

# Histopathological findings in stranded *Caretta caretta* (Linnaeus, 1758) on the Paraná coast, Southern Brazil

## Achados histopatológicos em *Caretta caretta* (Linnaeus, 1758) encalhadas no litoral do Paraná, sul do Brasil

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### Highlights

80.0% of *Caretta caretta* stranded along the Paraná coast are impacted by bycatch. Pneumonia, splenitis and liver degeneration are the main histological findings. The main cause of death in these stranded animals is anthropogenic interaction.

### Abstract

*Caretta caretta*, one of the five sea turtle species in Brazil, is currently listed as globally endangered. While health assessments are crucial for monitoring population status, they are infrequent and lack systematic implementation. Evaluating the health condition of sea turtles can occur through examinations of both live and deceased animals. Carcasses play a pivotal role in identifying diseases and potential threats impacting the species. Therefore, this study presents the histopathological findings from 20 dead stranded *C. caretta* along the Paraná coast between 2015 and 2021. All individuals underwent measurements, and necropsies were conducted. The mean curved carapace length (cm) was  $67.3 \pm 6.9$  (49.4 – 81.5). Body condition was classified as good in 13 animals, fair in four, poor in two, and undetermined in one. Fourteen females, four males, and two undetermined sexes were registered, with a mean age of  $15.8 \pm 3.89$  (ranging from 9 – 24) years. Evidence of anthropogenic interaction (10 bycatch interactions, 6 marine debris, 2 oil interactions combined with marine debris) was noted in 80.0% of the animals. Histological changes primarily affected the liver, lungs, spleen, and kidneys, with prominent alterations such as inflammatory infiltrate (interstitial pneumonia, heterophilic splenitis, nephritis, endocarditis), congestion, and hepatic and renal hydropic degeneration. This study significantly contributes to our understanding of pathological findings in stranded wild *C. caretta* on the

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Paraná coast, offering valuable insights into the health of these animals and aiding in the conservation efforts to protect their populations.

**Key words:** Loggerhead turtle. Sea turtle. Anthropogenic interaction. Pathology.

## Resumo

*Caretta caretta* é uma das cinco espécies de tartarugas marinhas encontradas no Brasil e atualmente está ameaçada de extinção em todo o mundo. As avaliações de saúde são fundamentais para monitorar a situação dessa população, no entanto não são realizadas sistematicamente resultando em escassez de dados. O estado de saúde das tartarugas marinhas pode ser investigado por meio de animais vivos e mortos. As carcaças são essenciais para a identificação de doenças e outras ameaças potenciais que afetam as espécies. Portanto, este estudo relata os achados histopatológicos de 20 *C. caretta* encalhadas mortas no litoral do Paraná, entre 2015 e 2021. Todos os indivíduos foram medidos e autopsiados. O comprimento curvo médio da carapaça (cm) foi de  $67,3 \pm 6,9$  (49,4 – 81,5); a condição corporal foi boa em 13 animais, regular em quatro, ruim em dois e indeterminada em um. Foram registrados 14 animais do sexo feminino, quatro do sexo masculino e dois de sexo indeterminado, com média de idade de  $15,8 \pm 3,89$  (variação de 9 – 24) anos. Evidências de interação antrópica (10 interações de captura incidental, 6 detritos marinhos, 2 interações de óleo concomitante a detritos marinhos) foram registradas em 80% dos animais. As alterações histológicas acometeram principalmente fígado, pulmões, baço e rins, sendo as principais alterações infiltrados inflamatórios (pneumonia intersticial, esplenite heterofílica, nefrite, endocardite), congestão, degeneração hidrópica hepática e renal. Este estudo contribui para o conhecimento dos achados patológicos em *C. caretta* encalhadas no litoral do Paraná e com informações importantes sobre a saúde dos animais, sendo que os dados obtidos podem colaborar na conservação das populações.

**Palavras-chave:** Tartaruga cabeçuda. Tartaruga marinha. Interação antropogênica. Patologia.

## Introduction

The *Caretta caretta* species is distributed in tropical, subtropical (Tacchi et al., 2019), and temperate waters (Baldi et al., 2023). However, over the past decade, there has been a notable expansion of this species observed in the Black Sea and Western Mediterranean regions (Girard et al., 2021; Zinenko et al., 2021; Mancino et al., 2022; Cardona et al., 2024). It is one of the five sea turtles inhabiting the Brazilian coast (Lutz et al., 1997). The loggerhead (*C. caretta*) is characterized by a large head in proportion

to its body, a carapace that ranges from brown to red-brown, and a plastron exhibiting various shades of yellow (Wyneken, 2001).

