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Microplastics contamination in fish, water, and sediment surrounding Ubatuba beaches, Southeastern Brazil

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Microplastics (MPs) contamination is a well-established impact in oceans, but integrated approaches combining simultaneous analyzes of biotic and abiotic components are scarce. This study addresses this gap, demonstrating *Atherinella brasiliensis* (fish species) ingestion of MPs and comparing with the contaminant presence in water and sediment. Three Ubatuba beaches (exposed, calm and sheltered estuary) were surveyed for fish, water, and sediment components in summer and winter. Environmental data evidenced spatial and seasonal differences (PCA/ANOVA). Presence of synthetic particles (SPs) in fish was high (~38%). Maximum concentrations occurred in the estuary, for water (490 SPs/m^3), and in the exposed beach, for sediment (62 SPs/50g). Fibers format predominated in all components. Fish preference for blue color seems to occur. Significant statistical relationships were determined for fish length and SPs size and between SPs concentrations in water and fish. The chemical identities (µ-FTIR spectra) polypropylene, polyethylene, polyamide, polyester, and cardboard/cellulose predominated. Influences of local hydrodynamics (*e.g.*, SPs sizes) and reduction in tourism during Covid-19 epidemic (*e.g.*, less SPs in summer) are discussed. This study confirms environmental contamination by SPs (mostly MPs) in Ubatuba beaches, affecting fish through direct water column ingestion. Urgent actions from authorities and changes in local user's habits are crucial.

Keywords: *Atherinella brasiliensis*, Cardboard/Cellulose fibers, Sandy beaches, Stomach contents, Synthetic polymers.

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Contaminação por microplásticos (MPs) é um impacto bem estabelecido nos oceanos, embora sejam escassos enfoques combinando análises simultâneas dos componentes bióticos e abióticos. Este estudo foi direcionado para esta lacuna, demonstrando a ingestão de MPs por *Atherinella brasiliensis* (espécie de peixe) e comparando com a presença do contaminante na água e sedimento. Três praias de Ubatuba (exposta, calma e estuário abrigado) foram amostradas para os componentes peixe, água e sedimento durante o verão e o inverno. Dados ambientais evidenciaram diferenças espaciais e sazonais (ACP/ANOVA). A presença de partículas sintéticas (PSs) em peixes foi alta (~38%). Concentrações máximas ocorreram no estuário, para água (490 PSs/m^3), e na praia exposta, para sedimento (62 PSs/50g). O formato fibra predominou em todos os componentes. Preferência dos peixes pela cor azul parece ocorrer. Relações estatísticas significativas foram determinadas para comprimento dos peixes e tamanho das PSs e entre concentrações de PSs na água e nos peixes. As identidades químicas (μ-FTIR spectra), polipropileno, polietileno, poliamida, poliéster e celulose/papelão predominaram. Influências da hidrodinâmica local (*e.g.*, tamanhos das PSs) e da redução do turismo durante a epidemia de Covid-19 (*e.g.*, menos PSs no verão) são discutidas. Este estudo confirma a contaminação por PSs (a maioria MPs) nas praias de Ubatuba, afetando os peixes através da ingestão direta na coluna d'água. Ações urgentes por parte das autoridades e mudanças de hábitos dos usuários locais são cruciais.

Palavras-chave: *Atherinella brasilienesis*, Conteúdo estomacal, Fibras de papelão/celulose, Polímeros sintéticos, Praia arenosas.

INTRODUCTION

The invention of plastic radically changed our consumption behavior and ushered in the era of the disposables. Its low cost, versatility and resistance were decisive for the exponential growth in the manufacture and use (Cole *et al*., 2011). Nevertheless, the massive consumption of these synthetic polymers, associated with the inappropriate disposal, generated an enormous environmental contamination of global proportions, with the accumulation of about 5 million tons in nature, especially in the oceans (Jambeck *et al*., 2015; Silva-Cavalcanti *et al*., 2017; Blettler *et al*., 2018; Olivatto *et al*., 2018). Presently, contamination with plastic residuals is found even in the most isolated environments on Earth, including deserts, top of high mountains, artic snow and deep oceans (Macleod *et al*., 2021). Recent reviews for the marine megafauna show that all groups, including turtles, cetaceans and fishes, are considerable affected by either ingestion or entanglement (Caron *et al*., 2018; Rezania *et al*., 2018; Moore *et al*., 2020; López-Martínez *et al*., 2021; Ugwu *et al*., 2021).

Microplastics (MPs) in the marine environment are even more pervasive than larger plastic waste, reaching, through their ingestion, all levels of the food webs (Wright *et al*., 2013; Ivar do Sul, Costa, 2014). It is widely accepted that MPs correspond to particles < 5mm (Thompson, 2015; Fu *et al*., 2020; Kavya *et al*., 2020; Tirkey, Upadhyay, 2021;

Ugwu *et al*., 2021). Currently, this component, MP, is numerically the most abundant kind of plastic in the oceans, found even in the most remote seas and high depths (> 1,500 m) areas (Cincinelli *et al*., 2017; Barrett *et al*., 2020; Bos *et al*., 2023). The quantities, inevitably, tend to increase as large, single items of plastic end up degrading into millions of smaller fragments (Law, Thompson, 2014). Factors such as sunlight, temperature, ultraviolet radiation, associated to material intrinsic characteristics, oxidize the polymers matrix causing its degradation (Mailhot *et al*., 2000; Wagner *et al*., 2014).

