Novel insights into the dynamics of green turtle fibropapillomatosis

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ABSTRACT: Outbreaks of fibropapillomatosis (FP), a neoplastic infectious disease of marine turtles, have occurred worldwide since the 1980s. Its most likely aetiological agent is a virus, but disease expression depends on external factors, typically associated with altered environments. The scarcity of robust long-term data on disease prevalence has limited interpretations on the impacts of FP on turtle populations. Here we model the dynamics of FP at 2 green turtle foraging aggregations in Puerto Rico, through 18 yr of capture-mark-recapture data (1997–2014). We observed spatiotemporal variation in FP prevalence, potentially modulated via individual site-fidelity. FP expression was residency dependent, and FP-free individuals developed tumours after 1.8 ± 0.8 yr (mean ± SD) in the infected area. Recovery from the disease was likely, with complete tumour regression occurring in 2.7 ± 0.7 yr (mean ± SD). FP does not currently seem to be a major threat to marine turtle populations; however, disease prevalence is yet unknown in many areas. Systematic monitoring is highly advisable as human-induced stressors can lead to deviations in host–pathogen relationships and disease virulence. Finally, data collection should be standardized for a global assessment of FP dynamics and impacts.

KEY WORDS: Fibropapillomas · *Chelonia mydas* · Mark-recapture · Disease dynamics · Emerging disease · Puerto Rico · Green turtle · Population dynamics

INTRODUCTION

Emerging diseases in marine ecosystems have increased over the past few decades (Harvell et al. 1999, 2004, Maynard et al. 2011). Climate change and anthropogenic pressure (e.g. habitat degradation, pollution) appear to contribute to marine wildlife disease outbreaks either by depressing host resistance or facilitating pathogen transmission (Harvell et al. 2004). Examples include recent outbreaks of infectious coral diseases worldwide (Maynard et al. 2011), the Caribbean-wide mass mortality of the long-spined sea urchin (Chiappone et al. 2002), mass mortalities of seals due to morbillivirus infection (Jensen et al. 2002), and several infectious neoplastic dis-

eases associated with novel viral pathogens in marine mammals (Bossart 2007).

Fibropapillomatosis (FP) is an infectious neoplastic disease of marine turtles. It was first described in 1938 in a green turtle captured in Florida (Smith & Coates 1938), but since the 1980s, disease outbreaks in the wild have been increasingly reported (Jacobson et al. 1989, Williams et al. 1994, Work et al. 2004, Foley et al. 2005). The tumours can be both external and internal and, though benign, depending on site and size they can hamper vital activities such as feeding, vision and swimming, and impede organ function (Herbst 1994, Herbst & Klein 1995). Neritic juveniles and subadults are the most susceptible life stages, whereas in adults the disease is rare (Herbst

& Klein 1995, Work et al. 2004, Foley et al. 2005). Although more frequent among green turtles (Hirama & Ehrhart 2007), FP has been reported in all species of hardshelled sea turtles (Herbst 1994, D'Amato & Moraes-Neto 2000, Guillen & Villalobos 2000). A novel alphaherpesvirus, the chelonid herpesvirus-5 (ChHV5), has been consistently detected by PCR analysis in tumour tissue samples from sea turtles (Quackenbush et al. 1998, Herbst et al. 2004, Ene et al. 2005, Patrício et al. 2012), and acknowledged as the most likely aetiological agent of FP (Herbst et al. 2004). However, recently, ChHV5 has been detected in several individuals not expressing visible tumours (Page-Karjian et al. 2012, Alfaro-Núñez et al. 2014).

Anthropogenically altered environments are associated with high FP prevalence (Herbst 1994, Aguirre & Lutz 2004, Van Houtan et al. 2010), implying that factors in these environments promote disease outbreak, e.g. facilitating virus transmissibility, and/or enhancing disease expression (Keller et al. 2014). A strong spatial heterogeneity observed in the distribution of ChHV5 variants in Florida, USA, along with sympatric species of marine turtles sharing virus variants suggests local infection after recruitment to coastal habitats (Ene et al. 2005). Transmission routes remain unclear, but may involve the direct contact between super spreaders and naïve individuals (Work et al. 2015).

