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A simple and low-cost methodology for plastic detection in marine fish in Brazil

Metodologia simples e econômica para detecção de plástico em peixes marinhos no Brasil

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ABSTRACT

The existence of plastic in marine environment is a current reality. The increasing presence of this anthropogenic material in the oceans is due to improper waste disposal. The contaminant can sometimes be ingested by marine animals, either primarily or through the consumption of animals that have ingested plastic. The impact of plastic residues ingestion includes gastrointestinal tract obstruction, malnutrition, and even intoxication. Considering that plastic ingestion is a significant cause of death among marine animals, a methodology for quantifying the ingestion of this pollutant is necessary to determine the extent of marine pollution. The methodology presented in the study is simple, low-cost, and reproducible in less equipped laboratories. The technique was conducted for the qualitative identification of plastic presence in the gastrointestinal tract of fish originated from Ubatuba-SP region, demonstrating effectiveness. The sampling included 104 specimens from 15 species and the pollutant was observed in 17.3% of the total.

Keywords: Pollution Effects; Qualitative Technique; Cost-Effectiveness; Plastic;Fish

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RESUMO

A existência de plástico no ambiente marinho é uma realidade atual. A presença desse material nos oceanos se mostra cada vez maior devido ao descarte incorreto do lixo. O contaminante pode por vezes ser ingerido por animais marinhos, seja primariamente ou através do consumo de animais que tenham se alimentado de plástico. O impacto da ingestão de resíduos plásticos inclui a obstrução do trato intestinal, subnutrição e a intoxicação dos animais. Considerando que a ingestão de plástico é uma causa de óbito entre os animais marinhos, mostra-se necessária uma metodologia de quantificação da ingestão desse poluente para determinar a causa de morte do animal e a extensão da poluição marinha. A metodologia apresentada no trabalho é simples, barata e reproduzível em laboratórios menos equipados. A técnica foi utilizada para a identificação qualitativa da presença de plástico no trato gastrointestinal de peixes originários da região de Ubatuba-SP, se mostrando efetiva. A amostragem contou com 104 exemplares e foi observada a presença do poluente em 17,3% do total.

Palavras-chave: Poluição; Técnica Qualitativa; Custo-Benefício; Plástico; Peixe

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INTRODUCTION

Since the past century, when plastic began to be introduced in industrial scale, it is estimated that around 8.3 billion tons of the material have been manufactured and about two-thirds of this total, 6.3 billion tons, have become waste (GEYER; JAMBECK; LAW, 2017). The oceans have become a significant depository for all types of plastic ever manufactured. By the year 2019. The equivalent of 170 trillion plastic litter particles was estimated to be found in the largest ocean basins (ERIKSEN et al., 2023).

The attention should be directed to microplastics, small plastic particles with dimensions smaller than five millimeters (OLIVATTO et al., 2018), which, for the most part, are results of photochemical and abrasive degradation of larger plastics, referred to as secondary microplastics (SOBRAL; FRIAS; MARTINS, 2011). However, they can also be manufactured to be incorporated into formulations of cosmetics and personal hygiene products, referred to as primary microplastics (OLIVATTO et al., 2018).

Microplastic particles can be found extensively in the marine environment; the denser particles tend to deposit in the sediment, while those with lower density tend to float to the water surface (NAKAJIMA, et al., 2019). The small dimensions, attractive coloration, and floating capacity are factors that lead marine animals to ingest the pollutant (OLIVATTO et al., 2018).

Arthropods, filter feeders, echinoderms, zooplankton, and phytoplankton are the most vulnerable individuals to ingest microplastic (LUSHER et al, 2017). However, fish, turtles, sea birds, and marine mammals may also consume the particles directly or through the consumption of other organisms that may have fed on the residue or be contaminated with it, including algae (SETÄLÄ et al., 2018).

