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Baseline

Postmortem investigations on leatherback sea turtles (*Dermochelys coriacea*) stranded in the Canary Islands (Spain) (1998–2017): Evidence of anthropogenic impacts

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ABSTRACT

Opportunities for postmortem studies on leatherback sea turtles (*Dermochelys coriacea*) are infrequent due to their predominantly pelagic life history. In this study, the pathological findings and causes of mortality of 13 leatherback turtles stranded in the Canary Islands, Spain, from 1998 to 2017, are described. In addition, concentrations of Se, As, Cd, Pb, Hg, 15 rare earth elements (REE) and other 4 minor elements (ME), 41 persistent organic pollutants, and 16 polycyclic aromatic hydrocarbons in hepatic samples from 5 leatherbacks were determined. 84.62% of the turtles died possibly due to anthropogenic causes (entanglement/fishing interaction - 46.15%; boat strike - 23.07%; plastic ingestion - 15.38%). Although Se, As, and Cd were found at higher hepatic concentrations than those reported for leatherbacks from other locations, no acute lesions were detected. This is the first report of exposure to REE-ME in sea turtles. Organic contaminant hepatic concentrations were generally low or undetectable.

Two families and seven species of sea turtles are currently recognized (Pritchard, 1997). The globally distributed leatherback sea turtle (*Dermochelys coriacea*) is the most ancient extant species, the sole living member of the family Dermochelyidae, and the largest living chelonian (Pritchard, 1997). It is listed as Vulnerable according to the IUCN Red List, but there are important differences among the seven subpopulations of leatherbacks recognized in different ocean basins (Wallace et al., 2013). Leatherbacks belonging to the East Pacific Ocean, West Pacific Ocean, Southwest Atlantic Ocean, and Southwest Indian Ocean subpopulations are considered Critically Endangered, whereas leatherbacks from the Northeast Indian Ocean and Southeast Atlantic Ocean subpopulations are included in the category Data Deficient (IUCN, 2020). Leatherbacks observed around the coasts of the Canary Islands belong to the Northwest Atlantic Ocean subpopulation, considered Endangered (IUCN, 2020).

Major recognized global threats to leatherbacks have an anthropogenic origin: incidental capture in fishing gear targeting other species (fisheries bycatch) (Gilman and Huang, 2017; Hamelin et al., 2017), direct utilization of turtles or eggs for human use (Spotila et al., 2000), and coastal development affecting critical turtle habitat (Wallace and Saba, 2009). When a new assessment framework was developed to define the global conservation priorities using categories of paired risk and threats scores for all subpopulations, marine pollution and debris was only scored as affecting three populations of leatherbacks (Wallace et al., 2011); in fact, there are fewer studies investigating inorganic elements and organic contaminants in leatherback turtles than in other sea turtle species such as loggerhead turtles (*Caretta caretta*) and green turtles (*Chelonia mydas*) (Orós et al., 2009; Perrault, 2014; Cortés-Gómez et al., 2017).

When compared with other sea turtle species such as loggerheads and green turtles, there are few reports describing necropsy findings in leatherback turtles (Stacy et al., 2015; Ferguson et al., 2016; Santos-Costa et al., 2020). Opportunities for necropsy and other postmortem studies are infrequent due to their predominantly pelagic life history, and the subsequent difficulty in accessing fresh carcasses (Stacy et al., 2015).

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Since 1994, the Veterinary Faculty at the University of Las Palmas de Gran Canaria (ULPGC), in collaboration with the Tafira Wildlife Rehabilitation Center (TWRC) (Cabildo de Gran Canaria), has been conducting a survey on the pathology and causes of mortality among sea turtles stranded on the coasts of the Canary Islands. Because the most common species around the Canary Islands is the loggerhead turtle, the majority of postmortem studies were focused on this species (Orós et al., 2005; Camacho et al., 2013a; Inurria et al., 2019).

The aim of this study was to describe the pathological findings and causes of mortality of leatherback sea turtles stranded on the coasts of the Canary Islands, Spain, from 1998 to 2017. In addition, chemical analyses were carried out to determine the concentrations of Se, As, Cd, Pb, Hg, 15 rare earth elements (REE) and other 4 minor elements (ME), 41 persistent organic pollutants (POPs), and 16 polycyclic aromatic hydrocarbons (PAHs) in hepatic samples from 5 leatherback turtles.

A total of 17 leatherback turtles were submitted to the TWRC from 1998 to 2017. Four turtles were excluded from the study due to their advanced autolytic status. Except for 3 turtles that died during the rehabilitation period (< 36 h), the rest of the animals were already admitted dead.

Necropsies and pathological studies were performed using the procedures previously described (Orós and Torrent, 2001; Orós et al., 2005). Samples from gross lesions and from spleen were cultured on a variety of selective and non-selective media (Oxoid Ltd., Basingstoke, UK), including blood agar, Mac-Conkey agar, Baird Parker agar for staphylococci, and Sabouraud Dextrose agar for fungi and yeasts. Bacteria were identified based on the biochemical profile (API 20 E, API 20 NE, and API 20 Staph, BioMérieux, Marcy-l'Étoile, France).

