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BESIDES MINING, DAMS, NON-NATIVE SPECIES... THE INGESTION OF ANTHROPOGENIC DEBRIS BY FISHES IN THE DOCE RIVER BASIN, SOUTHEASTERN BRAZIL

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Abstract: The Doce River basin is impacted by mining, dams, introduction of non-native species, and other human actions. Here we show an additional threat — the ingestion of anthropogenic objects by the freshwater fishes of the region. We collected 234 specimens at five sites across the Doce River basin. We found that seventeen specimens (7.3% of the total) of five fish species ingested anthropogenic debris (including those from fishing activities). Our findings show the need for new studies, especially to evaluate drifting residues in the main river channel. In addition, our results suggest the need for actions to reduce the pollution and other anthropic impacts in the Rio Doce basin.

Keywords: Fishhook; Fishing; Fishing line; Gum; Microplastic

INTRODUCTION

Mining, construction of dams, and introduction of non-native species seem to be the main negative impacts on the Doce River basin, in Brazil (Latini & Petreri Jr 2004, Barros *et al.* 2012, Fernandes *et al.* 2016, Fragoso-Moura *et al.* 2016, Coelho *et al.* 2018, Salvador *et al.* 2020, Souza *et al.* 2021). For instance, the rupture of a tailing dam in the city of Mariana strongly impacted the ecosystem of the region (Escobar, 2015, Fernandes *et al.* 2016). The mud wave had an acute impact when it reached the river (Fernandes *et al.* 2016) and now represents a chronic problem for aquatic biodiversity. However, additional threats are certainly occurring and need to be documented for the Doce River basin.

Ingestion of anthropogenic debris — *i.e.*, material of human origin such as microplastics, hooks, among others — by freshwater fishes has been documented for numerous hydrographic basins (Norén 2007, Azevedo-Santos *et al.* 2019). In southeastern Brazil, there are reports from

Paraíba do Sul (Lima *et al.* 2021) and the upper Paraná River (*e.g.*, Urbanski *et al.* 2020). However, there is no information about the ingestion of anthropogenic residues by fish assemblages of the Doce River basin. Considering the potential negative effects resulting from this behavior — *e.g.*, contamination (Azevedo-Santos *et al.* 2021) —, this is an important gap in the knowledge for the watershed. Here we report the presence of anthropogenic debris in the stomach contents of fishes from the lower Doce River, in the Espírito Santo State, southeastern Brazil.

MATERIAL AND METHODS

Study area

The Doce River basin — with an area of ~8,350,000 ha — is a large and important drainage in Brazil (Coelho, 2007 *apud* Coelho, 2009). It is important to human populations by providing several ecosystem services (*e.g.*, Viana 2016). Currently, however, it is one of the Brazilian basins most negatively affected by human activities (*e.g.*, Latini & Petreri Jr 2004, Fernandes *et al.* 2016).

The Doce River basin can be regionalized as "upper, middle, and lower" portions (Coelho, 2007 *apud* Coelho, 2009, p. 133). According to Vieira (2010), the upper portion comprises all courses above the mouth of the Matipó River (~19° 53.215'S, 42° 33.129'W). The same author stated that the middle portion is from the confluence of the Matipó and the Doce rivers to Aimorés, in Minas Gerais State, and Baixo Guandu, in Espírito Santo State (~19° 29.485'S, 41° 2.632'W), and the lower portion extends from there to the Atlantic Ocean (~19° 36.519'S, 39° 48.150'W). Our five sampling sites for this study are located in the lower portion of this river basin (Fig. 1; Table 1) and include lotic and lentic ecosystems in three municipalities of the State of Espírito Santo, southeastern Brazil (Table 1).

Methods

Fish were collected in 2021 (January, April, September and November) and 2022 (January, March, and April) with IBAMA permit SISBIO - 64191-12. The specimens were captured with cast nets (40 and 80 mm of mesh - between opposite knots) and gill nets with different mesh sizes (40, 50, 60, and 80mm - between opposite knots). Cast nets were used during the day and gillnets were installed at 4 pm and removed at 8 pm (for each site and month). After sampling: (i) fishes were anesthetized; and (ii) all specimens were submersed in 10% formalin, which was also injected in their abdominal cavity (in order to avoid deterioration of their gut contents).