Among the deleterious effects of the microplastic consumption include the induction of intern and extern lesions, ulcers, blockage of the gastrointestinal tract that can result in pseudo-satiety and, consequently, physiological stress, changes in feeding patterns and reduction in fertility, fecundity, and progeny survival rates (GUZZETTI et al., 2018; WRIGHT; TROMPSON; GALLOWAY, 2013). Furthermore, digestive systems filled with the residue can have lower nutrient assimilation capacity, consequently reducing the growth rate, energy reserves, the ability to avoid predators, and the capacity to search for food (GUZZETTI et al., 2018). Animals that consume plastic

have lower life expectancies and survival rates, which can, over the long term, lead to the collapse of certain populations (SANTOS, 2006).

Microplastics are also potential vectors of toxic composts for marine animals, including chemical additives added to plastic during its manufacturing process, ambient contaminants that bind to plastic during its degradation in the marine environment (such as pesticides, herbicides, persistent organic pollutants – or POPs, and hydrocarbons) and heavy metals (SOBRAL; FRIAS; MARTINS, 2011). Intoxication can lead to metabolic and reproductive activity alterations, reduced immune response, cellular oxidative stress, and inflammation, effects that occurs directly in the animal that ingested the plastic or can be transferred through the food chain (JOVANOVIĆ, 2017). Intoxication can also induce carcinogenesis (GUZZETI et al., 2018).

Considering the significant ecological impacts of plastic pollution on the marine population, it is important to have methodologies available for the systematic determination of the presence of plastic contamination in the digestive tract of these animals, as Al Mamun et al. (2023), considering not only the health of these individuals but also the fact that they may eventually be consumed by humans.

Currently, there is a wide variety of methodologies available in the market for the identification and quantification of microplastic in various types of samples, ranging from the simple to the most elaborate ones, employed exclusively in scientific experiments. In this context, density separation is one of the most employed methodologies for extracting microplastic from sediments and sand samples (LUSHER et al, 2017). Some studies suggest elutriation as a step to be considered in sample processing, aiming to reduce its mass and the amount of flotation medium required for density separation (CLAESSENS et al., 2013). Nuelle et al. (2014) suggested utilizing the fluidized bed technique for the same purpose as elutriation, decreasing the sample mass for density separation step, conserving the solute.

Coppock et al. (2017) developed a customized equipment made of PVC pipe and a spherical valve, aiming the optimization of density separation, allowing it to be performed in a single step, without the necessity to repeat the process. Nakajima et al. (2019) also developed an improved density separation method based on the Utermöhl's sedimentation chamber concept, reducing the required amount of supersaturated solution,

and minimizing the loss of plastic particles during sample processing, due to the fact the equipment lacks valves and is entirely made of glass.

Felsing et al. (2018) also suggested separating microplastic from dry sediment and sand samples by exploiting the electrostatic behavior of the plastic, a technique that reduces the sample mass to subsequent density separation.

Grbic et al. (2019) suggested the magnetic extraction of smaller microplastic particles, usually not recovered through density separation, by adding hydrophobic iron nanoparticles to the samples, that bind to particulate plastic, allowing its extraction with a magnet. The technique performs less efficiently for particles with a dimension larger than 1 millimeter.

The technique is developed through the dissection of the digestive tract (LUSHER; MCHUGH; THOMPSON, 2013) or the entire animal (LUSHER et al., 2017), followed by visual identification and manual microplastic extraction using a microscope, fine tweezers, and dissecting needle. However, there is a possibility that microplastics may be embedded in the tissue, making them difficult to detect by this method (MILLER; KROON; MOTTI, 2017). Considering this, digestion can be considered as a more reliable methodology, being conducted through the use of acids, alkalis, oxidative agents, and enzymes (LUSHER et al., 2017).

Despite being more reliable, some steps in digestion methodologies, when reproduced in laboratory, have been demonstrated to induce alterations in the integrity of microplastic particles. Claessens et al. (2013) proved that the use of HNO_3 and high temperatures promote the melting of polystyrene spheres and the disintegration of nylon fibers, a prevalent pollutant in all studies. On the other hand, the use of enzymes ensures that there is no microplastic loss during its processing, but it is difficult to reproduce on a large scale and requires a significant amount of time for execution (LUSHER et al., 2017).