Hepatic concentrations of 24 inorganic elements, including Se as essential trace element, the four major toxic elements (As, Cd, Pb, Hg) from the ATSDR Substance Priority List (ATSDR, 2019), 15 rare earth elements (Ce, Dy, Er, Eu, Gd, Ho, La, Lu, Nd, Pr, Sm, Tb, Tm, Yb, Y) and other 4 minor elements (Ga, In, Nb, Ta) were determined. A total of 57 analytes belonging to three relevant groups of organic contaminants were also selected for this study. The 23 organochlorine pesticides (OCPs) and metabolites included were the diphenyl-aliphatics (methoxychlor, *o,p'*-DDT, *p,p'*-DDT, *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDD, *p,p'*-DDD, and dicofol); the persistent and bioaccumulative contaminant hexachlorobenzene (HCB); the four isomers of hexachlorocyclohexane (α-, β-, δ-, and γ-HCH); the cyclodienes heptachlor, dieldrin, aldrin and endrin, chlordane (*cis*- and *trans*-isomers) and mirex; endosulfan (α- and β-isomers) and endosulfan sulfate. With respect to the polychlorinated biphenyls (PCBs), 12 dioxin-like congeners (IUPAC numbers# 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189), and six non-dioxin-like congeners (IUPAC numbers# 28, 52, 101, 138, 153 and 180) were included. Finally, we also included the 16 EPA (Environmental Protection Agency) priority PAHs, often targeted for measurement in environmental samples (naphthalene, acenaphtylene, acenaphtene, fluorene, anthracene, phenanthrene, fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, dibenz[*a,h*]anthracene and benzo[*ghi*]perylene).

For the analysis of inorganic elements, pure standards of elements in acid solution (5% HNO3, 100 mg/L) were purchased from CPA Chem (Stara Zagora, Bulgary). Two standard curves (ten points, 0.005–20 ng/mL) were made to avoid interferences between elements: a) one using a commercial multi-element mixture (CPA Chem Catalog number E5B8•K1.5N.L1, 100 mg/L, 5% HNO3) containing all the essential elements and main heavy metals; and b) other multi-element mixture tailor-made in our laboratory from individual elements (CPA Chem), which contained the REE and ME, as previously reported (Henríquez-Hernández et al., 2017). The liver samples were acid digested using a Milestone Ethos Up microwave (Milestone, Bologna, Italy). All the samples were processed in duplicate, and for this two 150 mg-portions of each turtle's liver were carefully weighted into the digestion vessels, and 3.5 mL of Milli-Q water and 1.mL of concentrated sub-boiling HNO_3 (65%) were added to each sample. Digestion conditions were according to the following program: Step 1: 1800–100–5 [power (W) – temperature (°C) – time (min)]; Step 2: 1800–150–5; Step 3: 1800–200–8, and Step 4: 1800–200–7. After cooling, the digested samples were transferred into 50 mL plastic bottles and diluted up to 7.5 mL with Milli-Q water. Finally, an aliquot of each sample was taken and the internal standards [ISTD solution was composed by Sc (scandium), Ge (germanium), Rh (rhodium), and Ir (iridium) at a stock concentration of 20 mg/mL each] were added for the analysis. Blanks were prepared in the same way as the sample.

An Agilent 7900 ICP-MS (Agilent Technologies, Tokyo, Japan) with standard nickel cones, MicroMist glass concentric nebulizer, and Ultra High Matrix Introduction (UHMI) system was used for these measurements. The Integrated Sample Introduction System (ISIS) was configured for discrete sampling. The UHMI system was operated in robust mode. The 4th generation Octopole Reaction System (ORS4) was operated in helium (He) mode to reduce polyatomic interferences. A tuning solution consisting in a mix of cesium, cobalt, lithium, magnesium, thallium, and yttrium was used before the analysis for optimization of instrumentation. Quantification of the elements was made in the MassHunter v.4.2. ICP-MS Data Analysis software (Agilent Technologies).

The entire procedure was validated prior to its use in the analyses of samples. Recoveries obtained ranged from 89 to 128% for REE and other elements used in high tech devices, and from 87% to 118% for ATSDR's toxic heavy elements and trace elements. Linear calibration curves were found for all elements (regression coefficients ≥0.998). Instrumental LODs and LOQs were calculated as the concentration of the element that produced a signal that was three and ten times higher than that of the averaged blanks, respectively (Supplementary Table 1). The accuracy and precision of this method was assessed using fortified alkaline solution (0.05, 0.5, and 5 ng/mL) in substitution of sample. In general, the calculated relative standard deviations (RSD) were lower than 13% for all the elements at the lowest level of fortification. The precision improved at the highest level of concentration, as it was lower than 5% for all elements.