According to the latest Red List assessment by the International Union for Conservation of Nature [IUCN] (2022), this species faces the threat of extinction and is classified as vulnerable. The risk of extinction is linked to anthropogenic impacts, including fishing activities, collisions with ships and boats (Work et al., 2010; Orazé et al., 2019), and ingestion of debris (Nelms et al., 2016). Furthermore, various diseases can adversely affect the health of sea turtles, including

infectious diseases, leading to population decline (Orós et al., 2005; Reis et al., 2010; Wallace et al., 2011).

Numerous studies have investigated the health of wild loggerheads (Stamper et al., 2005; Day et al., 2010; Rousselet et al., 2013; Stacy et al., 2018) examining various aspects such as contaminants (Keller et al., 2006; Bucchia et al., 2015), causes of stranding and mortality (Orós et al., 2016), and the presence of parasites (Werneck et al., 2017). However, studies specifically focusing on systemic histopathological aspects in this species are limited. Most existing research has primarily documented the occurrence of parasites (Santoro et al., 2010, 2017; Stacy et al., 2010; Werneck et al., 2017), with only a few reporting pathological findings in stranded *C. caretta*, such as those in Spain (Orós et al., 2005). Recently, septicemic listeriosis was reported in a loggerhead stranded along the Adriatic coast (Di Renzo et al., 2022). In Brazil, specific conditions like fibropapillomatosis (Rossi et al., 2015) and phaeohyphomycosis (Domiciano et al., 2014) have been described.

Conducting pathological studies on stranded sea turtles is imperative for sea turtle conservation, playing a vital role in advancing our understanding of both animal and environmental health. Gross examination and histopathological analysis offer valuable insights into the overall health of animals and contribute to identifying the primary causes of morbidity or mortality in a specific region (Tagliolatto et al., 2019). Therefore, the aim of this study is to document the histopathological findings in 20 stranded *C. caretta* along the Paraná coast in South Brazil.

## Material and Methods

The specimens analyzed were found stranded or were obtained from accidental/incidental capture in fishing nets, along the coast of Paraná state (25°44' S 48°29'W), South Brazil, throughout the monitoring period from 2015 to 2021. These specimens were collected as part of the monitoring efforts conducted by the Santos Basin Beach Monitoring Project (PMP-BS). The PMP-BS is a program required by the Brazilian Institute for the Environment and Renewable Natural Resources (IBAMA) for the environmental licensing process for the production and flow of oil by Petrobrás in the Pre-Salt Pole in the Santos Basin.

The autopsies of the sea turtles were conducted at the PMP-BS base, situated at the Center for Studies of the Sea (UFPR/ Pontal do Paraná-PR). The decomposition state of the carcasses was categorized as 'fresh' (organs exhibiting no visible alteration in color and texture), 'moderate' (organs with some degree of color and texture alteration, and reduced presence of putrefaction gas), and 'advanced' (organs displaying altered color and texture, affected by putrefaction gas) (Domiciano et al., 2022). The curved carapace length (CCL) (cm) of each specimen was measured. Body condition was assessed and categorized as 'poor,' 'fair,' or 'good' following the criteria established by Thomson et al. (2009), and the sex of each turtle was recorded. Age determination was estimated through histological processing of cross-sections of the humerus, evaluating the total number of growth marks on each bone (Andrade et al., 2016).

