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First case report of fibropapillomatosis tumor regression identified through photoidentification and histopathology in a *Chelonia mydas* in Itapirubá, Santa Catarina, Brazil

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ABSTRACT

Background: Fibropapillomatosis (FP) is a tumor disease primarily affecting juvenile sea turtles, often characterized by external growths that can regress spontaneously. This study reports the first documented case of total tumor regression in a free-living green turtle (*Chelonia mydas*) with FP in southern Brazil.

Case Description: A juvenile green sea turtle (*Chelonia mydas*) was captured and recaptured on Itapirubá Beach, Santa Catarina, Brazil, showing signs of tumor regression with a period of 302 days between captures. At the first capture, photographs of the head and other regions were taken for photoidentification, along with documentation of fibropapilloma sites and tumor scoring. Tumor samples from the initial capture were histopathologically confirmed as fibropapillomas. At the recapture, the turtle showed a slight increase in carapace length, remaining classified as a juvenile. Tumors observed during the first capture were absent at recapture, with only scars remaining in the affected areas. Photoidentification confirmed the recapture, facilitating case monitoring. Tumor regression in this turtle is likely linked to various environmental and ecological factors.

Conclusion: Spontaneous regression of FP tumors remains a crucial indicator in the health monitoring of sea turtle populations. This is the first documented case of FP regression in this region of Brazil, suggesting that the turtle's increasing age and reduced exposure to anthropogenic pressure may have contributed to the tumor's regression. Although clinical follow-up of free-living sea turtles is challenging, reports of FP tumor regression are vital for understanding the health dynamics of sea turtle populations.

Keywords: Marine turtle, Skin tumor, Follow-up, Coast, Diagnosis.

Introduction

Fibropapillomatosis (FP) in sea turtles is an epidermal neoplasm characterized by the presence of arborized papillary projections, supported by an abundant fibrovascular base, which presents with variable external morphology, location, color, and texture (Work *et al.*, 2017; Rossi *et al.*, 2021). The development of these tumors is proven to be related to chelonian herpesvirus type 5 (ChHV5), but its transmission process and specific role in oncogenesis and tumor progression are still unclear (Farrell *et al.*, 2021).

There are environmental factors such as variations in sea surface temperature, salinity, and the discharge of effluent nutrients from surrounding rivers, which are correlated with a higher incidence of the disease (Manes *et al.*, 2022), with pollution being one of the main factors. However, the role of these factors in the development of the disease is not yet well established (Lawrance *et al.*, 2018).

The diagnosis of FP is performed through histopathological analysis and identification of the virus by the Polymerase Chain Reaction (PCR)

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technique (Work *et al.*, 2017). Some studies carried out both in Brazil and in other countries report spontaneous regression of FP (Hirama and Ehrhart, 2007; Guimarães *et al.*, 2013; Tagliolatto *et al.*, 2016); however, such findings are still scarce, especially in southern Brazil. This is believed to be due to the difficulty of capturing and recapturing free-living sea turtles (Tagliolatto *et al.*, 2016). Therefore, this study aimed to report the first case of total spontaneous regression of FP in a free-living green turtle captured and recaptured on the beach of Itapirubá, Santa Catarina, Brazil.

Case Details

The capture area was at Itapirubá beach in Santa Catarina, Brazil, using a 50 m long, 3.2 m high holding and/or trawl net with 0.30 m mesh for capture procedures (Martinez-Souza *et al.*, 2013). In August 2022, a juvenile green sea turtle weighing 32 kg with a 63 cm curved carapace length (CCL) was captured (Fig. 1), showing seven small skin tumors, two on the neck (Fig. 2a), and five on the right front fin (Fig. 2b), all under 4 cm with mild scores, and photographs were taken for identification purposes (Reisser *et al.*, 2008). Tumor presence sites were recorded, and scores were determined according to established protocols, based on the following tumor size categories: A (<1 cm), B (1–4 cm), C (4–10 cm), and D (>10 cm) (Work and Balazs, 1999; Rossi *et al.*, 2016). The fibropapilloma

