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Short Communication

Marine pollution between gyres: plastic debris in marine turtles and dolphins in French Guiana, Equatorial Atlantic

**Flore Emonnot[1], Blandine Siegrist[1], Amandine Bordin[2,3], Virginie Dos Reis[1,3]
Damien Chevallier[3,4], Yannick Estevez[5] & Benoit de Thoisy[1,3,6]**
[1]Kwata NGO, Cayenne, French Guiana
[2]Réserve Naturelle Nationale de l'Ile du Grand-Connétable, Rémire-Montjoly, French Guiana
[3]Réseau Echouages de Guyane, Cayenne, French Guiana
[4]BOREA, MNHN, CNRS 8067, SU, IRD 207, UCN, UA, Schœlcher, Martinique
[5]UMR EcoFoG, CNRS / AgroParisTech / Cirad / INRA / Université des Antilles
Université de Guyane, Cayenne, French Guiana
[6]Institut Pasteur de la Guyane, Cayenne, French Guiana
Corresponding author: Benoit de Thoisy (benoit@kwata.net)

ABSTRACT. Plastic pollution has not raised much attention until the 2000s, despite being manufactured for about a century. It is now considered one of the most substantial environmental issues. Here we investigate the presence of plastic contamination in 34 stranded animals on the coast of French Guiana, South America. Here we present information highlighting the magnitude of plastic contamination in marine coastal and pelagic tropical marine vertebrates on the Equatorial Atlantic coast of South America. All four species studied here are protected and emblematic vertebrates of the region, with a fragile conservation status, including the olive ridley turtle *Lepidochelys olivacea*, the green turtle *Chelonia mydas* and the leatherback turtle *Dermochelys coriacea*, and a small cetacean, the Guiana dolphin *Sotalia guianensis*. Macroplastics (polypropylene, polyethylene, terephthalate, and polystyrene) were detected in four juvenile green turtles. Microplastics (polypropylene, polyethylene, terephthalate, polystyrene, nylon, acrylates, polycarbonates) were found in 13 individuals (two dolphins, six olive ridleys, four green turtles, and the leatherback turtle). The sampled species have different diets, distribution patterns, and ages, suggesting widespread plastic pollution. The study area is located far from the oceanic gyres. However, they are influenced by the North Brazilian Current, the Amazon River, and other rivers of the Guianas.

Keywords: macroplastics; microplastics; *Lepidochelys*; *Chelonia*; *Dermochelys*; *Sotalia*

The Anthropocene began when human activities became the dominant cause of recent environmental changes (Lewis & Maslin 2015). Its "Great Acceleration" in the 1950s is undoubtedly associated with the industry and development of novel materials, such as plastics, considered a stratigraphic indicator of the Anthropocene (Zalasiewicz et al. 2016). The limited management and overuse of plastics have contaminated the planet, impacting ecological communities, biological dynamics, and processes, likely extending to a global system scale (Villarrubia-Gómez et al. 2018). Macro- and microplastics are widely reported in marine invertebrate species (Foley et al. 2018), fishes, marine birds, mammals, and reptiles (Kuhn & Van Freneker 2020, Lipej et al. 2022, Lopes et al. 2022, So et al. 2022). However, data remains scarce in several geographic areas, including the Equatorial Atlantic (Lopez-Martinez et al. 2021). Long-lived species, par-

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ticularly top predators, can be bio-indicators of habitat contamination (Diepens & Koelmans 2018).

In the western Equatorial Atlantic Ocean, between the North Atlantic and the South Atlantic Gyres, the coastal region of the Guianas extends from the Amazon to Orinoco rivers under the influence of the Amazon plume and the North Brazil Current. This coastal region has physicochemical estuarine-like characteristics (Anthony et al. 2013), directly influencing local marine biota's biodiversity and distribution (Spalding et al. 2007).

French Guiana coastline represents critical nesting sites for three species of marine turtles: the leatherback turtle *Dermochelys coriacea*, the green turtle *Chelonia mydas*, and the olive ridley turtle *Lepidochelys olivacea*. The leatherback is a pelagic species that migrates to the North Atlantic after nesting in French Guiana (Fossette et al. 2008). The olive ridley is a carnivorous species living in coastal waters (Chambault et al. 2017), with rookeries on the Cayenne beaches (Kelle et al. 2009), to then migrate westward to the large river mouth of the Guianas shield (Chambault et al. 2016). The green turtle is also a neritic species, with females nesting in French Guiana to migrate eastward to seagrass beds in the northeast of Brazil (Baudouin et al. 2015). Immature greens are regularly observed in the Guianan littoral rocky areas (UICN France 2017).

