Vol. 121: 233–240, 2016
doi: 10.3354/dao03020

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published October 27

Characterization of fibropapillomatosis in green turtles *Chelonia mydas* (Cheloniidae) captured in a foraging area in southeastern Brazil

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ABSTRACT: Fibropapillomatosis (FP) is a multifactorial disease that affects all species of marine turtles, including green turtles *Chelonia mydas* (Linnaeus, 1758). It is characterised by the development of internal or external tumours that, depending on their locations and sizes, may intensely impact the health condition of sea turtles. The goal of this study was to characterise the disease in *C. mydas* found in a foraging area in southeastern Brazil, evaluate the prevalence in this region, and correlate presence and absence, size, body distribution, number of tumours, and disease severity with biometric variables of the captured green turtles. Between 2008 and 2014, the prevalence rate of FP was 43.09%, out of 246 green turtles. The size of the animals with FP was relatively greater than animals without tumours, and the prevalence of FP increased with animal size, peaking in the 60–80 cm size class. From 2013 to 2014, gross evaluation of fibropapillomas was performed. The number of tumours per turtle ranged from 1 to 158. The size of tumours ranged from <1 cm (Size A) to >10 cm (Size D); Size A tumours and turtles slightly affected by the disease (Score 1) predominated. Tumour progression (72.1%) and regression (32.8%) were seen in some recaptured individuals (n = 61). Moreover, 24.6% of these turtles showed both progressions and regressions of tumours.

KEY WORDS: Disease · Fibropapilloma · Tumour · Prevalence · Regression · Progression

INTRODUCTION

Fibropapillomatosis (FP) is a multifactorial infectious disease affecting marine turtles, including the green turtle *Chelonia mydas* (Barragan & Sarti 1994, Herbst 1994, Limpus & Miller 1994, Aguirre et al. 1999, Lackovich et al. 1999, D'Amato & Moraes-Neto 2000, Huerta et al. 2002). The disease is characterised by the development of single or multiple tumorous masses in the ocular region, bucal cavity, skin, carapace, plastron or internal organs (Jacobson et al. 1989, Balazs 1991, Herbst 1994, Aguirre et al. 2002, Work et al. 2004). Tumours may contribute to progressive debilitation and eventually death of marine turtles, depending on tumour size and location (Foley et al. 2005). The etiology of FP is not completely known, but it is associated with a specific herpesvirus and a series of environmental and biological stressors, such as temperature changes, chemical contaminants, bacterial infection and ectoparasites (Aguirre & Lutz 2004, Foley et al. 2005, Hirama & Ehrhart 2007, Page-Karjian et al. 2014). Tumours

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may regress, progress or remain stable in size and quantity (Jacobson et al. 1989, Herbst 1994, Guimarães et al. 2013). The biological mechanisms causing progression or regression of the disease are still unknown.

This study aimed to characterise FP in *C. mydas* in a foraging area within tropical southeastern Brazil. Our objectives were to: (1) measure the prevalence rate of FP over 7 yr and the prevalence variation between turtle size classes; (2) correlate severity of disease and the presence of ocular tumours to body condition; and (3) quantify progression and regression of the disease in individual turtles.

MATERIALS AND METHODS

Study site

The study was conducted in the coastal zone of Itaipu, Niterói, state of Rio de Janeiro, Brazil (22° 53′ 14″ S, 43° 22′ 48″ W). The region is partially sheltered by coastal islands and connected to a coastal lagoon complex (Piratininga-Itaipu Lagoon) (Fig. 1), which drains sewage-contaminated waters into the ocean (Lema 2012, Weber et al. 2013). In addition, Itaipu lies within the entrance to Guanabara Bay, which is seriously affected by the release of domestic and industrial effluents from several sources (Amador 1997, Lema 2012). Being a sheltered coastal environment protected by islands and enriched by the presence of a lagoon complex, the region attracts many foraging juvenile green turtles.

