SRAS Final Report

Microplastic Characterization in Stomach Contents of the American Crocodile *(Crocodylus acutus*) from Cozumel, Mexico

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Abstract

Microplastic pollution is an emerging threat to coastal ecosystems, yet its occurrence in apex reptilian predators remains poorly documented. This study assessed the presence, abundance, and characteristics of microplastics in stomach contents of the American crocodile (*Crocodylus acutus*) from Laguna Colombia, located in the Punta Sur Ecological Park, Cozumel, Mexico. Stomach flushing was conducted on 18 individuals captured during June 2024. Recovered particles were visually classified using stereomicroscopy and chemically identified by ATR–FTIR spectroscopy. Microplastics were detected in 77.8% of the sampled crocodiles, with abundances ranging from 0 to 39 items per individual (mean ± SD: 10.4 ± 11.7). No significant differences in abundance were observed among sex, age class, body length, or weight. A total of 187 particles were identified, dominated by fragments (70.1%), followed by semirigid foams, films, and fibers. Most particles measured 2–5 mm and were predominantly yellow. Chemical analysis revealed 18 polymer types, with EPDM (ethylene: propylene: diene monomer) as the most frequent. The high diversity of polymers suggests multiple anthropogenic sources associated with maritime and terrestrial activities. These results represent the first record of microplastic ingestion in wild *C. acutus* in Mexico and highlight the species' potential as a sentinel for monitoring plastic pollution in tropical coastal lagoons.

1. Introduction

Marine pollution is one of the most pressing issues in the current eco-environmental crisis. The quantity and variety of pollutants entering the ocean, either directly or indirectly, are intrinsically linked to human activities and societal development. It is estimated that 80% of marine pollution originates from land-based activities, such as tourism, industry, and inadequate waste management (Diez et al., 2019; UNEP, 2016, 2021a). Among the primary pollutants are solid wastes (plastic bags, bottles, food packaging, and discarded fishing gear, flip-flops commonly worn by tourists), which not only affect marine species and biodiversity but also damage the visual appeal of oceans and have negative repercussions on the health of ecosystems, animals, and humans (UNEP, 2021b).

Among emerging pollutants, plastics and microplastics stand out due to their persistence, bioaccumulation potential, and toxicity to organisms. These materials, particularly microplastics, have become a global problem due to their ubiquity and ease of transport through ocean currents (Tambutti et al., 2022; UNEP, 2021a). The Caribbean is the second most plastic-polluted sea on the planet, a situation attributed to five key factors: i) high population growth, ii) urbanization, iii) tourism development, iv) elevated demand and production of plastic products, v) poor or absent government policies for land-use planning, vi) lack of an integrated waste management system and vii) inadequate management of marine ecosystems (Diez et al., 2019)

In this context, the American crocodile (*Crocodylus acutus*) emerges as a key ecological indicator due to its position in the food chain as an apex predator, its wide distribution in coastal ecosystems, and its exposure to both aquatic and terrestrial pollutants. Studying the presence of contaminants in this species not only provides valuable information about their impact on local fauna but also reflects the health status of the ecosystems they inhabit.

The objective of this study was to determine the abundance and distribution of microplastics in stomach flushings of *C. acutus* in the Mexican Caribbean. The results contribute to a better understanding of the effects of plastic pollution on coastal ecosystems and serve as a basis for the development of conservation and sustainable management strategies.

2. Material and Methods

2.1. Study area

The Punta Sur Ecological Park, also known as Laguna Colombia, is one of the most significant protected natural areas on Cozumel Island, located in the state of Quintana Roo, Mexico (INEGI, 2023). Situated at the southernmost tip of the island, this park covers an area of approximately 1,000 hectares and is characterized by a diverse range of ecosystems, including mangroves, wetlands, coastal zones, coral reefs, and pristine beaches (INEGI, 2023). These ecosystems support a rich biodiversity, making the park a critical site for ecological research and conservation efforts (Figure 1).