More recently, another concern is related to contamination of synthetic/semi-synthetic fibers, originated from manufacture cardboard, mechanically or chemically treated, which is used for packaging a wide variety of commercial products. These fibers will completely break down into organic matter within months or maybe years. However, they can also be seen as environmental contaminants, due to the presence of harmful mineral oils from the printing inks used on cardboard (Geueke *et al*., 2018; Coltro *et al*., 2021).

Oceans circulation patterns play an important role in the dispersion of pollutants, which can sink rapidly or remain floating for long periods, depending on the polymer densities. Floating marine litter is commonly transported by currents and winds before accumulating in sheltered shore areas or lose buoyancy and sink (Thiel *et al*., 2003). In coastal areas the beach profiles and morphodynamics influence on the MPs abundance, with dissipative conditions favoring higher compared to reflective ones (Tsukada *et al*., 2021).

Due to their ubiquitous distribution, fish are severely affected by plastic residuals, whose ingestion is scientifically demonstrated (Carpenter *et al*., 1972; Hoss, Settler, 1990; Kubota, 1990; Laist, 1997; Boerger *et al*., 2010; Silva-Cavalcanti *et al*., 2017; Blettler *et al*., 2019). Higher frequency of plastic ingestion is reported for sharks and rays (Laist, 1997), but teleost fishes are also susceptible to ingestion of these products (Carpenter *et al*., 1972; Kubota, 1990; Laist, 1997; Boerger *et al*., 2010; Blettler *et al*., 2019).

Fish mainly ingest plastic fragments that are similar in color size and shape to their natural food particles (Carpenter *et al*., 1972; Boerger *et al*., 2010). Recent studies highlight the importance of MPs measurements in biota and abiotic matrices, in order to identify risk assessments and transference routes of these emerging pollutants (and associated adsorbed contaminants) (Karlsson *et al*., 2017; Ferreira *et al*., 2020; Tien *et al*., 2020). In an extensive study carried out in the Mediterranean Sea, Güven *et al*. (2017) found higher contamination rates in fish sampled in area with higher number of plastic particles in water and sediment.

The beach silverside *Atherinella brasiliensis* (Quoy & Gaimard, 1825), selected for our study, is an abundant small coastal fish, with a maximum total length of 160 mm, widely distributed in the Western South Atlantic from Venezuela to southern Brazil (Figueiredo, Menezes, 1978). It is a generalist species, feeding on plant material, small fish, benthic invertebrates, crustaceans and some insects (Contente *et al*., 2011), being one of the most common species in the intertidal zone of sandy beaches of Ubatuba (Gondolo *et al*., 2011). Due to its varied diet, it is predictable that this species is directly affected by synthetic particles occurring in both water column and sediment.

The main objective of this research was to verify the ingestion of MPs by *A. brasiliensis* in the beaches of Ubatuba (Southeast Brazil), in summer and winter seasons. As a popular touristic destination, the quantities of MPs should increase in summer. We also intended to characterize, simultaneously, contamination of this emerging pollutant in water and sediment, and possible relations between MPs concentration if fish and in the abiotic components. The study was carried out in three adjacent beaches in a conspicuous gradient varying from low energy reflective to high energy dissipative, hypothesizing that local hydrodynamics influences the MPs distribution in the abiotic components, with higher concentrations and larger sizes in reflective and the opposite in dissipative.

MATERIAL AND METHODS

Study area. The study area is in the city of Ubatuba, northern coastal area of State of São Paulo, Southeastern Brazil. The sandy beaches studied herein, located in the Ubatuba Bay, were Barra Seca and Perequê-Açú (23º25'04"E/45º02'52"S and 23º25'05"W/45º02'55"S, respectively) (Fig. 1). Due to its larger size and different local hydrodynamics, Perequê-Açú was divided into two sampling areas: Perequê "Brava" (dissipative of high energy) and Perequê "Calma" (moderate hydrodynamics). The beach Barra Seca, East-West oriented, is an estuarine beach, classified as reflective of low energy with an approximate average slope of 5.1° and with a high risk of coastal erosion (Souza, 2012). It is characterized by calm waters, influenced by a freshwater river discharge, much used by local fishermen, moderate frequency of tourists and little urbanization. The Perequê-Açú, an urbanized beach, is oriented towards the NE, is classified as low-energy dissipative with an average slope of the stretch around 4.5° (Souza, 2012), more exposed to the action of the tides and with high risk of coastal erosion.

Sampling methods. Samplings were performed in the intertidal zones of the selected beaches during two fieldworks, one in summer and another in winter, in January and July 2021, respectively. The samples were standardized for the full and new moon periods, when there is higher tidal amplitude, allowing periods of very low tide, when collection of fish was facilitated. Fish were collected with a beach seine (9 m long and 1.5 m high, with internode distance of 5 mm). Fishing effort was performed as many times as necessary to obtain 20 individuals in each sampling area and in each seasonal period. Individuals were euthanized with Eugenol solution and fixed with 4% formaldehyde. Voucher specimens of the examined species are deposited in the scientific collection of the Laboratório de Ictiologia de Sorocaba, São Paulo, Brazil (LISO): LISO 999, LISO 1000, LISO 1001, LISO 1002, LISO 1003, and LISO 1004.