Turtle capture and measurements

Turtles were captured incidentally on the artisanal beach-seine fishery from July 2008 to November 2014, and intentionally, during 8 d each month in January, April, July and October 2013 and 2014. We used a special beach-seine net of 100 m, with diagonal mesh sizes of 50 mm (between knots) in the wings and 25 mm in the cod end.

Captured green turtles were tagged with 2 metallic tags (Inconel, National Band and Tag, USA) provided by Projeto TAMAR-ICMBio (Brazilian National Marine Turtle Conservation Project). Tags were placed in the proximal position of the foreflippers, between the first and second scale. Digital photographs of the dorsal and ventral regions, head and post-orbital scales were taken for additional photo-identification of individuals.

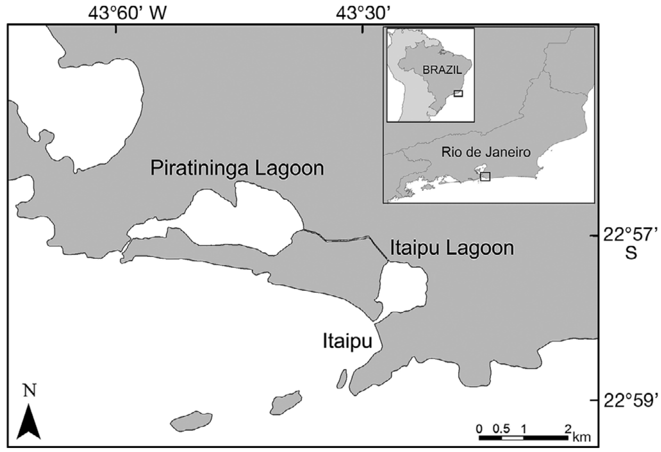

Fig. 1. Coastal zone of Itaipu, state of Rio de Janeiro, Brazil, with the Piratininga-Itaipu lagoon complex

The curved carapace length (CCL, cm) was measured with a flexible measuring tape (Bolten 1999), and used as the standard size measurement of green turtles in this study. The animals were weighed with a digital scale (body mass, kg). The CCL was then converted to straight carapace length (SCL) to calculate the body condition index (BCI = body mass/ $SCL^3 \times 10\,000$) (Bjorndal & Bolten 1989, Bjorndal et al. 2000).

Prevalence of fibropapillomatosis

All green turtles captured from July 2008 to November 2014 were examined for presence and absence of external tumours. To estimate prevalence rate, we used presence and absence data of the last catch of each turtle (each turtle was counted only once, regardless of recaptures), and divided the number of green turtles with FP by the total number of captures of green turtles from 2008 to 2014 and within a year to compare temporal variation from 2008 to 2011, when sampling effort and capture method were the same.

Macroscopic evaluation of fibropapillomatosis

Tumours measurements (number, body distribution and size) were recorded only for turtles captured between April 2013 and November 2014. The tumours were grouped into 4 approximate sizes, using the maximum diameter, following Work & Balazs (1999): A, ≤1 cm; B, >1–4 cm; C, >4–10 cm; and D, >10 cm.