Laguna Colombia is part of the Cozumel Reef National Park, which was designated a Ramsar site (RAMSAR, 2025), recognizing its status as a wetland of international importance due to its ecological value. It is adjacent to the Mesoamerican Barrier Reef System, the second-largest coral reef system in the world, further highlighting its significance for marine and coastal conservation (WWF, 2025). The park serves as a key habitat for various species, including sea turtles, which are monitored as part of ongoing conservation programs. Additionally, efforts are underway to restore mangrove habitats and promote environmental education among local communities and visitors (INEGI, 2023).

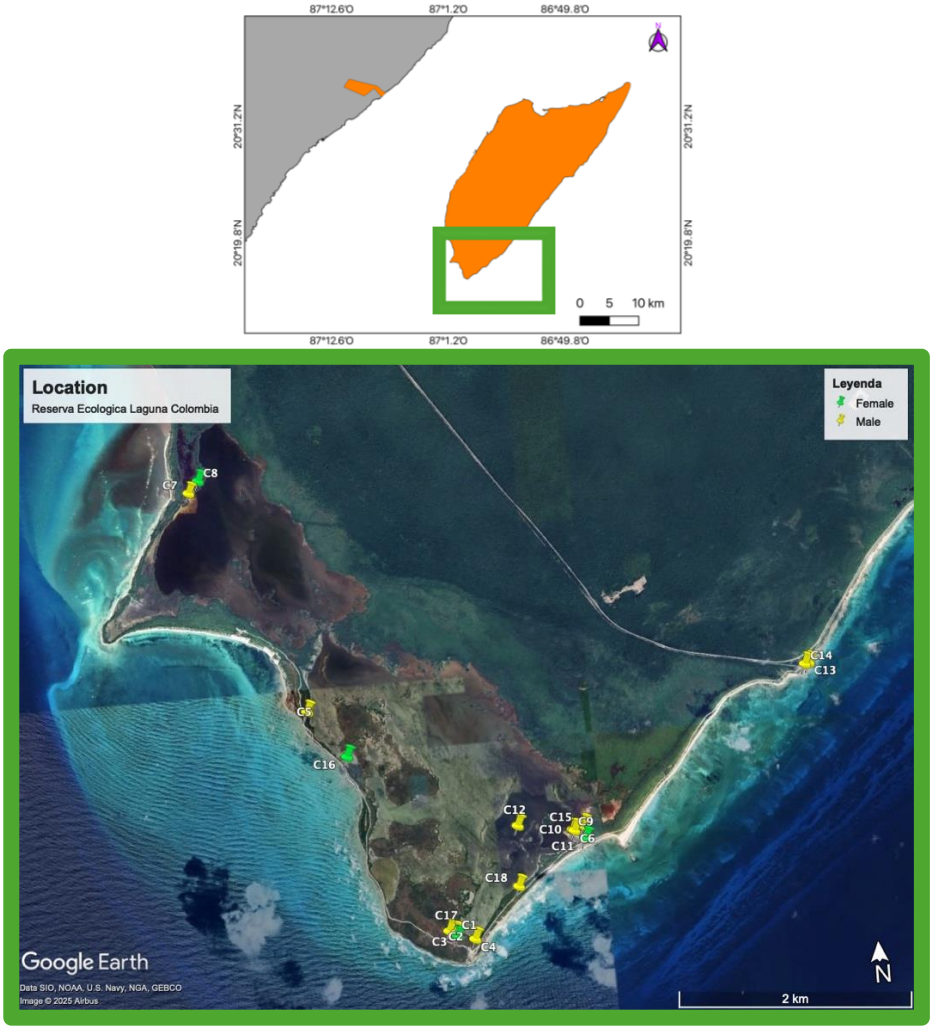


Figure 1. Study area and sampling location.