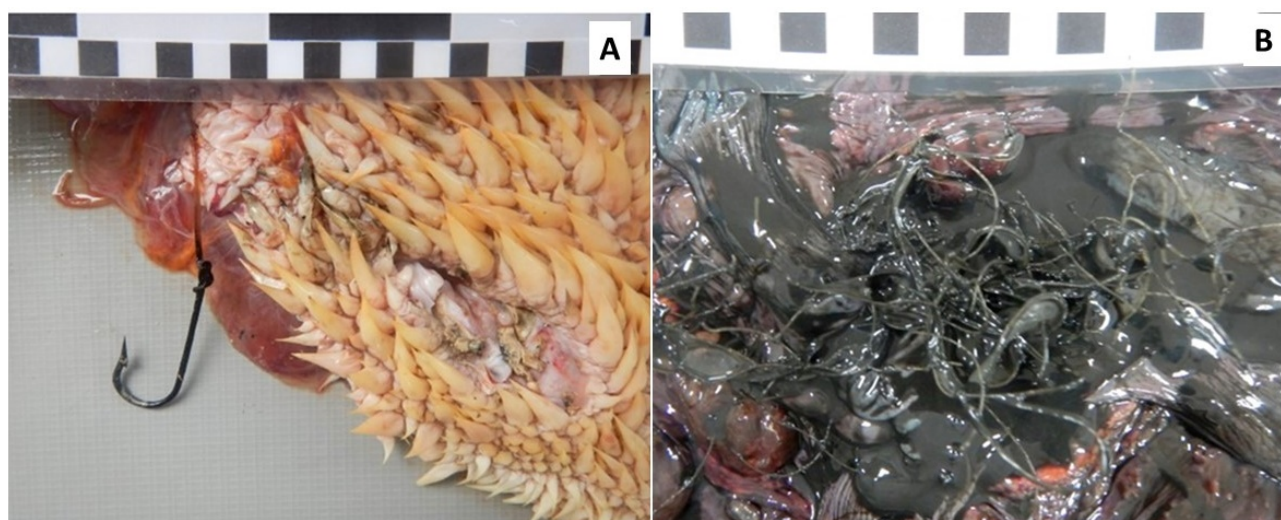
Evidence of anthropogenic interactions (marine debris, oil, or/and bycatch) was registered. Indicators of bycatch interaction encompassed erosions, abrasions, and/or lacerations around the neck, flippers, and carapace. Data were retrieved from the SIMBA database (Sistema de Informação Monitoramento da Biota Aquática [SIMBA], 2021).

Tissue samples, approximately 2 cm<sup>3</sup> each, were extracted from the lung, heart, spleen, liver, gastrointestinal tract, kidney, brain, bladder, and skin, and then fixed in a 10% buffered formalin solution. The tissue samples underwent routine processing, and histological slides were stained with hematoxylin and eosin before being examined under an optical microscope. Fragments for histopathological analysis were exclusively sampled from carcasses in a fresh or moderate state of decomposition.

## Results and Discussion

In the period of the study, 305 *C. caretta* were found stranded on the Paraná coast in different stages of carcass decomposition. From these, 20 (6.56%) were considered in fresh or moderate decomposition state of the carcass and were sampled for histopathological analysis.

Overall, the mean curved carapace length (CCL) (cm) was  $67.3 \pm 6.9$  SD (49.4 – 81.5). Fourteen females, four males, and two undetermined sexes were registered. The visual body condition was good in 13 animals, fair in four, poor in two, and in one undetermined. The mean age was  $15.8 \pm 3.89$  SD (09 - 24 years). Evidence of anthropogenic interaction was registered in 16 animals (80.0%); in ten bycatch interaction was detected (Figure 1A), in six marine debris (Figure 1B), and in two animals oil interaction (concomitantly with marine debris) was observed (Figure 1B).



**Figure 1.** Evidence of anthropogenic interactions registered in *Caretta caretta* in the Paraná coast. A. Presence of hook in the esophagus. The mucosa shows an ulcerated area. B. Presence of nylon line in the gastrointestinal tract accompanied by content like oil.