A fully validated method was employed for the analysis of organochlorine pesticides and PCBs (Luzardo et al., 2014b). The scope of the method was subsequently extended to PAHs and validated in-house (in terms of accuracy, precision and recovery) (Luzardo et al., 2014a). For the extraction, 1 g of liver was homogenized with 4 mL of ultrapure water, and from this homogenate 1 mL was taken, to which 10 μL of the P-IS were added. In this method, a matrix-matched calibration curve was used, so the matrix (chicken or beef liver tested negative for the analytes of interest) was prepared in the same way, but the 1-mL aliquots were fortified to 12 increasing concentrations of the POPs mixture (0.05 to 50 ng/mL). After this, 2 mL of acidified acetonitrile (0.5% formic acid) were added, shaken vigorously (30 sg), and subjected to an ultrasound bath for 20 min (Selecta, Barcelona, Spain). After this time, 480 mg of anhydrous magnesium sulphate and 120 mg of sodium acetate were added to each tube and shaken vigorously again (90 sg). The samples were centrifuged at 4200*g* for 5 min at 2 °C and the supernatant was collected and filtered through 0.2 μm (Chromafil PET-20/15, Macherey-Nagel, Düren, Germany) to be used directly for chromatographic analysis, without any additional purification steps. An Agilent 1290 UHPLC (Agilent Technologies, Palo Alto, USA) coupled to an Agilent 6460 triple-quadrupole mass spectrometer was used to separate and detect the analytes. Validation parameters are given in Supplementary Table 2. Chromatographic and acquisition conditions and basic procedural details can be found in Rial-Berriel et al. (2020).

The mean ± standard deviation of the straight carapace length (SCL), curved carapace length (CCL) and curved carapace width (CCW) were 147.70 ± 23.09 cm (range, 123–210 cm), 149.10 ± 22.50 cm (range, 127–213 cm) and 86.50 ± 13.60 cm (range, 74.40–120 cm), respectively. On the basis of SCL and sexual maturity estimated by gonadal visualization, all specimens were adult or subadult, and all the turtles

Table 1
Pathological and microbiological findings and suspected causes of death in 13 leatherback sea turtles (*Dermochelys coriacea*) stranded in the Canary Islands.

Turtle	Gross lesions	Histological lesions	Microbiology	Suspected cause of death
1	Ulcerative/purulent dermatitis (neck/flipper)	Purulent dermatitis Interstitial pneumonia	*Vibrio alginolyticus* (lung)	Entanglement
2	Ulcerative/purulent dermatitis (flipper) Ileocecal diverticulitis	Purulent dermatitis Edema (lung) Ileocecal diverticulitis	*Proteus* spp. (ileocecal diverticulum)	Entanglement
3	Multifocal granulomatous hepatitis / splenitis / pneumonia Ileocecal diverticulitis	Multifocal granulomatous hepatitis / splenitis / pneumonia Renal thrombosis Ileocecal diverticulitis	*Serratia marcescens* (liver, spleen, lung) *Morganella morganii* (ileocecal diverticulum)	Septicemia
4	Ulcerative/purulent dermatitis (neck/flipper)	Purulent dermatitis Edema (lung)		Entanglement
5	Ulcerative/purulent dermatitis (flipper) Ileocecal diverticulitis	Purulent dermatitis Edema (lung) Hydropericardium Ileocecal diverticulitis	*Proteus* spp. (ileocecal diverticulum)	Entanglement
6	Skull fractures/Brain hemorrhage Ileocecal diverticulitis	Brain hemorrhage/acute inflammation Ileocecal diverticulitis	*Morganella morganii* (ileocecal diverticulum)	Boat strike
7	Ulcerative/purulent dermatitis (flipper)	Purulent dermatitis Edema (lung) Hydropericardium		Entanglement
8	Traumatic injury (carapace)	Necrosis (lung)		Boat strike
9	Intestinal obstruction (plastic bag)	Edema (gastrointestinal serosa)		Plastic ingestion
10	Intestinal perforation	Fibrinous intestinal serositis/celomitis		Plastic ingestion
11	Skull fractures/Brain hemorrhage	Brain hemorrhage/acute inflammation		Boat strike
12	Fibrinopurulent perihepatitis Ileocecal diverticulitis	Fibrinopurulent perihepatitis Acute interstitial nephritis Ileocecal diverticulitis	*Morganella morganii* (liver, ileocecal diverticulum)	Septicemia
13	Ulcerative/purulent dermatitis (flippers) Hook injury (flipper) Small plastic piece (stomach)	Purulent dermatitis Edema (lung) Hydropericardium		Entanglement/fish hook

were female. Pathological and microbiological findings are given in Table 1. Suspected causes of death (Table 1) were entanglement ($n = 6$) (Fig. 1), boat strike ($n = 3$) (Fig. 2), plastic ingestion ($n = 2$) (Fig. 3), and septicemia (n = 2) (Fig. 4). Ileocecal diverticulitis (without intestinal obstruction) was observed in 5 turtles (Fig. 5).

Hepatic concentrations of Se, As, Cd, Pb, and Hg are given in Table 2. Among the ATSDR list of toxic elements, As and Cd were the ones with the highest concentrations.

Hepatic concentrations of rare earth elements and other minor elements (Ga, In, Nb, and Ta) are given in Table 3. The mean concentration of cerium was the highest, doubling the mean concentration of the next element, lanthanum.