2.2. Sample collection

Samples were collected in Laguna Colombia, Cozumel, between June 19 to 26, 2024. With the respective permit for the collection of collection by the Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT; Permit No. SPARN/DGVS/08829/24), crocodiles were captured manually or with a lasso, depending on their size, and physically restrained to obtain stomach content samples using the gastric lavage method described by Gonzalez-Jauregui et al. (2019) as briefly outlined below (Figure 2). For each crocodile, morphometric measurements included total length (TL, cm), head length anterior (LHC A, mm), head length dorsal (LHC D, mm), orbital head length (LHO 2, mm), interorbital length (LIO 2, mm), cranial length (LCr, mm), maximum cranial width (ACrMd or CW, mm), head height (mm), tail base perimeter (mm), and body weight (kg) were taken prior to sample collection.

The crocodile then has its jaws manually opened and a short PVC tube was inserted and, secured with red electrical tape. The crocodile's head was then elevated at a 30-degree angle from the longitudinal axis of its body. A spoon lubricated with mineral oil was then inserted through the PVC tube into the stomach (using gentle rotational movements) to help dislodge larger particles during stomach pumping. Once the spoon reached the lower end of the

stomach, a silicone hose, also lubricated with mineral oil, was placed directly into the stomach. A funnel was connected to the other end of the hose and deionized water was used to fill the crocodile's stomach. The tube, spoon, and hose used were selected according to the size of the crocodile. Once the stomach was full of water, the hose was removed, and the crocodile was tilted at a 45-degree angle over a collection bucket fitted with a stainless-steel sieve on top. The procedure was repeated four times until the expelled content consisted only of water meaning the stomach was empty. The samples were collected in glass jars and kept refrigerated during the analysis process.

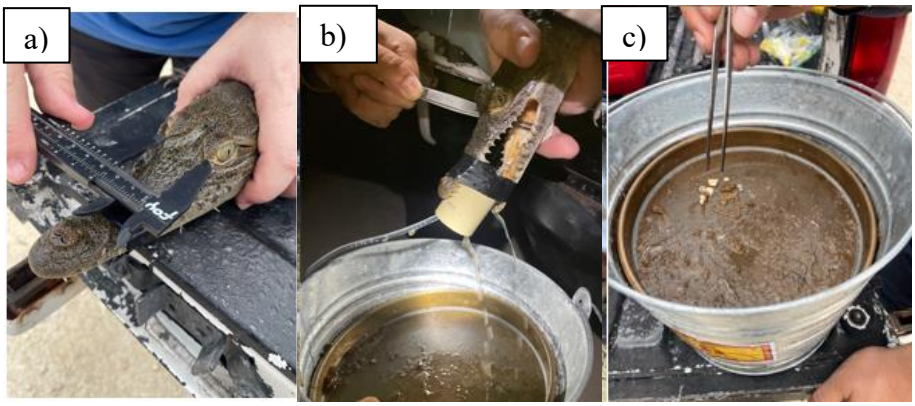


Figure 2. Stomach flushing procedure used to obtain stomach contents from *C. acutus* in Laguna Colombia, Cozumel. a) Crocodile restrained for morphometric measurements. b) Introduction of deionized water into the stomach using a hose connected to a funnel as part of the lavage process. c) Recovery and inspection of expelled stomach contents collected in a sieve (0.100 mm).

2.3. Microplastic Identification

2.3.1. Physical Characterization

Physical characterization of microplastics (MPs) was conducted at the Laboratory of Ecology and Molecular Biology, Universidad de Quintana Roo. We followed the proposed by Escalona-Segura et al. (2022) and Rossi et al. (2024), based on direct observation under a stereomicroscope (Leica® EZ4) to detect suspected microplastic particles. Selected particles were cleaned in 70% alcohol and classified according to shape, color, and size.

2.3.2. Chemical Identification

Each suspected particle was analyzed using Fourier Transform Infrared Spectroscopy (FTIR) with a Nicolet iS5 spectrophotometer (Thermo Fisher Scientific™), operating at a spectral resolution of 4 cm^{-1} with 3–5 scans per sample. Automatic atmospheric adjustment, baseline correction, and ATR correction were applied. Polymer identification was performed by comparing spectra against specialized reference libraries (HR Hummel Polymers and Additives Library, HR Rubber Compounds Library, and Sprouse Polymers ATR Library)

and validated using OMNIC™ Specta software when necessary. Spectral mapping was applied to selected particles, achieving >80% concordance.

