

RESEARCH ARTICLE

Prevalence of fibropapilloma in *Chelonia mydas* (Testudines, Cheloniidae) juveniles and environmental quality of their habitat at north of Villa Clara, Cuba

Prevalencia de fibropapilomas en juveniles de *Chelonia mydas* (Testudines, Cheloniidae) y calidad ambiental de su hábitat en el norte de Villa Clara, Cuba

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Abstract

In Cuba, juvenile green turtles affected by fibropapillomatosis have increased; however, neither animals' life quality nor their habitat quality has been evaluated. Therefore our objectives were to assess green turtles' habitat quality in the Wildlife Refuge "Lanzanillo-Pajonal-Fragoso" and to evaluate the severity of the fibropapillomatosis and the body condition of affected sea turtles. From October/2013 to December/2019, 48 juvenile green turtles stranded or entangled were spotted (19 were measured). Spearman correlation was used to determine annual variation of juveniles' size. Body condition was assessed based on plastron characteristics and muscle mass. In ten individuals, tumors were counted according to their size and position. Foraging habitat quality was assessed with the Ecological Evaluation Index and the Ecological Status Index using the proportion of opportunistic and late successional macroalgal groups. The presence/absence of seven heavy metals (cobalt, chromium, iron, germanium, mercury, manganese and nickel) was determined in *Thalassia testudinum* and *Syringodium filiforme* using X-ray fluorescence spectrometry. Habitat quality was moderate to high according to the ecological index and the presence of Mn, Hg, Fe and Cr in more than 30 % of seagrass samples. Fibropapillomatosis prevalence was of 69 % (28/41) in stranded individuals and 43 % (3/7) in entangled. The severity score was very high in the 52 % of animals (13/25). Tumors predominated in the anterior region of their body. Habitat quality may be related with the presence of fibropapilloma since most of afflicted sea turtles were found in stations with moderate quality and the presence of heavy metals.

Keywords: green turtle, fibropapillomatosis disease, heavy metals, Cuba

Resumen

En Cuba han aumentado los juveniles de tortuga verde con fibropapiloma pero no se ha evaluado su calidad de vida ni la del ambiente donde viven. Por ello, nuestros objetivos fueron determinar la calidad del hábitat de tortuga verde en el RF "Lanzanillo-Pajonal-Fragoso" y evaluar la severidad de la fibropapilomatosis y la condición corporal de las tortugas afectadas. Entre octubre/2013 y diciembre/2019 se evaluaron 48 juveniles de tortuga verde (19 fueron medidos). Se aplicó la correlación de Spearman para determinar la variación anual de sus tallas. La condición corporal se evaluó mediante las caracterís-

ticas del plastrón y la masa muscular. En diez individuos se contaron los tumores por tamaño y posición en el cuerpo. Se evaluó la calidad del hábitat mediante el Índice de Evaluación Ecológica y el de Estatus Ecológico. Se determinó la presencia/ausencia de siete metales pesados (cobalto, cromo, hierro, germanio, mercurio, manganeso y níquel) en pastos marinos mediante espectrometría por fluorescencia de rayos X. La calidad del hábitat es de moderada a alta según los índices ecológicos mientras que se encontró Mn, Hg, Fe y Cr en más del 30 % de las muestras de pastos marinos. La prevalencia de fibropapiloma en individuos varados fue del 69 % y del 43 % en los capturados por pescadores. El índice de severidad fue muy alto en el 52 % de los animales. Los tumores predominaron en la región anterior del cuerpo. La mayoría de los individuos con tumores se encontró en las estaciones que presentaron una calidad moderada y la presencia de metales pesados.

Palabras clave: Tortuga verde, fibropapilomatosis, metales pesados, Cuba

Introduction

Sea turtles are listed as endangered species mainly due to human activities (Wallace *et al.*, 2011). Factors such as historical turtle overfishing, the destruction of feeding and nesting habitats and fisheries bycatch cause considerable mortality (a rate of 0.31 according to Hays *et al.* 2003 and 0.23 in Casale *et al.* 2004) provoking population declines worldwide (Lemons *et al.*, 2011). In addition to these anthropogenic actions, oceanic pollution and diseases such as fibropapillomatosis (FP) represent an additional threat to these species (Herbst, 1994; Aguirre *et al.*, 1998), as the development of tumors can reach dimensions large enough to preclude visibility, locomotion and feeding (Aguirre & Lutz, 2004).

Fibropapilloma tumors can be distinguished because they are the result of a proliferation of epidermal cells and dermal fibroblasts unlike papillomas (just epidermal cells) or fibromas (dermal fibroblasts) (Smith & Coates, 1938 in Foley *et al.*, 2005). Also, it is becoming an increasingly frequent disease making it the most significant neoplastic disease of reptiles (Herbst, 1994).

It is associated with the presence of a herpesvirus identified as one of the etiological agents of the disease (Klein *et al.*, 1998; Lackovich *et al.*, 1999; Work *et al.*, 2009); although the only presence of the virus does not imply the development of tumors (Alfaro-Núñez *et al.*, 2014). Environmental stressors like dietary conditions (Hall *et al.*, 2007; Van Houtan *et al.*, 2010, 2014) and environmental quality (Dos Santos *et al.*, 2010) may triggers tumors growing. In particular, some heavy metals and organic pollutants, can immunologically depress the organism, facilitating the expression of the herpes virus (Adnyana *et al.*, 1997).

Healthy sea turtle population require good environmental conditions for optimally develop, therefore, the condition of associated ecosystems is key for the health of sea turtle populations. One of those ecosystems is seagrass bed, which is very sensitive to changes in the environment, specifically, to human impacts (Short & Wyllie-Echeverria, 1996). Since it is a key habitat for many species, marine turtles included, it is essential to survey any disturbance that can threaten its biodiversity. Macrophytes are good indicators of habitat quality because there are opportunistic and sensitive species with different responses to stress that can be evaluated (Fytianos *et al.*, 1999; Juanes *et al.*, 2008; González-Díaz *et al.*, 2018). According to Orfanidis *et al.* (2014) a more predictive approach might be achieved using functional classifications of indicator species to ensure that the same level of ecological quality is equally represented everywhere. This is the reason why Orfanidis indexes (Orfanidis *et al.*, 2001, 2003) are so useful and appropriate for seagrass beds evaluation because they are based on morphofunctional groups rather than particular species.

In Cuba, specifically to the north of the province of Villa Clara, in the Wildlife Refuge “Lanzanillo-Pajonal-Fragoso”, juvenile green turtles (*Chelonia mydas*, Linnaeus 1758) are frequently observed with presence of tumors like fibropapillomas (FP). This site is a developmental area for this species, although it is contaminated

by sources such as local agriculture, porcine farms and even a soda chlorine factory in Sagua la Grande Bay that is a mercury pollution source (Montalvo *et al.*, 2008). Considering the levels of contamination, the impact it might be having in the development of tumors in marine turtle juveniles in the region, an environmental quality evaluation is needed to determine threats for green sea turtles' development. An increase in the number animals affected with tumors might be indicating the existence of an environmental problem that will affect, not only turtles, but the entire ecosystem.