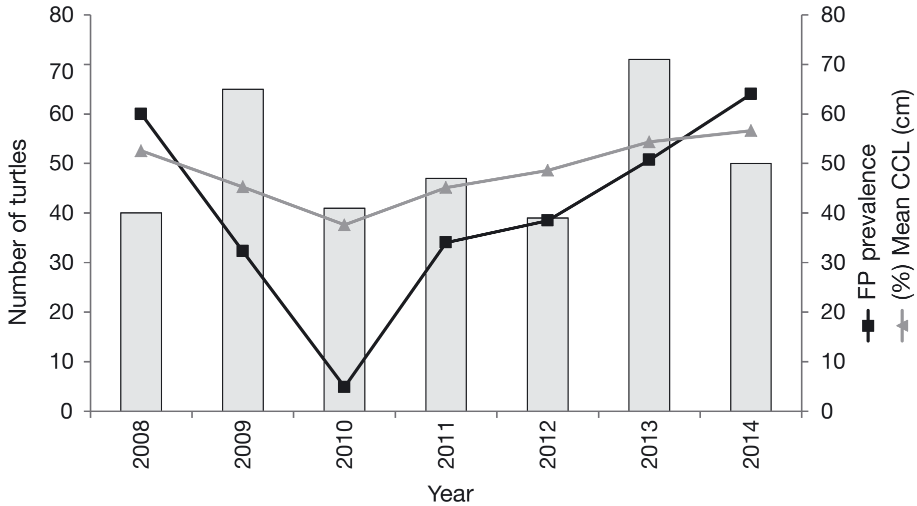

Fig. 2. Prevalence rate variation of fibropapillomatosis (FP) in *Chelonia mydas* (black line), mean curved carapace length (CCL, cm) (grey line) and number of turtles captured (bars) per year (2008–2014). Recaptures are not included

Tumour scores

An index of FP severity (FP score) was assigned to each individual, using the number of tumours and tumour size class as indicators. FP scores were: 0, non-afflicted; 1, lightly afflicted; 2, moderately afflicted; and 3, heavily afflicted (Balazs 1991, Work & Balazs 1999).

Progression and regression of fibropapillomas

We assessed FP progression and regression by comparing photographs of captured and recaptured individuals between 2008 and 2014. The appearance of new tumours and any increase in tumour size were considered a progression, and the tumour disappearance and any decrease in size were considered a regression. Animals affected by FP were handled separately from non-affected individuals, using separate materials.

Statistical analysis

Differences between annual prevalence of FP and between green turtle size classes were compared using Pearson's chi-square (χ^2). The Mann-Whitney-Wilcoxon median test was used for comparing size differences between animals with and without tumours.

Differences in animal sizes among tumour scores were assessed through the Kruskal-Wallis median test, followed by a Dunn post-hoc test for multiple comparisons. The difference between BCI of individuals with and without ocular tumours was evaluated with the Mann-Whitney-Wilcoxon median test.

The level of significance for all statistical tests was $\alpha = 0.05$, and all analyses were computed in R 3.1.1 software (R Core Team 2014).

RESULTS

Prevalence of fibropapillomatosis

Between July 2008 and November 2014, a total of 246 green turtles were captured in the study area. The prevalence rate of FP was 43.1% (106 out of 246), with annual prevalence decreasing significantly from 2008 to 2010 and increasing again towards 2014 (χ^2 = 59.5, df = 6, $p < 0.05$). The annual prevalence of FP accompanied the mean size of green turtles caught each year (Fig. 2).

The CCL of captured green turtles ranged from 28.0 to 81.5 cm (average = 48.7 ± 13.8 cm). Animals affected by FP ranged in size from 35.0 to 81.5 cm (average = 60.2 ± 11.3 cm), whereas non-affected animals ranged from 28.0 to 79.5 (average = 40.4 ± 8.2 cm). The median size of green turtles with FP was significantly higher than animals without FP (W = 13404.5; $p < 0.05$). The prevalence of FP increased with animal size, peaking in the 60–80 cm CCL class (χ^2 = 195.47, df = 6, $p < 0.05$) (Fig. 3).