2.3.3. Quality Control

Equipment blanks were conducted by pumping distilled water through the gastric lavage system without a crocodile. Blank samples were processed identically to biological samples and examined under a stereomicroscope. No microplastics were detected in blanks. To assess procedural contamination, the lavage hose material was analyzed by ATR-FTIR. Spectra indicated Tygon-type polymers and plasticized elastomers, which did not match EPDM, the most frequently identified polymer in stomach samples, confirming that the equipment was not a source of contamination.

All recovered particles were preserved in 70% alcohol, stored in sealed containers, and refrigerated until analysis. Samples were dried at 40 °C prior to inspection. Strict contamination control protocols were implemented throughout laboratory procedures, including cleaning surfaces and instruments with alcohol, rinsing tools before handling, wearing cotton lab coats and nitrile gloves, minimizing sample exposure time, and storing materials in covered glass Petri dishes.

2.4. Data Analysis

Microplastic abundance was expressed as the number of polymer-confirmed particles per individual (items ind^{-1}) and analyzed in relation to sex and age of *C. acutus*. Data normality was evaluated using the Shapiro–Wilk test. As distributions were non-normal ($p < 0.05$), sex differences were assessed using the Wilcoxon test, and age-group differences using the Kruskal–Wallis test. Spearman's rank correlation was applied to examine associations between microplastic abundance and morphometric variables (body weight and total length). All analyses were conducted in R (R Core Team, 2025) using RStudio v4.5.1 (Posit team, 2025), with statistical significance set at $p < 0.05$. Microplastics were also categorized by shape, color, size, and polymer type. Descriptive statistics were calculated, and results are reported as mean ± standard deviation (SD).

3. Results

A total of 18 crocodiles were captured, comprising 13 males (72.2%) and 5 females (27.8%). Microplastics were detected in 77.8% of individuals (14/18). Total length ranged from 40.3 to 315 cm (mean 131.0 ± 59.8 cm; median 123.3 cm), and body weight ranged from 0.17 to 158 kg (mean 15.0 ± 36.1 kg; median 4.79 kg). The discrepancy between mean and median values indicates a right-skewed distribution influenced by a single large adult specimen. Most individuals were subadults (44.4%) and juveniles (38.9%), while adults and yearlings

accounted for 11.1% and 5.6% of the sample, respectively (Table 1). No mesoplastics (>5 mm) were found in any stomach content samples.

3.1. Abundance of MPs

Microplastic abundance per crocodile varied between 0 and 39 items ind^{-1}, with a median of 6.5 (mean 10.4 ± 11.7; n = 18) (Table 1). Data were not normally distributed (Shapiro–Wilk, p = 0.004). Although females showed a higher median abundance than males, the difference was not statistically significant (Wilcoxon test, p = 0.254). Similarly, no significant differences were detected among age classes (Kruskal–Wallis test, p = 0.597).

Spearman correlation analyses indicated no significant relationships between microplastic abundance and total length (p = 0.531) or body weight (p = 0.669). Overall, microplastic ingestion appeared unrelated to sex, age, or body size.