In Cuban waters the prevalence of FP based on sea turtles captured by turtle fishery were quantified between 1983 and 1996 (Moncada & Prieto, 2000). However, habitat quality was not evaluated, nor the body condition of the affected animals. Evaluation of the etiological factors related to this disease, such as

habitat quality (Keller *et al.*, 2014), is very useful in understanding the prevalence of the disease. Thus, the objectives of this study were to assess 'green turtles' habitat quality in the Wildlife Refuge "Lanzanillo-Pajonal-Fragoso" with the Ecological Evaluation Index and Ecological Status Index and the presence of heavy metals in the two most abundant seagrass species and second, to estimate the prevalence and severity of the fibropapillomatosis and the body condition of affected sea turtles.

Materials and methods

Study site

The study area is located on the northeast platform of Cuba (Fig. 1), in the stretch of the Sabana-Camagüey archipelago corresponding to the province of Villa Clara (22°54'-22°32' N & 79°46'-79°34' W). It is a Wildlife



Fig. 1. Location of sampling stations and turtle reports through the stranding network in the bays of Nazábal and Juan Francisco during the period from October 2013 to December 2019. E1: Canal de Córdoba, E2: Jácate, E3: Cubereta, E4: Juan Francisco. Red stars: turtles with fibropapilloma tumors, green stars: turtles without tumors.

Refuge and covers an area of 87,070 ha (76,490 marine and 10,580 terrestrial). The territory offers favorable habitat for a high biodiversity of marine and terrestrial species of high conservation interest (manatee, sharks, endemic tree rats, aquatic birds).

Data collection

Analysis of FP prevalence and turtles' body condition

From October 2013 to December 2019, a total of 48 green turtle juveniles were found. Stranded turtles were spotted by volunteers of the marine turtle stranding network in the Wildlife Refuge during their daily monitoring or entangled in fishermen gillnets. Around 20 km of the coastline were covered. Once a stranding or an entangled sea turtle was detected, volunteers immediately informed a specialist from the protected area for the external evaluation of the turtle. Individuals that were found alive or with incipient decomposition were measured (19 in total) notch to tip with a metric tape (1 mm error) to determine curve carapace length (CCL) and width (CCW). Measured turtles were classified into five equivalent size categories: 20-30 cm,

30.1-40 cm, 40.1-50 cm, 50.1-60 cm, and >60 cm for frequency analysis. Both, alive and dead individuals were processed onsite, and the recording protocol included photograph, morphometric measurements (when equipment was available on site) and immediately released to water in case they were alive (mostly entangled in fishermen nets).

Thomson *et al.* (2009) criteria were used to assess the body condition of 41/48 of the juveniles (Fig. 2), based on plastron characteristics and muscle mass, since body mass and size of all the animals was not obtained. Individual sea turtles were considered 'poorly nourished' when they presented a concave plastron and scarce muscle mass; 'thin', when the plastron was slightly sunken and little loss of muscle mass; 'normal' or 'good' when the plastron was aligned and 'robust' when the animal had evidence of fat accumulation (Thomson *et al.*, 2009).

Also, for ten animals with more than five tumors, FP tumors size (straight diameter) was measured as in Fig. 3 and then, classified into three categories according to their straight diameter following Work and Balazs (1999) criteria: (small) <4 cm, (medium) 4-10

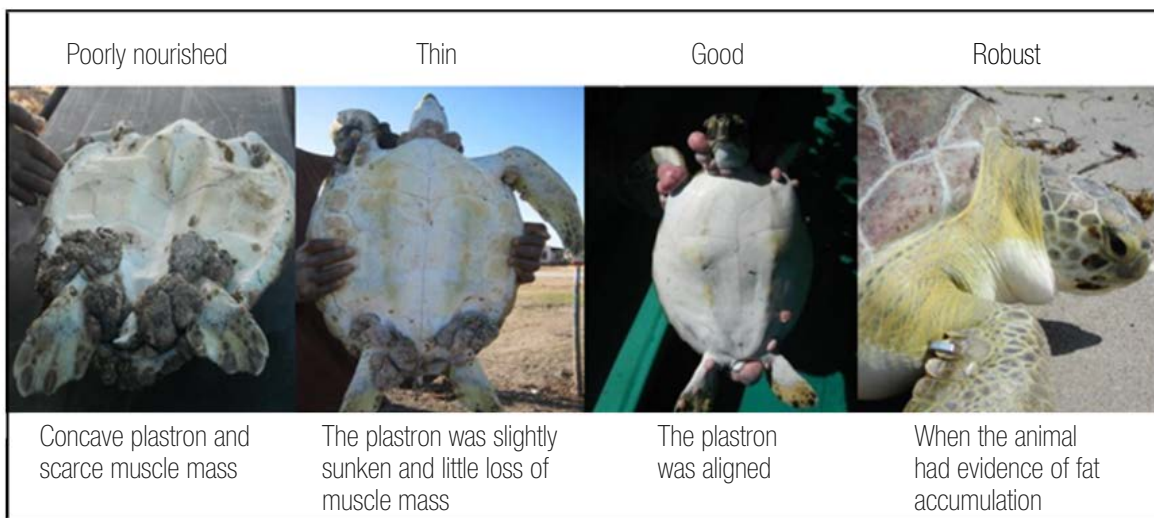


Fig. 2. Classification of body condition used for green turtle juveniles found with the stranding network in of Nazábal and Juan Francisco bays.



Fig. 3. Example of small tumor measurement.

and (large) > 10 cm. The number of each category was counted per regions of the body: ‘anterior region’, which includes the anterior flippers and the pectoral region; ‘posterior region’ that includes the rear flippers and the surrounding soft tissue; head and finally carapace and plastron because it has been recorded in these regions as well, especially in the growing lines (Jacobson *et al.* 1989). According to the number and size category of the tumors, a qualitative index of FP severity was assigned to each individual sea turtle (Table 1).

Table 1. Severity score determination considering the number of tumors per size category following criteria of Work & Balazs (1999).

Tumor size categories	Severity score (FPS) classification according to the number of tumors			
	0	1	2	3
(small) <4 cm	0	1-5	>5	>5
(medium) >4–10 cm	0	0	1-3	>4
(large) >10 cm	0	0	0	>1

This index ranges from FPS0 (without tumors) to FPS3 (highly tumorized) depending on the number and size of tumors counted in each individual (Balazs 1991; Work & Balazs, 1999). Tumors counting was done on site but verified after by photographic analysis (to verify that the number and size classification were correct), so accurate evaluation depended on having enough and good quality photographs of the individuals, as well as the decomposition advance of the carcass.

