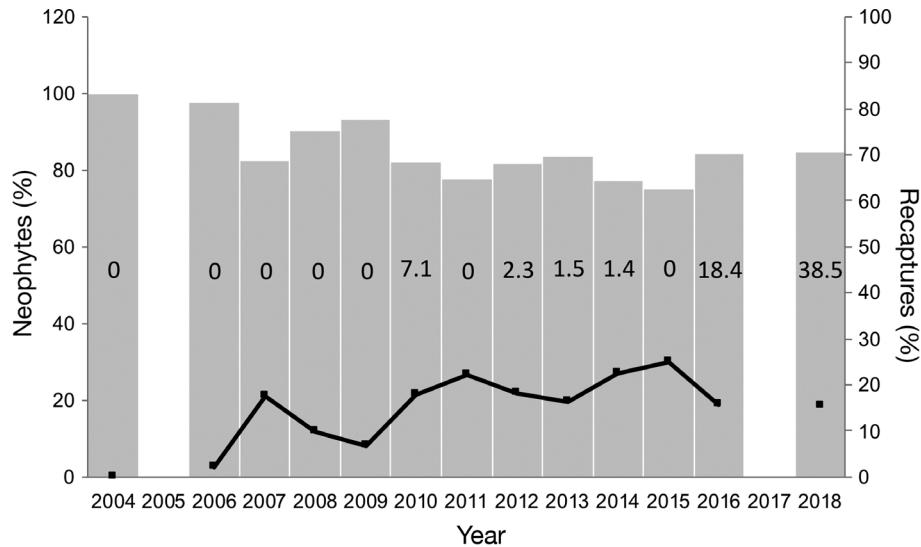


Fig. 2. Frequency of neophyte green turtles *Chelonia mydas* captured (bars), frequency of recaptures (line), and the annual percentage prevalence of fibropapillomatosis (FP) (numbers inside the bars) in neophyte green turtles in Akumal Bay, Quintana Roo, Mexico, during 2004–2018. A notable increase in the prevalence of FP can be observed in 2016 and 2018

tumor was observed on the mouth, carapace, or plastron (Fig. 3).

Based on recapture data, 21% of turtles exhibited tumor regression, with all regressing turtles going from tumor score 1 to score 0; in all cases, tumors were in the conjunctiva or periocular tissues. The mean CCL at recapture of turtles with regression was 74.5 ± 4 cm (SD).

In both GAMs, size of sea turtles (CCL, cm) and year were covariates that together explained, with statistical significance, the occurrence and severity of FP of green turtles in Akumal Bay (Table 2). FP prevalence and tumor score were explained by 28 and 31%, respectively. Season and year (as categorical covariates) and CPUE were not explanatory. There was an increasing trend in both FP prevalence (Fig. 4A,B) and severity (Fig. 4C,D) with CCL and years. There was a noticeable increase in FP after 2015, reaching a peak in 2018. Severity of FP (tumor score) tended to be higher in smaller individuals (<40 cm CCL), and tumor score remained constant in sea turtles >60 cm CCL (Fig. 4C).

4. DISCUSSION

Long-term monitoring of FP in sea turtle populations is crucial for understanding the dynamics of the disease and elucidating its epizootiology and pathogenesis (Chaloupka et al. 2009). The prevalence of FP varies by geography. For instance, in some regions, such as Hawaii and Puerto Rico, a rapid increase in prevalence of FP was observed, reaching a maximum peak (>90% in Hawaii), followed by a slow decrease in case numbers (Chaloupka et al. 2009, Patrício et al.

2016). In Brazil, a sustained increase has been reported (Tagliolatto et al. 2016), while a high prevalence has been observed in Florida, where it has remained stable (Foley et al. 2005). Our monitoring project is the first of its kind in Mexico, and the results demonstrate that at one site in the Yucatan peninsula, FP prevalence has undergone a recent, steady increase at least through 2018, the last year monitored.

One potential explanation for the increase in FP prevalence is that the population structure of the green sea turtles of Akumal Bay has changed over the years, mainly in the last decade. Size of the captured specimens significantly increased over time, especially in 2015 and 2016, and larger turtles in Mexico seem more prone to have FP, a phenomenon similar to that seen in green turtles from Brazil (Tagliolatto et al. 2016). In contrast, in other regions like Florida, Texas, Puerto Rico, and Brazil, medium-sized turtles were the most affected by the disease (Herbst 1994, dos Santos et al. 2010, Shaver et al. 2019). In places such as Puerto Rico and Brazil, where small turtles (30–45 cm CCL) predominate and where turtles affected with FP are in the medium size range (40–60 cm CCL), it appears that turtles acquire the disease shortly after recruitment to coastal foraging grounds, which would explain why FP affects mainly juveniles (Herbst 1994, Patrício et al. 2016, Tagliolatto et al. 2016). In Akumal Bay, the green turtle population consists of individuals between 25 and 85 cm CCL (Labrada-Martagón et al. 2017), with the highest number of turtles in the medium to large size classes (50–80 cm CCL) that possibly develop tumors after recruitment in the bay. The hypothesis that larger turtles acquire FP in Aku-

