Title: Blue nests: the use of plastics in the nests of the crested oropendola (*Psarocolius decumanus*) on the Brazilian Amazon coast

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Abstract

Birds have been impacted by plastic pollution via entanglement, accidental ingestion, and exposure to chemical contaminants. However, plastics were also observed as a nesting material for several species. For the first time, we describe the occurrence and composition of plastics in the nests of the crested oropendola (*Psarocolius decumanus*) in three sites on the Amazon coast. Plastics were present in 67% of abandoned, fallen nests. At the mangrove site, all nests contained plastics, while at the grassy clearing and the fishermen's village, plastics were present at 35.3 and 90% of the nests, respectively. Blue fibers and ropes were the main plastics observed, probably derived from discarded fishing gear. Of 79 analyzed fibers, 97.5% were composed of Polyethylene, and 83.5% contained Cobalt Phthalocyanine as an additive. Our results showed a widespread use of plastics by *P. decumanus*, which may increase the exposure of juveniles to potential contaminants.

Keywords: Icteridae; plastic debris; marine litter; fishing gear; ghost fishing; Salinópolis

The Brazilian Amazon coast is a highly dynamic ecosystem, where multiple interactions occur between the great Amazon delta, the Atlantic coast, and the adjacent sea (Nittrouer *et al*., 2021). A myriad of ecosystems coexist in the area, like sandy beaches, rocky outcrops, vegetated dune-beach ridges, mangroves, estuarine channels, and muddy flats (Pedrini *et al.*, 2021). The biota from the Amazon coast is also diversified, where many residential and migratory birds are benefited by the provided ecosystem services (Owuor et al., 2024).

The region bears a diversity of both marine and continental birds (Lees et al., 2014). Such animals are probably already impacted by plastic pollution, since plastic debris and microplastics are widely distributed in the Amazon biome and, particularly on the Amazon coast (Morais *et al*., 2024), posing a potential threat to the local biota (Pegado *et al*., 2018; Morais *et al*., 2020).

Recently, the first official record of synthetic fibers in the structure of nests of the crested oropendola, *Psarocolius decumanu*s (Pallas, 1769), was published by Hoyos et al. (2021). *P. decumanus* occurs from the south of Central America to South America, except in Chile and Uruguay (e.g., Ridgely & Tudor, 2009). The bird inhabits humid forests, mangroves, dry forests, clearings, and agricultural areas (Van Perlo, 2009). Despite the least concerning status, geographic distribution, conservation, and population dynamics in Brazil are controversial and demand a new synthesis (IUCN, 2020; SIBBr, 2020). In the Brazilian Amazon, studies on *P. decumanus* are limited to checklists and geographic distribution (Sanaiotti & Cintra, 2001; Lees et al., 2014) or a few biological and ecological aspects like the mapping of nesting sites (Lopes et al., 2023) and the description of the entomological fauna associated with the nests (Torres, 2001).

Psarocolius decumanus is a tropical icterid bird, with a dark brown color with a yellow tail and yellowish-white beak; the adults measure between 35 and 48 cm (Van Perlo, 2009). The bird is known for its elaborate, long nests, which are suspended from palm leaves or branches of trees and composed of natural fibers from dry leaves, orchid roots, fungi elongated rhizomorphs (Sick, 1984), and even plastics (Hoyos et al., 2021).

Several birds often incorporate synthetic materials into their nests, such as plastic fibers and ropes (Jagiello et al., 2019), a frequently observed behavior in many seabird species (Tavares et al., 2016; Lavers et al., 2013; O'Hanlon et al., 2019; Delgado et al., 2020; among others), but still poorly described for birds with a wider distribution towards coastal and continental areas like *P. decumanus*.

It is suggested that the use of anthropogenic materials in the composition of nests can lead to greater predation, as these nests are more easily detected (Møller, 2017). Additionally, synthetic materials can cause entanglement and death of birds (Blettler & Mitchell, 2021), and they have other detrimental effects on the organism and its offspring (e.g., Teuten et al., 2009). Moreover, the presence of toxic plastic additives and adsorbed contaminants in these materials poses a significant health risk since such contaminants were reported in eggs from seabirds (van der Schyff et al., 2021).

Here, we evaluated for the first time in the scientific literature the types and frequency of plastics from 36 abandoned, fallen nests of the crested oropendola, *Psarocolius decumanus* (Passeriformes: Icteridae) distributed at three sites on the Brazilian Amazon coast during field observations between 2022 and 2023. We also analyzed the chemical composition of a subset of samples to avail the composition and the presence of potential chemical contaminants in these plastic debris.

The area is located on the Atlantic coast of Pará state, which has a strong seasonality marked by the pluviosity regime and the Amazon River plume variability throughout the year. The heterogeneous coast is composed of sandy beaches, rocky outcrops, dunes, and typical coastal vegetation locally described as "restinga," mangroves, tidal channels, and muddy flats (Pedrini *et al*., 2021).