tumor index (FPI) was then calculated using the formula $FPI = 0.1 \times NA + 1 \times NB + 20 \times NC + 40 \times ND$ (Rossi *et al.*, 2016). Based on this index, tumors were categorized using the Fibropapillomatosis Score Southwest Atlantic system: mild ($FPI < 40$), moderate ($40 < FPI < 120$), or severe ($FPI \geq 120$). Biopsies were taken from larger, less adherent tumors, fixed in 10% formalin for 48 hours, and routinely processed for histopathological evaluation, assessing criteria such as anatomical location, size, color, texture, consistency, surface characteristics, and skin integrity (Page-Karjian, 2019).

In May 2023, the same turtle was recaptured in the same area, confirmed through photo-comparison of post-orbital scales (Fig. 1), weighing 31.8 kg with a 63.4 cm CCL, showing no FP tumors but scar tissue in their place, with reddish scars on the fin region and black scars on the neck, maintaining a similar shape to the previous FP sites, indicating total regression of FP tumors (Fig. 2c and d).

The tumors presented as smooth and whitish on the fin, while appearing black on the neck, measuring approximately 1.8×0.7 cm. They featured a white base with a blackened apex, soft and irregular surfaces that were predominantly flat, along with areas of ulceration. Microscopic examination revealed significant connective tissue proliferation in the dermis (Fig. 3a and b), forming a cellular mantle interspersed with foci



Fig. 1. Green sea turtle (*Chelonia mydas*), captured (a) and recaptured (b) after 302 days, at Itapirubá beach (SC), with tumor regression. Photoidentification of the post-orbital scales on the right side, confirming that it was the same animal.



Fig. 2. At capture: presence of two tumors on the right cervical dorsum (a) and five tumors in the ventro-cranial region of the right anterior fin (b). Recapture: black scars in the neck where there were tumors (c) and reddish scars approximately maintaining the shape of the insertion of FP tumors.

of neovascularization (Fig. 3a, c, d), and surrounded by a moderate inflammatory infiltrate consisting of lymphocytes, heterophils, and macrophages (Fig. 3b–d). Notable findings included intense inflammation, mild liquefactive necrosis, and edema at the epidermal junction. The stratum corneum exhibited both orthokeratotic and parakeratotic hyperkeratosis, while the stratum spinosum demonstrated papillomatous acanthosis, edema, liquefactive necrosis, and lymphocyte exocytosis.

Ethical approval

The procedures for capturing and collecting biological samples were carried out with prior federal authorization by the Biodiversity Authorization and Information System (SISBIO), under numbers 7860 and 51465, and by the Ethics Committee on the Use of Animals of the Federal Fluminense University (CEUA/UFF) under No. 8308100221.

Discussion

The comparison of photographs enabled the confirmation of both the capture and recapture of the green turtle, as well as clear documentation of FP tumor regression. Photoidentification, a validated technique for systematically identifying individual sea turtles based on their unique markings and features (Reisser *et al.*, 2008), has proven to be an invaluable tool in the study of FP, allowing for accurate detection and evaluation of tumor growth and regression

(Guimarães *et al.*, 2013). To the best of the authors' knowledge, this is the first documented case of total FP regression in a green sea turtle along the southern coast of Brazil. In contrast, two cases of FP regression were previously documented using photoidentification in the state of Rio de Janeiro, both occurring within 188 days, a shorter timeframe than observed in this study (Guimarães *et al.*, 2013; Tagliolato *et al.*, 2016). Nonetheless, identifying cases of FP regression in turtles remains challenging due to the difficulties in monitoring these animals in the wild.