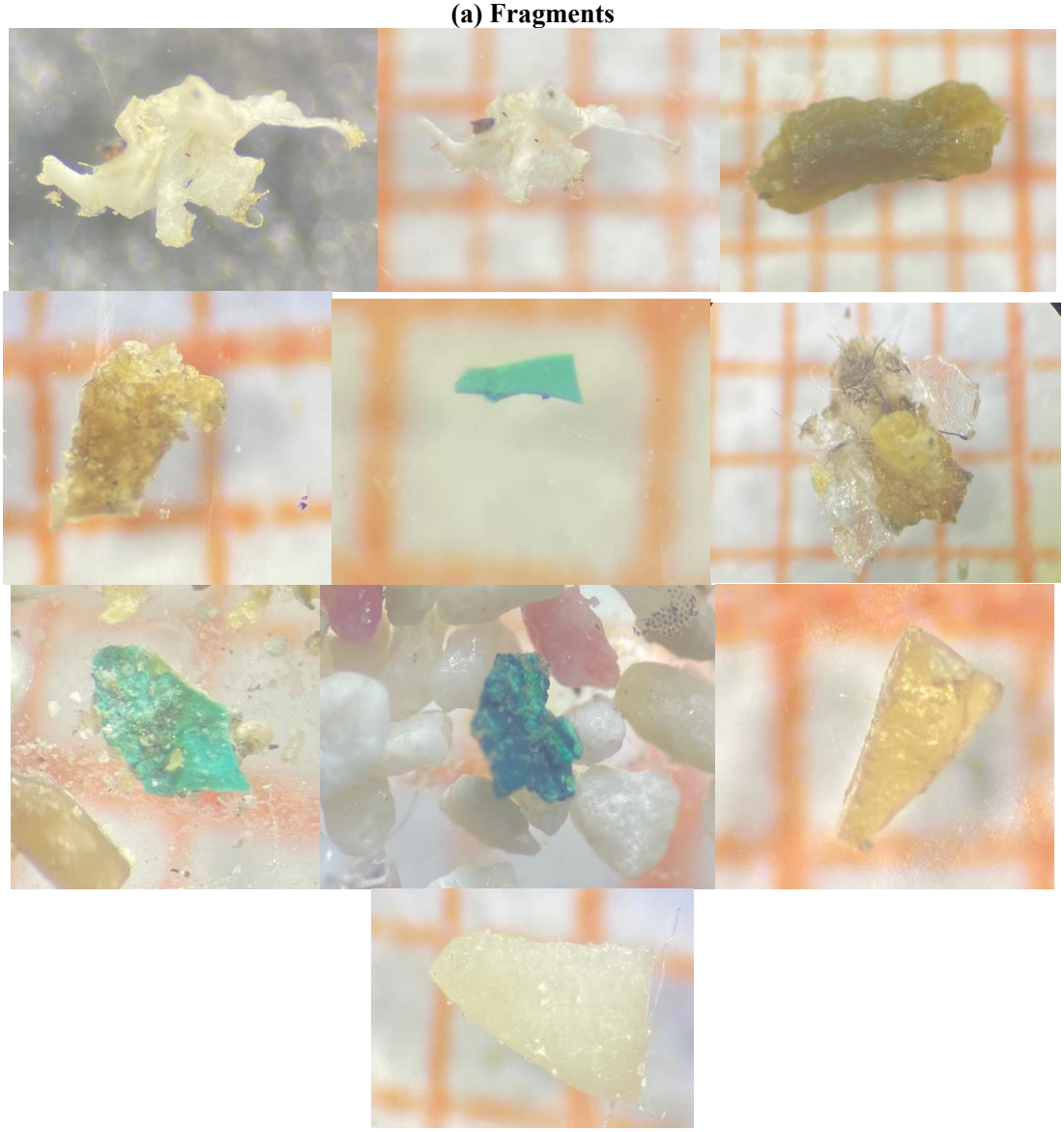
Table 1. Abundance of Mps found in crocodiles from Laguna Colombia, Cozumel

ID crocodile	MPs Abundance (items ind^{-1})	Sex	Total Length (cm)	Weight (kg)	Age Class
1	11	Male	101	2.7	Juvenile
2	24	Female	161	12.31	Subadult
3	21	Female	132	6.23	Subadult
4	39	Male	125.5	4.93	Subadult
5	4	Male	99	2.98	Juvenile
6	13	Male	139	8.11	Subadult
7	8	Male	68.7	0.97	Juvenile
8	33	Female	118.3	4.2	Juvenile
9	12	Male	96	2.45	Juvenile
10	7	Male	315	158	Adult
11	6	Female	186.5	19.8	Adult
12	4	Male	165	14.29	Subadult
13	0	Male	40.3	0.17	Yearling
14	2	Male	62	0.63	Juvenile
15	0	Male	121	4.4	Subadult
16	0	Female	118	4.64	Juvenile
17	0	Male	149.5	9.48	Subadult
18	3	Male	161	13.64	Subadult