In each of the three sampling areas, five superficial (0 – 1 m) water samples were collected in equidistantly distributed points for synthetic particles analyses. A 10L steel bucket was used for filtering a total volume of 100 L, through a 60 µm plankton net. Subsequently, the samples were stored in glass vials and fixed with 4% formalin. Five superficial sediment samples (0 – 5 cm), obtained at the same points with a steel collecting shovel, were also stored in glass vials to avoid contamination and immediately refrigerated.

FIGURE 1 | The three sampling areas: Barra Seca Beach (red line) and Perequê-Açú Beach (Brava, orange line, and Calma, green line) in Ubatuba, São Paulo State, Brazil. Source: QGIS.

Environmental variables. Simultaneously, at each of these five points, *in situ* measurements of temperature, pH, electrical conductivity, salinity, dissolved oxygen, redox potential, suspended solids and turbidity, were obtained with a multiparameter probe, Horiba U52.

Laboratory fish analyses. Fish were transferred to 70% ethanol solution in the laboratory, measured and weighed. The gastrointestinal contents were removed and preserved in 70% ethanol. Purification was performed overnight at room temperature (20 to 25C°), to eliminate organic residues from the synthetic particle through a digestion with trypsin proteolytic enzyme (SIGMA-ALDRICH), obtained from swine pancreas. The use of the digestive enzyme trypsin is a proved valid method to extract MPs from biological samples, reducing considerable amount of biomass (88%) with no changes in the synthetic polymers (shape, color, size) (Courtene-Jones *et al*., 2017). Posteriorly, the whole sample (solution of the digested material) was analyzed, including the fibers measurements, at a DI–724 stereomicroscope (DIGILAB) and a Zeiss Discovery V20 stereomicroscope attached to a digital camera.

The debris found were classified by size, color and shape (fiber, fragment or pellet). The identification followed visual criteria to determine if the particles are synthetic: 1, absence of cellular and organic structures; 2, identifiable and homogeneous colors; 3, fibers of the same thickness having a three-dimensional bending to exclude the biological origin (Norén, 2007; Hidalgo-Ruz *et al*., 2012). The largest longitudinal dimension was considered to determine the particle size. The number of ingested particles was quantified for each analyzed animal collected in the distinct sampling areas and seasonal periods. Glass vials, covered by aluminum foil, were used for the storage of the fibers found in the samples (Sarijan *et al*., 2018).

In order to avoid contamination, clean cotton aprons and surgical procedure gloves were used during the laboratory procedures, and the work surfaces and utensils used were sterilized, as well as chemical solutions for laboratory (not commercial) analyses (Silva-Cavalcanti *et al*., 2017). To assess external contamination (*i.e*., airborne plastic fibers *sensu* Zhang *et al*., 2020), an open petri dish with ethanol was placed adjacent to the microscope. Eventual contamination was subtracted from the microscopic counts.

Laboratory sediment and water analyses. For water, aliquots from the five collection points of each beach were integrated, totaling three final samples per period. The trypsin enzyme (SIGMA-ALDRICH) was added to the material to digest the organic material and facilitate the identification of the synthetic particles. After digestion, these integrated samples were passed through metal sieves, to avoid plastic contamination, with 0.125, 0.250, 0.5, and 1.0 mm of mesh size (Rowley *et al*., 2020), and the retained material separated per fractions size was recovered for analysis.

To account for debris in the sediment, an aliquot of 25 g (dried sediment) of each individual sample was separated and integrated with the others of the same area, totaling thus three final samples of 125 g. Due to a fungus contamination of the trypsin enzyme available in the laboratory, it was necessary to use a different procedure to remove the excess of organic matter in the sediment samples. Purification was carried out to eliminate organic residues using hydrogen peroxide (H_2O_2) on a heating plate at 60°C, until digestion of most organic materials. Comparative tests with distinct sample purification methods for MPs analyses showed that hydrogen peroxide (15 to 35%) is an efficient way for oxidation of organic material (Schrank *et al*., 2022). Subsequently, the material was filtered through a series of stainless sieves (0.125, 0.18, 0.25, 0.5, and 1.0 mm), followed by washing the sediment with distilled water to remove residues from the solution. After this step, a saturated NaCl solution was added to the remaining sediment for flotation of the synthetic debris, stirring for 1 – 2 min and then waiting for 3 min (Bettler *et al*., 2019). The NaCL solution is a tested method for recovery of PS (polystyrene), PA (polyamide), PP (polypropylene), PVA (polyvinyl alcohol) and PE (polyethylene), also easy to manipulate, having low chemical hazards and allowing the use FTIR/Raman (Miller *et al*., 2017). Finally, the material present in the samples' supernatant was recovered using a sterilized metal spoon and stored for synthetic debris analyses.