The study of stranded turtles has provided insight into the spatiotemporal trends of FP prevalence in eastern USA and in Hawaii (Work et al. 2004, Foley et al. 2005, Chaloupka et al. 2008b); however, this could give biased estimates of FP trends if turtles with FP have mainly stranded as a consequence of advanced disease, leading to an overrepresentation of severely afflicted animals and potentially missing mild FP states. Alternatively, analyses of capture-mark-recapture (CMR) records can generate reliable estimations of disease incidence (LaPorte et al. 1992). CMR data have been widely applied to assess key population dynamic parameters of sea turtle populations, i.e. survival, abundance and somatic growth (Bjorndal et al. 2000, Chaloupka & Balazs 2005, Patrício et al. 2011, 2014), but rarely used to evaluate disease dynamics (but see Chaloupka et al. 2009). Overall, long-term data on chronic wildlife disease prevalence among live individuals are still scarce (Harvell et al. 2002, Lloyd-Smith et al. 2005, Chaloupka et al. 2009).

At Puerto Rico, reports of FP from occasional stranded turtles date back to 1985 (Williams et al. 1994, Ortiz Rivera et al. 2002). Since 1997, 2 foraging grounds for immature green turtles, Tortuga Bay and Puerto Manglar, have been monitored annually through CMR. FP was first observed in 2000 and has been present since. Here, we modelled the dynamics of FP disease on these coastal turtle aggregations through the analyses of 18 yr (1997–2014) of live CMR records. We investigated the effects of body size, year and abundance on FP risk, and estimated for the first time the periods from recruitment to expressing FP, and from FP expression to complete recovery.

MATERIALS AND METHODS

Study site and sampling

Puerto Manglar (18.30° N, 65.25° W) and Tortuga Bay (18.32° N, 65.23° W) are foraging grounds for immature green turtles, located on the islands of Culebra and Culebrita, respectively, which lie east of the main island of Puerto Rico (see Fig. 1 in Patrício et al. 2011). Puerto Manglar is a mangrove-lined bay, bordered by *Rhizophora mangle* (red mangrove), surrounded by wetlands and minor residential development. Maximum depth is 5 m, and the water has high turbidity (Diez et al. 2010). Tortuga Bay is located at the uninhabited island of Culebrita, managed by the US Fish and Wildlife Service as part of the Culebra National Refuge. A sandy beach surrounds the bay, underwater vegetation is sparser than at Puerto Manglar, water transparency is greater, and depth goes to 12 m (Diez et al. 2010). Turtles were captured with an entanglement net 200 m long and 5 m deep (nylon twine, 25 cm stretch mesh), deployed for ~1 h in areas <5 m deep using a 7 m long motor boat. Swimmers snorkelled continually along the net to extract entangled turtles. Turtles were tagged in the front flippers with 2 external tags (inconel and/or plastic tag) plus 1 internal passive integrated transponder (PIT) tag. Multiple tagging (i.e. flipper tags plus PIT tag) plus photo identification (facial profile photographs; Reisser et al. 2008) of each captured turtle assured that we were able to correctly identify all unique individuals throughout our CMR program. Straight carapace length (SCL, from the nuchal notch to the posterior tip) was measured to the nearest 0.1 cm. All individuals were examined for the presence of cutaneous or conjunctival FP (Brooks et al. 1994) and were assigned a tumour score (1−3; Work & Balazs 1999). Turtles were kept covered with wet towels, and handling time was minimized to 15 min per individual, after which they were released near their capture location. Overall sampling effort ranged from 5 to 16 net sets yr^{-1}, with

5.9 ± 3.5 net sets yr^{-1} (mean ± SD) in Tortuga Bay and 6.6 ± 3.6 net sets yr^{-1} in Puerto Manglar.

Data set

From 1997 to 2014 (except 1999), we recorded 764 capture events, 443 at Puerto Manglar, corresponding to 218 unique individuals, and 321 at Tortuga Bay, comprising 143 individual turtles (Table S1 in the Supplement at www.int-res.com/articles/suppl/m547p247_supp.pdf). Mean yearly individual captures at both sites corresponded to a proportion of 0.39 ± 0.15 (mean ± SD) of the estimated annual abundance (range: 0.13–0.68; see Patrício et al. 2014 for abundance estimates).