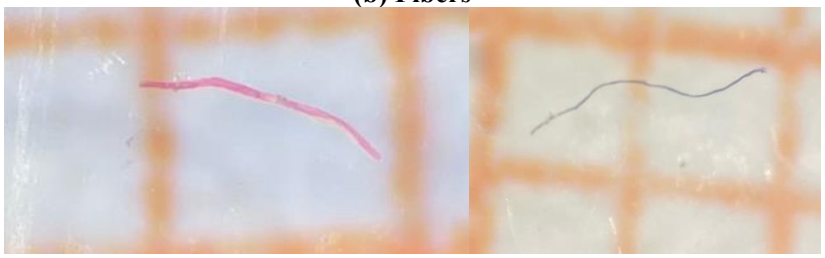
3.1. Characterization of MPs

A total of 187 microplastic particles were recovered from the stomach contents of 14 out of 18 crocodiles. Fragments were the dominant morphotype (70.1%, n = 130), followed by semirigid foams (12.3%, n = 23), films (7.5%, n = 14), and fibers (7.0%, n = 13). Less frequent categories included fiber aggregates (2.7%, n = 5) and spheres (0.5%, n = 1) (Figure 3 and 4a).

(a) Fragments



(b) Fibers





(c) Spherical



(d) Fiber's conglomerate



Figure 3. Shapes of microplastics found in crocodiles.

The microplastics exhibited a variety of colors (Figure 4c), with yellow being the most frequent (49.5%, n= 93), followed by white (26.1%, n= 49), gray (8.5%, n= 16), red (4.3%, n= 8), black (3.7%, n= 7), transparent (3.2%, n= 6), blue (2.1%, n= 4), green (1.1%, n= 2), and both orange and multicolor accounting for 0.5% each (n=1) (Figure 4c). Size analysis (Figure 4b) revealed that microplastics were mostly in the 2-5 mm range (52.9% =99), followed by 1-2 mm (26.7%, n= 50), 0.3-1 mm (14.4%, n= 27) and <0.3 (5.9%, n= 11)