Sampling took place at three distinct sites from the Pará state coast, Brazilian Amazonia (Fig.1). The first is a mangrove patch associated with the Maçarico sandy beach at Salinópolis municipality. The typical mangrove trees were *Avicennia germinans* (L.) L. and *Laguncularia racemosa* (L.) C.F. Gaertn, where discarded fishing nets accumulated on roots (Fig. 2). The second area was a modified grassy clearing with some vacation houses and the presence of palm trees. The area is only a few meters from dune-beach ridges associated with the Farol Velho beach, also located at Salinópolis.

Finally, a fishermen village located at Maiandeua island, inside the Algodoal-Maiandeua Environmental Protection Area (municipality of Maracanã) was sampled. The traditional village has a low density of civil constructions, most of them with vegetated gardens. Mangrove and vegetated dunes are also present around the village. Abandoned and fallen nests of *P. decumanus* were collected by active search in each sampling site. Field sampling and observations were conducted between September 2022 and September 2023. Only abandoned, discarded nests were sampled, individually placed in boxes, and transported to the laboratory.

At the laboratory, each nest was visually inspected for the detection of plastic debris. The presence of plastics was evident in some of the nests due to the large number of plastic fibers and the blue color (Fig. 2). For all the nests, the potential plastic fibers, ropes, and films were separated from the organic matter, with the aid of a stereomicroscope. Particles below 5 mm in length (microplastics) were not analyzed. The isolated plastics were then classified by type and color, according to GESAMP (2019).

To confirm the polymeric nature of the potential plastic fibers, 79 filaments from the three different sites were removed from the nests and analyzed by RAMAN spectroscopy: 13 items from Farol Velho, 35 from the fishermen's village, and 31 from the mangrove patch. The labRAM HR Evolution equipment (HORIBA) was used with lasers of different wavelengths (473, 532, 633, and 785 nm) and a 50x long-range objective (NA=0.55). The resistance of each material was previously tested to obtain the best signal for the spectra without damaging the material. For the final spectrum measurement, the spectral region from 200 to 3200 cm^{-1} was used, allowing observation of the common polymer fingerprint region and the C-H stretching region.

After optimizing parameters (e.g., integration time, number of accumulations, and slit diameter), it was ensured that there was no detector saturation across the entire spectrum collection region, and the signals were typically collected with a total time between one and five minutes. A filter was used to automatically identify and remove any spikes due to cosmic rays by comparing the spectra from different accumulations. A baseline was applied, and a noise filter was used through the micro-Raman software tools (Labspec) or a Matlab® code. The compounds in the spectra were identified using the Knowitall® program's database (Wiley, 2024).

Plastics were observed in 24 of the 36 analyzed nests. All nests from the mangrove area contained plastics (n = 9), classified as fibers, tangled fibers, and ropes. Blue was the dominant color, followed by green, black, and transparent fibers. For the grassy clearing, six of 17 nests contained plastics, and all of them were blue or black fibers on tangled fibers. At the fishermen's village, plastics were present in nine of 10 nests. Fibers, tangled fibers, and ropes were present in the nests. Blue, followed by green were the dominant colors, with rare occurrences of black, white, and transparent fibers. Blue was always the dominant fiber color (Fig. 2, 3).

From the 79 analyzed fibers, 77 were successfully identified as polyethylene, one as polypropylene, and one as cellulose (Fig. 3). Density was determined through RAMAN spectroscopy for 46 fibers, from which 25 were made of high-density, four of intermediate density (0.945) and 17 of low-density polyethylene.

All six identified dyes were classified as organic pigments, which were detected in 75 fibers (94.9%). Among the fibers, 72 (91.1%) contained blue dyes and three (3.8%) contained green dyes. Cobalt Phthalocyanine was the most common dye, present at 66 fibers, followed by Hostasol Green G-K with three occurrences, while Irgazin Blue (Phthalocyanine), Indanthren Dark Blue, and Copper Phthalocyanine occurred in two fibers each. Sepisol Fast Blue occurred at a single fiber (Fig. 3). For a single fiber, two dyes were simultaneously identified: Hostasol Green G-K and Irgazin Blue (Fig. 4). The detected additives are widely used in industrial applications; all of them are dyes for plastics, nylon, cotton, and other materials. Five are known to be toxic for the biota, at least at a low toxicity level (Table 1).

This study provides evidence of the widespread usage of polyethylene fibers by the crested oropendolas (*P. decumanus*) for nest construction along the Brazilian Amazon coast. The likelihood of plastic being used in nest construction depends not only on the abundance of debris near the nest but also on the availability of natural nesting material (Lavers et al., 2013; Witteveen et al., 2017). Consequently, the prevalence of plastics across all three sites (mangrove, grassy clearing, fishermen village) suggests this behavior may be increasingly common and linked to nylon fiber availability in the surrounding environment.