The microscopic procedures, and criteria for synthetic particles classification, previously described for fish, were also applied for sediment and water samples. For sediments, results of synthetic particles contamination were expressed per/50 g, and for water, per/m^3. The granulometric analyses of the sediment samples were performed as described by Hakanson, Jansson (1983), after complete drying at 50°C for approximately ten days. A mechanical agitator was used to separate the grains, with sieves of sizes 53 μm (silt and clay), 125 μm (fine sand), 250 μm (medium sand), 0.5 mm (coarse sand) and 1.0 mm (very coarse sand), according to the Wentworth (1922) scale. After separation, the material retained on each sieve was weighed and the percentage of each fraction was determined. For this analysis, only samples collected during summer were used.

Chemical identification of the polymers. A random sample of 65 polymers, retained from the analyses of the distinct compartments (water, sediment and fish contents), was considered for individual chemical identification using μ-FTIR (Fourier Transformed Infrared) spectroscopy (Cincinelli *et al*., 2017; Jung *et al*., 2018; Primpke *et al*., 2018; Tirkey, Upadhyay, 2021). The analyses were performed using a Vertex 70/Bruker spectrometer, wavelength from 4000 to 400 cm^{-1}, 4 cm^{-1} resolution, and 65 scans. The open access reference database Open Specy was used for the matches (https://openanalysis.org/openspecy/) (Cowger *et al*., 2021).

Data analysis. To verify possible relationship of fish biometry and ingestion of SPs we performed correlation analyses (Pearson; $p < 0.05$) (Sigma Plot 14) and linear regression model (PAST) (Hammer *et al*., 2001) considering: data of fish weight and length *versus* number and size of ingested particles (Fig. **S1**). Fish biometric characteristics were tested for statistical differences ANOVA (R Cran Project Software) considering both, seasons and beaches. For each season (summer and winter), the chi-squared test was applied for verification of associations between MPs quantities in water, sediment and fish. Significance of differences were determined using the Monte Carlo post hoc test (PAST) (Hammer *et al*., 2001). The average values (and the standard deviation) were calculated for the environmental variables considering the five distinct points per area in each seasonal period. For a proper characterization of the beach's particularities, an ANOVA (Sigma Plot 14) was performed to verify significant statistical differences ($p < 0.05$), for each measured environmental variable (mean and standard deviation values), per area/period. A principal component analysis (PCA) (Pearson correlation) (Sigma Plot 14), using *in situ* measurements obtained with the water probe, was performed for ordination of the distinct sampling areas. Except for pH, data were previously $\log_{10} +1$ transformed.

RESULTS

Synthetic debris in fish. Considering the whole dataset, 46 of the 120 individuals exhibited synthetic polymers in their gastrointestinal tract, corresponding to a contamination of 38.3%. In summer, except for one fragment, the synthetic particles were fibers. The blue color predominated, followed by transparent (Fig. 2). Size ranged from 0.41 to 5.68 mm (Fig. 3). All ingested particles in winter were fibers, predominately blue, followed by transparent (Fig. 2). Particles sizes ranged between 0.67 and 8.43 mm (Fig. 3). Contamination was higher, in fish from the estuarine Barra Seca beach compared to the others sites, and did not change seasonally, 45% of the individuals in summer and winter as well. In the exposed beaches the proportion of contaminated fish was twice higher in winter (45% in Perequê Brava and 50% in Perequê Calma) compared to summer (25% in both beaches).

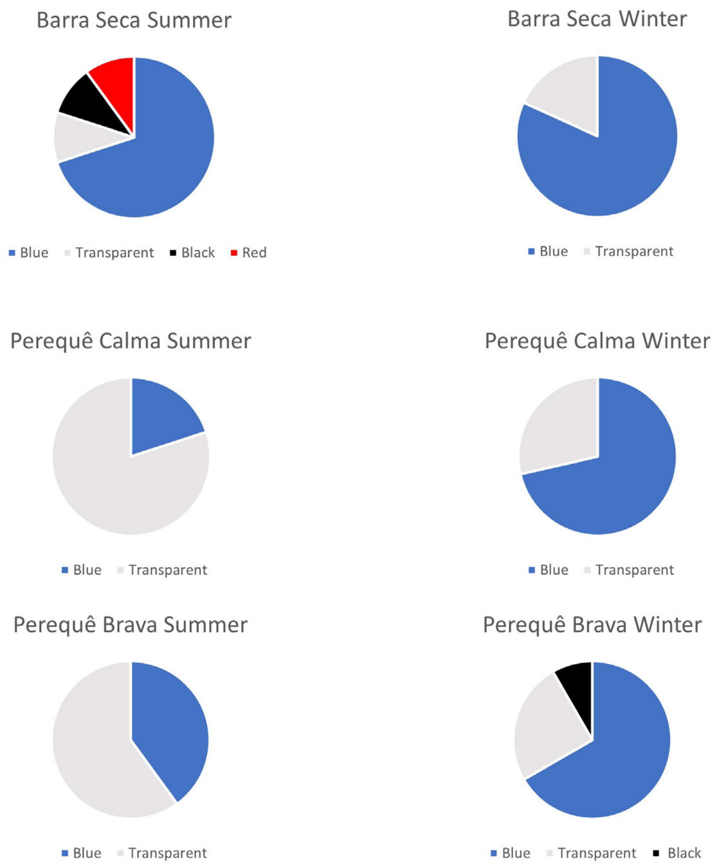

FIGURE 2 | Representation of colors percentage of the synthetic particles in the gastrointestinal tract of *Atherinella brasiliensis*, from sandy beaches of Ubatuba, Brazil (summer, January/2021, and winter, July/2021).