A recent study, conducted by Clere et al. (2022) in New Zealand, detected plastic contamination in 75% of 155 samples of commercial marine fish examined, using primarily a Raman spectroscopy, a nondestructive technique to obtain data from molecules. Therefore, despite the innovation of the method, the cost of the equipment required for replication has to be evaluated.

In the majority, the described techniques require expensive equipment and inputs that are difficult to acquire, especially considering the reality of marine life conservation projects in the country. Additionally, systematic evaluations of the presence of plastic and microplastic can help the clear understanding of the availability of pollutants for ingestion and health risks posed to marine animals, allowing also to provide data that reveals the true extent of pollution. In that way, the development of a low-cost, easily replicable, and efficient technique for recovering plastic from organic matter is necessary.

MATERIALS AND METHODS

The methodology used in the present study, adapted from Martins e Carreira (2017) e Masura et al. (2015), is based on the dissolution of all organic matter from the gastrointestinal tract, leaving only the plastic contaminant of the sampling and other inorganic components, as sand.

For method validation, it was used the intestinal content of *Oreochromis niloticus* (Nile tilapia) mixed with a known amount of particulate plastic types, including polyvinyl chloride (PVC), polypropylene (PP), polyethylene terephthalate (PET), high-density polyethylene (PEAD) and ethylene-vinyl acetate (EVA), all with particle dimensions smaller than 5 millimeters, obtained through granulometry by sieving with sieves. 10 grams of organic matter and 100 milligrams of a mixture of plastic (equivalent to 1% of the total sample) were also used and weighed with an analytical balance. The sample was homogenized and transferred to a 100 milliliters capacity beaker.

Subsequently, 15 milliliters of a 30% hydrogen peroxide solution and 15 milliliters of a 0.05 mol aqueous solution of iron II were added to the beaker. Subsequently, the mixture was left on the table at room temperature for 5 minutes. After 5 minutes, a magnetic stirring bar was added to the beaker, and it was transferred to a heating plate with a magnetic stirrer, where it remained under controlled temperature of 75ºC, in a water bath, covered with a watch glass for 30 minutes.

Whenever the reaction seemed to have the potential to exceed the volume of the beaker, distilled water was added to retard the process. At the end of 30 minutes, if the presence of organic material was still observed, an additional 15 milliliters of hydrogen peroxide was added to repeat the process until all visible organic material was eliminated.

After the chemical digestion, the material was filtered with a 300-microns mesh, and subsequently, the remaining residue was passed through a paper filter. In this step, it was already possible to notice the presence of plastic by naked eye in all intentionally contaminated samples. For confirmation of its nature, all recalcitrant residue was disposed on a Petri dish and observed with the aid of a stereomicroscope and sterilized fine-tipped anatomical forceps.

To prevent the contamination of airborne microplastic fiber, appropriately sterilized glassware covered with crepe paper, nitrile gloves, and cotton lab coats were used. All the equipment used in the processing was cleaned before use and covered after processing.

The technique was applied to the fish collected in the city of Ubatuba. 2 trips were made between August and December of 2021 to obtain a total of 104 fish, averaging 52 samples for each trip. The fish were collected from fishermen at the Mercado Municipal de Ubatura-SP and Entreposto de Itaguá. The collected species included mullet (*Mugil liza*), smooth weakfish or cuvier (*Cynoscion leiarchus*), marine catfish *Bagre bagre*, acoupa weakfish (*Cynoscion acoupa*), shorthead drum (*Larimus breviceps*), southern kingcroaker (*Menticirrhus americanus*), puffer fish *Lagocephalus laevigatus*, marine catfish *Cathorops spixii*, cutlassfish (*Trichiurus lepturus*), sciaenid *Paralonchurus brasiliensis*, remo flounder (*Oncopterus darwinii*), pompano (*Trachinotus carolinus*), bluewing searobin (*Prionotus punctatus*), and gray triggerfish (*Balistes capriscus*).