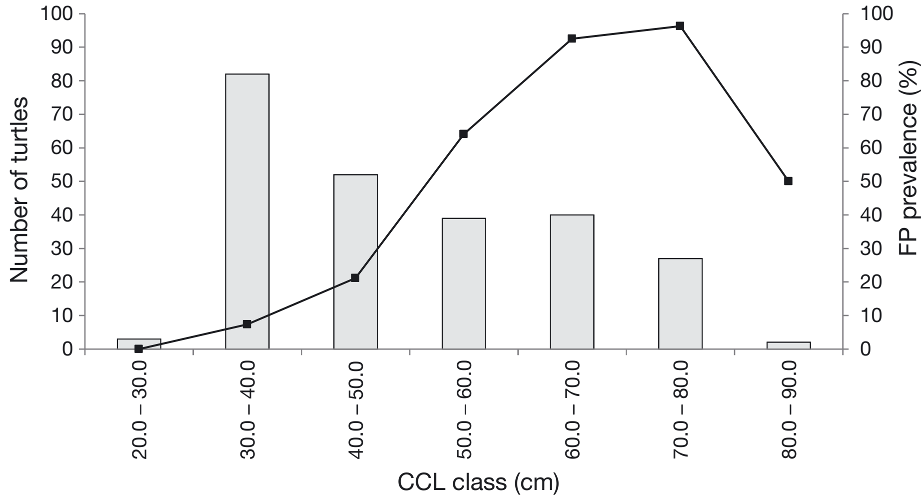

Fig. 3. Prevalence rate variation of fibropapillomatosis (FP) in *Chelonia mydas* (line) and number of turtles captured (bars) in each curved carapace length (CCL, cm) size class

Macroscopic evaluation of fibropapillomatosis

Between April 2013 and November 2014, we documented the actual location of tumours in 42 turtles. Most animals showed fibropapillomas distributed in the anterior section of the body (85.7%), followed by the posterior section (76.2%), carapace or plastron or both (66.7%) and ocular regions (61.9%), including the eyelid, periocular surfaces and the cornea. Nearly all animals (90.5%) showed tumours of the size class A (range: 1–85 tumours per individual), followed by B (80.9%) (range: 1–61 tumours per individual), C (59.5%) (range: 1–13 tumours per individual) and D (16.7%) (range: 1–5 tumours per individual) (Fig. 4).

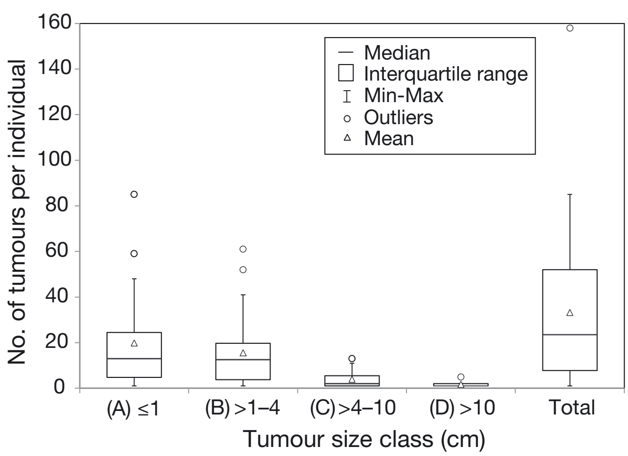

Fig. 4. Number of tumours per individual of *Chelonia mydas* in different tumour size classes: A (≤1 cm) = 38, B (>1–4 cm) = 34, C (>4–10 cm) = 25, D (>10 cm) = 7, and total (n = 42)

Tumour scores and ocular tumours

Of the 76 green turtles examined, 44.7% were classified with tumour score 0, followed by 21.0% with score 1, 19.7% with score 2 and 14.5% with score 3.

The average size (CCL) of green turtles with tumour score 0 was 45.1 ± 10.9 cm (range: 33.0–79.5 cm); with score 1 was 56.7 ± 11.6 cm (range: 36.5–74.0 cm); with score 2 was 65.1 ± 8.2 cm (range: 50.5–78.5 cm); and with score 3 was 67.8 ± 9.4 cm (range: 45.5–78.5 cm). The categories of tumour scores increased with turtle size (Shapiro-Wilks $W = 0.94$, $p < 0.05$; Kruskal-Wallis = 34.43, df = 3, $p < 0.05$); however, we found no significant difference in CCL between scores 2 and 3 turtles (Dunn post-hoc test for multiple comparisons: $p > 0.05$).

Out of 76 green turtles examined, 34.2% showed ocular tumours (eyelid, periocular surfaces and cornea) of Sizes A and B, ranging in number of tumours from 1 to 7 (average: 2.9 ± 1.6). The average BCI of green turtles with ocular tumours was 1.45 ± 0.12 kg cm^{-3} (range: 1.28–1.83 kg cm^{-3}) and those without 1.39 ± 0.22 kg cm^{-3} (range: 1.14–2.75 kg cm^{-3}). The median BCI was significantly higher ($W = 386.5$, $p < 0.05$) for individuals with ocular tumours.