Hepatic concentrations of POPs and PAHs detectable in at least one turtle are given in Table 4. The rest of pollutants analyzed and not expressed in the Table had values below detection limit. Among the POPs, the highest values were those detected for *p,p'*-DDE, followed by PCB 138. In the case of PAHs, detectable concentrations of naphthalene and fluoranthene were only found, respectively, in two turtles (leatherbacks #9 and #12).

Our study confirms two relevant aspects derived from the pelagic habits of this sea turtle species and its physiological characteristics. Firstly, the extreme difficulties to carry out a successful rehabilitation in these animals have been in evidence; it was not possible to rehabilitate any of the three leatherbacks that were admitted alive, and they died in a short time. However, during a similar period of time (1998–2014), 86.29% of loggerhead turtles admitted alive to the TWRC were successfully released (Orós et al., 2016). Due to their pelagic habits, when they strand on the coasts, leatherbacks often do so either dead or in terminal condition; in addition, the size and weight of these animals makes it extremely difficult to handle and house them in adequate facilities in rehabilitation centers. Secondly, the difficulties to carry out postmortem studies on a high number of animals are also highlighted, as stated by other authors (Stacy et al., 2015; Ferguson et al., 2016).

In our survey, 46.15% of leatherbacks ($n = 6$) died possibly due to entanglement in fishing gear. Although interactions with the Canarian artisanal fishery are infrequent, potential interactions between leatherbacks and fisheries can take place in waters off due to use of 'trasmallos' (as in loggerheads, with 50.81% of strandings caused by entanglement) (Orós et al., 2016), a fishing net used to catch several fish species. Entanglement was a significant cause of injury in several surveys (Stacy et al., 2015; Hamelin et al., 2017; Archibald and James, 2018). More recently, in an unusual mortality event in Brazil involving 23 leatherbacks, cardiorespiratory collapse by asphyxia due to entanglement in nets was the most likely cause of death (Santos-Costa et al., 2020); the most prevalent lesions were cutaneous lesions around the neck and flippers, generalized congestion, and pulmonary edema (Santos-Costa et al., 2020).

In our survey, only the turtle #13 had hook-compatible lesions in a front flipper. Bycatch in pelagic longline fisheries is one of the most important anthropogenic threats to leatherback turtle populations (Gilman and Huang, 2017; Hamelin et al., 2017). An international survey showed that 50,000 leatherbacks were likely taken as pelagic longline bycatch in 2000 (Lewison et al., 2004). Leatherbacks are more likely to be foul-hooked in the head, shoulders, flippers, or carapace than to swallow hooks, while ingestion of hooks is more frequent in other sea turtle species (Blades et al., 2019). Indeed, during a similar period of time (1998–2014), 221 loggerheads were admitted to the TWRC due to ingestion of hooks and monofilament lines (Orós et al., 2016).

Several strategies have been implemented in various countries to mitigate this bycatch. Large circle hooks have been shown to reduce bycatch primarily by reducing the chances of a turtle swallowing the hook (Gilman and Huang, 2017; Blades et al., 2019). Use of fish bait (vs. squid) has been found to significantly reduce the bycatch probability (Gilman and Huang, 2017; Swimmer et al., 2017). Bycatch reduction may also be gained for leatherbacks by mitigation of color of lightsticks in longline gear (Swimmer et al., 2017) or using light sources flickered at >16 Hz (Crognale et al., 2008).

In our survey, 23.07% of leatherbacks ($n = 3$) died due to boat strikes. During a similar period of time (1998–2014), 97 loggerheads

Fig. 1. (A) Leatherback turtle (#5) showing ulcerative and purulent skin lesions (arrow) around the left front flipper caused by entanglement in fishing gear. Inset: detail of the lesion from a dorsal view. (B) Leatherback turtle (#13) showing a penetrating injury (arrow) in the left front flipper possibly caused by a hook.

Fig. 2. Severe skull fractures in a leatherback turtle (#11) possibly caused by boat strike.

were admitted to the TWRC due to boat strikes (Orós et al., 2016). Involvement of vital organs such as lungs and kidneys (because the anatomical location, dorsally attached to the carapace), and brain, explains the generally poor prognosis for turtles with severe traumatic injuries in the carapace and skull (Orós et al., 2005). The prevalence of boat strike injuries varies according to the subpopulations analyzed: whereas boat strikes were the main causes of leatherback strandings in Algeria (Belmahi et al., 2020), in a recent study on characterization of watercraft-related mortality of sea turtles in Florida, authors estimated that 4–6 leatherbacks died annually due to boat strike injuries; however, taking into account that only about 10–20% of turtles that died likely washed ashore, authors suggested that the overall annual mortality may be 5–10 times greater than that represented by strandings (Foley et al., 2019).