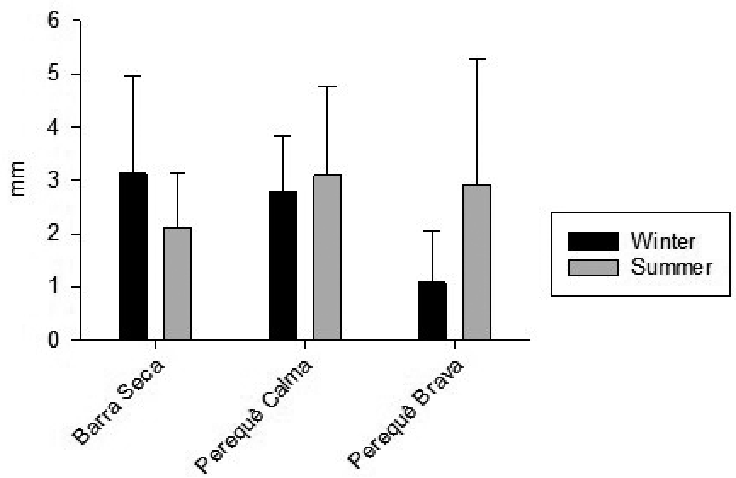

FIGURE 3 | Mean values and standard deviation of the synthetic particles in the gastrointestinal tract of *Atherinella brasiliensis*, from sandy beaches of Ubatuba, Brazil (summer, January/2021, and winter, July/2021).

Fish weight and size were lower in summer, with means of 2.7 g and 60.7 mm, respectively, compared to the winter, with means of 4.8 g and 71.2 mm, respectively. Differences were significant among seasons for size ($F_{1,44} = 4.677$, $p = 0.036$) (Tabs. **S2**, **S3**). The mean size of the ingested synthetic particles was similar between seasons: 2.5 mm in summer and 2.7 mm in winter, considering all analyzed fish. Fish from Barra Seca in summer and Perequê Brava in winter exhibited higher means, 3.1 mm; while lower means were observed for Perequê Calma in summer, 1.06 mm, and Barra Seca in winter, 2.12 mm (Fig. 3). In terms of the correlation and linear regression analyses results for fish biometry (weight and length *versus* MPs number and size, only the one between fish size-polymers size was positively significant ($R^2 = 0.09$, $F_{1,44} = 4.37$, $p = 0.042$) (Fig. **S1**).

Synthetic debris in water. The amount of synthetic debris considerably differed among beaches (Fig. 4A; Tab. **S4**). The maximum value occurred in Barra Seca, 490 particles/m^3 in the summer; and the minimum value occurred in Perequê Brava, 300 particles/m^3, also in summer. Perequê Calma exhibited intermediate values. In terms of particle size, larger dimensions (between 0.5 and 1.0 mm) predominated in Barra Seca; there was a higher variation in Perequê Brava and lower variation in Perequê Calma. Fibers widely predominated among the synthetic particles in the water samples. Fragments were only present in Perequê Calma in small percentages (8 to 9%). Pellets were not observed. In terms of color, transparent synthetic particles prevailed, followed by blue. Red and black also occurred, mainly in the summer.

Synthetic debris in the sediment. Quantities of synthetic debris in the sediment samples varied between 27 particles/50g (Barra Seca, summer) and 62 particles/50g (Perequê Brava, winter) and were more homogeneous in winter compared to summer (Fig. 4B; Tab. **S5**). In Barra Seca and Perequê Brava beaches, amounts of MPs were considerable higher in winter. In Perequê Calma seasonal variation was not evident. Concerning the different sizes, there was no tendency of spatial or temporal variation. As in the water column, most synthetic particles forms were fibers, followed by fragments and no pellets were found. In general, there was the predominance of the transparent color, followed by blue, and a lower frequency of red, black, and green.

Associations between MPs quantities in water, sediment and fish. The Chi squared test allowed for the correlation between water, sediment and fish, for both stations (summer and winter) as well for the sites (Barra Seca, Perequê Calma, Perequê Brava) (Tab. 1). For summer, there was significant ($p < 0.002$) associations between synthetic particles in water and fishes in Barra Seca, and higher proportions of synthetic particles concentrations in the sediment of Perequê Calma beach (Tab. 1). There was no significant associations in the winter.