Progression and regression of fibropapillomas

Between 2008 and 2014, we analysed 61 of 140 recaptured individuals for progression and regression of fibropapillomas. Tumour progression or development was observed in 44 individuals (72.1%).

Of these, 27 were FP free at capture, but showed tumours at recapture. The remaining 17 animals had tumour progression with 3 showing new tumours, 3 showing increased tumour size and 11 showing both increase in number and size of tumours (Fig. 5a).

Twenty (32.8%) turtles showed signs of tumour regression. In 2 cases, total remission of the disease was observed in 327 and 367 d. Six animals showed total regression of one or more tumours, 5 turtles showed partial regression of one or more tumours, and 7 showed both total and partial regression (Fig. 5b).

Fifteen (24.6%) individuals showed both tumour progression and regression, of which 10 occurred during a single recapture and the remainder occurred over different recapture events (Fig. 5c).

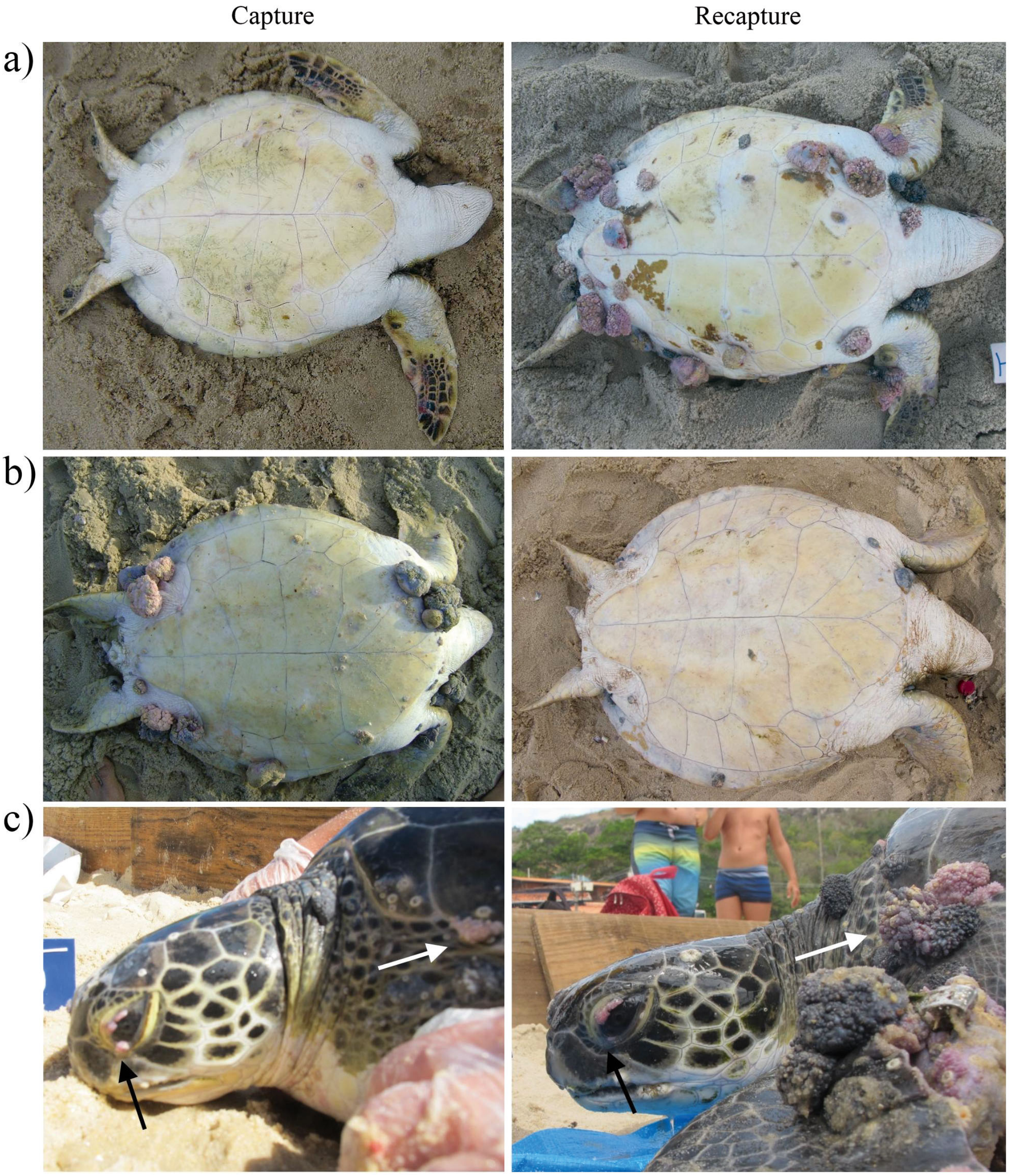

Fig. 5. Progression and regression of fibropapillomas. (a) Tumour progression (619 d between capture and recapture of the individual). (b) Tumour regression (925 d between capture and recapture of the individual). (c) Both tumour progressions (foreflipper; white arrows) and regression (ocular region; black arrows) (187 d between capture and recapture of the individual)

DISCUSSION

Prevalence of fibropapillomatosis

Prevalence of FP encountered here (43.1%) was high compared with other areas in Brazil, where general prevalence is relatively low (15.4%; Baptistotte 2007). The only prevalence that exceeded the findings in Itaipu was in the Espírito Santo Bay, where 58.3% of turtles manifested the disease (Santos et al. 2010). However, compared with some regions in other countries, the prevalence found in this study was lower than average, e.g. Hawaiian Islands: 0 to 92% (Balazs 1991); Florida: up to 72.5% (Lackovich et al. 1999); Western coast of Florida (Gulf of Mexico): 51.9% (Foley et al. 2005); Indian River Lagoon, Florida: 61.6% (Hirama & Ehrhart 2007); Moreton Bay, Australia: up to 70% (Aguirre et al. 2000).

