

# Post-mortem investigations on a leatherback turtle *Dermochelys coriacea* stranded along the Northern Adriatic coastline

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**ABSTRACT:** Leatherback sea turtles *Dermochelys coriacea* are regularly reported in the Mediterranean Sea but rarely reach the northern Adriatic Sea. In the summer of 2009, a well-preserved carcass of an adult female of this species was found dead along the coast of Lido di Venezia. A complete necropsy was carried out, along with evaluation of levels of tissue trace elements. The post-mortem revealed acute severe bacterial gastroenteritis caused by *Photobacterium damsela* ssp. *piscicida*, an opportunistic agent that infected an apparently debilitated animal weakened by ingested plastic debris. High levels of heavy metals (Hg, Pb, Cd and As) found in the liver and kidneys might have contributed to the animal's demise. These findings support previous indications that marine debris is one of the major threats to marine animals, particularly for critically endangered species such as the leatherback turtle.

**KEY WORDS:** Leatherback turtle · *Photobacterium damsela* ssp. *piscicida* · Marine debris · Adriatic Sea

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## INTRODUCTION

The leatherback sea turtle *Dermochelys coriacea* (Vandelli, 1761) is a circumglobal species with the largest distribution of all reptiles (James et al. 2005). It is listed as critically endangered in the International Union for Conservation of Nature Red List of Threatened Species (Sarti Martinez 2000) because of human impacts on its population health worldwide (Spotila et al. 1996). Besides loggerhead (*Caretta caretta*) and green (*Chelonia mydas*) turtles, which reproduce in the Mediterranean, leatherbacks are regularly present in this basin, most likely originating from Atlantic populations (Casale et al. 2003). In particular, the number of animals stranded in the Adriatic Sea, mainly in the southeastern basin, dur-

ing the summer seems to be higher than the Mediterranean average (Lazar et al. 2008). They are rarely reported to reach the northern coastline. Herein, we report the main findings of a post-mortem investigation carried out on a leatherback sea turtle stranded along the northern Adriatic coastline.

## MATERIALS AND METHODS

On 9 June 2009, an adult female leatherback sea turtle (total length: 178 cm; curved carapace length: 138 cm; mass: 199.5 kg) was found dead on the sandy beach of Lido di Venezia, Venice, Italy (45° 24' 44" N, 12° 22' 33" E). The preservation status of the carcass was good, and a detailed post-mortem examination

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was performed at the Faculty of Veterinary Medicine of Padova within 24 h of recovery of the stranded animal. Tissue samples were preserved in 10% neutral buffered formalin for histopathological examination, refrigerated for microbiological and parasitological examinations or frozen for biomolecular and ecotoxicological investigations. For the histopathological examination, the tissues were embedded in paraffin, sectioned (4 µm thickness) and routinely stained with hematoxylin and eosin. Selected tissue sections from the main organs were also stained using different histochemical staining techniques (Periodic acid-Schiff, Gram and Giemsa staining) to detect biological agents. Danscher's auto-metallographic technique (Danscher & Moller-Madsen 1985) and the rhodizonate method for lead salts were used on hepatic and renal tissue to detect inorganic mercury and lead particles, respectively (Lillie 1954). During necropsy, intestinal swabs were collected for standard microbiological analyses. The samples were inoculated onto sheep-blood agar (tryptic soy agar supplemented with 5% defibrinated sheep blood) and incubated aerobically at 28 and 37°C for 24 to 48 h. The isolated colonies were biochemically identified using API 20NE (bioMérieux). To confirm the identity of the isolates, a molecular analysis was performed. The total DNA of the isolates was extracted by heating a selected colony at 100°C for 10 min and emulsifying in 100 µl of sterile nuclease-free water; this was used as a template for further molecular investigations.

Parasitological examinations were performed on all the major organs. The collected parasites were stored in 70% ethanol and studied by light microscopy after staining with borax-carmin and clarifying in Amman's lactophenol.

Trace elements in the liver, kidney, skin and muscle tissues were analyzed at the University of Bologna, Italy. Subsamples (0.7 g) of each tissue were digested with a Milestone MLS 1200 Mega microwave oven using 4 ml of nitric acid and 1 ml of hydrogen peroxide. Measurements of the concentrations of trace elements (namely, arsenic, lead, chromium, copper, manganese, iron, selenium, zinc, cadmium, nickel and mercury), were performed by inductively coupled plasma optical emission spectrometry using a Perkin Elmer Optima 2100 DV instrument coupled with a CETAC U5000AT+ ultrasound nebulizer for mercury. Two blanks were run during each set of analysis to check for purity of the trace elements, and the accuracy of the method was verified using reference materials (lyophilized mussel; CRM 278, Community Bureau of Reference, BCR, Brussels). All of the reference material values were within the certified limits.