Environmental quality assessment

An assessment protocol for seagrasses (Martínez-Daranas *et al.*, 2013) was used to characterize their potential as foraging and residency habitat (Fig. 1), especially close to the stranding events (although the animals might develop the tumors in a different area). In the area where stranded juveniles were detected, four stations were evaluated: two in Nazábal bay (Canal de Córdoba and Jácate beach) and two in Juan Francisco bay (Punta Cubereta and Juan Francisco beach). In each station the environmental quality of the area was semi-quantitatively evaluated by using two indices: Ecological Evaluation Index (EEI) and Ecological Status Index (ESI). To estimate these indices, angiosperms and macroalgae were classified into two ecological groups (Orfanidis *et al.*, 2001, 2003): group I (species that indicate good environmental conditions) included leathery, calcareous, crusted macroalgae as well as seagrasses and group II (opportunistic species that indicate deteriorated environments) which included foliose, filamentous and fleshy macroalgae (Table 2). From the average value of the relative abundance of the species in each of the groups, a habitat classification was established according to the criteria of Orfanidis *op. cit.* The higher is the abundance of the first ecological status groups species, the better are the ecological status of the ecosystem.

To determine heavy metal in each station, three transects of 50 m length were placed parallel to the shore and at 10 m of distance from each other. In each

Table 2. Criteria for habitat classification according to the ecological evaluation index (EEI) and ecological status index (ESI) considering the abundance of the ecological status groups (ESGs). Modified from Orfanidis *et al.* (2003). Ecological status group (ESG) I: seaweed species with a thick or calcareous thallus, low growth rates and long life cycles (late successional); ESG II: includes sheet-like and filamentous seaweed species with high growth rates and short life cycles (opportunistic).

Average cover ESG I (%)	Average cover ESG II (%)	ESC	EEI
0-30	0-30	Moderate	6
	>30-60	Low	4
	>60	Bad	2
>30-60	0-30	Good	8
	>30-60	Moderate	6
	>60	Low	4
>60	0-30	High	10
	>30-60	Good	8
	>60	Moderate	6

transect, 20 shoots of the most abundant angiosperms (*Thalassia testudinum* K.D. Koenig and *Syringodium filiforme* Kützing) were randomly collected. There were eight samples of *S. filiforme* and 12 of *T. testudinum*. The samples were processed in the laboratory of Chemical Analysis of the Faculty of Chemistry, Havana University to determine the presence of seven heavy metals (Co: Cobalt, Cr: chromium, Fe: Iron, Ge: germanium, Hg: Mercury, Mn: manganese and Ni: nickel), using the technique of X-ray fluorescence spectrometry. Samples were washed with distilled water and dried in an oven at 70 °C for 48 hours. Then they were homogenized in an Agate mortar until it became a fine powder and then passed through a 125 µm sieve. For each 3.60 g of pulverized plant material, 0.40 g of boric acid was added as binder material. Finally, material was pressed for 2 min at 15 tons of pressure and kept in a desiccator until analysis to prevent rehydration. The X-ray fluorescence spectrometry technique (model ESLX 30-150 N° 319 of CANBERRA) was used to determine heavy metal presence using a radioisotopic

source of direct excitation and central configuration of plutonium (^{238}Pu). Finally, a presence/absence matrix was built for the seven metals determined in the 20 samples since quantification of metal concentration was not possible. Animal tissue was not analyzed because the proper protocol was not available in the lab.

Statistical analysis

The number of individuals with tumors grouped by trimester, per size class (20-30 cm, 30.1-40 cm, 40.1-50 cm, 50.1-60 cm and >60 cm) and considering the FP severity scores were obtained. We determined the mean and standard deviation of CCL of juvenile sea turtles. Temporal trend in the size of juveniles was determined with a Spearman nonparametric correlation (N=19 in six years). The number of all tumors and the number of large tumors between the two most abundant body condition classes (thin+badly nourished and good+fat) were compared using a Mann-Whitney U test. Relative frequency of heavy metals was determined dividing the number of observations with detected metals between the total samples per seagrass species (eight for *S. filiforme* and 12 for *T. testudinum*). Only non-parametric statistical tests were used because of the data are not normally distributed and sample size. A level of significance of 5% was considered for all tests. The tests were carried out in the STATISTICA 7.0 program.

Results

Prevalence of FP and severity of the disease

Temporal distribution of turtle reports as result of the daily patrolling of the marine stranding volunteers network in Lanzanillo-Pajonal-Fragoso is presented in Figure 4. Sea turtles were incidentally captured or found in two locations: Juan Francisco bay (n=11) and Nazábal bay (n=37), mainly during autumn-winter season (October to March). Fishers accidentally caught 15 % (7) of studied sea turtles juveniles in gill nets and the remaining 85 % (41) animals were found

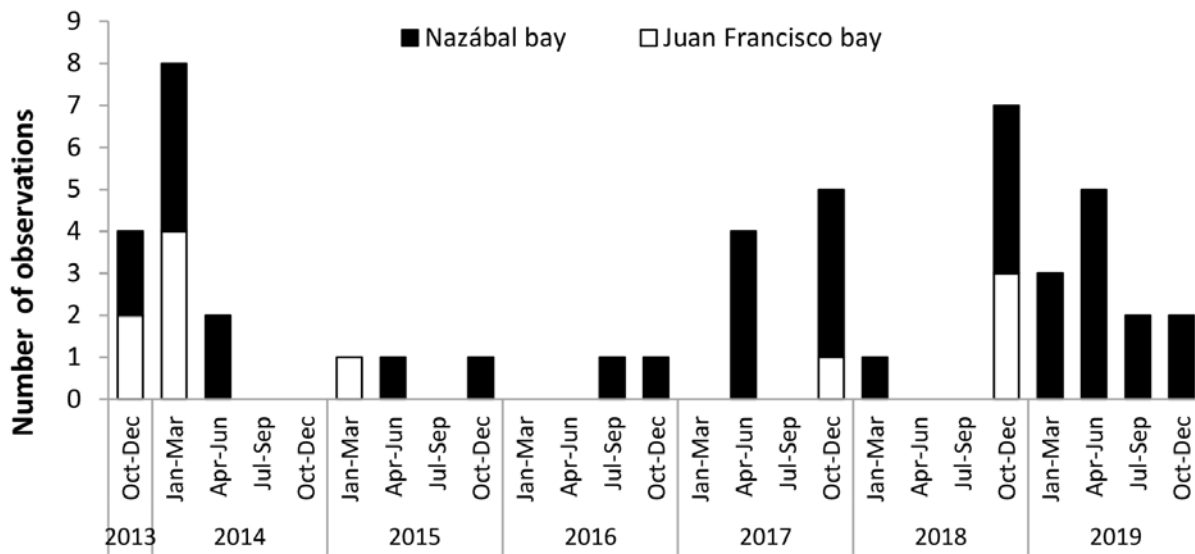


Fig. 4. Temporal distribution of turtle reports through the stranding network in Nazábal and Juan Francisco bays during the period from October 2013 to December 2019.

near the coast or stranded (dying or already dead). Prevalence of FP was higher in Cubereta (32 %) followed by Canal de Córdoba and Juan Francisco with 16 % each (Fig. 1). The rest were found outside of these areas.