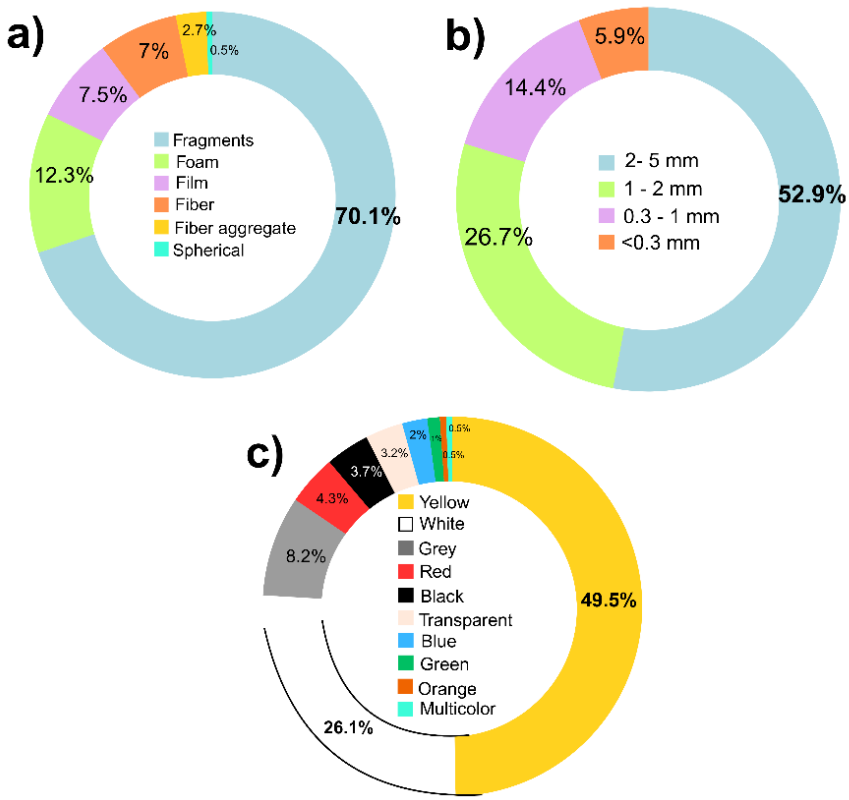


Figure 4. Physical characterization of microplastics (MPs) found in stomach contents of *Crocodylus acutus* (n= 187 particles), in Cozumel Island, Mexico. MPs were classified according to: (a) morphotype, (b) range size, and (c) color.

3.3. Chemical characterization

A total of 187 suspected microplastic particles were visually detected and analyzed by ATR-FTIR. All were successfully identified at the polymer level. Polymer identification was achieved through FTIR-ATR spectral matching with reference libraries, with similarity indices between 70–100%, ensuring reliable classification.

In total, 18 different polymers were identified (Figure 5). The most abundant was ethylene propylene diene monomer (EPDM) (39.0%= 73), followed by polyvinylidene fluoride (PVDF) (13.9%= 26), polyamide (PA6 /PA6.6) (12.8%= 24), polyethylene (PE) (9.1%= 17), poly vinylidene chloride methylacrylate 9:1 (PVDC-MA) (5.3%=10), poly styrene methyl methacrylate acrylonitrile (PS-MMA-AN) (4.3%=8), alkyd resin (3.7%=7), cellophane (3.2%=6), polyamide 6 + polyamide 6.6 (2.1%=4), poly vinyl acetate ethylene (PVAc-E) (1.0%=2), and chlorinated polyvinyl chloride (CI-PVC) (1.0%=2). The remaining polymers, including ethylene vinyl acetate (EVA), poly carbonic acid, polyethylene mix (PE-Carbonix Mix), poly trimellitamide imide (PTI), poly acrylonitrile (PAN), poly ethylacrylate (PEA), and polymer of phthalimide were each represented by a single particle (0.5%, n =1).