## RESULTS

External examination revealed poor body condition and bloody discharge from the mouth and the nostrils, along with epidermal bruises. In addition, severe oedema and several petechiae in the subcutaneous tissues and the internal viscera were seen. A moderate amount of sero-hemorrhagic fluid with fibrin was found in the coelomatic cavity. Upon examination of the gastrointestinal tract, acute severe diffuse hemorrhagic gastroenteritis was observed in association with the presence of a plastic fragment perforating a fold of the gastric mucosa (Fig. 1). As reported in Table 1, 14 different foreign bodies were found in the alimentary tract, mainly represented by floating plastic fragments, obstructing the intestinal lumen (Fig. 1). Upon microscopic investigation, diffuse and severe hemorrhagic mucosal necrosis with low-grade heterophilic inflammation was observed in the esophagus, stomach and upper intestine with

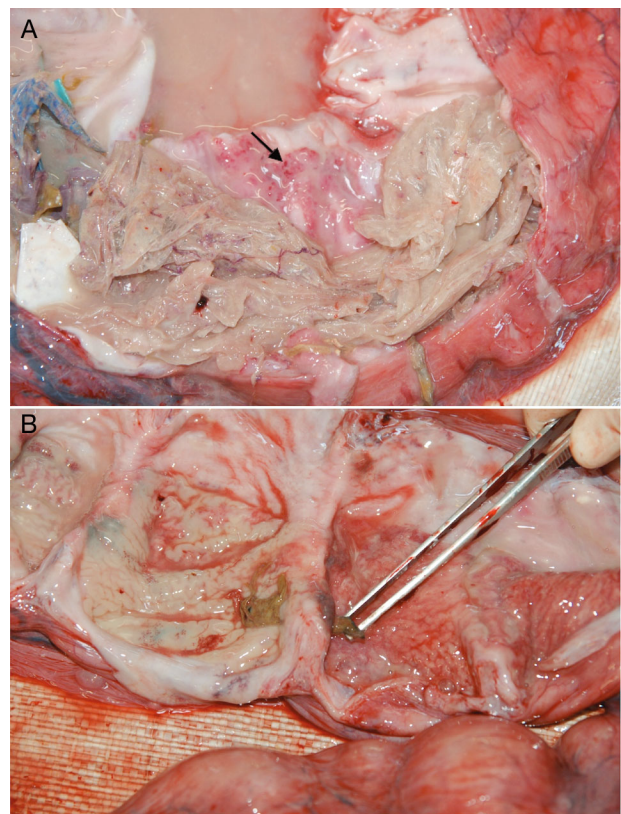


Fig. 1. Foreign bodies found during gross examination in the gastro-intestinal tract of the stranded *Dermochelys coriacea*. (A) Multiple pieces of plastic obstructing the intestinal lumen associated with hemorrhages, ulceration and severe diffuse edema of the enteric mucosa (arrow). (B) A rigid plastic fragment perforating the gastric wall

multiple foci of hepatic, renal and cardiac necrosis and moderate to severe melanomacrophage hypertrophy and hyperplasia in the liver (Fig. 2). Chronic mild multifocal bronchopneumonia was diagnosed during the histopathologic examination.

The microbiological and biochemical assays identified a hemolytic strain of *Photobacterium damsela*. To confirm the identity of the isolate belonging to this species and to characterize its subspecies (*P. damsela* ssp. *piscicida* or *P. damsela* ssp. *damsela*), a duplex PCR using 76a–76b and Ure5'–Ure3' primers was performed as previously described (Amagliani et al. 2009). In the reaction, a no-template control and a positive control (provided by the Istituto Zooprofilattico Sperimentale delle Venezie, Padova) were included. The duplex PCR assay confirmed the pres-

ence of *P. damsela* ssp. *piscicida*. Electrophoresis of the PCR products confirmed the expected amplification fragment of 297 base pairs (Fig. 3).

Twenty-three adult trematode specimens with morphological features overlapping those described by Almor et al. (1989) for *Pyelosomum renicapite* (Digenea; Pronocephalidae) were found in the intestine.