The average curved carapace length of the individuals (n=19) was 39.8 ± 16.1 cm and curved carapace width was 32.7 ± 13.6 cm. Six of the sea turtles were in the interval between 20-30 cm, two between 30.1 and 40 cm, three between 40.1 to 50 cm, five between 50.1

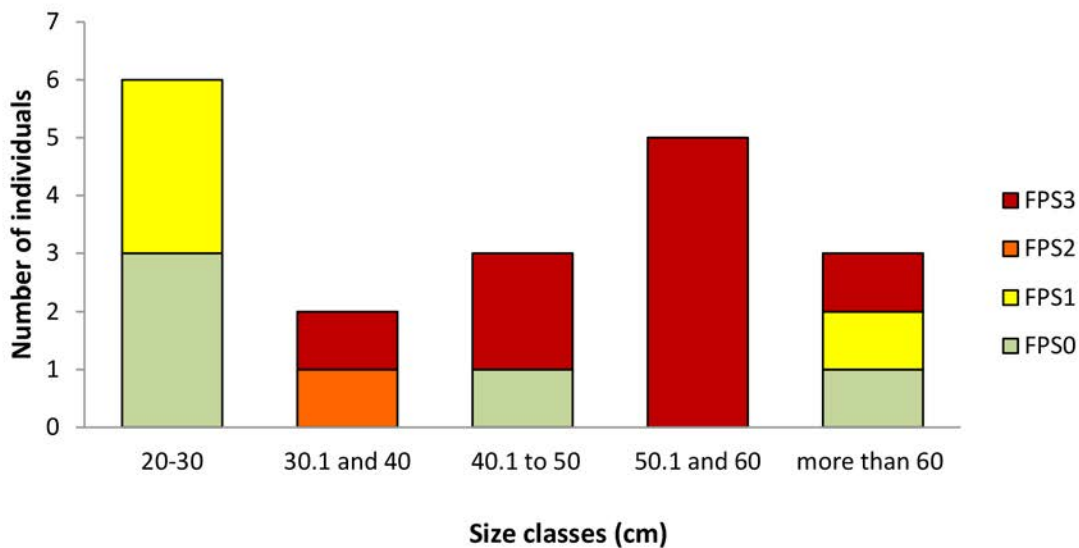


Fig. 5. Distribution of juveniles per size class and fibropapilloma severity score (FPS).

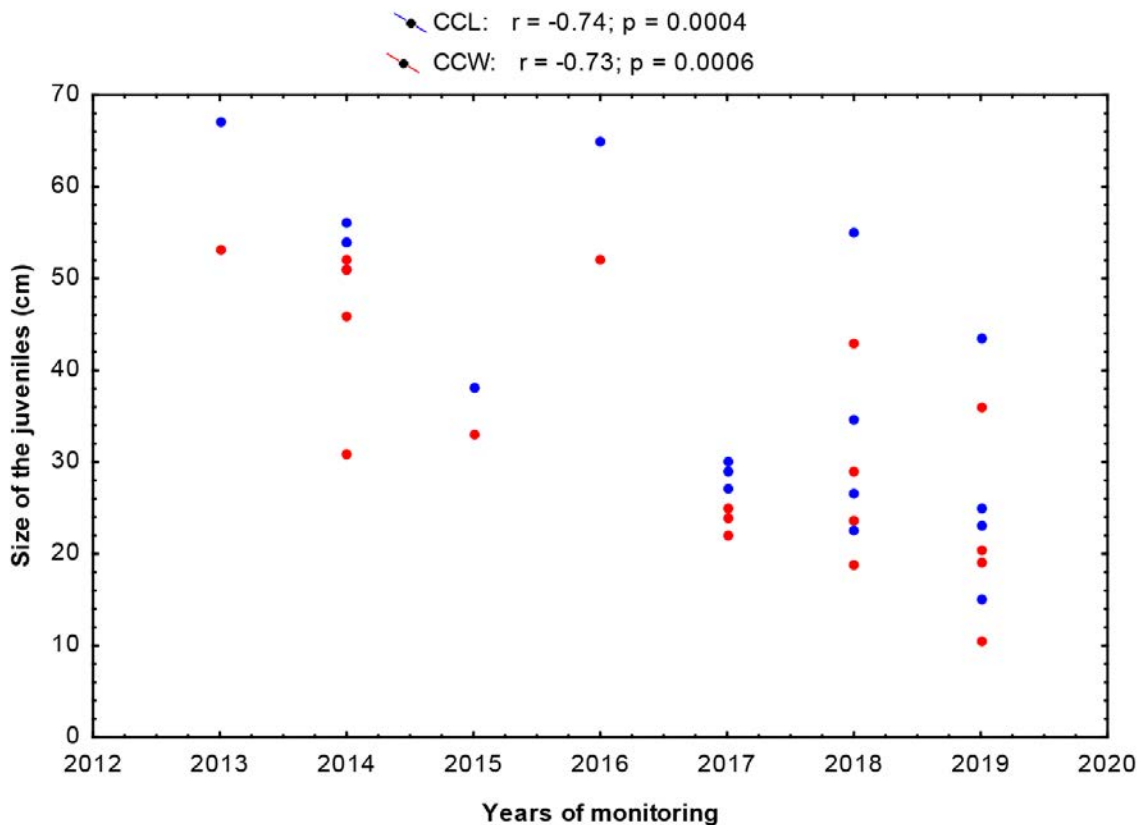


Fig. 6. Negative yearly trend of green turtle juveniles' curved carapace length (CCL) and width (CCW) during the period from 2013 to 2019.

and 60 cm and three with more than 60 cm (Fig. 5). A negative trend of the size of juveniles along the years was detected ($r=-0.74$; $p=0.004$; $n=19$) with a yearly decrease in the size of stranded and incidentally captured sea turtles with the disease (Fig. 6).

It was found that 65 % of all juveniles ($n=48$) presented evidence of tumors, prevalence in stranded individuals was 69 % (28/41) and 43 % (3/7) in individuals caught by fishermen. The anterior region was the most affected followed by the rear, head and carapace (Fig. 7). Medium-sized tumors were more abundant in the anterior region of the body. In contrast, large tumors were predominantly found in the anterior and posterior region of the body while in the head small tumors were more abundant.

According to the severity index, turtles with tumors were classified as follow: 13 turtles (52%) had the highest

score (FPS3) since their average number of tumors was high (35 ± 16), one (4%) had the second highest (FPS2) and 11 (44 %) the third (FPS1). The remaining turtles had the lowest score (FPS0=16) and seven could not be classified due to the condition of the carcass. Size distribution of the severity index is also presented in Figure 5. According to the body condition, the individuals did not have such a severe condition since 70 % (29/41) of the juveniles were in good external appearance and 2 % (1/41) were fat. The remaining 29 % were seven thin and three badly nourished.