In our survey, 15.38% of leatherbacks ($n = 2$) died due to digestive lesions caused by plastic ingestion. According to the Ocean Conservancy, 150 million tonnes of plastic are currently in the oceans, and, as stated by the World Economic Forum, eight million tonnes infiltrate the oceans per year (Aretoulaki et al., 2020). Especially in the case of leatherbacks, plastic may be mistaken for jellyfish (Mrosovsky et al., 2009). Ingestion of plastics can cause intestinal obstruction and other intestinal lesions, dietary dilution, malnutrition, and increased buoyancy resulting in poor health, reduced growth rates and reproductive output, or death; in addition, plastics can accumulate contaminants from the marine environment, such as heavy metals and PCBs (Nelms et al., 2016).

In a review of 408 necropsy records of leatherback turtles (1885–2007), plastic was found in the gastrointestinal tract in 34% of cases, although lethal effects were relatively infrequent (8.7%) (Mrosovsky et al., 2009). Other recent studies have not reported the ingestion of plastics, or only in very few animals, possibly due to the low number of leatherbacks analyzed (Clukey et al., 2017; Rizzi et al., 2019). The ingestion of significant quantities of plastic debris may not be lethal for leatherbacks, especially if it can be expelled (Barreiros and Barcelos, 2001); however, perforation of the gastric mucosa by a plastic fragment,

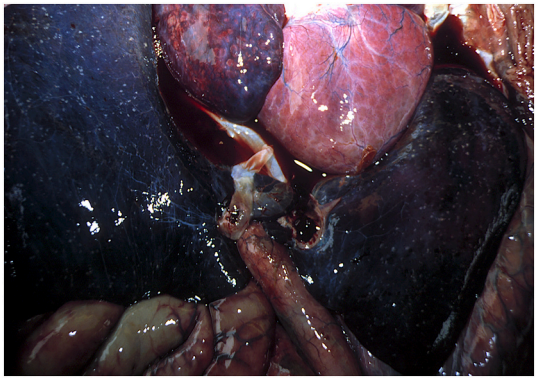

Fig. 4. Severe multifocal granulomatous hepatitis in a leatherback turtle (#3).

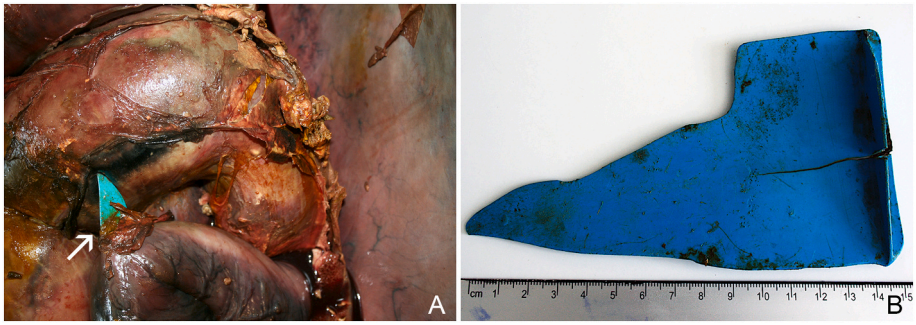

Fig. 3. (A) Intestinal perforation (arrow) in a leatherback turtle (#10) caused by a hard, sharp piece of plastic; note also the severe fibrinous intestinal serositis. (B) Note the size of the plastic piece causing the intestinal perforation.

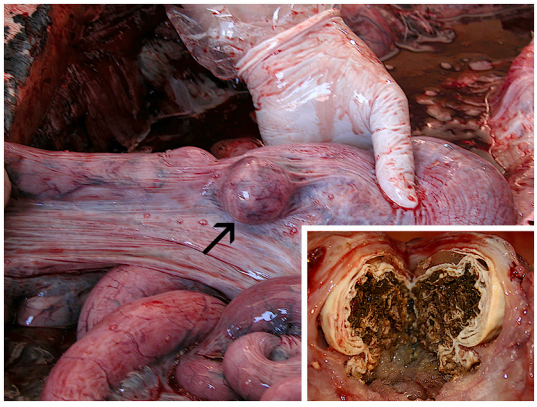

Fig. 5. Ileocecal diverticulum (arrow) in a leatherback turtle (#5). Inset: the diverticulum was filled with firm yellow caseous exudate. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Concentrations of inorganic elements (μg/g wet wt.) detected in liver samples from 5 adult female leatherback sea turtles (*Dermochelys coriacea*) stranded in the Canary Islands.

	Turtle 9	Turtle 10	Turtle 11	Turtle 12	Turtle 13	Mean
Essential trace element						
Selenium (Se)	29.5	11.55	13.28	22.61	11.18	17.62
ATSDR list of toxic elements						
Arsenic (As)	3.61	39.20	11.55	22.89	1.39	15.73
Cadmium (Cd)	25.56	15.05	7.04	7.24	3.78	11.73
Lead (Pb)	1.21	0.03	0.78	0.31	0.32	0.53
Mercury (Hg)	1.08	0.15	0.31	0.18	0.12	0.37

ATSDR: Agency of Toxic Substances and Disease Registry.

hemorrhagic gastroenteritis, and obstruction of the intestinal lumen by plastic fragments were reported in a leatherback stranded in Italy (Poppi et al., 2012).