In the laboratory, each fish was identified to the species level using the literature (*e.g.*, Britski *et al.* 1986, Fink, 1993, Vieira *et al.* 2014, Ota *et al.* 2018). With surgical scissors and scalpels, we opened the abdominal cavity and removed the stomach of the fishes. Contents were transferred to Petri dishes, where the anthropogenic debris (determined visually; *sensu* Norén, 2007) were separated from non-anthropogenic food items (*e.g.*, seed, fish, among others). All procedures (including analysis of the stomachs) occurred with caution to avoid external contamination with anthropogenic particles (*sensu* Silva-Cavalcanti *et al.* 2017). Analysis of the color were carried out with a stereomicroscope (Olympus SZ61) or, when possible, by naked eye. Moore's (2008) particle size classification was adapted by us as follows: micro (0.1 to 0.9 mm), meso (1.0 to 5.0 mm), and macro (greater than 5.0 mm). These sizes are based on the degree of difficulty to see the particle with the naked eye. Measurements were performed along the longest axis of the object.

RESULTS

There were captured 234 fish specimens in the five sampling sites during the studied period. Anthropogenic particles were found in the digestive tract of seventeen individuals (7.3 %) comprising five species: *Metynnis lippincottianus* (Cope 1870), *Oreochromis niloticus* (Linnaeus 1758), *Serrasalmus brandtii* Lütken 1875, *Trachelyopterus striatulus* (Steindachner 1877), *Pygocentrus nattereri* Kner 1858. Of these, *M. lippincottianus* had the largest number of specimens that ingested material of human origin (Table 2).

Individuals of *M. lippincottianus* and *O. niloticus* had fragments in the stomach that are apparently polyamide (Fig. 2a) from nets or other fishing gear. In addition, an individual of the *M. lippincottianus*, captured at S3, had gum in its digestive content (Fig. 2b). Two objects ingested by

Table 1. Sampling sites (for this study) along the lower Doce River basin, Espírito Santo State, Southeastern Brazil.

Site (Abbreviation)	Municipality	Coordinates (long, lat)	Brief description
Site 1 (S1)	Baixo-Guandu	-41.0035, -19.5092	Located in the channel of Doce River, in the Mascarenhas Reservoir, urban area of Baixo-Guandu (Fig. 1b). The region is used for amateur fishing.
Site 2 (S2)	Colatina	-40.4452, -19.4503	Located in the channel of Doce River. Although there is no major urban agglomeration nearby, the landscape of this region is characterized by coffee and banana plantations and sand mining. This stretch of river is near the ES-248 highway, which facilitates access to the river for amateur fishing (Fig. 1c).
Site 3 (S3)	Marilândia	-40.4421, -19.5293	Located in the Óleo Lake. This waterbody is widely used for recreation (fishing, bathing, watercraft) and there are residences and commercial establishments on its shore. Like site 2, this region is also located near the ES-248 highway (Fig. 1c).
Site 4 (S4)	Linhares	-40.2398, -19.4511	Located in the Palmas Lake. This waterbody has a less intense use for recreational activities. It is located in an agricultural landscape where tomato and pepper crops are cultivated (Fig. 1d).
Site 5 (S5)	Linhares	-39.9279, -19.4503	Located in the channel of Doce River. It is in the most downstream portion of the basin and ~ 25 km from the mouth of the Doce River (Fig. 1e). This site is embedded in a landscape matrix predominantly of cocoa cultivation. It has no urban agglomerations or highways in its vicinity. However, the entire basin drains to this point until it reaches the mouth of the Rio Doce.

S. brandtii and *T. striatulus* are very similar, with the exception of the size (Fig. 2c). One specimen of *P. nattereri* ingested a fishhook (Fig. 2d) and four other fragments associated with fishing activities (*e.g.*, Fig. 2e-f).

The colors of the anthropogenic particles ingested by the fish species were brown (2), silver (1), transparent (4), white (1), red (1), purple (1) and blue (7). According to their size, 47.1% of anthropogenic particles may be classified as meso and 52.9 % as macro-debris (Table 2).

DISCUSSION

Most of the species that consumed human debris, with the exception of *Trachelyopterus striatulus*, are non-native to the Rio Doce basin (*sensu* Vieira 2010, Jégu 2003). Studies published (*e.g.*, Ribeiro-Brasil *et al.* 2020, Santos *et al.* 2020, Garcia *et al.* 2020, Oliveira *et al.* 2020, Oliveira *et al.* 2021) show that numerous native freshwater fishes of the ecosystems are able of consuming plastics and other objects of human origin. Thus, we believe that other native species of the Rio Doce — besides *T. striatulus* — are also consuming anthropogenic materials. In addition, it should be considered that, once introduced, non-native species are incorporated into the food chain of other large native predators (*e.g.*, Almeida *et al.* 1997). Thus, non-natives (*e.g.*, *M. lippincottianus*) may transfer the anthropogenic fragments to their possible native predators, such as fish (*e.g.*, *Steindachneridion doceanum* (Eigenmann & Eigenmann 1889), *Hoplias* spp., reptiles [*e.g.*, *Caiman latirostris* (Daudin, 1802)], birds, and semi aquatic mammals from the Doce River basin.