Although the number of tumors was greater in thin and badly nourished individuals, there were no statistical differences (Mann-Witney $U_{(7,4)} = 0.50$, $p = 0.44$). However, when the tumors were large, statistical differences were observed (Mann-Witney $U_{(7,4)} = 7.5$, $p=$

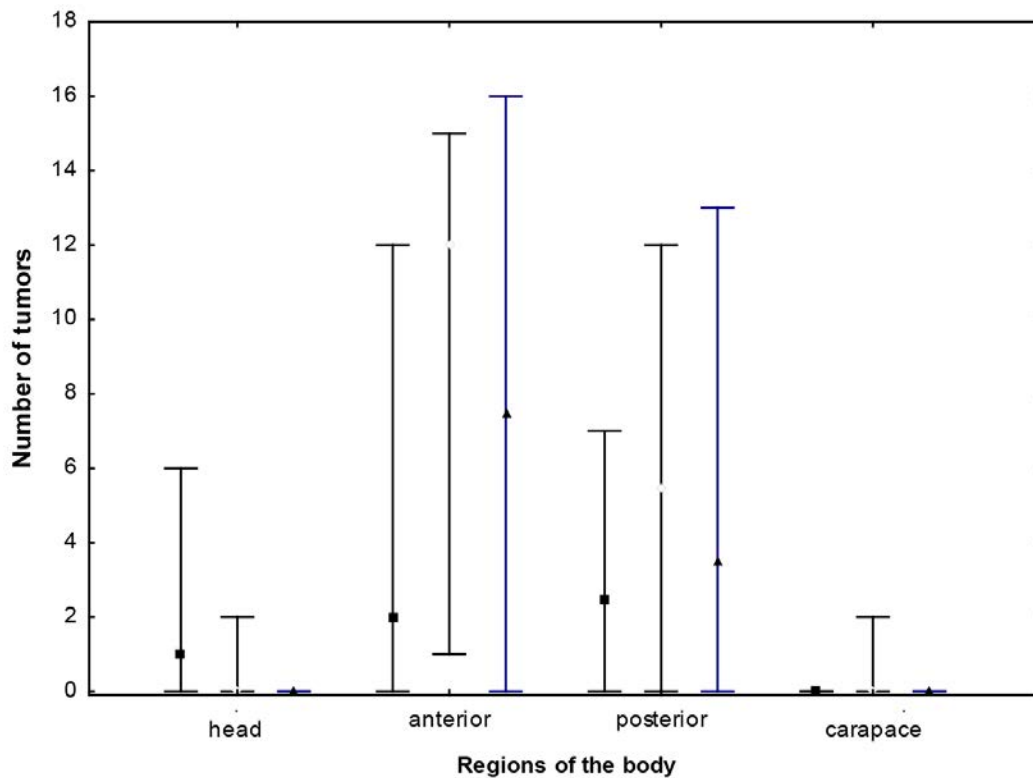


Fig. 7. Number of fibropapillomas tumors and their size found per body regions in juvenile green turtles at the “Lanzanillo-Pajonal-Fragoso” Wildlife Refuge. Work and Balazs (1999) criteria: small <4 cm, medium 4-10 cm and large > 10 cm. Central position represent the median and whiskers represent minum-maximum interval.

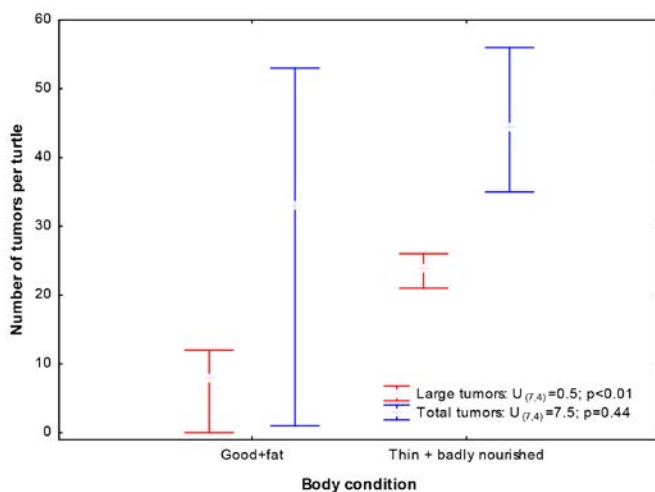


Fig. 8. Number of tumors in green turtle juveniles according to their body condition in the “Lanzanillo-Pajonal-Fragoso” Wildlife Refuge. Central position represent the median and whiskers, non-outlier range.

0.006) between thin and badly nourished individuals and those with good body condition with higher frequency of tumors in the former (Fig. 8).

Environmental quality of seagrass

The Ecological Status Index evaluated in the study area ranged from high, in those stations with the highest percentages of coverage of seagrass species, to moderate, where the percentage of coverage was lower (Table 3). This index showed an improvement in the quality of seagrass beds during the second sampling period for all stations, except in Juan Francisco where, despite a decrease in coverage, stayed in the category of High. Playa Jácate was the area with the lowest scores of the index and changed from moderate to good unlike the other three.

Four heavy metals (chromium, iron, mercury and manganese) appeared in more than 30 % of samples of *T. testudinum* and *S. filiforme* (Fig. 9). Manganese was the most frequent among the stations, followed by mercury. In the case of chromium and iron were abundant only in *T. testudinum*. Punta Cubereta and Playa Jácate had the highest number of observation of heavy metals.

Discussion

The prevalence of FP and severity of the disease

Our results coincide with Foley *et al.* (2005), who reported that the frequency of FP presence increased in the months of autumn and winter (Fig. 4). Herbst (1994) proposes that this temporal pattern could be because tumors should grow more rapidly in the hottest months of the year, so that they reach a size large enough to cause simultaneous stranding at the end of summer. However, the frequency of standings per trimester varied within this period, thus suggesting that other factors besides temperature should be considered in this study case like interaction with humans or predators, debris ingestion, among others.

The average size of the individuals affected by the disease is similar to what was found in Florida (Foley *et al.*, 2005) and Brazil (Baptistotte, 2007; Dos Santos *et al.*, 2010), where the majority of turtles with FP showed a range in CCL between 40 and 70 cm. In Brazil, it was noted that researchers rarely encounter individual sea turtles in the size classes of 20-30 cm and > 80 cm of CCL. In our case, the size class 20-30 cm was frequent but with small size tumors. If we consider the hypothesis that FP is triggered by some factor or combination of factors to which the turtles are exposed after their recruitment to neritic zones (Herbst & Klein, 1995; Dos Santos *et al.*, 2010), then it is possible that environmental factors in the area are rapidly affecting recruits since they are developing the disease at small sizes. However, in Indonesia an increase in FP

incidence was found in individuals from 80 to 85 cm CCL (Adnyana *et al.*, 1997), and in Hawaii, the incidence increased with carapace length (Balazs, 1991; Chaloupka & Balazs, 2005). In the same way, we found the highest frequency of FPS3 scores in the size interval of 50.1 to 60 cm. The differential manifestation of the disease between the populations of the Pacific and the Atlantic oceans could be related to a genomic difference of the supposed causative agent of the disease, the CFPHV herpesvirus. Greenblatt *et al.* (2005) reported the existence of four varieties of CFPHV of which the Atlantic group has a 0.98 % genetic divergence of the middle and West Pacific groups, which could help to explain the differences in the manifestation of the disease between the Atlantic and the other two regions of the Pacific.