Chemical identification of the polymers. Considering the 65 polymers that were analyzed for chemical identification, 59 (90.8%) resulted as synthetic polymers, mostly plastic but there was also a considerable proportion of cardboard/cellulose fibers (9% of the identified synthetic polymers). Polypropylene (PP) and polyethylene terephthalate (PET) predominated, corresponding to 50% and 28% of the MPs, respectively,

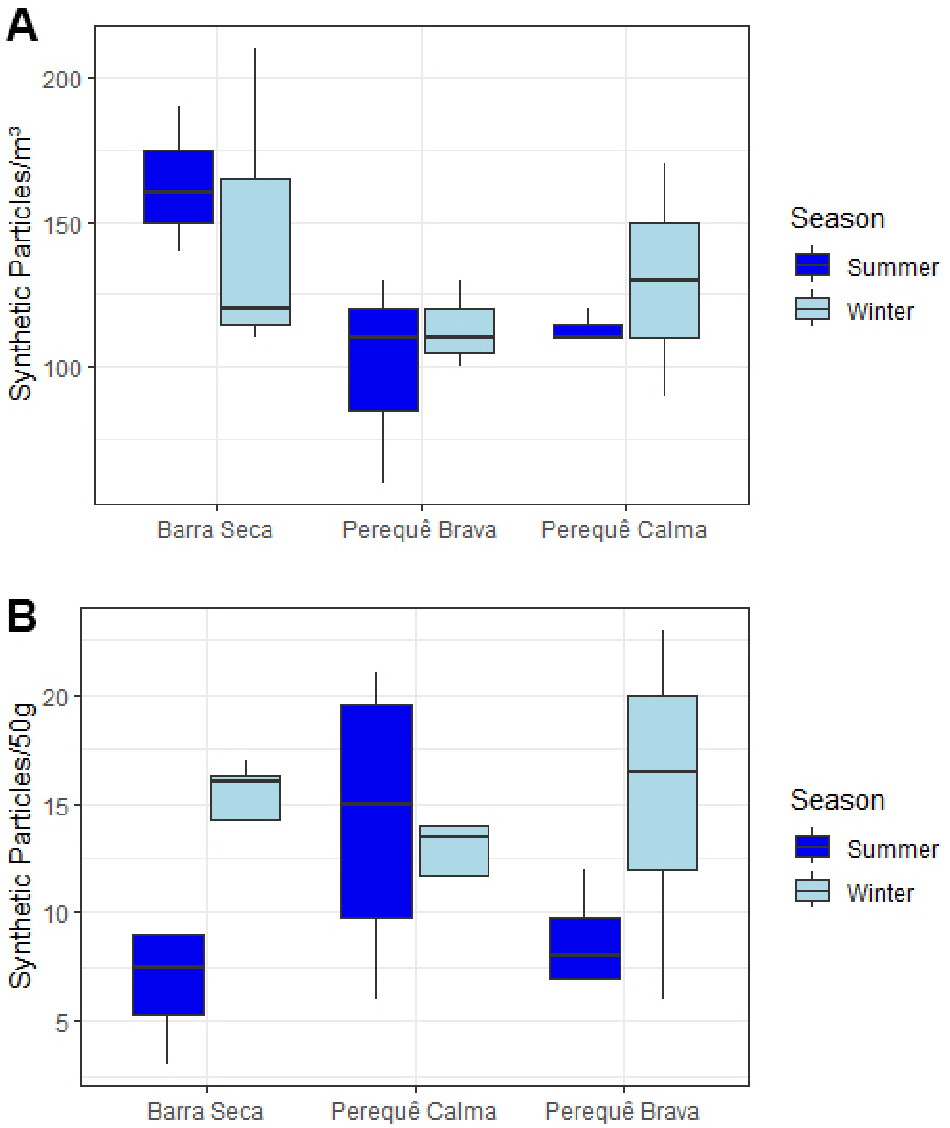

FIGURE 4 | Box Plot representation (median and standard deviation) of synthetic particles quantities in the water (**A**) and sediment (**B**) samples from sandy beaches of Ubatuba, Brazil (summer, January/2021, and winter, July/2021).

TABLE 1 | Association of synthetic polymers concentration among biotic and abiotic components using the chi-squared test. In bold significant results (Monte Carlo *post hoc* significance test) ($p < 0.002$).

Site	Sediment	Water	Fish
Summer			
Barra Seca	0.23	**0.43**	**0.47**
Perequê Brava	0.29	0.27	0.26
Perequê Calma	**0.48**	0.30	0.26
Winter			
Barra Seca	0.34	0.38	0.33
Perequê Brava	0.37	0.29	0.30
Perequê Calma	0.29	0.33	0.37

followed by polyamide (11%) and polyester (11%). Some selected absorbance spectra are presented in the Figs. **S6**, **S7**, **S8**, **S9**, **S10**, **S11**, **S12**, **S13**. It is important to mention that the analyses were inconclusive for part of the results, with low correlation values (r < 0.6) between the obtained and the reference spectra for 28% of the analyzed particles. This fact may be associated to the growth of algae and fungi around the fibers as samples have been stored for several months until the chemical analyses.

Environmental variables. The granulometric analysis showed a high percentage (> 99%) of sand for the three beaches. Percentage of finer particles are higher in Perequê Calma, followed by Barra Seca and Perequê Brava (Tab. 2).