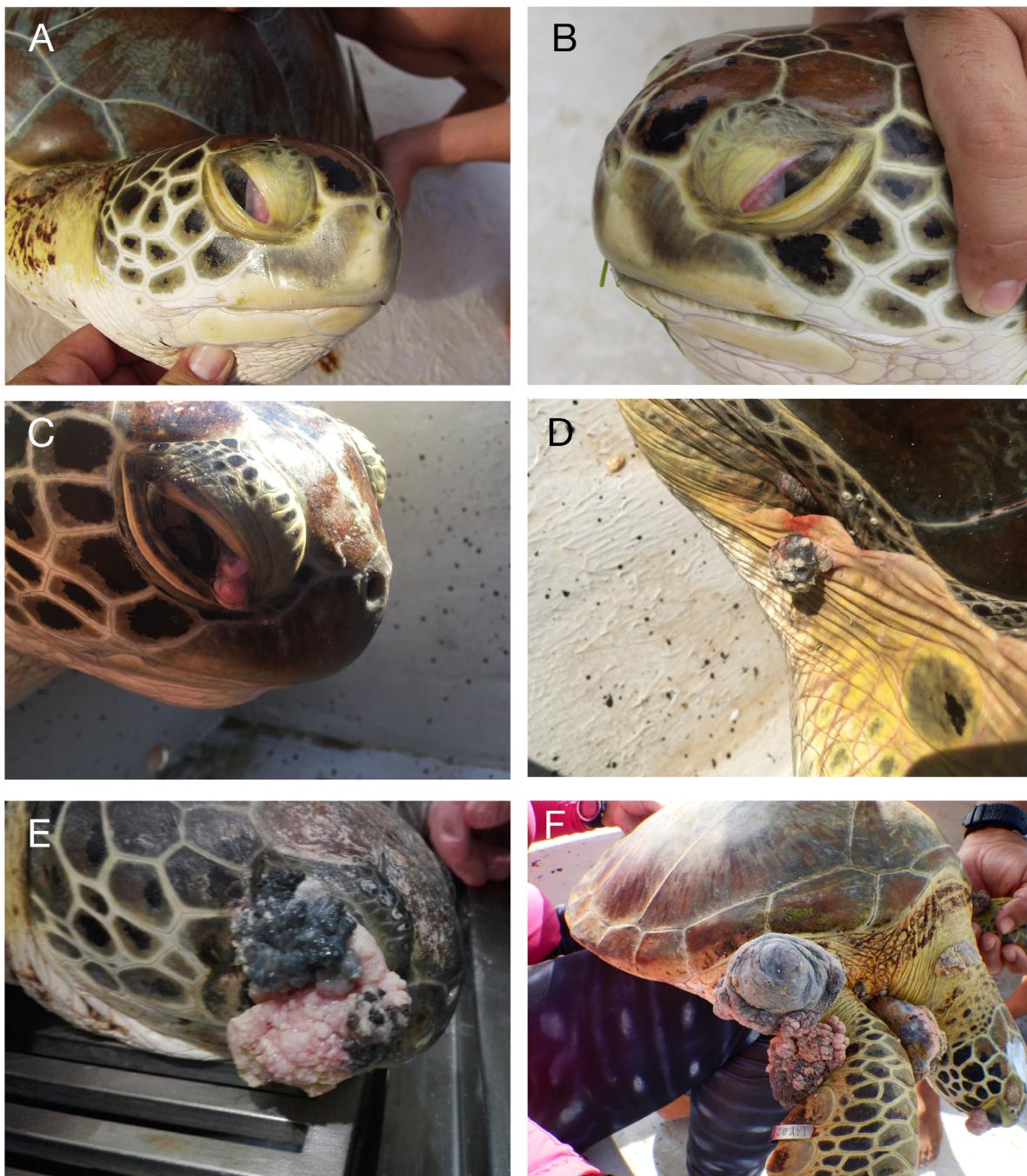


Fig. 3. Increasing severity of fibropapillomatosis (FP) over time in 5 different turtles (panels A & B show the same turtle). (A,B) First tumors detected in 2008 were restricted to the conjunctiva. (C,D) Marked increase in tumor size, involving eyes and neck skin, detected in 2015. (E,F) Detection of specimens severely affected by FP in 2018, encompassing eyes and external soft tissues, as well as weakness and emaciation