The prevalence of tumors found (65 %) is higher than 0.6 % previously reported by Moncada & Prieto (1999) in Cuban waters 21 years ago. This difference could be because the results were based on fishing reports, where animals are expected to be healthy while stranded animals are likely to be sick. In addition, Work *et al.* (2004) propose that adults with tumors are infrequent because their immune systems develop defenses and tumors eventually disappear. On the contrary, Foley *et al.* (2005) analyzed the reports of sea turtle stranding from 1980 to 1998, finding an incidence of 22.6 %, which is much lower than that found in our study. Another possible explanation for this difference is that, in Florida, at least 43 % of the stranding can be attributed to other anthropogenic causes such as the interaction with fishing gear (Bellido *et al.*, 2010) and with boats (Bacon *et al.*, 2006). Our study area is protected therefore, fishery and vessels interaction are infrequent so they should not have a great impact on turtles mortality in the area (hence only 15 % of turtles were entangled in fishermen nets) and none of stranded individuals had signs of fishery interactions (injuries, hooks, nets residues). In addition, the difference in the spatial and temporal magnitude of both studies, 18

years in a radius of 200 km around the peninsula for the case of Florida, should not be ruled out, while in Villa Clara the FP has been studied by only six years in a much smaller area. So it will be necessary to continue studies to determine if these levels of damage are maintained and if the same happens on living individuals with a capture-mark-recapture program in the area.

The presence of larger tumors in the anterior regions is similar to the results obtained by Dos Santos *et al.* (2010) in Brazil, and in Hawaii by Work *et al.* (2004), where the number of tumors was significantly higher in the anterior region than in the posterior region. This result could be due to the turtles' behavior or the factors involved in the disease etiology and transmission. Work *et al.* (2004) propose that turtles may come into contact with infected material as they explore their environment since it is known that the virus can remain infectious in seawater (Curry *et al.*, 2000). While other authors (Lu *et al.*, 2000) suggest that the manifestation of the disease in the anterior region could be related to some cleaning fish that behave as vectors of the virus. They found CFPHV gene material in *Thalassoma duperrey*.

Although the predominance of turtles with the highest severity index has been found in studies based on turtle captures (Dos Santos *et al.*, 2010) and

stranded individuals (Work *et al.*, 2004), the degree of severity found in the turtles of Villa Clara is superior to all the other reports since the FPS2 is practically absent. In concordance, the turtles exhibited a higher average number of tumors per turtle (35.38 ± 15.61). For example, in Indonesia (Adnyana *et al.*, 1997) it was found that most turtles had between one and ten tumors, while in Hawaii (Work *et al.*, 2004) they found 12-24 tumors per turtle. Perhaps this difference is because stranded turtles tend to have a greater number of tumors than those that are in water.

The higher frequency of large tumors in thin turtles, compared to turtles in good body condition, is a reflection that in animals with a more significant number of large tumors their feeding abilities may be compromised (Herbst, 1994). This limitation in the development of their vital functions may result in the eventual stranding of sick individuals, as evidenced in our study.

Environmental quality of seagrass

It has been reported an association between the conservation status of the sea turtle feeding area and the incidence of FP. Dos Santos *et al.* (2010) evaluated two different areas using the ecological evaluation index (EEI) as reference, one with a high incidence of

Table 3: Indicators of the ecological evaluation index (EEI) and ecological status index (ESI) following the methodology of Orfanidis *et al.* (2001, 2003) per station during two sampling periods. Ecological status group (ESG) I: seaweed species with a thick or calcareous thallus, low growth rates and long life cycles (late successional); ESG II: includes sheet-like and filamentous seaweed species with high growth rates and short life cycles (opportunistic).

Station	Sampling period	ESG I (% cover)	ESG II (% cover)	ESI	EEI
Canal de Córdoba	October	1.52	0	Moderate	6
	April	66.57	0	High	10
P. Jácate	October	28.13	0	Moderate	6
	April	50.08	0.7	Good	8
P. Cubereta	October	6.75	0	Moderate	6
	April	77.87	0	High	10
P. J. Francisco	October	94.16	0	High	10
	April	6.2	0	High	10

FP and another without it. This study resulted in a lower environmental quality where there was a higher incidence of disease. In the present study, there is a high severity and prevalence in an area of moderate to high quality, however, a high number of stranding were close to playa Jácate, where the ecological conditions were the worst.

Two factors potentially bias these results. First, an accurate population assessment concerning sea turtle distribution and quantity of juveniles is fundamental to allow sampling outside the foraging area; second, studying the prevalence and severity of FP just in stranded individuals overestimate the prevalence of the disease in the population. Therefore, studying the characteristics of the foraging population is of vital importance to correctly assess the impact of FP in the region. In the same way, it is important to evaluate the severity of the disease in individuals caught at random, in order to avoid overestimation.

Despite the good quality of the habitat determined by the ecological index, four heavy metals were found in a frequency higher than 30 % (Mn, Hg, Fe and Cr) of the marine angiosperm samples. The resulting different abundance between each heavy metal may be the result of differences in bioavailability in the study area. Ralph *et al.* (2006) stated that the complex nature of seawater determines that some elements have low bioavailability. The lack of knowledge of baseline concentrations prevents any assertion in the study area, therefore, short term studies to determine metals concentration in different matrixes (sediment, seagrass species and turtles' blood and muscle tissue) is mandatory. However mercury is considered one of the most toxic metals (Marques-Junior *et al.*, 2009). Ralph *et al.* (2006) proposed that the damage caused by these metals could be reversible if they are removed so, even with high bioavailability in the environment, a recovery is possible (Ward, 1987). A possible explanation is that metals identified in the area do not reach toxic levels. Heavy metals concentration

in seagrass species needs to be quantified in future studies.

It is not clear whether the incidence of FP in the area is related to the presence of heavy metals as some authors suggest (Adnyana *et al.*, 1997) until it is known whether these are in toxic levels or not. Despite this, it is not discarded as a possible triggering factor of the FP occurrence in these sea turtles. Several adverse effects of heavy metals on marine turtles are known such as damages to the endocrine system that affects growing, development and reproduction and to the immune system facilitating the expression of FP (Adnyana *et al.*, 1997; Storelli *et al.*, 2005).

In most cases, the severity was very high, particularly in the anterior region, which compromised the body condition of the individuals, mostly thin or badly nourished. Presence of fibropapilloma was related with habitat quality in the area since most of sick turtles were found in the stations with moderate quality and the presence of several heavy metals (Jácate and Cubereta).

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Competing Interests

The authors declare there are no competing interests.