Seven out of nine variables from the water column showed significant statistical difference between the study areas, for both seasonal periods (Tab. **S14**). The only exceptions were the oxygen and the turbidity, for both periods. Higher differences were observed between Perequê Brava and the other two areas, Perequê Calma and Barra Seca (Tab. **S14**). The variance explained by the PCA analysis (Fig. 5; Tab. **S15**) was high, 85.01% (PC1 62.45%, PC2 22.56%), considering the first two components. For both seasonal periods, but especially in summer, the estuarine beach Barra Seca exhibited a more differentiated positioning (higher temperature) compared to the other more exposed sites (Perequê Calma and Perequê Brava) (higher turbidity, salinity/conductivity, redox and oxygen).

TABLE 2 | Granulometric characteristics of the sediment from beaches of Ubatuba, Brazil.

Site	Texture classification	Grain size (mm)	% Sand	% Silt+Clay
Barra Seca	Medium sand	2.32	99.43	0.56
Perequê Calma	Fine sand	1.79	99.52	0.48
Perequê Brava	Medium sand	2.90	99.85	0.14

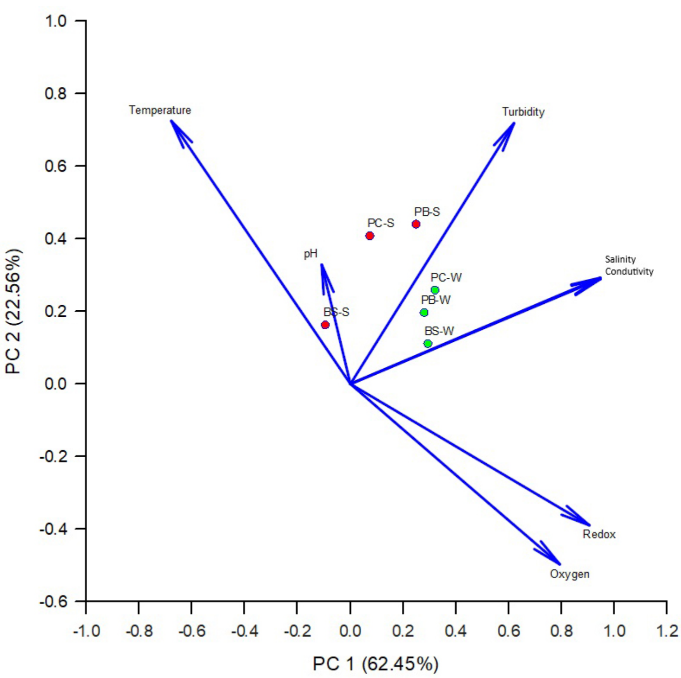

FIGURE 5 | Graphic representation of the Principal Component Analysis (PC1 and PC2) based on environmental variables measured in sandy beaches of Ubatuba, Brazil. Red circles = summer (S); Green circles = winter (W); BS = Barra Seca; PC = Perequê Calma; PB = Perequê Brava.

DISCUSSION

The study evidenced the environmental contamination with synthetic polymers, mostly microplastics, in the sandy beaches of Ubatuba region, SE Brazil. Ingestion of synthetic polymers by *Atherinella brasiliensis* was verified in all sampling sites. The degree of fish contamination by these synthetic polymers reached 38% of the analyzed individuals. This high contamination may be associated with the fact that *A. brasiliensis* is a generalist species, feeding on insects, plant material, crustaceans and other fishes, exhibiting an opportunistic behavior according to the availability of food items (Contente *et al*., 2011; Chagas, Costa Junior, 2013).

A statistical positive correlation between the quantities of SPs in fish and in water was seen for the sheltered (estuarine) beach Barra Seca during summer. In this site, where SPs reached maximum concentrations, approximately half of the individuals collected in both seasons were contaminated. Probably, the differentiated local hydrodynamics – a reflective low energy beach, with more homogenous waters mass, favor the continuous permanence of these emergent contaminants in the water column.

The consumption of synthetic particles by *A. brasiliensis* reinforces the hypothesis that the artificial debris suspended in the water column are ingested together with natural food items (Hoss, Settler, 1990; Browne *et al*., 2010). Synthetic polymers may resemble the alimentary items of the fish species, by either shape, size, or color, influencing their accidental ingestion (Carpenter *et al*., 1972; Boerger *et al*., 2010).

We also found a statistical positive correlation between fish length and size of the ingested particles, what can be considered as an evolutionary trap. In this case, the selection of larger “food” items may result in a maladaptive feeding behavior taking to the ingestion of low-quality food items (Santos *et al*., 2020).

The present study corroborates the general pattern of synthetic debris consumption by aquatic fauna, predominantly transparent and blue particles (Tanaka, Takada, 2016; Güven *et al*., 2017; Kor, Mehdinia, 2020). In our case, colored synthetic particles seem to be important, as most ingested ones were blue, despite the predominance of transparent fibers in the water and sediment samples. This probably occur due to the fish visual orientation during feeding process, showing a higher consumption of items that are more easily discriminated.

In terms of synthetic polymers forms, fibers widely prevailed in the analyzed gastrointestinal tracts (98.1%), as already seen by other authors (Güven *et al*., 2017; Pennino *et al*., 2020). This form of synthetic polymer, which also prevailed in the water and sediment samples, is common, especially in the water column, due to its lower density compared to seawater (Erni-Cassola *et al*., 2019).