Linear mixed effects modelling

Body condition indices (BCIs) have been used to describe the well-being of several wild species (Stevenson & Woods 2006). We calculated the BCI for each capture as follows: BCI = weight/SCL^3 (Bjorndal et al. 2000). A tumour score (TS; Work & Balazs 1999) was assigned to each capture of an FP turtle. We analysed the relationship between BCI and having FP using the data set of all captures (n = 764), with linear mixed effects analysis using lme4 (Bates et al. 2015) implemented in R v.3.1.2 (R Development Core Team 2008). FP presence was included in the model as a fixed effect and turtle identity as a random effect. Similarly, within the group of captures corresponding only to turtles with FP, we assessed the relationship between TS (fixed effect) and BCI, also using turtle identity as a random effect. p-values for fixed effects were obtained by likelihood ratio tests of the models with the effect against models without it. Residual plots were visually inspected to detect deviations from homoscedasticity or normality.

Non-linear modelling

We applied generalized additive mixed modelling (GAM), available from package mgcv (Wood 2015) and applied in R v.3.1.2 (R Development Core Team 2008), to assess the relationship between FP presence and 3 potential explanatory covariates: SCL, sampling year, and annual abundance. GAMs are a semi-parametric form of generalized linear models that use smooth functions to fit the data, thus allowing for nonlinear relationships between the response and explanatory variables (Hastie & Tibshirani 1995), and perform well with binary responses (Wood 2015). A range of different models was tested, including different combinations of the potential predictors, until only significant covariates were kept. GAMs had a binomial error distribution and logit link. Model selection was based on Akaike's information criterion (Sugiura 1978) and smoothing selection performed with restricted maximum likelihood estimation (Corbeil & Searle 1976). Annual aggregation abundance estimations were extended to 2014 using the same methods as used previously (Patrício et al. 2014).

RESULTS

Prevalence

FP was first observed in Puerto Manglar in 2000, with FP prevalence peaking in 2003, when 75% of individuals captured presented tumours. Disease prevalence slowly decreased until 2007, and has since remained low (Fig. 1). At Tortuga Bay, FP was not observed until 2005, and prevalence peaked in 2009 at 33%. FP has persisted since, albeit with a low prevalence (Fig. 1). At Puerto Manglar, 21% of the
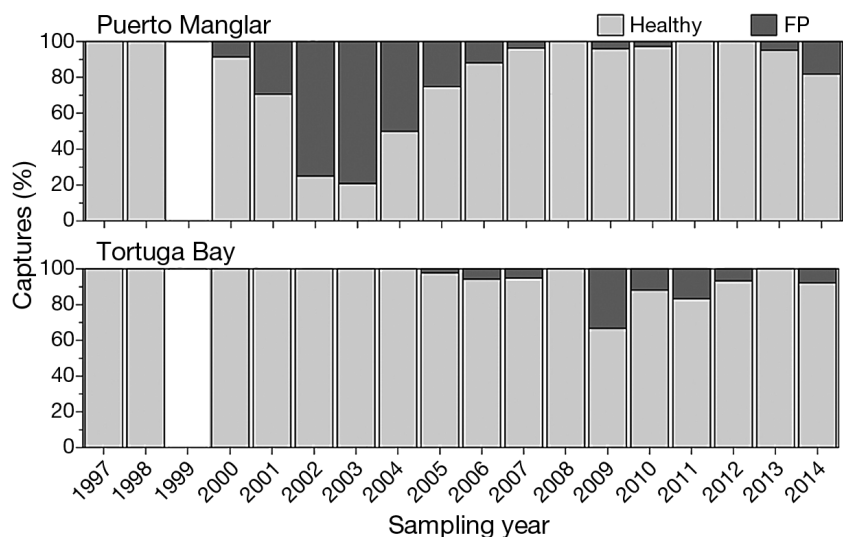

Fig. 1. Percentage of captures of healthy green turtles (light grey) and those with fibropapillomatosis (FP; dark grey), at 2 juvenile turtle foraging grounds, Tortuga Bay (N = 321) and Puerto Manglar (N = 443), Puerto Rico, throughout 18 yr of capture-mark-recaptures

Table 1. Summary of linear mixed effects models fitted to captures of immature green turtles from Puerto Rican foraging grounds. BCI = body condition index, FP = fibropapillomatosis, ID = turtle ID, TS = tumour score