Among marine mammals, the Guiana dolphin *Sotalia guianensis* is endemic to the eastern coast of South and Central America, occurring in warm and shallow waters in estuaries and river mouths (De Jesus-Lobo et al. 2021). In French Guiana, it is observed along the entire coast, on a strip area between 30 km offshore and 15 km upstream of the rivers (Bordin et al. 2022).

These four species use the coastal areas of the Guianas, either during the breeding period (female sea turtles) or year-round (Guiana dolphins and immature sea turtles). Those areas also host most of the human activities with potential sources of plastic pollution. These sources are well described for the Caribbean's large marine ecosystem (La Daana et al. 2022), including land-based plastic sources associated with domestic, industrial, agricultural, and recreational activities (e.g. tourism, limited waste management, and nearshore maritime activities). These sources likely threaten contamination for large vertebrates, yet unexplored. Here we aimed to explore macro and microplastic contamination on 34 stranded animals of these four species, to understand how bioecological patterns and geographic distribution of the different life stages of these species may influence the risks of plastic ingestion.

Strandings of 0-5 leatherbacks (nesting females), 15-20 green turtles (nesting females) and 5-20 olive ridleys (nesting females), and 2-6 dolphins are reported for the Guianan coastline annually (Dars et al. 2021). The 34 fresh specimens (26 turtles and 8 dolphins) used in this study were collected between 2012 and 2019. For small specimens, i.e. olive ridley and green turtles, carcasses were returned to the Institut Pasteur de la Guyane, Cayenne Laboratory. The gastrointestinal tract was removed, tied on the field, and collected for further analysis for larger specimens such as the leatherbacks and dolphins. At the laboratory, digestive tracts were separated into three parts (esophagus, stomach, and intestines), and gut contents, if any, were stored individually and kept frozen. Samples used for the research of macro and microplastics are summarized in Table 1. Not all organs were available for each specimen due to some cold storage limitations: suspected degraded and contaminated samples were discarded. Necropsies were conducted under the licenses R03-2018-06-05-008 (BS) and R03-2016-03-31-003 (BdT).

We used the curved carapace length (CCL) as a proxy to determine the life stages of turtles that were not nesting. For green turtles, the CCL threshold was 105 cm (Frazer & Ladner 1986, Bjorndal et al. 1995). We considered the specimen adult for olive ridleys when CCL >60 cm (Reichard 1993, Martinez-Vargas et al. 2022). For the Guiana dolphins, females above 165 cm and males above 170 cm (total body length) were considered mature (Weber & Monteiro-Filho 2002).

During all the laboratory work, a white cotton gown and nitrile gloves were used for each sample to avoid contamination. Glass containers and stainless-steel utensils were cleaned with a 70% ethanol solution before each use. Illustrates the workflow (adapted from Crawford & Quinn 2016) for sample examination and macro- and microplastic analysis.

Macroplastics

Marine debris items were visually separated from diet items, washed *quantum sufficit* with Milli Q water, and dried overnight at 70°C. Polymer composition was identified through Fourier Transform Infrared Spectroscopy (FTIR) (Fig. 1) using a Bruker Tensor 27, equipped with a diamond lens Attenuated Total Reflectance module (ATR). Spectra were compared to Bruker's online database to identify the nature of the polymers. A database recording the spectra of known objects like nitrile gloves, cotton gowns, blue polypropylene caps, fishing lines, Ziploc® used to store organs, and polyethylene garbage bags was created to ensure no contamination occurred during the analysis.

Table 1. Species and organs used for macro- and microplastic screenings. n: number of individuals.