mal Bay is anecdotally supported by a case of a 53.7 cm turtle found dead on the beach of Akumal Bay on 18 February 2019, with multiple tumors larger than 5 cm in the eyes and neck; this animal had been previously captured in 2016 (53 cm CCL) with no tumors. The prevalence of FP, observed mainly in

subadult turtles (60–80 cm CCL) in Akumal Bay, suggests that unlike other regions like Florida or Hawaii where turtles develop FP as juveniles, development of FP in Akumal Bay occurs much later, probably because the proportion of turtles <50 cm has been low since the start of monitoring in Akumal Bay (2004),

Table 2. Summary of generalized additive models of prevalence of fibropapillomatosis (FP) in green sea turtles *Chelonia mydas* captured in Akumal Bay, Quintana Roo, Mexico. Fitted models are shown after model selection for fibropapillomatosis presence and number of tumors. CCL: curved carapace length; AIC: Akaike's information criterion; edf: estimated degrees of freedom

Model	N	R^2	AIC	Deviance explained (%)	Fixed effects			Variable	Smooth terms			
					Estimate	SE	p		edf	χ^2	p	
Presence FP ~ CCL + Year ^a	462	0.28	144.5	34.5	Intercept	-4.20	0.53	<0.001	CCL	2.23	9.39	0.02
Tumor Score ~ CCL + Year ^b	462	0.31	178.4	43.1	Intercept	-4.11	0.50	<0.001	Year	3.80	36.3	<0.001

^aBinomial error distribution and logit link function; ^bPoisson error distribution and log link function