The macroscopic and microscopic characteristics of the tumors were consistent with those previously described in the initial report by Smith and Coates (1938) and closely resembled findings from other studies on FP in green turtles, loggerhead turtles, and olive ridley turtles (Page-Karjian *et al.*, 2014; Rossi *et al.*, 2015; Reséndiz *et al.*, 2022). Based on these evaluations, along with their epithelial and stromal proliferation, the cutaneous tumors collected were determined to be consistent with FP. Although there are limited studies characterizing the anatomic-histopathology of regressing FP tumors, previous research on green turtles has indicated a more favorable prognosis for those with only flat FP lesions compared to those exhibiting verrucous morphology, with over 50% of the former group experiencing spontaneous regression (Page-Karjian *et al.*, 2014). However, it should be noted that flat tumors cannot be

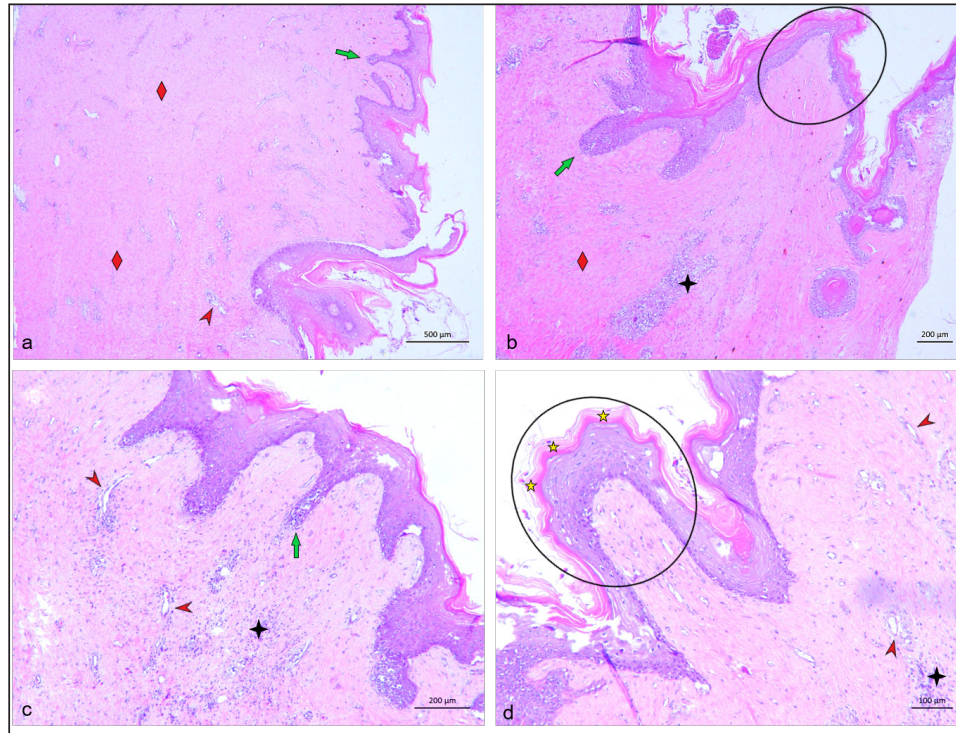


Fig. 3. Microscopy of the FP tumors showing proliferation of fibrous connective tissue (red rhombi), neovascularization (red arrow head), papillary projections towards the interior of the dermis (green arrow), papillary projections (rounded areas), inflammatory infiltrate (4-pointed black star), and parakeratosis (yellow star).

definitively interpreted as a sign of tumor regression (Bennett *et al.*, 1999; Page-Karjian *et al.*, 2014).

The tumor score serves as a reliable indicator for predicting potential regression of FP. Supporting this, research indicates that 72% of cases showing tumor regression were classified as mild (Bennett *et al.*, 1999). Furthermore, it has been suggested that turtles with less severe FP may undergo spontaneous tumor regression, as seen in the current case (Hirama and Ehrhart, 2007). However, we advise caution in generalizing this observation, as lighter FP tumors might also suggest that the animal is still in the early stages of disease progression.

The turtle in this report was classified as juvenile, nearing the sub-adult phase (Chaloupka and Limpus, 2005), a critical stage when turtles begin to migrate to new feeding areas (Bolten, 2003). The 302-day interval between captures supports the notion that the turtle was transitioning from the juvenile neritic zone into the sub-adult phase.