In our survey, 15.38% of leatherbacks (n = 2) possibly died due to septicemia. During a similar period of time (1998–2014), 103 loggerheads were admitted to the TWRC due to infectious diseases, and they had a high unassisted mortality rate (25.49%) during the hospitalization period (Orós et al., 2016). *Serratia marcescens* has been associated with hepatic lesions in loggerheads (Orós et al., 2005); more recently, it has also been isolated from captive green turtles with ulcerative stomatitis (Vega-Manriquez et al., 2018). *Morganella morganii* has been isolated from cloacal samples of rehabilitated green turtles in Australia (Ahasan et al., 2017) and loggerheads in Italy (Pace et al., 2019). It was also isolated from three leatherbacks with large intestinal diverticulitis (Stacy et al., 2015).

Solitary large intestinal diverticulitis was a very frequent necropsy finding in leatherbacks in North America (Stacy et al., 2015); it was unrelated to the cause of death or clinical disease in any of the cases, although authors suggested the possibility of perforation or obstruction (Stacy et al., 2015). Two of the turtles with ileocecal diverticulitis in our survey (leatherbacks #3 and #12) died due to septicemia, but only in the leatherback #12 the microorganism isolated from the diverticulum and the hepatic and renal lesions was the same, *Morganella morganii*.

Therefore, when causes of mortality were established in our survey, 84.62% of the turtles died possibly due to anthropogenic causes (entanglement/fishing interaction - 46.15%; boat strike - 23.07%; plastic ingestion - 15.38%); obviously, the absence of cases of predation as a natural cause of death skews our results. When 1860 loggerhead turtles admitted to the TWRC during a similar period of time (1998–2014) were analyzed, anthropogenic causes of mortality were detected in 71.70% of the turtles, although in 20.4% of the total admitted turtles the cause of death could not be determined (Orós et al., 2016).

There are very few reports of pollutants in liver samples from leatherbacks due to limited opportunities for necropsy (Davenport and Wrench, 1990; Godley et al., 1998; Caurant et al., 1999; Mckenzie et al., 1999; Orós et al., 2009; Perrault, 2012; Poppi et al., 2012). The number of studies for the detection of pollutants in blood from leatherbacks is greater, although if compared to other sea turtle species, they are still scarce (Perrault et al., 2011; Keller et al., 2012; Perrault et al., 2013).

In addition, several reasons make it difficult to investigate the effects of environmental pollutants on sea turtles: (i) sea turtles often harbor various pollutants from the sea and their synergistic effect is unknown in most cases, (ii) the biological effects of pollutants are a function of the concentration or contaminant burden and it is difficult to detect the

Table 3
Concentrations of rare earth elements (RRE) and other minor elements (ME) (ng/g wet wt.) detected in liver samples from 5 adult female leatherback sea turtles (*Dermochelys coriacea*) stranded in the Canary Islands.

	Turtle 9	Turtle10	Turtle 11	Turtle 12	Turtle 13	Mean
Rare earth element						
Cerium (Ce)	27.64	1.36	12.66	6.82	5.32	10.76
Dysprosium (Dy)	0.71	0.04	0.15	0.12	0.09	0.22
Erbium (Er)	0.41	0.02	0.07	0.06	0.04	0.12
Europium (Eu)	0.19	0.01	0.06	0.04	0.03	0.07
Gadolinium (Gd)	1.03	0.06	0.28	0.18	0.14	0.34
Holmium (Ho)	0.16	0.01	0.03	0.03	0.02	0.05
Lanthanum (La)	15.68	0.70	6.44	3.37	2.96	5.83
Lutetium (Lu)	0.04	0.001	0.02	0.005	0.003	0.01
Neodymium (Nd)	5.55	0.31	2.12	1.32	0.10	1.88
Praseodymium (Pr)	1.47	0.09	0.58	0.35	0.29	0.55
Samarium (Sm)	0.83	0.05	0.28	0.18	0.13	0.29
Terbium (Tb)	0.13	0.01	0.03	0.02	0.02	0.04
Thulium (Tm)	0.05	0.003	0.01	0.007	0.005	0.01
Ytterbium (Yb)	0.24	0.01	0.05	0.03	0.03	0.07
Yttrium (Y)	6.36	0.32	1.20	0.10	0.66	1.73
Minor element						
Gallium (Ga)	2.86	2.90	1.41	1.17	0.89	1.85
Indium (In)	0.05	0.08	0.05	0.02	0.01	0.04
Niobium (Nb)	0.37	0.44	0.22	0.06	0.12	0.24
Tantalum (Ta)	0.02	0.02	0.01	0.007	0.01	0.01
∑REE-ME	63.79	6.43	25.67	13.89	10.87	24.13

Table 4
Concentrations of persistent organic pollutants (POPs) and polycyclic aromatic hydrocarbons (PAHs) (ng/g wet wt.) detected in liver samples from 5 adult female leatherback sea turtles (*Dermochelys coriacea*) stranded in the Canary Islands.