Toxicological examination revealed high hepatic and renal levels of Hg, Pb, Cd and As, but extremely low levels of Fe and Zn. The findings of the complete analysis of the trace elements are summarized in Table 2. Under microscopic observation using specific staining, Hg and Pb concentrations were mostly

Table 1. Marine debris found in the stomach of the stranded leatherback sea turtle (type and dimensions)

Type of marine debris	Dimensions (cm)
Hygienic pad	15 × 5
Plastic pieces	16.5 × 13
	6.5 × 12
	21 × 4
	10 × 12
	4 × 6
	3.5 × 3
Shopping bag parts	4.5 × 2
	40 × 20
	20 × 4
Plastic can parts	4.5 × 3.5
	5 × 1.5
Small rope	19
	23

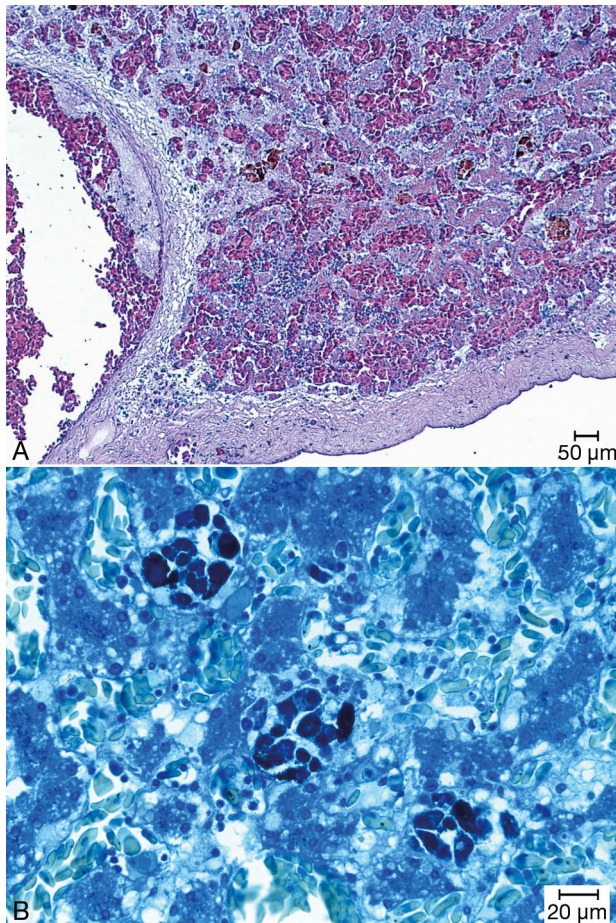


Fig. 2. Microscopic changes in melanomacrophages in the liver of the stranded *Dermochelys coriacea*. (A) Hyperplasia and hypertrophy in hepatic melanomacrophages are evident (hematoxylin & eosin, 100×). (B) A histochemical technique specific to inorganic Hg confirms the presence of this metal in melanomacrophages, indicated by black cytoplasmic deposits (Danscher's staining, 400×)

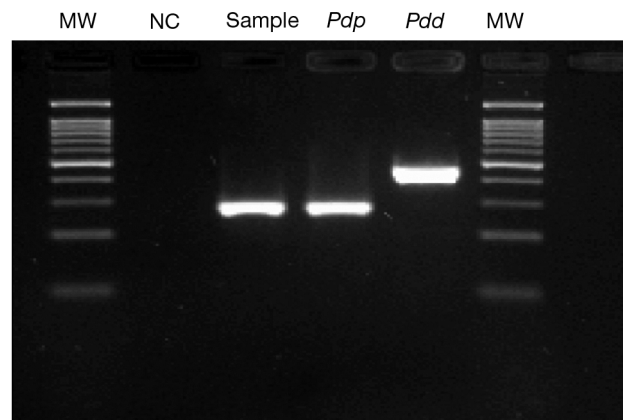


Fig. 3. Agarose gel electrophoresis of PCR amplified products. Lane MW: DNA molecular weight marker (100 bp ladder); Lane NC: no-template control; Lane sample: *Photobacterium damsela* ssp. *piscicida* (leatherback turtle isolate); Lane Pdp: *P. damsela* ssp. *piscicida* (positive control); Lane Pdd: *P. damsela* ssp. *damsela* (positive control)



Table 2. Heavy metal concentrations ( $\mu\text{g g}^{-1}$  wet weight) in tissues of the stranded *Dermochelys coriacea*

Metal	Organ			
	Liver	Kidney	Muscle	Skin
Co	0.5	0.83	0.32	0.5
Cr	0.95	0.16	0.13	0.18
Mn	3.75	0.89	0.2	0.25
As	2.13	3.75	11.84	2.65
Se	12.57	14.57	5.94	6.7
Cu	7.57	1.47	0.54	0.25
Zn	13.5	5.7	7.3	3.98
Fe	0.77	521	80.95	8.4
Hg	20.4	1.95	0.8	0.7
Pb	16.37	8.42	0.13	0.76
Cd	5.68	40.81	0.26	0.49

localized in the cytoplasm of the hyperplastic melanomacrophages in the liver (Fig. 3) and in the epithelial cells of the renal tubules.

