Marine Pollution Bulletin xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Pathologies of the digestive system caused by marine debris in *Chelonia mydas*

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ARTICLE INFO

Article history:
Received 13 July 2016
Received in revised form 3 December 2016
Accepted 3 January 2017
Available online xxxx

Keywords:
Chelonia mydas
Impaction
Digestive tract
Marine debris

ABSTRACT

The growth of human population and deficient pollution control measures pose significant challenge to the environment. Despite conservation efforts, all sea turtle species are at some risk of extinction. The present study investigated the effect of marine debris on the gastrointestinal tract of green turtles in southeastern Brazil. Of the 777 animals evaluated, 290 showed marine debris in one segment of the gastrointestinal tract. The presence of these materials in the gastrointestinal tract may be harmful, independent of the segment involved, and increases the risk of impaction. Marine debris has become a significant hazard to *Chelonia mydas* in the region surveyed, causing perforation, rupture, or fecal impaction that, when not treated, is potentially fatal, exposing the intestine to bacterial infection.

1. Introduction

Exploitation of marine resources and intense maritime traffic exacerbate the pollution of oceanic and coastal waters. One of the main threats to the marine fauna is the contamination of beaches and seas with several toxic compounds such as oil and sewage (Pough et al., 2003). In addition to typical pollution issues, sea turtles are increasingly threatened by anthropic activities such as unregulated urban development in coastal areas; these activities have led to considerable depletion of the populations of these animals.

Increasing efforts have been made in recent decades toward the preservation of sea turtles and their habitats. Initiatives based on improving awareness are useful tools to underscore the importance of turtle species, which have lived in oceans for over 150 million years (Marcovaldi and Marcovaldi, 1999).

Garbage has ceased to be a problem associated exclusively with urban environments. Hazardous pollutants of marine ecosystems, plastics, and other types of solid waste may block the digestive tract of sea animals, causing erosion, ulcers, or necrosis. Indirectly, waste may affect lipid metabolism, extend intestinal transit times, worsen the accumulation of gas, and make fluctuation more difficult (Lutz and Musick, 1997).

The Cheloniidae family includes six species, namely *Caretta caretta*, *Chelonia mydas*, *Eretmochelys imbricata*, *Natator depressus*, *Lepidochelys kempii*, and *Lepidochelys olivacea* (Marcovaldi and Marcovaldi, 1999). In Brazil, the most common species is the green sea turtle *C. mydas* (Fidelis et al., 2005). Although the species has been listed as endangered worldwide (IUCN, 2016), in Brazil, *C. mydas* has been protected by federal laws since 1989 and has been classified as vulnerable to extinction in the list of threatened species published by the Brazilian Ministry of the Environment (MMA, 2003). The species uses the ocean islands Fernando de Noronha, Trindade, and Atol das Rocas as oviposition sites, but juveniles have been observed along essentially all the country's coast (Marcovaldi and Marcovaldi, 1985).

The present study identified and characterized the anatomopathological findings associated with gastroenteric changes caused by marine debris in *C. mydas* on the Brazilian coast.

2. Materials and methods

The tissues for analysis were obtained after the necropsy of *C. mydas* individuals that died on beach or after unsuccessful treatments. Samples were collected from animals living in seawaters adjacent to a 650-km strip of the Brazilian coast, between the municipalities of Arraial do Cabo (22°57′58″S, 42°01′40″W, state of Rio de Janeiro) and São Mateus (18°42′58″, 39°51′32″W, state of Espírito Santo). All samples were collected over a 3-year period (between 2010 and 2013).

Samples of viscera of the digestive system were collected and fixed in 10% buffered formalin during necropsy. Subsequently, samples were cleaved and dehydrated in a solution of alcohols, clarified in xylol, embedded in paraffin, sectioned (5 μm), stained with hematoxylin–eosin, mounted on slides, and inspected under a microscope (Nikon Eclipse 80i) using specific software (NIS Elements).

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http://dx.doi.org/10.1016/j.marpolbul.2017.01.009
0025-326X/

Gender, measurements, and presence of impaction and of marine debris were recorded. All foreign material and the respective segment of the gastrointestinal tract where it was detected were identified.

The occurrences of impaction in animals that had some kind of marine debris or other material in the gastrointestinal tract were compared using the chi-square test at 5% significance in a specific software (SAEG, version 9.1).

Binary logistic regressions were constructed to calculate the odds ratio between the occurrence of impaction in a given organ (large intestines or others) and the kind of waste causing it (plastic or other materials). The regression analyses were performed using an appropriate software (Minitab, version 15.1.1.0).

Table 1 lists studies discussing marine debris ingestion. To obtain a reliable and representative comparison, only manuscripts that analyzed the complete gastrointestinal system of at least 15 green turtles were considered.

3. Results

In total, 777 green turtles were included, 586 (75%) of which were females and 176 (23%) were males. The gender of 15 animals (2%) could not be identified due to the advanced state of putrefaction. In addition, 769 turtles were juveniles, as indicated by the curved carapace length (CCL), which varied between 26 and 85 cm, whereas eight were adults (four males and four females), with CCL between 90.3 and 118.9 cm.

Marine debris was found in the gastrointestinal tract of 290 of 777 turtles (37%). Plastic was the most common material and was observed in 195 of these 290 animals (61%), followed by nylon (n = 74, 23%), fishing line (n = 35, 11%), rope (n = 4, 1%), fabric (n = 3, 0,9%), string (n = 2, 0.6%), fishhook (n = 2, 0.6%), expanded polystyrene (n = 2, 0.6%), fiber of piassava plant species (n = 2, 0.6%), aluminum parts (n = 1, 0.3%), and rubber parts (n = 1, 0.3%).