	Turtle 9	Turtle 10	Turtle 11	Turtle 12	Turtle 13	Mean
POPs[a]						
p,p'-DDD	BDL	0.21	BDL	BDL	BDL	0.04
p,p'-DDE	4.67	11.23	0.32	9.63	4.11	5.99
Hexachlorobenzene	1.17	0.97	BDL	3.21	2.11	1.49
β-hexachlorocyclohexane	BDL	1.07	BDL	BDL	BDL	0.21
PCB 52	BDL	3.13	BDL	BDL	1.15	0.86
PCB 138	1.11	BDL	BDL	BDL	2.13	0.65
PCB 153	2.09	BDL	0.87	BDL	2.11	1.01
PCB 180	4.88	1.17	2.18	0.45	2.91	2.32
BDE 99	BDL	1.23	BDL	BDL	BDL	0.25
PAHs[b]						
Naphthalene	2.63	BDL	BDL	BDL	BDL	0.526
Fluoranthene	BDL	BDL	BDL	13.44	BDL	2.69

BDL: below detection limit.
[a] 23 organochlorine pesticides and 18 polychlorinated biphenyls were analyzed (see Material and methods).
[b] 16 polycyclic aromatic hydrocarbons were analyzed (see Material and methods).

effects if the animals die from another cause, and (iii) experimental studies are especially difficult to carry out in these protected reptiles (Orós et al., 2021).

Our results indicate that Se levels in leatherback turtles stranded in the Canary Islands are higher than those found in leatherbacks from other geographical areas (Table 5) (Davenport and Wrench, 1990; Godley et al., 1998; Perrault, 2012; Poppi et al., 2012). Se is a trace element naturally found in high-protein marine food sources, and it is considered an essential element. However, at high concentrations it can be toxic, and even fatal (Perrault et al., 2011). The studies of Se levels in leatherbacks have been aimed at trying to explain their low hatching and emergence success compared to other sea turtle species (Perrault et al., 2011, 2013; Perrault, 2014). Se serves to detoxify the Hg body burden, and a positive correlation of Se and Se/Hg with leatherback turtle hatching and emergence success has been found (Perrault et al., 2011). Accordingly, we found low Hg levels in our leatherbacks, although these levels were higher than those found in loggerhead sea turtles stranded in the Canary Islands (Torrent et al., 2004). Also, Pb levels found in our leatherback turtles were lower than those found in other studies (Table 5) (Godley et al., 1998; Poppi et al., 2012), including those carried out in the Canary Islands with samples from loggerheads (Torrent et al., 2004).

In our study, As concentrations were higher to those detected in leatherbacks from other geographical areas (Table 5) (Davenport and Wrench, 1990; Godley et al., 1998; Poppi et al., 2012) and similar to the mean As concentration found in loggerheads stranded in the Canary Islands (Torrent et al., 2004). However, whereas hepatic lesions such as severe diffuse vacuolar hepatic degeneration, and multifocal necrotizing hepatitis were reported in three loggerhead turtles stranded in the Canary Islands with high As hepatic concentrations (Torrent et al., 2004), no hepatic lesions attributable to As were found in our leatherbacks.

It is remarkable the high mean Cd concentration (11.73 μg/g) detected in our leatherbacks, especially if compared to other studies (Table 5) (Davenport and Wrench, 1990; Caurant et al., 1999; Poppi et al., 2012). Godley et al. (1998) also reported a high Cd hepatic concentration in a leatherback stranded in United Kingdom. A mean Cd hepatic concentration of 2.53 μg/g was reported in loggerheads stranded in the Canary Islands, without evidence of hepatic lesions (Torrent et al., 2004). Histological lesions attributable to Cd contamination were also not detected in the leatherback turtles in this study.

To the best of our knowledge, there are no reports of exposure to rare earth elements and other minor elements in sea turtles. REEs are a group of metals comprised of 15 lanthanides, yttrium, and scandium, which have been named "the vitamins of modern industry" due to their use in a wide range of industrial processes (Rim, 2016). The consequent generation of huge amounts of electronic waste represents an environmental problem that has been rarely analyzed in wildlife (Censi et al., 2013; Sánchez-Virosta et al., 2020). It is remarkable the high ∑REE-ME concentration (63.79 ng/g) found in the leatherback #9, especially Ce and La concentrations (27.64 and 15.68 ng/g, respectively). In addition, leatherbacks #9, #11, #12, and #13 had higher hepatic Ce concentrations than the maximum levels found in eagle owls (*Bubo bubo*) inhabiting polluted environments in southeastern Spain (Sánchez-Virosta et al., 2020). Several toxicity tests on REEs, mostly Ce, La, and Gd, have been conducted in laboratory animals, although long-term REE exposure studies have not been reported (Rim, 2016). Unfortunately, studies on REE-associated toxicity, especially for marine organisms, are very

Table 5
Concentrations of metals (mean ± SD, range, μg/g wet wt.) detected in liver samples from leatherback sea turtles (*Dermochelys coriacea*) from different geographical areas.