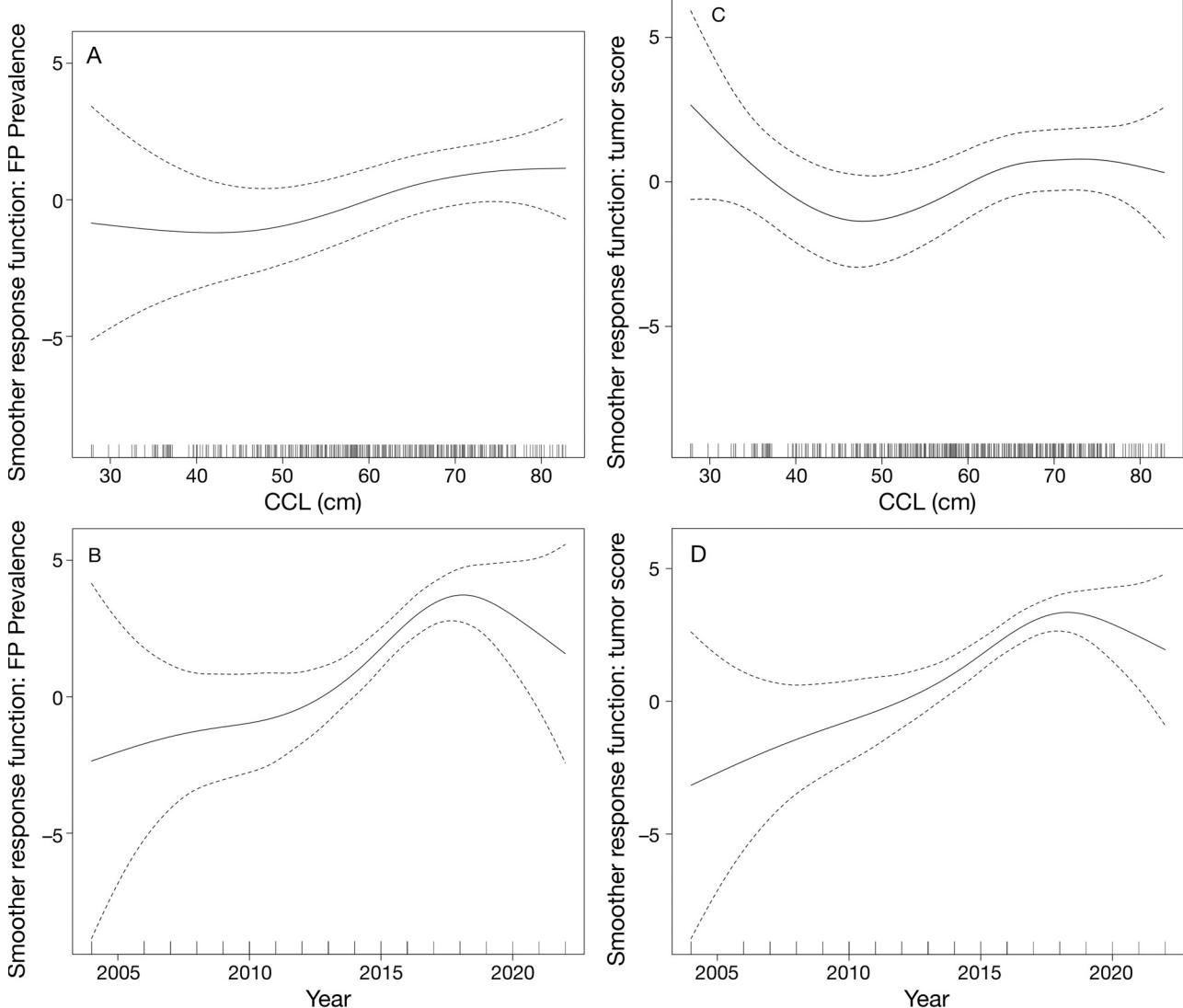


Fig. 4. Estimated smoothing curves by generalized additive models of (A,B) prevalence and (C,D) tumor score of fibropapillomatosis (FP) in green sea turtles *Chelonia mydas* captured in Akumal Bay, Quintana Roo, Mexico during 2004–2018, by size (curved carapace length, CCL; A,C) and year (B,D). Solid line is the smoother fit, dotted lines are 95 % confidence bands.

Vertical hatches (rug plot) along the x-axis in panels A and C represent covariate distributions

indicating that Akumal Bay has characteristics that are not conducive to allowing smaller turtles to thrive in that habitat (Fig. 2).

The increase in the prevalence and tumor score of FP observed, from 4.23 % in 2014 to 53.85 % in 2018 (Table 1, Fig. 4) coincides with the massive arrival of *Sargassum* to the Caribbean coasts, which began in 2014, an event that has caused drastic changes in coastal and neritic waters, including accelerating the already ongoing eutrophication process caused by tourism development (Barrera Escoria & Namihiira Santillán 2004, Foley et al. 2005, Gil et al. 2015, van Tussenbroek et al. 2017). Many studies have shown the relationship between habitat degradation (urban and maritime developments) and a higher prevalence of FP (dos Santos et al. 2010, Van Houtan et al. 2010). There is evidence of a relationship between tourism development in Akumal Bay and increased mortality and adverse effects to marine life such as corals and fishes, associated with a greater amount of organic matter and human aquatic activity (Baker et al. 2013, Gil et al. 2015). The unusual arrival of large quantities of *Sargassum* to the coasts of the Mexican Caribbean has greatly increased the amount of organic matter in the water, which is already contaminated by organic leachates from land (van Tussenbroek et al. 2017). This suggests that the altered environmental conditions of the last decade in this green turtle foraging area may have played a role in the dramatic increase in the prevalence of FP, although this remains to be confirmed. If prevalence levels of FP continue to remain as elevated as those seen in 2018, then those levels are at least as high as those reported at other turtle foraging sites in the Gulf of Mexico, Caribbean, and the Atlantic (Foley et al. 2005, Patrício et al. 2016, Tagliolatto et al. 2016, Shaver et al. 2019). However, while the severity of FP has increased in Akumal Bay over the years (Figs. 3 & 4b), the disease is generally much less severe than in other regions; for example, no individuals with tumors >10 cm (tumor score 3) were captured, in contrast to sites like Hawaii (Work & Balazs 1999). It is possible that the severity of disease in in-water studies is underestimated, because we have seen much larger tumor sizes in stranded turtles with FP from the region undergoing rehabilitation.

Sea turtle density was not related to prevalence of FP in a previous study of the disease in green turtles from Puerto Rico (Patrício et al. 2016), a finding that accords with those of this study which showed no relationship between CPUE and prevalence of FP. Neophytes are those turtles not previously tagged and thus assumed to have no known history of resi-

dence at the foraging site (Richardson et al. 1999). A major increase (39 %) in neophyte green turtles with FP was observed during the last 2 years of monitoring (2016 and 2018), suggesting a pulse of recruitment to Akumal Bay (Fig. 2). Neophyte green turtles with FP contributed 69.96 and 71.56 % of the total prevalence of FP in 2016 and 2018, respectively, thus explaining the high increase of FP in those 2 years (Fig. 2). If the trend of increasing annual percentage of FP depends on a large percentage of neophyte green turtles with FP, it will be very important to constantly survey in the next years to determine if this correlation is maintained, and more importantly, to determine if these neophyte turtles acquire FP in Akumal Bay or are already recruited with FP and where they come from, which will contribute a lot to a better understanding of the epidemiology of FP.