Dataset	Model	Mixed effects			Fixed effects			
		Covariate	Variance	SD	Covariate	Estimate	SE	t
All captures (n = 764)	BCI~FP+(1|ID)	Turtle ID (intercept) Residual	6.69×10^{-11} 1.01×10^{-10}	8.18×10^{-6} 1.01×10^{-5}	Intercept FP	1.32×10^{-4} -2.67×10^{-7}	6.09×10^{-7} 1.44×10^{-6}	216.61 -0.18
FP captures (n = 85)	BCI~TS+(1|ID)	Turtle ID (intercept) Residual	3.87×10^{-11} 1.35×10^{-10}	6.22×10^{-6} 1.16×10^{-5}	Intercept TS	1.37×10^{-4} -2.25×10^{-6}	3.91×10^{-6} 2.48×10^{-6}	34.91 -0.91

turtles (45/218) were observed with FP during the sampling period; 31 % of these were later observed in a fully recovered state. At Tortuga Bay, only 9 turtles were captured with FP (6 %), and none were observed to have recovered.

BCI

There was no effect of FP on BCI ($F_{1,763} = 0.80$, p = 0.37; Fig. S1a in the Supplement) and the effect of individual (i.e. turtle identity) accounted for negligible amounts of variance (see model summary in Table 1). For the 85 captures of turtles with external fibropapillomas (corresponding to 54 unique individ-

uals; 59 % with TS1, 36 % with TS2, and 5 % with TS3), the effect of individual on BCI was also negligible (Table 1), and there was no effect of TS on BCI ($F_{2,82} = 0.81$, p = 0.45; Fig. S1b).

FP risk

For Puerto Manglar, the minimal adequate GAM showed that both SCL (GAM edf = 2.75, ref.df = 3.48, $\chi^2 = 26.01$, p < 0.001, where edf is estimated degrees of freedom and ref.df the estimated residual degrees of freedom) and sampling year (GAM edf = 5.17, ref.df = 6.20, $\chi^2 = 71.25$, p < 0.001) were significant explanatory variables for FP risk, and the model containing these 2 covariates was a good fit, with $R^2 = 0.42$ (deviance explained = 40.4 %). The size-specific function was nonmonotonic, with the probability of having FP increasing first with SCL, plateauing around 57 to 59 cm SCL, then decreasing with increasing carapace length (Fig. 2a). The year-specific function was also nonmonotonic, with FP rapidly increasing to a peak in 2003, from then on decreasing and apparently stabilizing (Fig. 2b). For Tortuga Bay, the best minimal GAM also retained SCL (GAM edf = 1.00, ref.df = 1.00, $X^2 = 7.02$, p < 0.01), and sampling year (GAM edf = 2.18, ref.df = 2.74, $X^2 = 11.43$, p < 0.01). The model, however, had a lower fit ($R^2 = 0.18$, deviance explained = 28.3 %), probably due to a very small sample size of turtles with FP. According to the GAM, the probability of having FP in Tortuga Bay increased linearly with SCL (Fig. 2c). It also increased with year until 2009, plateauing thereafter (Fig. 2d). There was no significant effect of abundance on the presence of FP, at either site (Puerto Manglar: GAM edf = 1.00, ref.df
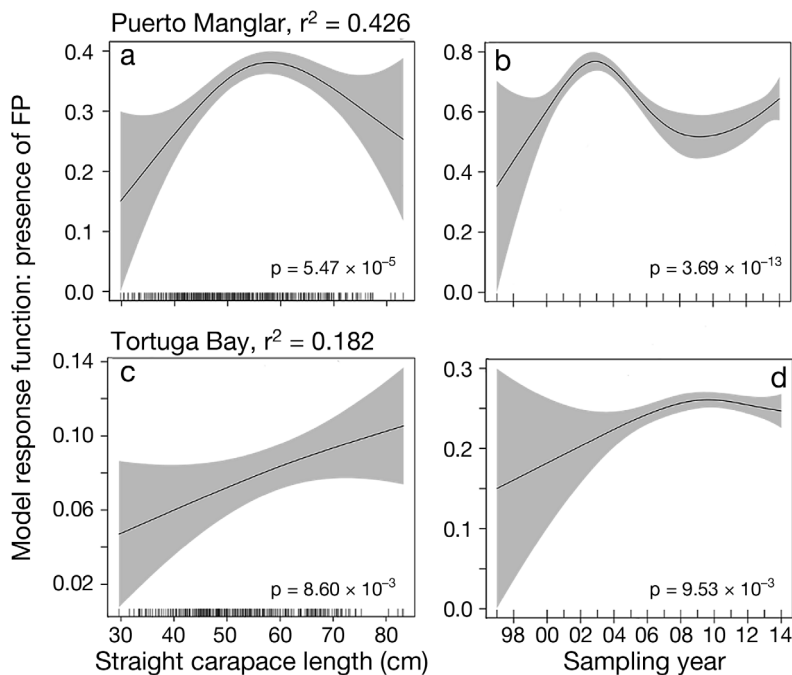