The amount of synthetic debris found in the water was similar between the two periods of the year in the most exposed beach (Perequê Brava), and higher in the intermediate beach (Perequê Calma) during winter. The initial hypothesis was that there would be less contamination in the winter, when the tourism is less intense and also due to the fact that the water column becomes more homogeneous with the entrance of coastal waters (Castro Filho *et al*., 1987; Pires-Vanin *et al*., 1993). However, the Covid-19 pandemic restrictions drastically reduced tourism in summer. In case of Barra Seca (reflective beach), the reduction of contamination in winter may be also associated to the seasonal decrease of rains in this season, reducing the litter washout from the adjacent watershed, since rivers are important pathways for introduction of plastic into the oceans (Santos *et al*., 2020).

There was an approximately two-fold increase in the deposition of synthetic polymers in the sediments during winter, compared to summer, in Barra Seca and Perequê Brava. Higher homogeneity of the coastal waters in the winter period could prevents the displacement of bottom synthetic particles. Additionally, higher deposition in Perequê Brava, during the winter, can also be related to the presence of a rocky shore. This geological structure acts as a physical protection of the shoreline and minimizes the transport of particles outside the bay (*e.g*., Pinheiro *et al*., 2019). Perequê Calma beach, with moderate hydrodynamics, showed lower seasonal variability of SPs in the sediment and statistical higher value compared to the other sites in summer. As previously mentioned for water data, the restricted hydrodynamics favors the deposition and permanence of microplastics (Tsukada *et al*., 2021). Higher deposition of synthetic polymers tends to occur in low energy beaches with fine sediments, decreasing exponentially with the increase of the grain size (Vermeiren *et al*., 2021; Wilson *et al*., 2021). There was no statistical association between SPs in sediments and fish ingestion.

Despite the beaches proximity, analyses using water data (PCA, ANOVA) showed significant differences. Distinctiveness among the studied environments was also evidenced through comparisons of the sediment granulometric characteristics. Therefore, small fish, such as the one selected in the study, are exposed to distinct physical and chemical local conditions and, probably, to distinct feeding resources as well. Despite data are limited to only two samplings, variability in weigh and length indicate possible population structuration – more juveniles (smaller individuals) during summer (statistically significant) and also more associated to the estuary. Despite the smaller sizes of fish in Barra Seca, spatial differences were not statistically significant.

Considering the polymers analyzed through μ-FTIR spectroscopy, we can assume that there was a good correspondence between the visual identification and the proportion that resulted as synthetic polymers (90.8%). Among the MPs (91% of the proved synthetic polymers), there was the predominance of polypropylene (PP) (50%) and polyethylene terephthalate (PET) (28%), followed by polyamide (11%) and polyester (11%). The fact that part (9%) of the analyzed polymers correspond to semi-synthetic cardboard cannot be neglected, but is also important to consider that identification through μ-FTIR has limitations, especially for semi-synthetic fibers (Tirkey, Upadhyay, 2021).

The synthetic debris contamination in Ubatuba beaches sediments was mostly represented by fibers (99.3%). The use of meshes ≤ 1 mm may have resulted in the exclusion of particles between 1 to 5 mm, and therefore, of "fragment" plastics common in sediments (Tsukada *et al*., 2021; Wilson *et al*., 2021). Further analyses, including larger fractions, between 1 to 5 mm, are necessary to verify whether MPs of the fragment type also occur, since they were not detected with the methodology applied in the present study.

Our work brought relevant data on the ingestion of synthetic polymers, mostly MPs, by the silverside *Atherinella brasiliensis*, as well as evidenced the contamination in the water column and superficial sediments. There was a positive correspondence (summer season) between SPs in water and fish and between fish size and SPs size. The hypothesis of higher SPs accumulation in summer was not proved. The hypothesis related to the hydrodynamics was partially demonstrated, with higher particles sizes and higher concentrations (summer season) in the reflective low-energy beach only for the water component.

The environmental contamination by plastic polymers in the region of Ubatuba, including the aquatic fauna, is a fact, requiring an intervention of the local authorities to reduce this kind of pervasive pollution. We expect that the results contribute to increase awareness about plastic contamination and help in the development of actions for the prevention and conservation of the coastal aquatic environments. The results can also be used as a basis for future studies in the area (*e.g.*, other fish species and invertebrate's contamination and food web transferences).

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AUTHORS' CONTRIBUTION

Esteban Jorcin Nogueira: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Visualization, Writing-original draft, Writing-review and editing.
Erminio Fernandes: Conceptualization, Methodology, Supervision, Writing-original draft, Writing-review and editing.
Marcos Gomes Nogueira: Formal analysis, Funding acquisition, Investigation, Methodology, Writing-original draft, Writing-review and editing.
Mauricio Cetra: Formal analysis, Methodology, Writing-original draft, Writing-review and editing.
George Mendes Taliaferro Mattox: Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Resources, Supervision, Visualization, Writing-original draft, Writing-review and editing.

ETHICAL STATEMENT

This research is registered at the Ethics Committee on the Use of Animals of Universidade Federal de São Carlos (UFSCar) (CEUA nº 5280201120) and collections were authorized by Instituto Chico Mendes, Ministério do Meio Ambiente (SISBIO 75633–1).

COMPETING INTERESTS

The author declares no competing interests.

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