References

- Adnyana, W., Ladds, P.W. & Blair, D. (1997). Observations of fibropapillomatosis in green turtles (*Chelonia mydas*) in Indonesia. *Aust. Vet. J.*, 75, 737-742.

- Aguirre, A.A. & Lutz, P.L. (2004). Marine turtles as sentinels of ecosystem health: is fibropapillomatosis an indicator? *EcoHealth*, 1, 275-283.
- Aguirre, A.A., Spraker, T.R. Balazs, G.H. & Zimmerman, B. (1998). Spirorchidiasis and fibropapillomatosis in green turtles of the Hawaiian Islands. *J. Wild. Dis.*, 34, 91-98.
- Alfaro-Núñez, A., Frost Bertelsen, M., Bojesen, A.M., Rasmussen, I., Zepeda-Mendoza, L., Tange-Olsen, M. & Pius Gilbert, M.T. (2014). Global distribution of Chelonid fibropapilloma-associated herpesvirus among clinically healthy sea turtles. *BMC Evol. Biol.*, 14(206), 1-11.
- Bacon, J. P., Gray, J.A. & Kitson, L. (2006). Status and conservation of the reptiles and amphibians of the Bermuda islands. *Appl. Herpet.*, 3, 323-344.
- Balazs, G. H. (1991). Current status of fibropapillomatosis in the Hawaiian green turtle, *Chelonia mydas*. In: G. H. Balazs and Pooley S. (eds.) *Research plan for marine turtle fibropapilloma*. (pp. 47-57). U.S. Department of Commerce, (NOAA-TMNMFS-SWFC-156), Washington, DC.
- Baptistotte, C. (2007). *Caracterização espacial e temporal de fibropapillomatose em tartarugas marinhas da costa brasileira*. Tesis de Doctorado. Universidad de Sao Pablo.
- Bellido, J. J., Báez, J.C., Castillo, J.J. Pinto, F. Martín, J.J. Mons, J.L. & Real, R. (2010). Loggerhead strandings and captures along the Southern Spanish coast: Body Size-Based Differences in Natural Versus Anthropogenic Injury. *Chel. Conser. Biol.*, 9(2), 276-282.
- Casale, P., Laurent, L., & De Metrio, G. (2004). Incidental capture of marine turtles by the Italian trawl fishery in the north Adriatic Sea. *Biol. Conser.*, 119(3), 287-295.
- Chaloupka, M. & Balazs G. (2005). Modelling the effect of fibropapilloma disease on the somatic growth rate dynamics of Hawaiian green sea turtles. *Mar. Biol.*, 147, 1251-1260.
- Curry, S. S., Brown, D. R., Gaskin, J. M., Jacobson, E. R., Ehrhart, L. M., Blahak, S., ... Klein, P. A. (2000). Persistent infectivity of a disease-associated herpesvirus in green turtles after exposure to seawater. *J. Wild. Dis.*, 36(4), 792-797.
- Den Hartog, C. & Phillips R.C. (2001). Common structures and properties of seagrasses beds fringing the coasts of the world. In K. Reise (Ed.), *Ecological comparisons of sedimentary shores. Ecological Studies* (pp. 195-212). 151 Springer-Verlag Berlin Heidelberg.
- Dos Santos, G.R., Silva, A., Torezani, E., Baptistotte, C., da Nóbrega, J., Antunes, P., ... Balazs, G.H. (2010). Relationship between fibropapillomatosis and environmental quality: a case study with *Chelonia mydas* off Brazil. *Dis. Aquat. Org.*, 89, 87-95.
- Dunson, W.A. & Travis J. (1991). The role of abiotic factors in community organization. *Amer. Nat.*, 138(5), 1067-1091.
- Foley, A.M., Schroeder, B.A. Redlow, A.E. Fick-Child K.J. & Teas W.G. (2005). Fibropapillomatosis in stranded green turtles (*Chelonia mydas*) from the Eastern United States (1980-1998): trends and associations with environmental actors. *J. Wild. Dis.*, 41, 29-41.
- Fytianos, K., Evgenidou, E. & Zachariadis, G. (1999). Use of macroalgae as biological indicators of heavy metal pollution in Thermaikos Gulf, Greece. *Bull. Environ. Contam. Tox.*, 62(5), 630-637.
- González-Díaz, P., González-Sansón, G., Aguilar Betancourt, C., Álvarez Fernández, S., Perera Pérez, O., Hernández Fernández, L., ... de la Guardia Llanos, E. (2018). Status of Cuban coral reefs. *Bull. Mar. Sci.*, 94(2), 229-247.
- Greenblatt R.J., Quackenbush, S.L., Casey, R.N., Rovnak, J. Balazs, G. H., Work, T. M., ... Sutton, C. A. (2005). Genomic variation of the fibropapilloma-associated marine turtle herpesvirus across seven geographic areas and three host species. *J. Virol.*, 79(2), 1125-1132.
- Hall, S.R., Sivars-Becker, L., Becker, C., Duffy, M.A., Tesier A.J. & Caceres, C.E. (2007). Eating yourself sick: transmission of disease as a function of foraging ecology. *Ecol. Lett.*, 10(3), 207-218.
- Hays, G. C., Broderick, A. C., Godley, B. J., Luschi, P., & Nichols, W. J. (2003). Satellite telemetry suggests high