Fig. 2. Graphical summary of generalized additive models fitted to an 18 yr green turtle mark-recapture dataset. Response variable: probability of fibropapillomatosis (FP) among immature green turtles from (a,b) Puerto Manglar and (c,d) Tortuga Bay foraging grounds, Culebra, Puerto Rico. Predictor variables: (a,c) straight carapace length and (b,d) year. p-values are displayed for significant effect of covariates in FP incidence

Table 2. Summary of generalized additive mixed models (GAM) fitted to captures of immature green turtles from 2 Puerto Rican foraging grounds, Puerto Manglar and Tortuga Bay, to model the relationship between fibropapillomatosis expression (FP, response variable) and straight carapace length (SCL) and sampling year (predictor variables or covariates). edf: estimated degrees of freedom of smooth term, ref.df: estimated residual degrees of freedom of smooth term (1 = linear)

Dataset/site	Model	Covariate	edf	ref.df	χ^2	p	R^2
Puerto Manglar (n = 443)	FP~SCL+Year	SCL	2.75	3.48	26.01	2.30×10^{-5}	0.42
		Year	5.17	6.20	71.25	4.02×10^{-13}	
Tortuga Bay (n = 321)	FP~SCL+Year	SCL	1.00	1.00	7.02	8.1×10^{-3}	0.18
		Year	2.18	2.74	11.43	8.0×10^{-3}	

= 1.00, χ^2 = 1.19, p = 0.276; Tortuga Bay: GAM edf = 1.00, ref.df = 1.00, χ^2 = 0.28, p = 0.595). See Table 2 for a GAM summary.

DISCUSSION

This study extends our knowledge on the dynamics of FP in green turtles by monitoring individuals through all stages of disease expression, i.e. prior to disease, diseased, and recovered, using long-term live CMR records. We observed the outbreak of an FP epidemic at Puerto Manglar in 2000, peaking in 2003, with 75% of the turtles exhibiting tumours. There was no evidence of disease-specific detectability at our study sites (Patrício et al. 2011), indicating no sampling bias or behavioural differences for FP turtles, so these are unbiased prevalence estimates (Jennelle et al. 2007). Located ca. 5 km away, Tortuga Bay appeared free of FP until 2005, thereafter FP prevalence remained low. This variability in FP prevalence between the 2 bays is consistent with the previously recognized individual turtle fidelity to foraging site (Hirama & Ehrhart 2007, Patrício et al. 2011). This attribute of behaviour could be an important factor limiting the spread of FP among foraging grounds, if highly infectious individuals responsible for disease transmission (super-spreaders; Work et al. 2015) stay resident.

High FP prevalence has been associated with anthropogenic change and habitat degradation (Williams et al. 1994, Van Houtan et al. 2010, Keller et al. 2014), and existing ChHV5 variants were shown to pre-date FP outbreaks (Herbst et al. 2004, Patrício et al. 2012), further implicating the environment as a factor in disease expression. Stress has also been posited as a risk factor (Lu et al. 2003). Puerto Manglar, where higher FP prevalence was observed, is potentially more anthropogenically altered than Tortuga Bay, which is located at an uninhabited island. An assessment of water quality in 2007 using DNA-markers

identified widespread human faecal contamination at Puerto Manglar, while at Tortuga Bay such contamination was only detected next to a boat (Diez et al. 2010). Additionally, nitrogen isotopic values (δ^{15}N) of macroalgae at Manglar suggested an intermediate level of wastewater impact (Diez et al. 2010). Ecological differences could also be a factor. Macroalgae and *Thalassia testudinum* dominate at Puerto Manglar, in contrast to the seagrasses *Syringodium filiforme* and *Halodule wrightii* at Tortuga Bay (Diez et al. 2010). Foraging aggregations of green turtles are, however, typically small (such as the ones in the present study) and demographic stochasticity alone (i.e. the probabilities of immigration, emigration, death, disease transmission and recovery) could affect FP prevalence (Lloyd-Smith et al. 2005).