The organ most frequently presenting marine debris was the large intestine (n = 250, 59.5%), followed by the stomach (n = 89, 21.19%), small intestine (n = 42, 10%), and esophagus (n = 39, 9%).

Of the 777 animals included in the present study, 65 were dead, although the causes of death were not specified in records. In addition to marine debris, these animals exhibited perforations by hard materials, perforation ulcers, and intestine rupture associated with necrosis caused by impaction. Of these 65 turtles, 11 had perforation caused by hard materials, whereas 54 had lesions associated with impaction.

The 11 animals showing perforation caused by hard materials died from celomitis followed by septic shock due to the entry of intestinal bacteria in the celomic cavity. The other 54 turtles exhibited intestinal compression caused by impaction, which initially induced the flattening (Fig. 1A) of the mucosa by compression and ischemia. Because of hypoxia, cells of the mucosa underwent degeneration and coagulative necrosis. The mucosa detached and formed an ulcer that allowed infectious agents into the intestinal tract. The ulcer enabled the proliferation of intestinal bacteria (Fig. 1B), thus enlarging the necrotic area and characterizing the inception of humid gangrene, which in some cases reached the serosa. Heterophils tried to combat the bacterial infection associated with the growing necrotic tissue, forming suppurative debris (Fig. 1C). The progression of the infection induced the formation of a crust of necrotic intestinal tissue with degenerated heterophils (Fig. 1D). These findings are compatible with ulcerous suppurative enteritis. Necrosis was observed on lamina propria and on the inner circular and outer longitudinal muscle layers. In more severe cases, the serosa was also affected. Such a condition can be fatal as bacteria invade the intestinal tissue and blood vessels. The presence of angiectasia was associated with the resumption of blood flow caused by inflammatory hyperemia.

Gross inspection of 54 animals with impaction revealed ulcers in 13 turtles (24%), rupture in 32 (59%), and obstruction by marine debris in 9 (17%). Microscopic analysis of samples collected from these 54 animals demonstrated that 26 turtles (48%) had mucosa compression, 25 (46%) had mucosa necrosis, 20 (37%) had ulcer, 17 (31%) had angiectasia, 16 (30%) had bacterial infections in the intestinal tissue, 8 (15%) had necrosis of muscle layers, and 2 (4%) had total necrosis of intestinal layers.

The results of the present study showed that the risk of impaction is 22 times higher in *C. mydas* when marine debris are present in the stomach compared with animals with no debris in the stomach ($P < 0.05$). Marine debris in each additional segment increases this risk by 6 times ($P < 0.001$). In addition, the presence of plastic and other debris in the digestive tract increases the probability of impaction by 12 and 11 times, respectively ($P < 0.001$).

The likelihood of impaction was the greatest in turtles with marine debris in the large intestine. Of 527 turtles that did not have any marine debris in the large intestine, only 5.5% (n = 29) showed impaction. Of the 250 animals with some kind of marine debris in the large intestine, 56% (n = 140) showed impaction.

Table 1
Studies describing the presence of marine debris in the gastrointestinal tract of *C. mydas*.

Reference	Total number of analyzed turtles	Turtles affected (%)	State/country
Macedo et al., 2011	36	44.4%	Bahia/Brazil
Reis et al., 2010	28	46.4%	Rio de Janeiro/Brazil
Bjorndal et al., 1994	43	56%	Florida/USA
Plotkin and Amos, 1990	15	46.7%	Texas/USA
Guebert-Bartholo et al., 2011	76	70%	Paraná/Brazil
Tourinho et al., 2010	34	100%	Rio Grande do Sul/Brazil
Present study	777	37%	Rio de Janeiro and Espírito Santo/Brazil

4. Discussion

In a study of the presence of marine debris in sea turtles conducted in north-central Rio de Janeiro, Reis et al. (2010) discovered that 46.4% of the animals examined had some kind of debris in the gastrointestinal tract. A similar result was observed for turtles on the coast of the metropolitan region of the state of Rio de Janeiro, where 59.2% of the 49 turtles examined had swallowed marine debris (Awabdi et al., 2013). In the state of Rio Grande do Sul, Brazil, Tourinho et al. (2010) discovered marine debris in the gastrointestinal tract of all 34 *C. mydas* examined. The present study included the largest number of *C. mydas* individuals thus far for research, and one of the interesting findings observed was that the percentage of animals that had ingested marine debris was the lowest in comparison with other studies (Table 1).

The ingestion of marine debris by turtles has been reported in an increasing number of scientific papers. However, some reports indicate that the percentage of animals with marine debris in the gastrointestinal tract is comparatively low, reaching 20% in Brazil (Santos et al., 2011) and 29% in Mexico (Seminoff et al., 2002). Interestingly, in one study in the USA, only 2% of turtles had marine debris in the gastrointestinal tract (Foley et al., 2007). In another investigation, the researchers did not observe marine debris in any of the animals examined (Hasbun et al., 2000); similar results were found in a study conducted in Oman (Ross, 1985). The presence of marine debris in the gastrointestinal tract of 37.3% of *C. mydas* individuals in the present work is similar to the value reported by Schuyler et al. (2013) in a literature review of studies that investigated the problem. Considering the region covered and methodology used, the study by Reis et al. (2010) found marine debris in 46.4% of the 28 *C. mydas* individuals from northern Rio de Janeiro coast, which is the closest value to the percentage observed in the present study.

Some research has solely investigated the gastric contents of green turtles. We used these data to draw a more complete comparison with the information obtained in the present study. For example, we

Please cite this article as: Jerdy, H., et al., Pathologies of the digestive system caused by marine debris in *Chelonia mydas*, Marine Pollution Bulletin (2017), http://dx.doi.org/10.1016/j.marpolbul.2017.01.009