- levels of fishing-induced mortality in marine turtles. *Mar. Ecol. Prog. Ser.*, 262, 305-309.
- Herbst, L.H. (1994). Fibropapillomatosis of marine turtles. *Ann. Rev. Fish Dis.*, 4(6), 389-425.
- Herbst, L.H. & Klein, J.P. (1995). Green Turtle Fibropapillomatosis: Challenges to Assessing the Role of Environmental Cofactors. *Environ. Health Perspec.*, 103(4), 27-30.
- Jacobson, E. R., Mansell, J. L., Sundberg, J. P., Hajjar, L., Reichmann, M. E., ... Ehrhart, L. M. (1989). Cutaneous fibropapillomas of green turtles (*Chelonia mydas*). *J. Comp. Pathol.*, 101(1), 39-52.
- Juanes, J. A., Guinda, X., Puente, A., & Revilla, J. A. (2008). Macroalgae, a suitable indicator of the ecological status of coastal rocky communities in the NE Atlantic. *Ecol. Indic.*, 8(4), 351-359.
- Keller J. M., Balazs, G. H., Nilsen, F., Rice, M., Work T. M. & Jensen B. A. (2014). Investigating the Potential Role of Persistent Organic Pollutants in Hawaiian Green Sea Turtle Fibropapillomatosis. *Environ. Sci. Technol.*, 48(14), 7807-7816.
- Klein, P.A., Curry, S., Brown, D.R., Homer, B.L., Garber, R.L., Mader, D.R., ... Lackovich, J.K. (1998). *Prevalence and cultivation of a chelonid herpesvirus associated with fibropapillomas of the green turtle, Chelonia mydas, and the loggerhead turtle, Caretta caretta, in Florida*. Gainesville, FL, Florida Cooperative Fish and Wildlife Research Unit, University of Florida.
- Lackovich, J.K., Brown, D.R., Homer, B.L., Garber, R.L., Mader, D.R., Moretti, ... Klein. P.A. (1999). Association of herpesvirus with fibropapillomatosis of the green turtle *Chelonia mydas* and the loggerhead turtle *Caretta caretta* in Florida. *Dis. Aquat. Org.*, 37(2), 89-97.
- Lemons, G., Lewison, R., Komoroske, L., Gaos, A., Lai, C., Dutton, P. ... Seminoff J.A. (2011). Trophic ecology of green sea turtles in a highly urbanized bay: Insights from stable isotopes and mixing models. *J. Exper. Mar. Biol. Ecol.*, 405, 25-32.
- Lu, Y., Yu, Q., Zamzow, J. P., Wang, Y., Losey, G. S., Balazs, G. H., ... Yanagihara R. (2000). Detection of green turtle herpesviral sequence in saddleback wrasse *Thalassoma duperrey*: A possible mode of transmission of green turtle fibropapilloma. *J. Aquat. Anim. Health*, 12(1), 58-63.
- Marques-Júnior, A.N., Moraes, R.B. & Maurat, M.C. (2009). Poluição marinha. In R.C. Pereira y A. Soraes-Gomez (Eds.) *Biologia Marinha. Interciencia*, (pp. 505-528). Rio de Janeiro.
- Martínez-Daranas, B., Macías Reyes, D. & Cano-Mallo, M. (2013). Protocolo para el monitoreo de los pastos marinos. Proyecto GEF-PNUD *Aplicación de un enfoque regional al manejo de las áreas marino-costeras en la región Archipiélagos del Sur de Cuba*. Centro Nacional de Áreas Protegidas. La Habana. Cuba. ISBN: 978-959-287-041-3.
- Moncada, F. & Prieto, A. (1999). Incidence of fibropapillomas in the green turtle (*Chelonia mydas*) in Cuban waters. In H. Kalb & T. Wibbels (Eds.) *Proceedings of the 19th Annual Symposium on Sea Turtle Conservation and Biology*. (pp. 40-41). U.S. Department of Commerce, NOAA Technical Memorandum NMFS-SEFSC-443.
- Montalvo, J. F., Martínez-Canals, M., Perigó, E., Puentes O. & García-García, N. (2008). Fuentes de contaminación de la zona costera de la provincia de Villa Clara, Cuba. *Contribución a la Educación y la Protección Ambiental*, 8, 154-161.
- Orfanidis S, Panayotidis, P. & Stamatis, N. (2001). Ecological evaluation of transitional and coastal waters: a marine benthic macrophytes-based model. *Medit. Mar. Sci.*, 2(2), 45-65
- Orfanidis S, Panayotidis, P. & Stamatis, N. (2003). An insight to ecological evaluation index (EEI). *Ecol. Indic.*, 3, 27-33
- Orfanidis, S., Dencheva K., Nakou, K., Tsioli, S., Papathanasiou, V. & Rosati, I. (2014). Benthic macrophyte metrics as bioindicators of water quality: towards overcoming typological boundaries and methodological tradition in Mediterranean and Black Seas. *Hydrobiologia*, 740, 61-78.
- Ralph, P. J. Tomasko, D. Moore, K. Seddon, S. & Macinnis-Ng C. M. O. (2006). Human Impacts on Seagrasses: Eutrophication, Sedimentation and Contamination. In

- A.W.D. Larkum, R.J. Orth, & C.M. Duarte (Eds.) *Seagrasses: Biology, ecology and conservation*. (pp. 567-593). Springer, The Netherlands.
- Short, F. T., & Wyllie-Echeverria, S. (1996). Natural and human-induced disturbance of seagrasses. *Environ. Conser.*, 17-27.
- Smith, G. M. & Coates. C. W. (1938). Fibro-epithelial growths of the skin in large marine turtles, *Chelonia mydas* (Linnaeus). *Zoologica*, NY 23: 93-98. In A.M. Foley, B.A. Schroeder, A.E. Redlow, K.J. Fick-Child & W.G. Teas (2005). Fibropapillomatosis in stranded green turtles (*Chelonia mydas*) from the Eastern United States (1980-1998): trends and associations with environmental actors. *J. Wildl. Dis.*, 41, 29-41.
- Storelli, M., Storelli, A., D'addabbo, R., Marano, C., Bruno, R. & Marcotrigiano, G. (2005) Trace elements in loggerhead turtles (*Caretta caretta*) from the eastern Mediterranean Sea: overview and evaluation. *Environ. Poll.*, 135(1), 163-170.
- Thomson, J. A., Burkholder, D., Heithaus, M. R., & Dill, L. M. (2009). Validation of a rapid visual-assessment technique for categorizing the body condition of green turtles (*Chelonia mydas*) in the field. *Copeia*, 2009(2), 251-255.
- Van Houtan, K.S., Hargrove, S.K. & Balazs, G.H. (2010). Land use, macroalgae and a tumor-forming disease in marine turtles. *PLoS ONE*, 5(9), e12900
- Van Houtan, K.S., Smith, C.M., Dailer, M.L. & Kawachi, M. (2014). Eutrophication and the dietary promotion of sea turtle tumors. *PeerJ*, 2, e602.
- Wallace, B.P., DiMatteo, A.D., Bolten, A.B., Chaloupka, M.Y., Hutchinson, B.J. ... Abreu-Grobois, F.A. (2011). Global conservation priorities for marine turtles. *PLoS ONE*, 6(9), e24510.
- Ward T.J. (1987). Temporal variation of metals in the seagrass *Posidonia australis* and its potential as a sentinel accumulator near a lead smelter. *Mar. Biol.*, 95, 315-321.
- Williams, S.L. (1987). Competition between the seagrasses *Thalassia testudinum* and *Syringodium filiforme* in a Caribbean lagoon. *Mar. Ecol. Progr. Ser.*, 35, 91-98.
- Williams, S.L. (1990). Experimental studies of Caribbean seagrass bed development *Ecological Monographs*, 60(4), 449-469.
- Work, T.M. & Balazs, G.H. (1999). Relating tumor score to hematology in green turtles with fibropapillomatosis in Hawaii. *J. Wildl. Dis.*, 35, 804-807.
- Work, T.M., Balazs, G.H., Rameyer, R.A. & Morris, R.A. (2004). Retrospective pathology survey of green turtles *Chelonia mydas* with fibropapillomatosis in the Hawaiian Islands, 1993-2003. *Dis. Aquat. Org.*, 62, 163-176.
- Work, T.M., Dagenais, J., Balazs, G.H., Schumacher, J., Lewis, T.D., Leong, J-A.C., Casey, R.N. & Casey, J.W. (2009). In vitro biology of fibropapilloma-associated turtle herpesvirus and host cells in Hawaiian green turtles (*Chelonia mydas*). *J.Gen. Virol.*, 90, 1943-1950.

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