Species (n, gender, and life stage)	Esophagus	Stomach	Intestine	Gut content
Chelonia mydas (n = 13, 6 mature -nesting-females and 7 immatures)	3	5	7	10
Lepidochelys olivacea (n = 12, mature, 11 nesting females and 1 male)	8	10	12	2
Dermochelys coriacea (n = 1, mature -nesting-female)	1	1	1	0
Sotalia guianensis (n = 8, mature, 7 males and 1 female)	0	8	0	0

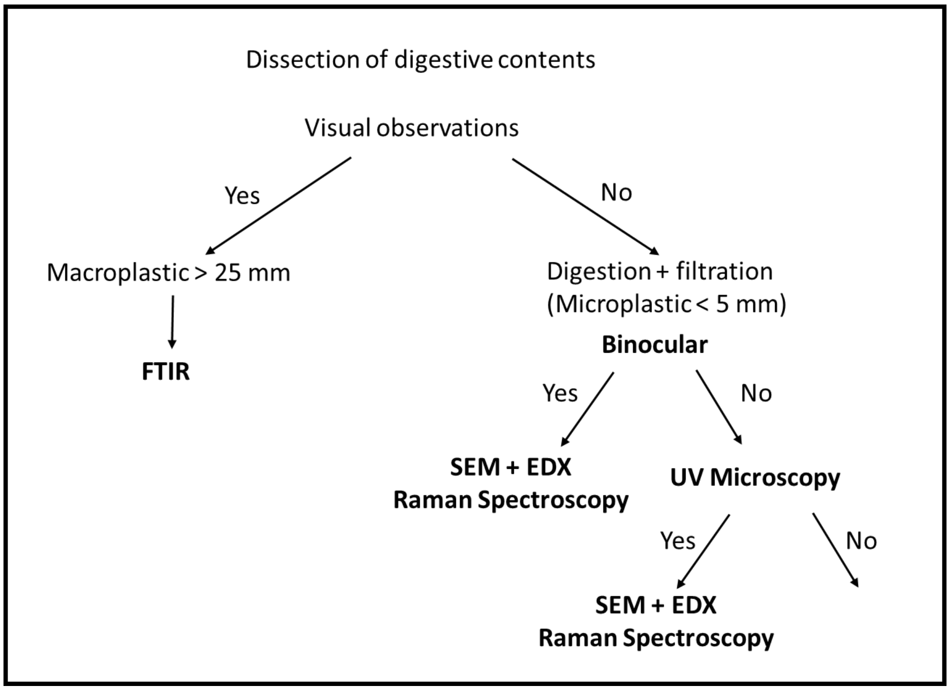

Figure 1. Summary of the workflow and tests carried out. (FTIR: Fourier-Transform Infrared Spectroscopy. SEM + EDX: Scanning Electron Microscope + Energy-Dispersive X-ray spectroscopy.

Microplastics

The content of each organ analyzed was collected within a cabinet biosafety, and a metal spatula was used to scrape off the walls of organs as well. Afterward, all contents were rinsed with a Milli-Q pipette over a 1 mm mesh sieve placed on top of a beaker, using a pestle when the content was too thick. To detect potential contamination during the lab work, we ran blank samples, i.e. a beaker containing Milli-Q, which were left exposed to air during the dissections. Several protocols exist to digest organic matter (Herrera et al. 2018). When the solutions obtained contained organic matter, a digestion process was conducted (Fig. 1). The alkaline digestion method was selected because of its higher efficiency than acid digestion and lower cost than enzymatic digestion (Cole et al. 2014, Herrera et al. 2018). After several preliminary tests (NaOH 10M, NaOH 1M, NaOH 2M, and NaOH 1M + SDS), NaOH 2M was used to lysis the samples for 24-48 h on a hot plate magnetic stirrer set at 70°C. After that, the solutions were filtered using the Buchner method under a fume hood (30 µm nylon filter, checked before use). Followed to the alkaline filtration, when the filter cake had a gelatinous consistency, ultrasonication (10 to 20 min) was performed (Cole et al. 2014), and then the sample was filtered again. Each filter was stored in a glass Petri dish to dry at room temperature, avoiding microplastic contamination. Microplastics were examined with an 20x binocular magnifier and an epifluorescence microscope (Axio Scope A1, Zeiss). The fragments, fibers, or beads suspected of being microplastics were photographed using the microscope

Table 2. Rate of contamination by microplastics (number of individuals contaminated with plastic class / total number of individuals contaminated). n: number of individuals.