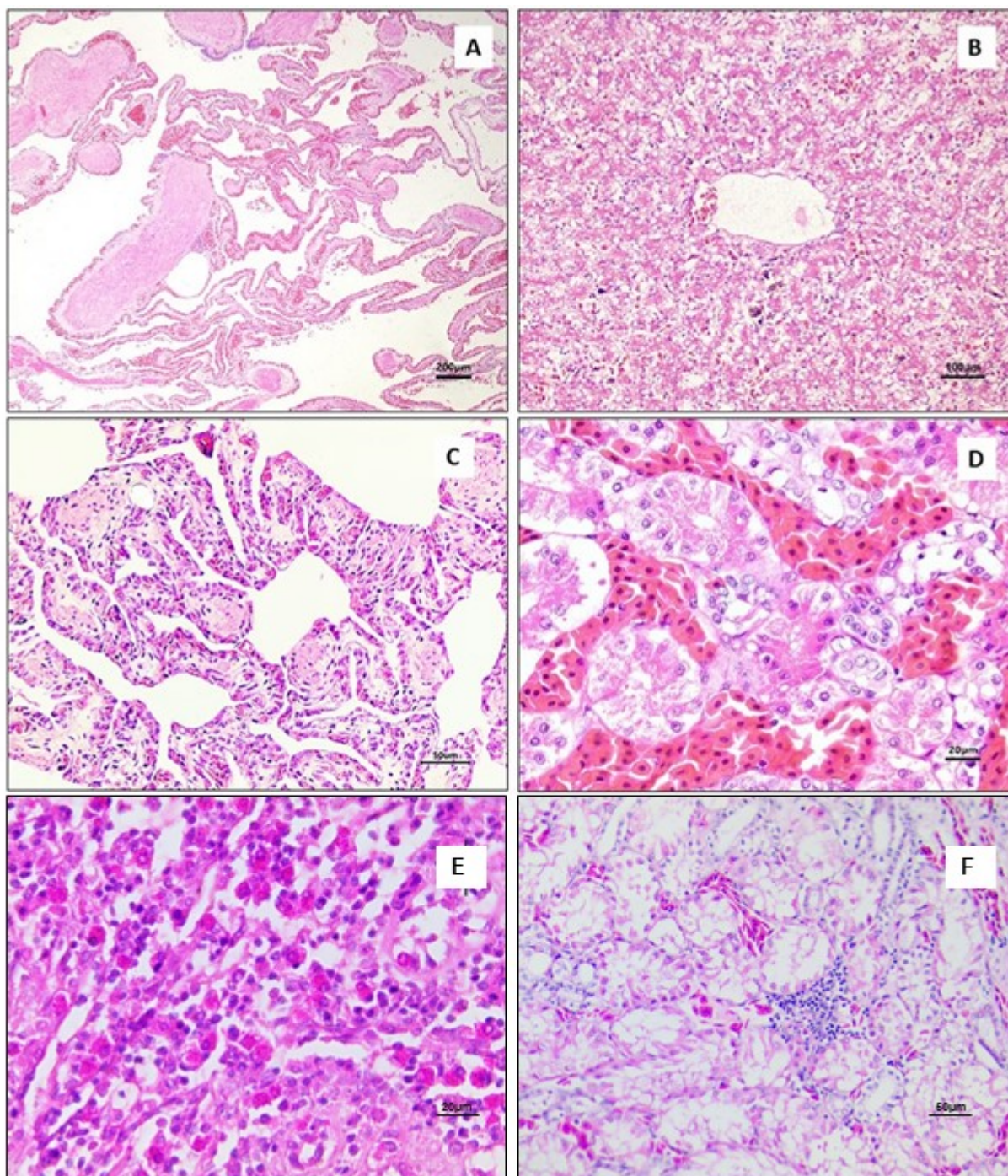
Table 1 lists the main histological findings observed in the studied loggerheads. Microscopic changes were detected mostly in the liver (100%), lungs (83.3%), spleen (83.3%) and kidneys (78.6%). The main changes observed in lungs, and spleen were inflammatory infiltrate and congestion (Figure 2A). The inflammatory infiltrate is mainly multifocal, while congestion appears

diffusely. Both changes showed mild to moderate intensity. Congestion was the main finding in brain and urinary bladder. In the liver and kidney, hydropic degeneration was the most frequent change (Figure 2B). Autolysis is frequently detected in gastrointestinal samples, brain and bladder limiting the number of histological analyses.

**Table 1**  
**Histopathological findings in different organs of stranded *Caretta caretta* on the Paraná coast**

	Lungs n 19	Kidney n 20	Spleen n 19	Liver n 20	Heart n 19	Skin n 13	Brain n 18	Bladder n 19
<b>Histological findings</b>								
Inflammatory infiltrate	11	3	9	1	2	3	0	1
Congestion	1	1	3	2	0	0	4	2
Hemorrhage	0	0	0	0	1	0	0	0
Degenerative changes	0	5	0	11	1	0	0	0
No changes	4	5	4	0	9	7	5	6
Autolysis	3	6	3	6	6	3	9	10





**Figure 1.** Histopathological changes in the lung (left) and liver (right) of *C. caretta* on the Paraná coast.

**A.** Diffuse lung congestion. HE. Bar = 200 µm. **B.** Hepatic hydropic degeneration. HE. Bar = 100 µm. **C.** Lungs. Moderate multifocal lymphocytic infiltrate in faveoli. HE. Bar=50 µm. **D.** Kidney congestion and cell tubular degeneration. HE. Bar = 20 µm. **E.** Spleen. Heterophilic infiltration within splenic cords. HE. Bar = 20 µm. **F.** Kidney. Focal interstitial lymphocytic infiltrate and tubular cell degeneration. HE. Bar = 50 µm.