Turtles did not appear to be diseased upon arrival at our study sites, supporting the hypothesis of local infection (Ene et al. 2005). Our model indicates that FP prevalence is low among smaller and larger individuals at Puerto Manglar, whereas medium-sized turtles are the most likely to show signs of the disease. Size distributions of healthy, FP and recovered individuals at this site indicate that FP appears at intermediate sizes and that only large turtles were seen recovered (Fig. 3). We believe that the size effect on FP expression observed in the GAM, and previously reported (Work et al. 2004, Foley et al. 2005, Patrício et al. 2014), is in reality the reflection of (1) residency plus tumour development, and (2) tumour regression. We estimate that it takes 1.8 ± 0.8 yr (mean ± SD, range: 1.0–3.4 yr; Fig. 4a) from recruitment to FP expression at Puerto Manglar, based on the records of 12 turtles, which were first captured healthy and later with fibropapillomas. These individuals were never missed for more than 1 yr in our CMRs and were first captured when FP was already present at the foraging ground (i.e. from 2000 onwards).

As FP prevalence at Puerto Manglar was greater earlier in our sampling period, sufficient time has elapsed to be able to observe recovery from the dis-
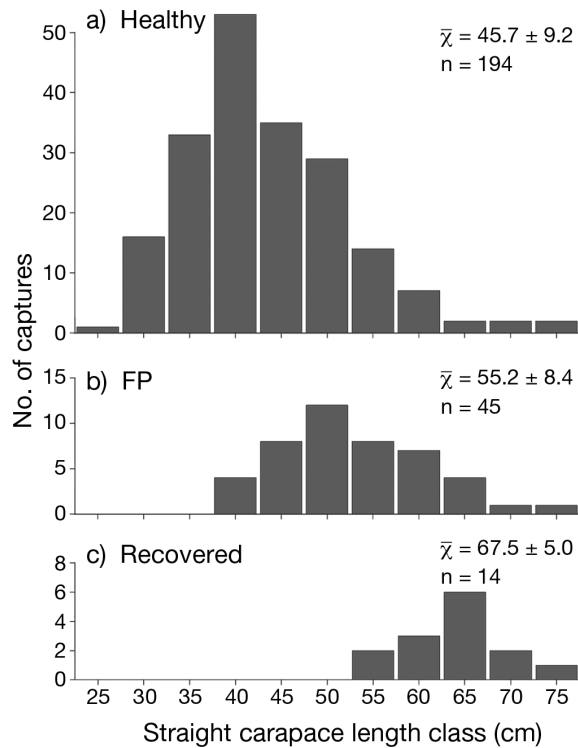
Fig. 3. Distribution of straight carapace lengths (SCLs) at first capture of green turtles: (a) healthy, (b) with fibropapillomatosis (FP), and (c) after recovery from FP at Puerto Manglar, Puerto Rico, throughout 18 yr of capture-mark-recaptures. Numbers on the *x*-axis represent the start of each 5 cm SCL class
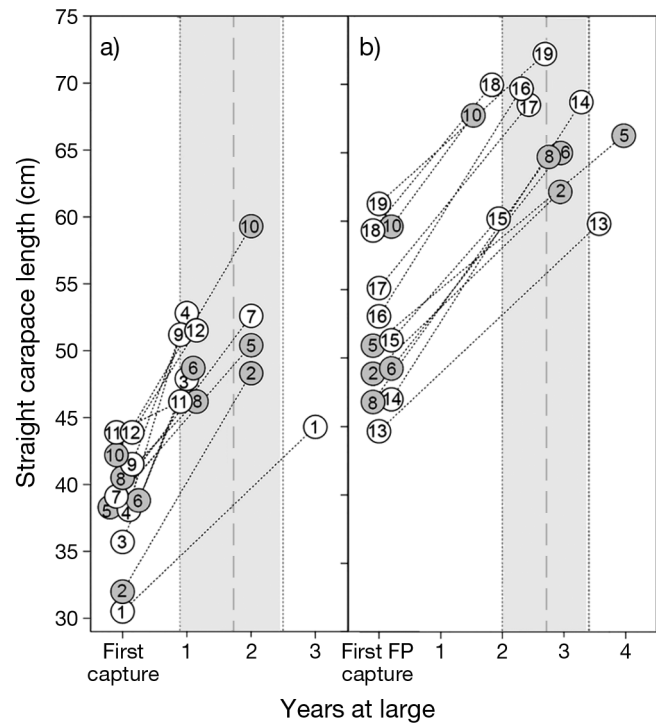