Green turtles typically reside at Akumal Bay for 1–4 yr, and a density-dependent effect on their somatic growth has been suggested for this foraging area (Labrada-Martagón et al. 2017). A study of seagrass dynamics of Akumal Bay in 2011 concluded that there was excessive foraging with no rotation of pastures and thus a reduced chance of seagrass bed recovery (Molina Hernández & van Tussenbroek 2014). Overgrazing of seagrass beds by sea turtles has been seen elsewhere. For instance, seagrass beds in marine protected areas are degraded by overgrazing in Indonesia and the Indian Ocean (Christianen et al. 2014). The CPUE data presented here agree with these previous data (Molina Hernández & van Tussenbroek 2014), observing a greater abundance of sea turtles over time. It is important to understand how a bay with excessive foraging supports a sea turtle population that appears to be steadily increasing. One possible explanation is that Akumal is experiencing increased migration of green turtles from elsewhere that are being displaced as result of poor foraging habitat. There has been a notable decrease in the quality and/or quantity of food available in other feeding grounds near Akumal Bay as a result of masses of *Sargassum*, which began arriving off the coast of Quintana Roo in 2014 leading to degradation of coral reefs and seagrass beds along the Mexican Caribbean coast (Molina Hernández & van Tussenbroek 2014). Moreover, in Akumal there is a shift in the dominant seagrass species, favoring a greater abundance of *Syringodium* and *Halodule*, and decreasing *Thalassia*, with an apparent change in the feeding preference of turtles foraging in the bay (van Tussenbroek et al. 2017, Christianen et al. 2021). Although it is tempting to incriminate higher densities of sea turtles as a factor contributing to FP, a reasonable

conjecture given that ChHV 5 is shed in tumors and the disease is likely transmitted by direct contact (Curry et al. 2000, Lloyd-Smith et al. 2005, Work et al. 2015), in our study, the covariates of relative turtle abundance turtle and season of capture did not significantly contribute to explaining prevalence of FP. Our findings accord with those elsewhere, for instance Puerto Rico, where no relationship between turtle density and FP was seen (Patrício et al. 2016).

The fact that year and size explained only 28 and 31 % of the variability in prevalence and severity of FP suggests that additional factors such as environmental variables (e.g. level of contaminants, eutrophication) or endogenous factors (e.g. metabolic and immune status) might be involved in the development of FP in green turtles in Akumal Bay, as suggested elsewhere (Aguirre & Lutz 2004, Foley et al. 2005, Jones et al. 2016). The immune response to FP differs among geographic areas and severity of disease can affect immune response (Work et al. 2001, 2019). How these factors play out in Akumal remains to be determined.

The locations of tumors on the eyes and soft tissues of the more cranial aspects of affected turtles in Akumal Bay reported here were consistent with the pathologic features reviewed in the literature (Herbst 1994, Work et al. 2004, Van Houtan et al. 2014, Jones et al. 2016). Notably, we saw no tumors on the carapace or plastron. Interestingly, we recorded a tumor regression rate of 21 % in recaptured turtles, similar to that found in other studies (Chaloupka et al. 2009, Machado Guimarães et al. 2013, Patrício et al. 2016). However, the results of the present study differ in terms of the size of the tumors, since tumor regression only occurred in all small tumors (<4 cm) present in the conjunctiva and periocular tissues of subadult turtles. Tumor regression likely occurred in <1 yr, as turtles were found to be tumor-free at the subsequent recapture (1 yr later).

5. CONCLUSIONS

Although FP does not seem to pose an immediate threat to sea turtle populations worldwide (Chaloupka et al. 2009, Jones et al. 2016), the increasing prevalence of FP in Akumal Bay is an important ecological signal that merits further investigation. Given the high visibility of this disease in a charismatic species like the green sea turtle in a nationally renowned ecological reserve frequented heavily by the public, a need exists to better understand underlying drivers of this trend.