Species	*Sotalia guianensis* (n = 8)	*Chelonia mydas* (n = 13)	*Lepidochelys olivacea* (n = 12)	*Dermochelys coriacea* (n = 1)
Polyethylene	1 / 2	1 / 4	2 / 6	
Polypropylene	1 / 2	1 / 4	2 / 6	1 / 1
Polyethylene terephthalate		2 / 4		
Polystyrene		1 / 4		
Nylon		1 / 4		
Acrylates		1 / 4		
Polycarbonates			2 / 6	1 / 1

camera, then mounted on double-sided carbon tape to be later analyzed with an environmental scanning electron microscope (ESEM) equipped with energy-dispersive X-ray spectroscopy (EDS) and facilitate the observation of the particles to be analyzed with the Raman spectrometer (Fig. 1). The microplastics found on the filters previously examined by binocular and microscope measured between 30 µm and 1 mm, making them quite complex to handle. Therefore, it was decided to use a microscope coupled to a Raman (Fig. 1) to detect particles from 1-2 µm in diameter (LabRAM HR Evolution from HORIBA-Jobin-Yvon). The different particles were first located on the carbon tape using the 10x lens, and then the revision was performed with the 50x lens. The supplementary data explains microscopic and chemical analyses in detail (doi: 10.6084/m9.figshare.23297732).

Macroplastics were detected in four immatures of green turtles. Polyethylene was present in all four individuals; polypropylenes and polyethylene terephthalate in three individuals, and polystyrene in two individuals. No macroplastics were found in other species. Microplastics were found in 13 individuals, including two dolphins, six olive ridleys, four green turtles, and the leatherback sampled (Table 2).

Our study's low number of samples requires a cautious interpretation of statistical tests. The rate of microplastic contamination did not differ among species (χ^2 = 4.0585, Monte Carlo P = 0.2385). In marine turtles, from a total of 20 intestines, nine were contaminated, although stomachs (2/24), esophagus (2/12), and gut content (2/12) were less contaminated (χ^2 = 9.1579, Monte Carlo P = 0.02). Among the contaminated green turtles, debris was found in immature individuals only.

Species here studied have regional conservation concerns. Green turtles and leatherbacks are listed as "Vulnerable" in the regional Red List, the olive ridley as "Near Threatened," and the Guiana dolphin as "Endangered" (UICN et al. 2017). The four species were found contaminated, although they have distinct bioecological characteristics, including foraging habits (i.e. carnivorous, herbivorous), habitat distribution (i.e. pelagic, neritic, coastal), and migration patterns (i.e. residents, migrants).

Given our low number of samples, the hypothesis on the origin of the contamination is cautious. Leatherbacks may contaminate either coastal waters during the nesting season or pelagic waters after post-nesting migration in the Northern Atlantic Gyre (Cózar et al. 2014). This plume and the one from the Northern Brazilian Current also influence turtles nesting in French Guiana and resident green turtles. Feeding and inter-nesting areas of olive ridleys are in the plume of the large rivers of the Guiana Shield (Chambault et al. 2016, 2017); thus, the contamination could have been at any place along their distribution. Similarly, the Guiana dolphin is a coastal species. For both carnivorous dolphins and ridleys, their contamination could be from their prey, either crustaceans or fishes (Colman et al. 2014, Rodrigues et al. 2020) in the neritic zone of the Guiana. The observed contamination of the immature green turtles could be related to the overlap of development areas and coastal islands and islets (UICN 2017), where they stay for several years at this life stage (Chambault et al. 2018, Siegwalt et al. 2020). Those areas are exposed to pollution from the French Guiana rivers -although unquantified- and under the influence of the Amazon plume that also contains plastic discharge (Lebreton et al. 2017). The low number of adult green turtles prevents us from interpreting any conclusions.

The impacts of plastic contamination still need to be fully elucidated. Microplastics dramatically modify the richness and diversity of microbiota, with detrimental impacts on health and physiology (Biagi et al. 2021).

Plastics in digestive contents, besides lethal obstruction of digestive tracts by macro-debris, may significantly lower body and health conditions and reduce energy intake for growth, reproduction, and reserve stocking, affecting ontogeny, development, and population dynamics (Marn et al. 2020).

This study highlights a growing concern about the magnitude of plastic contamination in marine ecosystems, stressing the extent of cryptic and unmanaged contamination that impacts key life stages (e.g. foraging, resting, reproductive) of threatened marine vertebrates.

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