Fig. 4. Straight carapace length at the first capture of resident green turtles at Puerto Manglar, Puerto Rico, that (a) were healthy and subsequently developed fibropapillomatosis (FP; n = 12), and (b) had FP and later recovered from the disease (n = 12). The *x*-axes show the time (in yr) for each transition. Circled numbers identify unique individuals, and grey circles highlight turtles for which both transitions were recorded (n = 5). Dashed vertical line: mean time for each transition (light grey bars: SD)

ease; a total of 31% of afflicted turtles were confirmed to have become tumour-free. This is likely a conservative estimate, nevertheless, as a previous analysis on the survival probability (φ) of turtles in the study aggregations found a much lower apparent survival among subadults (SCL ≥ 65 cm, φ = 0.529) compared to juveniles (SCL < 65 cm, φ = 0.832), most likely attributed to the permanent emigration of the larger turtles (Patrício et al. 2011). The mean SCL of turtles at first capture after disease recovery was 67.5 cm, well within the subadult category. Thus, FP regression is in reality probably even higher, as larger turtles are both recovering from FP and permanently leaving the foraging ground (Patrício et al. 2011, 2014). If turtles are likely to recover from FP and acquire immunity in the process, it could explain the rarity of the disease among adult turtles.

The time from FP expression to complete recovery was 2.7 ± 0.7 yr (mean ± SD, range: 1.5–4.0 yr, Fig. 4b), estimated for 12 individuals (of 14 confirmed to have recovered) never missed in the CMR analysis for a period longer than 1 yr. Evidence of high disease recovery at Puerto Manglar suggests that one factor

involved in disease fadeout could be herd immunity, as more turtles became resistant to FP, and the number of susceptible individuals decreased (Lloyd-Smith et al. 2005). The annual size structure of green turtles at Manglar appears to support this hypothesis, as there seems to have been very little recruitment between the peak years of the FP epidemic and its fadeout (Fig. 5, size class < 40 cm SCL), keeping the stock of vulnerable individuals low. If this is the case, the replenishment of susceptibles, by recruitment of new individuals to the forage aggregation could potentiate a new epidemic (Lloyd-Smith et al. 2005). Here, we observed from 2008 onwards an increase in the smaller size class (Fig. 5), indicative of recruitment, and indeed we detected a slight increase in FP prevalence in the last 2 sampling years at Puerto Manglar, attributed entirely to new individuals (i.e. first tagged in 2013). This could suggest that cyclic epidemics may occur at this site, depending on the immigration rate of individuals naïve to FP.