Acknowledgements. We thank the fishermen Rolando, Fávio, and Rene Figueroa for their support in the management of nets used to capture sea turtles in open waters; Centro Ecológico Akumal for support in field work, especially P. Sánchez Navarro, A. Lorences Camargo, M. Suárez, S. Sarre, H. Lizárraga, and M. Acévez; A. Orozco (Akumal Dive Center) and G. Arcila (Akumal Dive Shop) for their help providing boats for sampling; R. Castellanos Balam (El Colegio de la Frontera Sur) for assistance with field work; and K. A. Flores Cepeda, M. Acosta, M. Aguilar, and students from the Facultad de Agronomía y Veterinaria (UASLP) for help with sample collection. Allen Foley provided constructive comments on earlier versions of this manuscript. Funding for this project was provided in part by the Wildlife Conservation Society (Field Veterinary Program), the Wildlife Trust, the National Fish and Wildlife Foundation, Flora Fauna y Cultura de México, A.C. and CONANP (Programa de Conservación de Especies en Riesgo, (PROCER) 2015 and 2016, Fondo de Apoyo a la Investigación-UASLP (FAI-UASLP) 2017. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All samples were collected under permits from the Dirección General de Vida Silvestre, SEMARNAT. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the US Government.

LITERATURE CITED

- Aguirre AA, Lutz P (2004) Marine turtles as sentinels of ecosystem health: Is fibropapillomatosis an indicator? *Eco-Health* 1:275–283
- Alfaro-Núñez A, Frost Bertelsen M, Bojesen AM, Rasmussen I, Zepeda-Mendoza L, Tange Olsen M, Gilbert MTP (2014) Global distribution of chelonid fibropapilloma-associated herpesvirus among clinically healthy sea turtles. *BMC Evol Biol* 14:206
- Baker DM, Rodríguez-Martínez RE, Fogel ML (2013) Tourism's nitrogen footprint on a Mesoamerican coral reef. *Coral Reefs* 32:691–699
- Barrera Escoriaza G, Namihira Santillán PE (2004) Contaminación microbiológica en la zona costera de Akumal, Quintana Roo, México. *Hidrobiología* 14:27–35
- Bennett P, Keuper-Bennett U, Balazs GH (1999) Photographic evidence for the regression of fibropapillomas afflicting green turtles at Honokowai, Maui, in the Hawaiian Islands. In: Kalb H, Wibbels T (eds) Proc 19th Annu Symp Sea Turtle Conserv Biol. Tech Memo NMFS-SEFSC-443. US Department of Commerce, NOAA, Brownsville, TX, p 37–39 www.turtles.org/99fp.htm#honokowai
- Bjorndal KA, Bolten AB, Chaloupka MY (2000) Green turtle somatic growth model: evidence for density dependence. *Ecol Appl* 10:269–282
- Bjorndal KA, Bolten AB, Chaloupka M, Saba VS and others (2017) Ecological regime shift drives declining growth rates of sea turtles throughout the West Atlantic. *Glob Change Biol* 23:4556–4568
- Chaloupka M, Balazs GH, Work TM (2009) Rise and fall over 26 years of a marine epizootic in Hawaiian green sea turtles. *J Wildl Dis* 45:1138–1142
- Christianen MJA, Herman PMJ, Bouma TJ, Lamers LPM and others (2014) Habitat collapse due to overgrazing threatens turtle conservation in marine protected areas. *Proc R Soc B* 281:20132890