In the lungs the inflammatory response was the most frequent change, characterized by mild to moderate interstitial pneumonia (11/19, 57.89%) with lymphocytic and heterophilic infiltrate in the faveoli (Figure 2C). In the spleen, multifocal to diffuse heterophilic infiltrate was the main histological change (9/19, 47.37%). In kidneys, hydropic degeneration of tubular epithelial cells was the most frequent lesion (5/20, 25%) (Figure 2D) followed by chronic interstitial nephritis (3/20, 15%); in one animal urate deposition within the lumen of tubules was observed. Hepatocyte hydropic degeneration was frequently detected in the liver (11/20, 55%). In the skin, lymphocytic dermatitis was the detected change (3/13, 23.08%). In the brain, diffuse congestion predominates (4/18), whereas in the heart two animals showed lymphocytic endocarditis (2/19, 10.53%) and another one, cytoplasmic vacuolation of cardiomyocytes (1/19). In the urinary bladder, most of the viable samples showed no histological changes (6/19). Only one animal presented an inflammatory infiltrate within the urinary bladder mucosa.

In the stomach and intestines, 60% of the samples showed autolysis. In viable samples of the gastrointestinal system, inflammatory response was the main finding. Focal to multifocal lymphocytic and histiocytic infiltrate in the mucosa were detected in four samples of stomach, three of small intestine, and two of large intestine. In one animal showing moderate lymphocytic gastritis, *Sulcascaris sulcata* was identified in the stomach. The same animal also presented a moderate lymphohistiocytic colitis associated with *Eimeria* spp. and *Caryospora* spp.

Long-term field monitoring projects including *postmortem* studies contribute to the information on the diseases and causes of mortality among sea turtles. However, studies on pathological findings on stranded wild loggerheads in Brazil are scarce and focusing on single case reports (Domiciano et al., 2014; Rossi et al., 2015; Werneck et al., 2017; Oliveira et al., 2020; Oriá et al., 2021). In the current study, a six-year survey was conducted enabling the analysis of a larger number of specimens in which anthropogenic interactions and histological changes were evaluated.

In the present study, 93.44% of the stranded animals were severely autolyzed, precluding gross and histopathological analyses. Besides a daily systematic monitoring program that recovers stranded animals, a high level of autolyzed animals persists. Similar studies involving pathological findings in sea turtles reported minor occurrences of autolysis (41.3 and 7.53%, Orós et al., 1996, 2005). This difference is probably related to the fact that only dead animals were analyzed in our study, while in these previous studies, animals from rehabilitation centers were included.

In our study, the mean CCL characterized all the individuals as juveniles (Tagliolato et al., 2019), and females predominated. These findings are like a previous study with stranded loggerheads submitted to a rehabilitation center (Orós et al., 2016). The body condition was classified as good or fair in 17 (85%) animals. Apparently, the histological lesions detected in the animals do not result in visual changes in the volume of fat or skeletal muscle tissues.



Anthropogenic interaction was detected in 80% of the analyzed specimens, mainly with fishing gear and/or plastics. Bycatch interaction and ingestion of plastic have been reported as a serious cause of death or contribution to mortality in sea turtles, however the frequency was higher in our study when compared to previous studies in Italy (Casale et al., 2010), Canary Islands (Orós et al., 2016) and Balearic Islands in Spain (Solomando et al., 2022). Erosions, abrasions and/or lacerations around the neck, flippers and carapace were considered as evidence of bycatch interaction. In Brazil, some studies also observed anthropogenic interactions with pelagic longlines affecting loggerheads in the extreme South of Brazil (Duarte et al., 2011; Monteiro et al., 2016). A study showed external findings in 90% of stranded *Dermochelys coriacea* (Santos-Costa et al., 2020). In these cases, entanglement in fishing nets was established as the cause of death. Additionally, the ingestion of marine debris was observed in 69.7% of *C. mydas* in the same region (Guebert-Bartholo et al., 2011).

In this study, congestion was observed in multiple organs, ranging from 5% (lungs, kidneys) to 22% (brain) of the samples. The occurrence of congestion in various organs is a well-documented phenomenon in drowned animals, such as dolphins (Knieriem & Hartmann, 2001), seabirds (Simpson & Fisher, 2017), and green sea turtles (Jerdy et al., 2020). Given the documented evidence of anthropogenic interactions and the observed congestion in multiple organs, it is reasonable to consider bycatch and subsequent drowning as a primary cause of death among loggerheads in this region.