## DISCUSSION

The post-mortem studies carried out on this adult specimen of *Dermochelys coriacea* revealed acute severe hemorrhagic gastroenteritis associated with the presence of *Photobacterium damsela* ssp. *piscicida* isolated from the intestinal tract. Based on necropsies performed on sea turtles, several studies have reported a high frequency of inflammatory changes of the alimentary tract associated with a wide range of Gram-positive and Gram-negative bacteria (Orós et al. 2004, 2005). The present study adds to the existing literature, being the first to report in a marine turtle the presence of *P. damsela* ssp. *piscicida*, the disease-causing agent of the bacterial septicemia in marine fishes known as pasteurellosis or pseudotuberculosis (Romalde 2002). In contrast, the subspecies *damsela* has been described primarily in *D. coriacea* (Obendorf et al. 1987), and is also occasionally encountered in humans (Barber & Swygert 2000, Alvarez et al. 2006, Aigbivbalu & Maraqa 2009, Kim et al. 2009). The necro-hemorrhagic changes observed microscopically in the gastric, intestinal, hepatic and renal tissues during the post-mortem analyses suggest a pathogenic mechanism similar to that observed in fishes, where *P. damsela* ssp. *piscicida* induces the destruction of macrophages and neutrophils by the apoptogenic exotoxin AIP56 (do Vale et al. 2007). The piece of plastic found in the stomach penetrating the gastric mucosa, along with the huge amount of ingested plastic objects,

could be considered predisposing factors for the infection (Bugoni et al. 2001, Orós et al. 2004, 2005). The amount of plastic fragments found in the alimentary tract appeared to present an obstacle to food passage in the lumen, possibly causing the poor body condition and likely representing the primary cause of death (Lazar & Gracan 2011). The presence of the digenetic trematode *Pyelosomum renicapite*, often found in the alimentary tract of apparently healthy turtles (Threlfall 1979, Almor et al. 1989, Manfredi et al. 1996), was determined to be of little or no clinical significance.

The trace element analyses conducted on selected tissues of the stranded leatherback turtle showed elevated concentrations of toxic heavy metals (i.e. Pb, Hg, Cd and As) but significantly lower hepatic Fe levels compared with those indicated by (albeit scarce) existing data on *Dermochelys coriacea* (Davenport & Wrench 1990, Caurant et al. 1999, Godley et al. 1999, Deem et al. 2006, Guirlet et al. 2008, Innis et al. 2010, Harris et al. 2011). The data obtained for Se, Zn, Hg, Cd and Pb concentrations were also higher than those reported for loggerhead and green sea turtles from the Mediterranean area (Andreani et al. 2008).

In particular, renal and hepatic Cd concentrations were higher than the toxic threshold established for freshwater painted turtles *Chrysemys picta* (Rie et al. 2005). Despite marine turtles being considered organisms that accumulate higher Cd concentrations (Storelli et al. 2005) than freshwater turtles and terrestrial vertebrates, it is possible that toxicity occurred in this animal. The Hg and Se tissue levels were high. The low molar Hg/Se ratio (0.64) suggests short-term exposure to high levels of Hg, as long-term exposure yields a value close to 1 owing to the detoxifying action of Se (Storelli et al. 2005). The high concentrations of these heavy metals, specifically Hg and Pb, might have been caused by the ingested foreign bodies. Histochemical techniques specific for Hg and Pb showed an intense staining, confirming high levels of these 2 metals in the cytoplasm of hypertrophic and hyperplastic hepatic melanomacrophages. These cells are normally encountered in reptile livers, where they are able to phagocytize erythrocytes and foreign materials (Christiansen et al. 1996), and their hyperplasia and hypertrophy are considered a nonspecific response to emaciation, stress and chronic pathological processes (Flint et al. 2009). In this case, the hyperplastic response of this cellular population might have been caused by the poor body condition of the animal.

In conclusion, the results of the post-mortem studies conducted on this adult female leatherback sea

turtle suggest that the bacterial gastroenteritis was caused by an opportunistic agent that infected a debilitated animal weakened by the ingestion of marine debris. These observations are in accordance with previous studies on stranded turtles, which report marine debris as one of the major threats to marine animals, particularly for already-endangered species such as the leatherback sea turtle (Bugoni et al. 2001, Mrosovsky et al. 2009, Lazar & Gracan 2011). Finally, the present study contributes to the current knowledge on the pathology and ecotoxicology of leatherback sea turtles and underlines the necessity of performing post-mortem examinations routinely on stranded animals (Flint et al. 2009).

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