- Christianen MJA, van Katwijk MM, van Tussenbroek BI, Pagès JF and others (2021) A dynamic view of seagrass meadows in the wake of successful green turtle conservation. *Nat Ecol Evol* 5:553–555
- Cunningham AA, Daszak P, Wood JLN (2017) One Health, emerging infectious diseases and wildlife: two decades of progress? *Philos Trans R Soc B* 372:20160167
- Curry SS, Brown DR, Gaskin JM, Jacobson ER and others (2000) Persistent infectivity of a disease-associated herpesvirus in green turtles after exposure to seawater. *J Wildl Dis* 36:792–797
- da Silva CC, Klein RD, Barcaroli IF, Bianchini A (2016) Metal contamination as a possible etiology of fibropapillomatosis in juvenile female green sea turtles *Chelonia mydas* from the southern Atlantic Ocean. *Aquat Toxicol* 170:42–51
- Diario Oficial de la Federación (2016) ACUERDO por el que se establece con el nombre de Bahía de Akumal el área de refugio para la protección de las especies que se indican, la porción marina que se señala en el Estado de Quintana Roo. www.dof.gob.mx/nota_detalle.php?codigo=5428829&fecha=07/03/2016
- dos Santos RG, Martins AS, Torezani E, Baptista C and others (2010) Relationship between fibropapillomatosis and environmental quality: a case study with *Chelonia mydas* off Brazil. *Dis Aquat Org* 89:87–95
- Foley AM, Schroeder BA, Redlow AE, Fick-Child KJ, Teas WG (2005) Fibropapillomatosis in stranded green turtles (*Chelonia mydas*) from the eastern United States (1980–98): trends and associations with environmental factors. *J Wildl Dis* 41:29–41
- Gil MA, Renfro B, Figueroa-Zavala B, Penié I, Dunton KH (2015) Rapid tourism growth and declining coral reefs in Akumal, Mexico. *Mar Biol* 162:2225–2233
- Greenblatt RJ, Work TM, Balazs GH, Sutton CA, Casey RN, Casey JW (2004) The *Ozobranchus* leech is a candidate mechanical vector for the fibropapilloma-associated turtle herpesvirus found latently infecting skin tumors on Hawaiian green turtles (*Chelonia mydas*). *Virology* 321:101–110
- Greenblatt RJ, Quackenbush SL, Casey RN, Rovnak J and others (2005) Genomic variation of the fibropapilloma-associated marine turtle herpesvirus across seven geographic areas and three host species. *J Virol* 79:1125–1132
- Herbst LH (1994) Fibropapillomatosis of marine turtles. *Annu Rev Fish Dis* 4:389–425
- Herbst LH, Jacobson ER, Moretti R, Brown T, Sundberg JP, Klein PA (1995) Experimental transmission of green turtle fibropapillomatosis using cell-free tumor extracts. *Dis Aquat Org* 22:1–12
- Herbst LH, Jacobson ER, Klein PA, Balazs GH, Moretti R, Brown T, Sundberg JP (1999) Comparative pathology and pathogenesis of spontaneous and experimentally induced fibropapillomas of green turtles (*Chelonia mydas*). *Vet Pathol* 36:551–564
- Hirama S, Ehrhart LM (2007) Description, prevalence and severity of green turtle fibropapillomatosis in three developmental habitats on the east coast of Florida. *Fla Sci* 70:435–448
- Jones K, Ariel E, Burgess G, Read M (2016) A review of fibropapillomatosis in green turtles (*Chelonia mydas*). *Vet J* 212:48–57
- Keller JM, Balazs GH, Nilsen F, Rice M, Work TM, Jensen BA (2014) Investigating the potential role of persistent organic pollutants in Hawaiian green sea turtle fibropapillomatosis. *Environ Sci Technol* 48:7807–7816
- Labrada-Martagón V, Méndez-Rodríguez LC, Gardner SC, Cruz-Escalona VH, Zenteno-Savín T (2010) Health indices of the green turtle (*Chelonia mydas*) along the Pacific coast of Baja California Sur, Mexico. II. Body condition index. *Chelonian Conserv Biol* 9:173–183
- Labrada-Martagón V, Méndez-Rodríguez LC, Mangel M, Zenteno-Savín T (2013) Applying generalized linear models as an explanatory tool of sex steroids, thyroid hormones and their relationships with environmental and physiologic factors in immature East Pacific green sea turtles (*Chelonia mydas*). *Comp Biochem Physiol A Mol Integr Physiol* 166:91–100
- Labrada-Martagón V, Muñoz Tenería FA, Herrera-Pavón R, Negrete-Philippe A (2017) Somatic growth rates of immature green turtles *Chelonia mydas* inhabiting the foraging ground Akumal Bay in the Mexican Caribbean Sea. *J Exp Mar Biol Ecol* 487:68–78
- Lloyd-Smith JO, Cross PC, Briggs CJ, Daugherty M and others (2005) Should we expect population thresholds for wildlife disease? *Trends Ecol Evol* 20:511–519
- Machado Guimaraes S, Mas Gitirana H, Vidal Wanderley A, Monteiro-Neto C, Lobo-Hajdu G (2013) Evidence of regression of fibropapillomas in juvenile green turtles *Chelonia mydas* caught in Niterói, southeast Brazil. *Dis Aquat Org* 102:243–247
- Molina Hernández AL, van Tussenbroek BI (2014) Patch dynamics and species shifts in seagrass communities under moderate and high grazing pressure by green sea turtles. *Mar Ecol Prog Ser* 517:143–157
- Patrício AR, Diez CE, van Dam RP, Godley BJ (2016) Novel insights into the dynamics of green turtle fibropapillomatosis. *Mar Ecol Prog Ser* 547:247–255
- Perrault JR, Perkins CR, Ajemian MJ, Bresette MJ, Mott CR, Page-Karjian A (2020) Harmful algal and cyanobacterial toxins in foraging green turtles (*Chelonia mydas*) in Florida's Big Bend. *Toxicon X* 5:100020
- Quackenbush SL, Work TM, Balazs GH, Casey RN and others (1998) Three closely related herpesviruses are associated with fibropapillomatosis in marine turtles. *Virology* 246:392–399
- R Core Team (2016) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- Richardson JI, Bell R, Richardson TH (1999) Population ecology and demographic implications drawn from an 11-year study of nesting hawksbill turtles, *Eretmochelys imbricata*, at Jumby Bay, Long Island, Antigua, West Indies. *Chelonian Conserv Biol* 3:244–250
- Rodenbusch CR, Baptista C, Werneck MR, Pires TT and others (2014) Fibropapillomatosis in green turtles *Chelonia mydas* in Brazil: characteristics of tumors and virus. *Dis Aquat Org* 111:207–217
- Rossi S, de Queiroz Hazbassanov NGT, Sánchez-Sarmiento AM, Setim Prioste FE, Matushima ER (2016) Immune response of green sea turtles with and without fibropapillomatosis: evaluating oxidative burst and phagocytosis via flow cytometry. *Chelonian Conserv Biol* 15:273–278
- Santoro M, Morales JA, Rodriguez-Ortiz B (2007) Spirorchidiidiosis (Digenea: Spirorchidae) and lesions associated with parasites in Caribbean green turtles (*Chelonia mydas*). *Vet Rec* 161:482–486
- Shaver DJ, Walker JS, Backof TF (2019) Fibropapillomatosis prevalence and distribution in green turtles *Chelonia mydas* in Texas (USA). *Dis Aquat Org* 136:175–182