Lung lesions were frequently found in this study, similarly to that reported by other authors (Orós et al., 2005, 2016; Ciccarelli et al., 2020). Interstitial pneumonia was the main lesion, affecting 58% of the animals. Pneumonia was also reported in another study with *C. caretta*, but with a low frequency (23.6%) and mainly the granulomatous and bronchopneumonia types (Orós et al., 2005). This difference is probably related to the etiologic agents; bacteria are associated with bronchopneumonia while virus infections are related to interstitial pneumonia (Schumacher, 1997; Orós et al., 2005; Cubas et al., 2007). In stranded alive loggerheads, radiographic examination revealed increased pulmonary radiopacity, with areas of inhomogeneous parenchyma, and an interstitial pattern (Ciccarelli et al., 2020), characterizing interstitial pneumonia. No microbiological analysis was performed in this study; therefore, the cause of pneumonia was not established. Inhalation of water is considered one of the primary causes of bacterial and fungal pneumonia. Foreign bodies penetrating the trachea or bronchi, entanglement injuries around the neck or flippers can also contribute to a pulmonary inflammatory response (George, 1997; Ciccarelli et al., 2020).

In the present study, the main lesion in the liver was hydropic degeneration (55%), whereas Orós et al. (2005) observed mainly necrotizing and/or granulomatous hepatitis (27.95%) in loggerheads. In this previous study, hepatic vacuolar degeneration and lipidosis represent 13% of the histological findings. Similarly, hepatic lipidosis affected 18.1% of green turtles analyzed in Australia (Flint et al., 2010). In a study conducted by



Jerdy et al. (2020) in *Chelonia mydas*, hepatic hydropic degeneration was associated with death by asphyxia by drowning. Similarly, in our study 40% of the animals showing bycatch interaction (4/10) presented hydropic degeneration of hepatocytes.

In the spleen, heterophilic splenitis was the main histological finding (45%). The spleen is considered the most important organ for immunologic response in chelonians (Saad & Bassiouni, 1993; Kassab et al., 2009), and in many reptiles, the heterophil is the main cell involved in the inflammatory process (Zimmerman et al., 2010; B. Stacy et al., 2020). Multifocal necrotizing splenitis (7.53%) was the most frequent lesion in loggerheads stranded in Spain and was associated with septicemic process (Orós et al., 2005). Heterophilic splenitis was reported in a *C. mydas* showing septic arthritis (Guthrie et al., 2010) and in captive box turtles (*Terrapene carolina carolina*) infected with ranavirus (Sim et al., 2016). Apparently, heterophilic infiltrate in the spleen is related to a non-specific activation of the immune system.

Kidney alterations were observed in 45% of the analyzed animals, and degenerative changes are the most frequent lesion (25%). Inflammatory infiltrate (14%) characterized as interstitial and granulomatous nephritis, and abscesses was the main change in a previous study with loggerheads from a rehabilitation center (Orós et al., 2005). Tubular epithelial degeneration was also reported in a lower frequency (3.23%) by Orós et al. (2005) and Storelli and Zizzo (2014) in stranded *C. caretta*. In this study, few samples of

the urinary bladder were available for microscopic evaluation due to autolysis, and in the evaluated, most of them showed no changes. Conversely, in leatherback sea turtles from São Paulo, Brazil, coccidiosis was frequently detected (Santos-Costa, 2020).

Gastritis and enteritis associated with parasites are reported as incidental findings in stranded loggerheads (Orós et al., 2005; Storelli & Zizzo, 2014). In the present study, most samples from the digestive tract were autolyzed, but in one animal parasitic infection in the stomach (*Sulcascaris sulcata*) and colon (*Eimeria* spp. and *Caryospora* spp.) was related to an inflammatory response. A high frequency of ileal diverticulitis was reported in *Dermochelys coriacea* during an unusual mortality event in São Paulo, Brazil (Santos-Costa, 2020); however the aetiopathogenesis remained unclear.

## Conclusions

This long-term field monitoring of an endangered species has evidenced a high rate of anthropogenic interactions causing a growing concern regarding the conservation. The histological analysis of stranded *C. caretta* showed mainly inflammatory changes affecting multiple tissues, reinforcing the hypothesis that health impairment may have contributed to the entanglement. This study provides information of pathological findings as well as anthropogenic impacts affecting this population. Studies like this can provide important information about the health of animals, besides helping in the conservation of the populations.

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