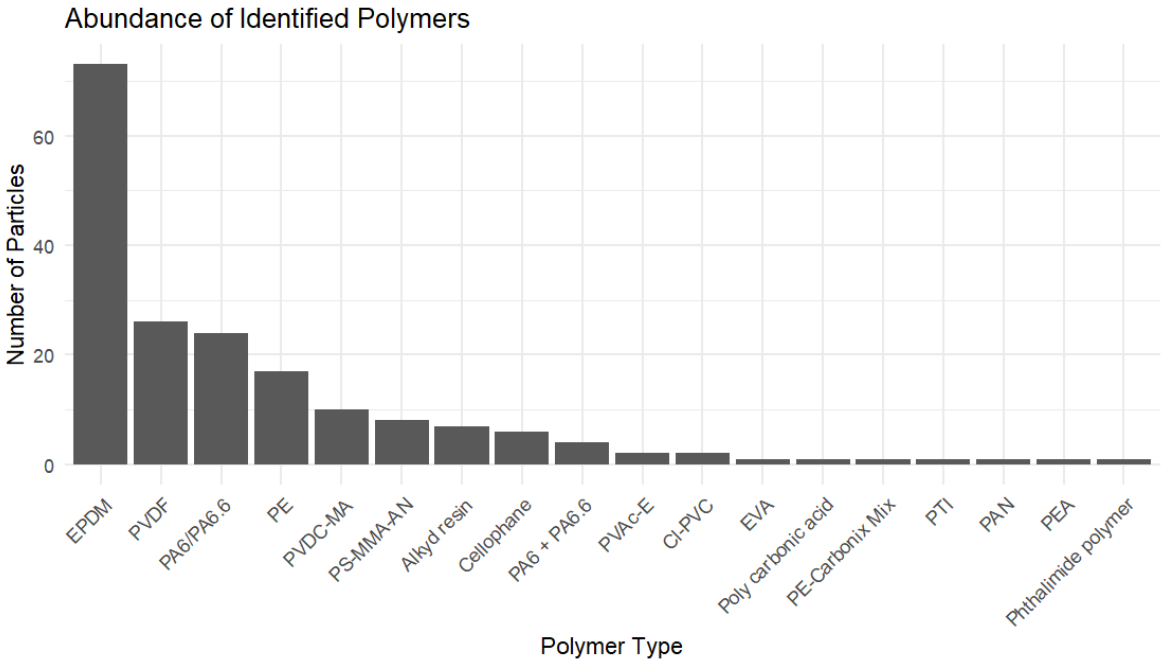


Figure 5. Plastic polymers identified in microplastics found in *C. acutus.*

4. **Discussion**

The detection of microplastics in *C. acutus* inhabiting Laguna Colombia demonstrates that plastic contamination extends into protected coastal lagoon ecosystems and is not restricted to open marine environments (Figure 6). The observed pattern is consistent with chronic, low-level contamination rather than isolated ingestion events. No significant differences in microplastic abundance were found across sex, age class, or body size, indicating relatively homogeneous exposure within the lagoon. The absence of particles in some individuals likely reflects behavioral or spatial variability, or rapid elimination, rather than absence of exposure. Given the limited sample size (n = 18), results should be interpreted cautiously.

The mean abundance recorded (10.4 ± 11.7 items ind^{-1}) is lower than some reports for marine turtles but broadly comparable to values documented in other large coastal reptiles (Ricardes Merino et al., 2022), suggesting similar exposure levels across taxa inhabiting coastal systems. Fragments were the dominant morphotype, consistent with secondary microplastics derived from the breakdown of larger debris (Caro-Martínez et al., 2025). The presence of films and semirigid foams reflects local environmental accumulation patterns. Particle sizes and color diversity indicate multiple anthropogenic sources and environmental weathering rather than laboratory artifacts (de Sá et al., 2018).

High polymer diversity was observed, with EPDM and fluorinated polymers (e.g., PVDF) predominating—materials commonly associated with industrial and maritime activities (Dallaev et al., 2022; Jiang et al., 2024). The detection of coating-related polymers (e.g., alkyd resins, PVDC-based materials) highlights non-conventional sources of microplastic pollution. According to the Polymer Hazard Index (PHI), several identified polymers (e.g., PVC, PVDC, PAN) fall into high-risk categories, indicating potential toxicological concern beyond simple particle presence (Lithner et al., 2011). These findings emphasize that ecological risk depends not only on quantity but also on polymer type and chemical properties.

A potential limitation of the methodology used herein known as the green methodological approach, which avoided chemical digestion, may have underestimated very small or embedded particles. Visual sorting under stereomicroscopy also limits detection of the smallest size fractions. Therefore, reported abundances should be considered conservative estimates. Future research should expand sample size, spatial and temporal coverage, and integrate trophic and long-term monitoring approaches.

As an apex predator, *C. acutus* may integrate contamination across trophic levels, reinforcing its value as a sentinel species for monitoring plastic pollution in tropical lagoons. Chronic exposure in insular systems may have cumulative long-term ecological implications.

5. Conclusions

This study provides the first record of microplastic ingestion in wild American crocodiles in Mexico, confirming plastic exposure in Laguna Colombia, Cozumel. The diversity of particle types and polymers highlights ecosystem permeability to land-based and maritime pollution sources and establishes a baseline for continued monitoring and management strategies in the Mexican Caribbean.



Figure 6. Presence of waste and macroplastics in some areas of Laguna Colombia

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