The fish were identified accordingly to the information gathered from local fishermen, weighted, measured, photographed, and had their gastrointestinal tract removed and weighted separately. Using the information, photos, and taxonomic books (FISCHER; PEREIRA; VIEIRA, 2011; MELO et al., 2015), the fish were classified according to their anatomomorphology, including species, feeding habit, mouth type, whether they were demensal or pelargic, and fishing strategy used in their capture. The gastrointestinal tracts were frozen for transportation and then thawed for processing in the laboratories of the University de Marília.

RESULTS AND DISCUSSION

104 fish were collected in 2 sampling periods (08/22/2021 and 12/21/2021), including 5 herbivorous and 99 carnivorous fish. In the herbivorous group, all fish presented contents in their gastrointestinal tract, while among the carnivores, only 54 animals had identifiable food remnants in their tracts. The gastrointestinal tracts were grouped according to their feeding habits into carnivores/ichthyophages and herbivores/filter feeders. The presence or absence of plastic in the tract and the percentage of contaminated fish within the samples were considered, relating the obtained data to the seasons in which the animals were collected.

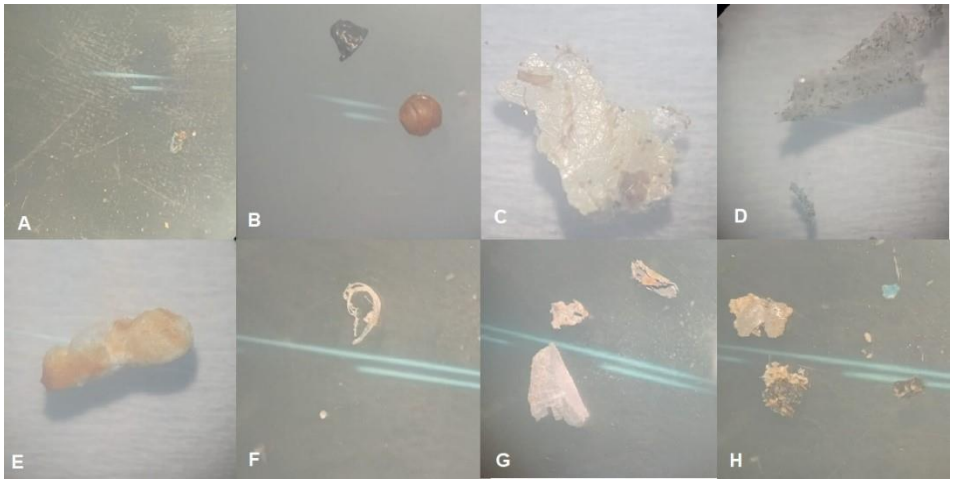
The presence of plastic of various dimensions and colors was observed in 18 of 104 samples collected in the period between August and December. The first collection occurred on august 22nd, which was obtained 58 samples, of which 11 were contaminated by plastic, corresponding to 18.96% of the first collection. Within the first sample, 5 out of 58 specimens were classified as herbivores, and of these, pollutant contamination was observed in 2, both belonging to the same species, *Mugil liza* (mullet), totaling 40% of the herbivore sample. 53 carnivore specimens were collected, and among these, plastic was observed in 9 individuals, of which 16.98% constituted the carnivore sample from the first collection. If considering that in these group 22 animals had no food in their gastrointestinal tract, the percentual of contaminated fish rises to 29.02%. Among the contaminated carnivores, 1 belonged to the species *Lagocephalus laevigatus* of puffer fish, 2 to the species *Bagre bagre* of marine catfish, 4 to *Paralonchurus brasiliensis* of siaenid, 1 to *Menticirrhus americanus* (southern kingcroaker) and 1 to *Micropogonias furnieri* (whitemouth croaker) (Graphic 1).