Pygocentrus nattereri has high potential to cut the line with its teeth which can explain the hook in its stomach. Fragments of fishing nets may also be ingested accidentally by *P. nattereri*. This

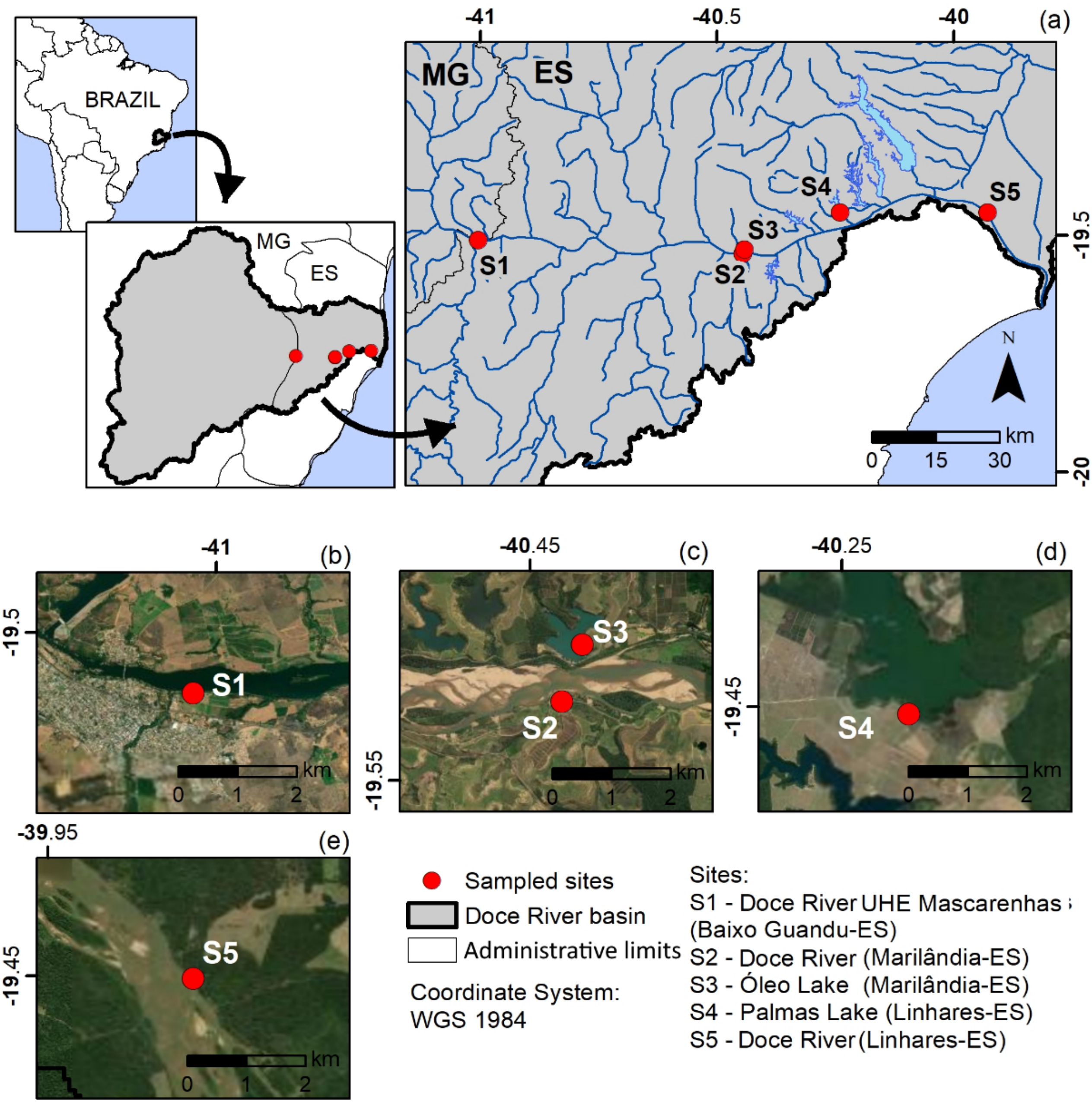

Figure 1. Sampling sites in the lower Doce River basin (a) with details of each (b-e).

species may have attacked fish caught in gillnets and ingested the prey together with net fragments. We also found gum inside the stomach of *Metynnis lippincottianus*. Gum was also found in waterfowl from the Brazilian coast (Brandão *et al.* 2011). We failed to find similar reports from freshwater fishes in the country.