Previous studies have shown that FP did not affect survival rates or somatic growth at Puerto Manglar
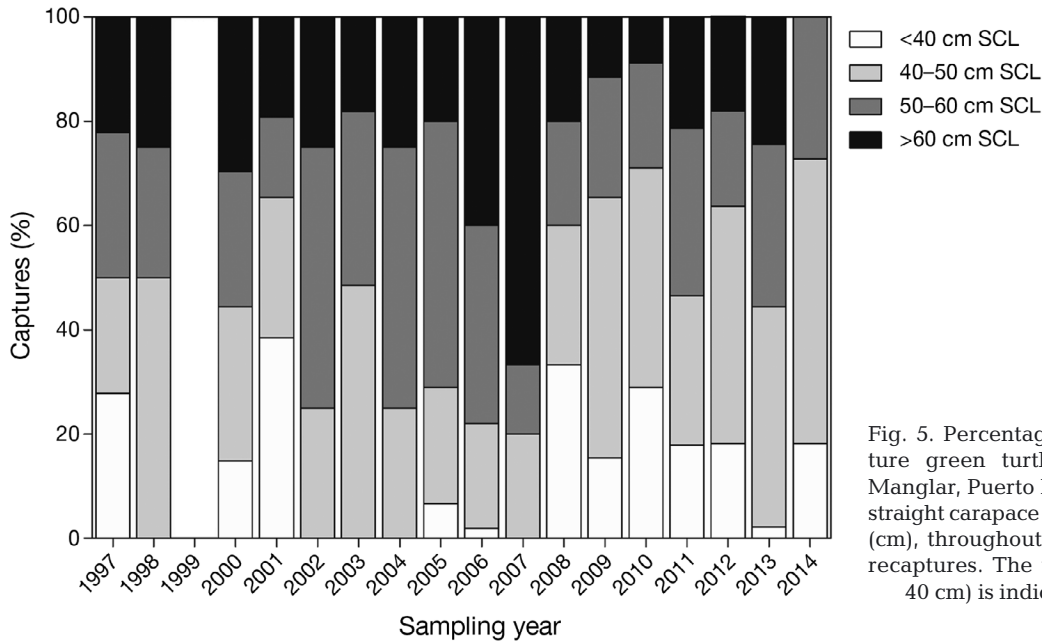
Fig. 5. Percentage of captures of immature green turtles foraging at Puerto Manglar, Puerto Rico, corresponding to 4 straight carapace length (SCL) size classes (cm), throughout 18 yr of capture-mark-recaptures. The white size class (SCL < 40 cm) is indicative of recruitment

and Tortuga Bay foraging grounds (Patrício et al. 2011, 2014). In Florida, FP was also shown to have no significant effect on somatic growth (Kubis et al. 2009), and in Hawaii, growth rates were only lower in severe cases of the disease (Chaloupka & Balazs 2005). Most FP turtles at our study sites were mildly to moderately affected, and we found no significant differences in mean BCI between healthy and afflicted turtles or among tumour scores, comparable to what was reported in Hawaii (Work et al. 2004). Similar to our evidence for a high rate of disease recovery, photo-identification of green turtles around the Hawaiian archipelago in a foraging ground in Maui revealed a regression rate of 32% (Bennett et al. 1999); in a different Hawaiian population, at Molokai, 13 to 18% annual recovery probabilities were estimated (Chaloupka et al. 2009). Tumour regression was further observed in Florida (22/24, 88%; Hirama & Ehrhart 2007), Brazil (2/8, 25%; Machado Guimarães et al. 2013), Australia (proportion undetermined; Limpus et al. 2005) and in olive ridley turtles from Costa Rica (20/42, 48%; Aguirre et al. 1999). Despite the FP epidemic at Puerto Manglar, a positive trend in aggregation size since the beginning of the CMR program was detected, with a mean annual increase of 10.9% (Patrício et al. 2014). Most remarkably, the once severely depleted Hawaiian green turtle population has recovered despite major FP outbreaks during the 1980s and 1990s (Chaloupka et al. 2009). Analogously, high FP prevalence in Florida has not halted population recovery (Chaloupka et al. 2008a). These optimistic findings suggest that

FP is not currently a major threat to marine turtle populations.

CONCLUSIONS AND MONITORING RECOMMENDATIONS

Anthropogenic activities, predicted to increase disease occurrence, are on the rise (Harvell et al. 2002, 2004). Human-mediated climate change may also increase disease prevalence in the marine environment (Harvell et al. 2002) or lead to deviations in host–pathogen relations and disease virulence. Additionally, selective harvesting of healthy individuals can increase FP prevalence in a population (Stringell et al. 2015). To better understand the dynamics of wildlife disease and attempt to predict outbreaks, it is essential to gather baseline data and to develop rapid response capability to identify, monitor and manage disease outbreaks as they occur (Harvell et al. 2004). FP disease monitoring can be easily integrated in already established population surveys; however, it is important to standardize the collected information. We suggest including the following data regarding disease presentation: number, size and location of tumours, weight of afflicted turtles, BCI, and presence of parasites. We also recommend more long-term monitoring, for reliable estimates of disease prevalence. The collection of biopsy samples from both affected and healthy tissues for molecular research is also desirable, as new molecular techniques are becoming available and may be key to

understand the evolution of ChHV5 and FP disease spread. A unified monitoring strategy could be achieved with little additional effort and would significantly improve our understanding of the implications of FP to marine turtle populations worldwide.

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