The high prevalence of FP in Itaipu, compared with other localities in Brazil, may be related to several environmental and biological factors as described in 'Study site', such as high nutrient pollution levels (Foley et al. 2005, Santos et al. 2010, Van Houtan et al. 2010, 2014), chemical contaminants (Adnyana et al. 1997, Silva et al. 2016), water temperatures (Herbst & Klein 1995, Page-Karjian et al. 2014), and the immunologic and physiologic status of turtles (Aguirre et al. 1995, Work et al. 2001). Furthermore, the high density of susceptible turtles in the area may also serve as a reservoir and potential vector for infectious viruses, facilitating the transmission of the disease (Herbst & Klein 1995, Curry et al. 2000). Nevertheless, Foley et al. (2005) observed that the high prevalence of the disease in Florida did not match the high density of green turtles.

Changes in the prevalence of FP over the years may be associated with the size classes of green turtles captured each year. Turtles caught in the study area were considered to be juveniles to sub-adults based on CCL <90 cm (Torezani et al. 2010). Turtles with FP in Itaipu had relatively higher CCLs than those without tumours, consistent with findings in various regions of the world (Aguirre et al. 1994, Foley et al. 2005, Baptistotte 2007). The prevalence of FP increased with turtle size up to 80 cm, decreasing thereafter. These results are also consistent with other studies, which, in general, showed the highest prevalence of the disease between 40 and 85 cm (Balazs 1991, Adnyana et al. 1997, Foley et al. 2005, Baptistotte 2007, Santos et al. 2010). The absence of FP in the smaller size classes and the higher prevalence of the disease in intermediate size classes may be explained by the hypothesis that the infectious agent associated with FP is acquired after the recruitment of juveniles into specific coastal areas (Herbst 1994, Ene et al. 2005, Rodenbusch et al. 2014). The lowest prevalence of FP in larger size classes may be explained by the capacity of larger turtles to overcome the disease, either by increasing the resistance conferred by age or by the fact that the disease is somehow self-limiting with increasing age (Bennett et al. 2000, Work et al. 2004, Foley et al. 2005, Hirama & Ehrhart 2007).