Here we just considered particles of anthropogenic origin (*sensu* Norén, 2007). Different methods can be used to detect tiny synthetic fibers (*e.g.*, Prata *et al.* 2019) as well as to identify their composition (*e.g.*, Lima *et al.* 2021, Andrade *et al.* 2019, Nematollahi *et al.* 2021, Savoca *et al.* 2021), but this was not possible for this study. Therefore, our methods may explain the predominance of macro-debris in our study. In addition, for the same reasons explained above, we believe the number of smaller anthropogenic particles found in stomachs of fish species may be greatly underestimated, and thus additional studies, focusing on the total assemblages, should occur for the Doce River basin.

The color of the material again consists of mostly blue, which converges with the data found in basins in Brazilian waters (Possatto *et al.* 2011, Ramos *et al.* 2012, Dantas *et al.* 2012). An

Table 2. Species and characteristics of anthropogenic debris ingested by fish in the Doce River basin, southeastern Brazil. Taxonomic classification follows Van der Laan *et al.* (2022)

Order/family/species	Site of collection	Number of individuals	Number of debris	Size of fragments (range – in millimeters)	Color of fragments
CHARACIFORMES					
Serrasalmidae					
Metynnis lippincottianus (Cope 1870)**	S1, S3, and S4	8	8	0.95-22.7 mm	3 blues, 1 white, 2 brown, 1 purple, and 1 red
Pygocentrus nattereri Kner 1858**	S1, S2, and S4	5	5	25.0-50.0 mm	4 transparent, and 1 silver
Serrasalmus brandtii Lütken 1875**	S2	1	1	15.2 mm	1 blue
SILURIFORMES					
Auchenipteridae					
Trachelyopterus striatulus (Steindachner 1877)*	S1, and S5	2	2	0.95-18.2 mm	2 blue
CICHLIFORMES					
Cichlidae					
Oreochromis niloticus (Linnaeus 1758)**	S2	1	1	1.0 mm	1 blue

*Native (*sensu* Vieira 2010)
**Non-native (*sensu* Jégu 2003, Vieira 2010)

explanation is that — as we visually identified the material — blue particles were easier to find in gastric contents and determined as from anthropogenic origin. However, a preference of the species from Doce River by blue anthropogenic material, as argued by Urbanski *et al.* (2020) for prochilodontids of the Tietê River basin, cannot be ruled out. This possibility mentioned by us to the Doce River is also supported, for instance, by the findings of Ríos *et al.* (2022), who exposed *Psalidodon eigenmanniorum* (Cope 1894) (Characiformes: Characidae) to fragments of six different colors. These authors found that this characid fish preferred yellow and blue particles. Therefore, this line of investigation is still open for freshwater fishes in the Doce River basin.

A number of negative effects of anthropic materials have been discussed around the world. Synthetic polymers (that include dozens of plastics), for example, can affect freshwater biodiversity in different ways (Jovanović *et al.* 2017, Azevedo-Santos *et al.* 2021). Metals (like the hook found herein) are of relatively less interest in the scientific community when compared to plastics. However, because they are often harder than many synthetic polymers (*e.g.*, fishing lines, styrofoam), they can cause much more intense physical damage to organisms that ingest them (this includes perforations of the digestive tract). The effect may reach other groups (*e.g.*, aquatic mammals) that feed on fishes with hooks in their stomach.

Figure 2. Some examples of anthropogenic debris found in fish species of lower Doce River basin: (a) line and (b) gum found in *Metynnis lippincottianus*; (c) plastic particle ingested by specimen of *Trachelyopterus striatulus*; (d) fishing hook, (e) line, and piece of fishing net from stomachs of specimens of *Pygocentrus nattereri*.

Our results show the need to intensify efforts for new studies of this type in the watershed, especially to include more native species. In addition, the presence of metals from the Fundão collapse (Escobar, 2015, Fernandes *et al.* 2016) or agrochemicals (Reis *et al.* 2017) suggest the need to investigate the possible synergetic effects with solid debris on the biodiversity of the Doce River. Finally, our findings point to the need for actions (*e.g.*, socio-educational measures) to reduce or even eradicate pollution with solid residues in the Doce River basin.

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