Despite this extended interval, the turtle's return to the same location highlights its fidelity to the study area, likely as it prepares for migration to deeper waters (Bolten, 2003). This behavior could influence tumor regression, as reduced exposure to coastal stressors and pollutants may occur when the turtle moves away from these areas (Bolten, 2003; Shaver *et al.*, 2019).

Research indicates that sub-adult and adult turtles migrating into deeper zones, which typically have lower pollutant densities, show lower incidences of FP (Jones *et al.*, 2016). As turtles move farther from the coast, their decreased exposure to environmental pollutants may enhance the likelihood of tumor regression.

Size and age also play significant roles in FP regression; sub-adults and adults rarely exhibit FP, indicating either recovery through acquired immunity before migration or a lack of exposure to the disease during their juvenile stages (Jones *et al.*, 2016; Kelley *et al.*, 2022). Recent studies reinforce this, showing higher FP rates in smaller turtles and lower rates with increased carapace length (Jones *et al.*, 2016; Kelley *et al.*, 2022). Although the study area is near ports with dredging activities and known pollution in adjacent regions (Righetti *et al.*, 2019; Ferreira *et al.*, 2021), it is designated as an Environmental Preservation Area (EPA), likely maintaining relatively low pollution levels that could contribute to the regression of FP tumors. Supporting this hypothesis, recent studies on trace elements in sea turtle blood from this area indicate low concentrations of these elements in the local population (Pérez *et al.*, 2023). However, research on organic compounds in *Sotalia guianensis* dolphins and in oysters (*Crassostrea gasar* and *Crassostrea rhizophorae*) has revealed significant concentrations

of these compounds along the coast of Santa Catarina, including southern areas close to the study site (Vidal *et al.*, 2023; Bastolla *et al.*, 2024; Guerreiro *et al.*, 2024), which may contribute to the prevalence of FP (Vilca *et al.*, 2018). Thus, it is very important to investigate the potential long-term impact of these compounds on the health of free-living sea turtles in this region.

The observed tumor regression may also be linked to the low incidence of FP in sea turtles from this region (Rossi *et al.*, 2019; Capri, 2022; Pérez *et al.*, 2023), which could facilitate the turtle's recovery. A lower presence of sick animals reduces the shedding of the ChHV5 virus in the water and the risk of infection and tumor development (Farrell *et al.*, 2021). This low occurrence is likely due to the study area's inclusion within the EPA and its overall environmental quality. The case of FP regression reported here is essential for a better understanding of this disease. The mechanisms of FP regression are not yet well determined (Guimarães *et al.*, 2013) and it is not clear whether the virus is lytic or latent in tumors (Farrell *et al.*, 2021). It demonstrates that exposure of data and reports on cases of this type are extremely necessary for the elucidation and understanding of the mechanisms involved in regression, as well as the factors that could help in the spontaneous recovery of FP, a disease so serious and common in sea turtles. Several factors may be related to the regression of these tumors and the environmental and immunological factors of the turtles are probably the most relevant. In addition, free-living sea turtles offer a great challenge to carry out clinical follow-up, which makes this study a valuable information tool.

Conclusion

This case of FP regression in a green sea turtle illustrates the importance of monitoring environmental conditions and biological factors influencing tumor recovery. The findings highlight the potential for spontaneous regression in less severe cases, particularly in areas with low pollutant exposure. This study contributes valuable insights into the dynamics of FP in sea turtles and emphasizes the necessity for ongoing research to understand the underlying mechanisms of tumor regression. Ultimately, it underscores the role of environmental quality in the health of marine species and the need for conservation efforts to protect these ecosystems.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Authors' contributions

YAAP carried out conceptualization, methodology, investigation, data curation, writing—original draft, writing—review and editing, visualization. SRL and MGC: carried conceptualization, validation, writing—review and editing. GMS: carried field methodologies for capture and data collection from the animal, investigation, resources, supervision, project administration, and funding acquisition. MJAH and TG: methodology, resources, project administration, funding acquisition. ETM, KVGCS, and NXA: conceptualization, methodology, validation, resources, supervision, project administration, and funding acquisition. All authors read and approved the final manuscript.

Data availability

All data were provided in the manuscript.

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