- Stevenson RD, Woods WA (2006) Condition indices for conservation: new uses for evolving tools. *Integr Comp Biol* 46:1169–1190
- Tagliolatto AB, Guimarães SM, Lobo-Hajdu G, Monteiro-Neto C (2016) Characterization of fibropapillomatosis in green turtles *Chelonia mydas* (Cheloniidae) captured in a foraging area in southeastern Brazil. *Dis Aquat Org* 121:233–240
- Van Houtan KS, Hargrove SK, Balazs GH (2010) Land use, macroalgae, and a tumor-forming disease in marine turtles. *PLOS ONE* 5:e12900
- Van Houtan KS, Smith CM, Dailer ML, Kawachi M (2014) Eutrophication and the dietary promotion of sea turtle tumors. *PeerJ* 2:e602
- van Tussenbroek BI, Hernández Arana HA, Rodríguez-Martínez RE, Espinoza-Avalos J and others (2017) Severe impacts of brown tides caused by *Sargassum* spp. on near-shore Caribbean seagrass communities. *Mar Pollut Bull* 122:272–281
- Vázquez-Sosa A, Martínez OF, Fraga Verdugo J (2015) Pueblos de apoyo en contextos turísticos. Akumal, enclave turístico de la Riviera Maya de Quintana Roo. *Temas Antropol Rev Cient Investig Reg* 37:121–139
- Work TM, Balazs GH (1999) Relating tumor score to hematology in green turtles with fibropapillomatosis in Hawaii. *J Wildl Dis* 35:804–807
- Work TM, Rameyer RA, Balazs GH, Cray C, Chang SP (2001) Immune status of free-ranging green turtles with fibropapillomatosis from Hawaii. *J Wildl Dis* 37: 574–581
- Work TM, Balazs GH, Rameyer RA, Morris RA (2004) Retrospective pathology survey of green turtles *Chelonia mydas* with fibropapillomatosis in the Hawaiian Islands, 1993–2003. *Dis Aquat Org* 62:163–176
- Work TM, Dagenais J, Balazs GH, Schettle N, Ackermann M (2015) Dynamics of virus shedding and *in situ* confirmation of chelonid herpesvirus 5 in Hawaiian green turtles with fibropapillomatosis. *Vet Pathol* 52: 1195–1201
- Work TM, Dagenais J, Weatherby TM, Balazs GH, Ackermann M (2017) *In vitro* replication of chelonid herpesvirus 5 in organotypic skin cultures from Hawaiian green turtles (*Chelonia mydas*). *J Virol* 91:e00404-17
- Work TM, Dagenais J, Willimann A, Balazs G, Mansfield K, Matthias A (2020) Differences in antibody responses against chelonid alphaherpesvirus 5 suggest differences in virus biology between green turtles from Hawaii and Florida. *J Virol* 94:e01658-19
- Zuur AF, Ieno EN, Walker NJ Saveliev AA, Smith GM (2009) Mixed effects models and extensions in ecology with R. Springer, New York, NY

Editorial responsibility: Dave Rotstein,
Olney, Maryland, USA

Reviewed by: A. Page-Karjian and B. Stacey, and previous
version reviewed in DAO by 3 anonymous referees

Submitted: December 7, 2021

Accepted: April 28, 2022

Proofs received from author(s): June 16, 2022