Macroscopic evaluation of fibropapillomatosis

Higher tumour occurrence on the front versus the back of turtles may be due to the significantly greater soft tissue surface area on the front. The same pattern has been previously observed (Work et al. 2004, Baptistotte 2007, Hirama & Ehrhart 2007, Santos et al. 2010). The predominance of small tumours is consistent with those observed in other regions in Brazil (Baptistotte 2007, Rossi 2007). According to Work et al. (2015), smaller tumours in turtles with FP may provide a renewable source of replicating virus (i.e. virus replication occurs in early tumour formation, becoming latent as they grow), explaining their predominance.

Tumour scores and ocular tumours

The predominance of Scores 1 and 2 among the turtles with FP is consistent with the findings in other studies (Work et al. 2003, Baptistotte 2007, Hirama & Ehrhart 2007, Deus Santos et al. 2015) but contradicts the findings from Espírito Santo Bay, where the turtles most severely affected by the disease predominated (Santos et al. 2010). The different manifestations of FP may be related to several factors, such as viral variants of the disease, associated with tumour morphology, size and severity (Rodenbusch et al. 2014), or may simply be a detectability or capture issue.

In the present study, animals with ocular tumours showed a higher BCI than animals without such tumours, contrary to the *a priori* hypothesis that ocular tumours may reduce and/or obstruct vision, and, consequently, cause disorientation and affect their potential ability to feed and avoid predators (Balazs 1991, Brooks et al. 1994, Adnyana et al. 1997, Flint et al. 2010). Several authors did not find significant differences in body condition related to ocular tumours (Baptistotte 2007, Hirama & Ehrhart 2007, Santos et al. 2010). These findings can be explained because most ocular tumours were small (A: ≤1 cm), and the number of ocular tumours per turtle was not large enough to impair vision in both eyes.

Progression and regression of fibropapillomas

Although turtles in our study had more tumour progressions than regressions, the rate of tumour regressions was relatively high compared with other studies, e.g. Florida: 16% (Ehrhart 1991); Hawaiian Islands: 23.1% (Bennett et al. 2000); Espírito Santo, Brazil: 8.1% (Santos et al. 2010) and 1.7% (Torezani et al. 2010); previous study in Itaipu: 25% (Guimarães et al. 2013).

The role of the virus in induction, growth and regression of tumours is still unclear. However, it is known that tumoured sea turtles are infected with the virus persisting in a latent state with rare replication (Greenblatt et al. 2004, Work et al. 2015) and, in some cases, viral DNA has been detected in non-tumoured turtles (Alfaro-Núñez et al. 2014). Our study is the first to document simultaneous progression and regression of tumours in FP-affected animals, which could enhance the role of the turtle immune system in the manifestation of FP. Therefore, future studies might focus on this host immune response in modulating progression or regression of tumours in green turtles.

Acknowledgements. We thank H. M. Gitirana for his contribution in the course of PROMONTAR Itaipu Project from 2008 to 2010. Thanks are also extended to the people of ECOPESCA laboratory, especially D. A. Borges, and every student and volunteer that helped in the fieldwork throughout the study. C.M.-N. and G.L.-H. hold research productivity fellowships from CNPq. A.B.T and S.M.G. hold a Masters and a PhD Fellowship from CAPES, respectively. Research was conducted under SISBio licenses 13488-1 (PROMONTAR Itaipu) and 40873-1 (Projeto Aruanã).

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Editorial responsibility: Stephen Feist, Weymouth, UK

Submitted: January 29, 2015; Accepted: May 18, 2016
Proofs received from author(s): September 20, 2016