The dominance of blue fibers across all locations suggests a common source, likely linked to discarded fishing gear, already reported for the study site (Martinelli Filho & Monteiro, 2019). This aligns with the observation of accumulated fishing nets in the mangroves where all nests contained plastic fibers (Fig. 2), and all except one nest from the fishermen's village contained the same fibers. The lower proportion of plastics in the nests from the grassy clearing also supports the idea of a lower occurrence of plastic fibers in the environment since the site was dominated by other types of litter and natural fibers.

Seabirds choose nesting materials primarily based on shape and color (Tavares et al., 2019). Studies have already shown that fishing lines and ropes were also among the most prevalent items in brown booby nests (Lavers et al., 2013; Tavares et al., 2016). Our analysis confirmed that polyethylene was the primary polymeric component (97.5%) of the analyzed fibers (Fig. 3). The dominance of high-density polyethylene suggests potential rigidity and durability of these fibers, possibly making them more suitable for nest construction, and an active selection and collection of such fibers by the birds. The choice for blue polyethylene fibers may be determined by the quality of material for nest construction, instead of the color, a hypothesis that demands future tests.

The Brazilian coast is widely impacted by plastic debris (Pegado *et al*., 2024), and some of these display potential hazards due to diverse associated contaminants (Cesar-Ribeiro *et al*., 2017). Moreover, the study site is known to bear moderate quantities of microplastics and fibers, mainly blue ones (Martinelli Filho & Monteiro, 2019). Adsorbed contaminants in microplastics were already reported for the Amazon coast, near the study area. Despite the relatively low concentrations, PAHs like naphthalene, phenanthrene, chrysene, pyrene, and fluoranthene are already adsorbed by microplastics in the region (Branco *et al*., 2023), which poses a potential risk of contamination by ingestion.

In a similar way, the fibers identified here also contain contaminants that are added during the industrial production of the colored nylon nets. Of the six identified dyes, five are known for at least a low toxicity degree in animal models, like rodents and microcrustaceans (*Daphnia magna*), but they have unknown effects on birds.While the range of toxicological effects on birds requires further investigation, previous studies have indicated potential health risks in animals, associated with some of these identified dyes (see Table 1).

The identification of Cobalt Phthalocyanine in a significant portion of the fibers (94.9%) raises concerns about potential contaminant exposure for *P. decumanus*. This dye is listed as hazardous by the 2012 United States Occupational, Safety and Health Administration (OSHA) Hazard Communication Standard. Potential health effects include respiratory sensitization, germ cell mutagenicity, and carcinogenicity. Other identified dyes, such as Indanthren Dark Blue and Copper Phthalocyanine, have also been linked to slight toxicity in rodents and aquatic life (see Table 1).

The potential transfer of contaminants from plastic fibers to the crested oropendola eggs and chicks is a significant concern. Studies have documented the presence of microplastics and associated contaminants in seabird eggs (van der Schyff et al., 2021), highlighting potential pathways for contaminant exposure in birds that utilize plastic as nesting materials.

The presence of tangled fibers and pieces of ropes could also lead to accidental entanglement of chicks or adults (Townsend & Baker, 2014), and such larger debris are also used by the crested oropendola. The ecological implications of incorporating plastics into nests require further study. While potential benefits like increased structural stability may be speculated, the increase in predation risk due to higher nest visibility was already observed (Corrales-Moya et al., 2023; Møller, 2017). At last, the effects of plastic additives on bird's health are yet to be investigated since it may pose an important risk to these birds during different stages of their life cycle.

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Table captions

Table 1: Name, synonyms, molecular formula, uses, toxicity and bibliographical references for the six dyes found in plastic fibers analyzed from nests of the crested oropendola (*Psarocolius decumanus*) in the Brazilian Amazon coast.

Figures captions

Fig. 1. Sampling sites for abandoned nests of the crested oropendola (*P. decumanus*) at Pará state, Brazilian Amazon coast.

Fig. 2. *P. decumanus* (A); An active blue nest (B); *P. decumanus* and nesting activity with incorporation of plastic fibers (C); A piece of entangled, discarded fishing net in the border of the mangrove patch by Maçarico beach (D).

Fig. 3. Color, material (polymeric composition), and additives for the 79 analyzed fibers removed from nests of the crested oropendola (*Psarocolius decumanus*). Transp: transparent; C. Phthal.: Cobalt Phthalocyanine; C. D. Blue: Cibanom dark blue; H. G. G-K: Hostasol green G-K; I. Blue: Irgazin blue; Host. Blue: Hostasol Blue; Ind. Dark Blue: Indanthren dark blue; N. blue: Neozapon blue.

Fig. 4. Examples of color, texture, RAMAN spectra and associated additives from fibers removed from nests of the crested oropendola (*P. decumanus*). A: the single polyethylene containing two different dyes: Polychloro copper phthalocyanine (Hostasol green G-K) and Phthalocyanine (Irgazin blue); B: a low density polyethylene containing Dinaphtho(1,2,3-cd:3',2',1'-lm)perylene-5,10-dione (Cibanon dark blue); C: the single registered polypropylene fiber; D: a high density polyethylene containg Cobalt Phthalocyanine.