Source	Number of turtles	Location	Se	As	Cd	Pb	Hg
Davenport and Wrench, 1990	1	UK	1.41[a]	0.58[a]	0.22[a]	0.12[a]	0.39[a]
Edmonds and Francesconi, 1994	1	Australia		1.2			
Godley et al., 1998	1	UK	6.5	2.6	28	4.3	0.37
Caurant et al., 1999	18	France (Atlantic)	–	–	6.84 ± 3.66 0.60–14.7	–	–
Perrault, 2012	14	USA (Atlantic)	8.34 ± 1.91 5.77–12.9	–	–	–	0.48 ± 0.38 0.07–1.44
Poppi et al., 2012	1	Italy (Adriatic)	12.57	2.13	5.68	16.37	20.4
This study	5	Canary Islands	17.62 ± 8.11 11.18–29.5	15.73 ± 15.59 1.39–39.20	11.73 ± 8.77 3.78–25.56	0.53 ± 0.46 0.03–1.21	0.37 ± 0.4 0.12–1.08

[a] Concentration in dry weight.

scarce, preventing an adequate comparison. Oral et al. (2010) demonstrated that Ce and La affect sea urchin embryogenesis at concentrations in the micromolar range. We do not know the possible effects on sea turtles, especially in these animals that died from other known causes, but their detection at relevant concentrations for the first time is worrying, possibly adding to the effects caused by other pollutants.

In our survey, organic contaminant hepatic concentrations were low or undetectable. As reported by Mckenzie et al. (1999) when analyzing two leatherbacks stranded in UK, *p,p'*-DDE was present at the greatest concentrations; individual leatherbacks from our study showed higher *p,p'*-DDE concentrations than those (6.5 and 1.7 ng/g wet weight) reported by Mckenzie et al. (1999). According to other surveys in sea turtles, *p,p'*-DDE is the pesticide found in the greatest concentrations due to its highly persistent nature (Monagas et al., 2008; Camacho et al., 2013b).

In our study, the predominant PCB congeners were PCB 180 and PCB 153. Mckenzie et al. (1999) reported PCB 153 as the one detected at the highest values. The only leatherback turtle previously studied in the Canary Islands showed much higher levels of PCBs (251 and 114 ng/g wet weight for PCB 153 and PCB 180, respectively) (Orós et al., 2009). Mean PCB concentrations in liver samples from 30 loggerheads stranded in the Canary Islands were also higher, reaching up to 915 ng/g wet weight for PCB 153 (Orós et al., 2009); although almost all loggerheads with severe septicemia had high levels of PCBs, it was difficult to establish a clear association between PCB concentrations and causes of death because no acute lesions exclusively attributed to PCBs were detected (Orós et al., 2009). In our study, with much lower levels of PCBs, associated acute lesions were not observed either, and chronic effects of PCBs are much difficult to demonstrate.

Camacho et al. (2012) reported highest blood concentrations of fluoranthene in loggerhead turtles stranded due to crude oil ingestion when compared to loggerheads stranded due to other causes. No signs of crude oil ingestion were observed in the leatherback #12, in which fluoranthene was detected (13.44 ng/g wet weight). Godley et al. (1998) reported very low or undetectable PAH concentrations in the liver of a leatherback turtle stranded in UK, with ∑PAH concentrations of 5.5 ng/g wet weight. Attending to the very low degree of biomagnification of PAHs, authors suggest that PAH concentrations in sea turtles are directly related to recent exposure to waters or food contaminated by PAHs (Camacho et al., 2013b). PAHs come from burning fossil fuels, not only from oil spills, and no hepatic lesions attributed to PAHs were detected in the leatherback #12.

As mentioned above, the biological effects of contaminants are a function of the concentration and, although contaminant concentrations measured in our leatherbacks did not produce acute toxic effects, sublethal or chronic effects may be occurring in this species. In addition, (i) there are evidences of maternal transfer of contaminants in this species (Perrault et al., 2011; Stewart et al., 2011), and (ii) physiological parameters in nesting leatherbacks (susceptible to be altered by low concentrations of some contaminants) correlate with hatching and emergence success (Perrault et al., 2012), already the lowest of all those reported for sea turtles.

In conclusion, this survey is the first focused on postmortem studies on leatherbacks stranded in the Canary Islands. Despite the low number of studied turtles in comparison with other species of turtles also analyzed in the Canary Islands, it is remarkable the negative anthropogenic impact, possibly causing the death of 84.625% of the stranded leatherback turtles. Although we found some inorganic elements (Se, As, Cd) at higher hepatic concentrations than those reported for leatherbacks from other geographical areas, no acute lesions were detected. This is also the first report of exposure to rare earth elements and other minor elements in sea turtles, and their detection at relevant concentrations in some of our turtles deserves to be studied in depth. Finally, organic contaminant hepatic concentrations were generally low or undetectable.

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CRediT authorship contribution statement

Jorge Orós: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Visualization, Writing - original draft. **María Camacho:** Formal analysis, Methodology. **Pascual Calabuig:** Methodology, Resources. **Cristian Rial-Berriel:** Formal analysis, Methodology. **Natalia Montesdeoca:** Formal analysis, Investigation. **Soraya Déniz:** Formal analysis, Methodology. **Octavio P. Luzardo:** Investigation, Resources, Writing - original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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