The second collection occurred on December 21st, of which 46 samples were collected, all related to fish with carnivore/ichthyophagous feeding habits. Among these, 23 fish exhibited an empty gastrointestinal tract with no visible food. 7 samples were contaminated with plastic, corresponding to 15.21% of the total sample from the second collection. However, if considering only the animals that had food in their tract, it can be considered that 30.43% were contaminated with plastic. Of these contaminated animals, 3 belonged to the species *Menticirrhus americanus* (southern kingcroaker) and 4 to *Micropogonias furnieri* (whitemouth croaker) (Graphic 2).

Considering the overall sampling, those contaminated by plastic correspond to 17.3% out of a 104 samples. Considering the feeding habits, contaminated herbivores corresponded to 40% of the herbivore sample, while the contaminated carnivores corresponded to 16.16% of the carnivore sample. However, if considering only the fish that had ingested any type of food, i.e., 54 animals, these percentage rises to 29.63%. Animals from 15 species were collected during the study, and plastic contamination was observed in 6 of them. The species with the highest observed contamination frequency was *Micropogonias furnieri* (whitemouth croaker).

During the study photographs were taken with the aid of a stereomicroscope of 8 from 18 samples contaminated with plastic. In A: blue-colored plastic fiber observed in a specimen of *Mugil liza*. In B: black-colored plastic and an orangish/colored sphere observed in a specimen *of Lagocephalus laevigatus*. In C: white-colored plastic observed in a specimen of *Paralonchurus brasiliensis*. In D: transparent-colored plastic in the center and 1 blue-colored plastic at the lower left corner observed in a specimen of *Mugil liza*. In E: polystyrene/styrofoam observed in a specimen of *Bagre bagre*. In F: transparent fiber observed in a specimen of *Micropogonias furnieri*. In G: plastic in 2 distinguishing colors, gray and white, observed in a specimen of *Micropogonias furnieri*. In H: plastic in 3 distinguishing colors, blue, gray, and black observed in a specimen of *Menticirrhus americanos* (Figure 1).

Figure 1 – Plastic contaminants from the gastrointestinal tract of fish samples observed in the study.



Source: created by the author, 2021.

Neves et al. (2015) conducted a study in Portugal, using visual inspection of the gastrointestinal tract as the pollutant recovery technique, where 19.8% of 263 samples were found to be contaminated with plastic, a similar result found in the present study. The authors also indicated the species *Scomber japonicus* (chub mackerel) as having ingested the highest plastic intake among the 26 species studied and suggested its potential as a monitoring indicator of the presence of plastic in the environment.

Lusher, McHugh e Trompson (2013) led a similar study in the Canal da Mancha, also employing as technique the recuperation of the pollutant by visual inspection of the gastrointestinal tract. In this study, 36.5% of 504 sampled fish were found to be contaminated. The disparity in the results obtained by the authors and those in the present study can be attributed to their larger sample size.

Despite being a small sample and corresponding to a short-term study in the region (between August and December), it was possible to prove the existence of marine plastic pollution and that marine animals are exposed to the pollutant to the extent of consuming it.

CONCLUSIONS

The methodology proved to be efficient, easily executable, accessible, and in a cost-effective way, enabling easy replication. Further studies must be conducted for a quantitative determination of the methodology, as the qualitative analysis has demonstrated its viability.

Through the digestion of organic matter in the gastrointestinal tract of the fish collected in Ubatuba, the technique enabled the detection of the presence of plastic pollution in 18 of 104 specimens, corresponding to 17.3% of the total sample. Considering that organic matter was found in the gastrointestinal tract of only 59 specimens, approximately one-third of the animals were found to be contaminated with plastic during their foraging activities.

Considering the obtained data in the study, it is possible to conclude that marine fish in the studied region are already interacting with extant plastic pollution, evidencing the environmental impact caused by plastic and microplastic in the marine environment.

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