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Microplastic contamination in natural mussel beds from a Brazilian urbanized coastal region: Rapid evaluation through bioassessment

M.F.M. Santana [a,*], L.G. Ascer [b], M.R. Custódio [b], F.T. Moreira [a], A. Turra [a]

[a] USP — University of São Paulo, Oceanographic Institute (IO), Department of Biological Oceanography, Praça do Oceanográfico, 191, 05508-120, Cidade Universitária, São Paulo, São Paulo, Brazil
[b] USP — University of São Paulo, Bioscience Institute (IB), Department of General Physiology, Rua do Matão, 14, 05508-090, Cidade Universitária, São Paulo, São Paulo, Brazil

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ABSTRACT

Microplastic pollution (particles <5 mm) is a widespread marine threat and a trigger for biological effects, especially if ingested. The mussel *Perna perna*, an important food resource, was used as bioindicator to investigate the presence of microplastic pollution on Santos estuary, the most urbanized area of the coast of São Paulo State, Brazil. A simple and rapid assessment showed that 75% of sampled mussels had ingested microplastics, an issue of human and environmental concern. All sampling points had contaminated mussels and this contamination had no clear pattern of distribution along the estuary. This was the first time that microplastic bioavailability was assessed in nature for the southern hemisphere and that wild *P. perna* was found contaminated with this pollutant. This is an important issue that should be better assessed due to an increase in seafood consumption and culture in Brazil and worldwide.

1. Introduction

Over the last few decades, plastic marine pollution has become ubiquitous across the globe (Barnes et al., 2009), with a current estimate of 5.35 trillion particles (~268,940 tons) floating on sea and ocean surfaces (Eriksen et al., 2014). Large-scale consumer use of plastic products and poor management practices (Jambeck et al., 2015) raise the potential risk of being lost to the environment during production, transportation, use and discard; once in the ocean, they are persistent pollutants, lasting hundreds to thousands of years (Moore, 2008; Barnes et al., 2009). Despite this clear potential for accumulation over time, the fate and consequences of plastic marine pollution are just beginning to be understood.

Among global marine plastic debris, 92.4% of the items are microplastics (Eriksen et al., 2014), particles with less than 5 mm diameter (Arthur et al., 2009). These microplastics could either be intentionally produced within this size range (primary microplastics) or originate from the fragmentation of larger plastic products (secondary microplastics) (Andrady, 2011; GESAMP, 2015). Microplastics are suggested to pose a special threat to marine ecosystems due to their high bioavailability, persistence, and capacity to adsorb and to be a vector of toxic substances to marine biota (Mato et al., 2001; Moore, 2008; Turra et al., 2014). Their small size makes them available for ingestion by a large number of organisms, including a variety of small invertebrates such as zooplankton (Cole et al., 2013), polychaetes (Besseling et al., 2013), bivalves (Browne et al., 2008; Van Cauwenberghe and Janssen, 2014), ascidians (unpublished data), echinoderms (Graham and Thompson, 2009) and sponges (unpublished data). As a consequence, physiological disturbances can occur, already described under laboratory conditions for some marine species (von Moos et al., 2012; Browne et al., 2013; Besseling et al., 2013; Rochman et al., 2014).

Microplastic ingestion by different marine groups and species also made this a plausible pathway for microplastics' transition among marine compartments (e.g. water column and bottom — Eriksen et al., 2014). Microplastic uptake could be responsible for plastic transference from the sea surface to the water column and sea bottom (via plastic rejection as feces and marine snow, Wright et al., 2013a), or to the trophic chains (via ingestion of contaminated prey by higher trophic levels (Murray and Cowie, 2011; Farrell and Nelson, 2013; Setälä et al., 2014; Santana et al., submitted for publication-a), broadening the risks of microplastic pollution to a wide range of marine organisms and ecosystems.

About eighty percent of plastics present in marine systems originate from land-based activities (Andrady, 2011). Therefore, densely urbanized coastal areas are both great sources and sinks of microplastics. Worldwide coastal populations contribute marine debris (including plastics) either through litter or inadequate disposal of wastes that eventually enter the ocean via rivers, wastewater outflows, etc. (Jambeck et al., 2015). Fifty percent of primary microplastics produced in the USA and used in cosmetics products, for instance, were

* Corresponding author.
E-mail address: marina.ferreira.santana@usp.br (M.F.M. Santana).

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estimated to pass through sewage treatment and reach marine environments (Gouin et al., 2011). Browne et al. (2011) reported eighteen shorelines along six different continents as contaminated with microplastics and found a positive relationship among these particles' abundances and densely populated areas, suggesting a high relevance of coastal cities to the input of microplastic marine pollution. Microplastics have also been reported in estuaries and sandy beaches all over the world (e.g. Cole et al., 2011; Lima et al., 2014; Lee et al., 2013; Turra et al., 2014; Vedolin, 2014; Gallagher et al., 2015). Coastal areas contain a wide variety of ecosystems (e.g. mangrove forests, estuaries, beaches and coral reef systems), many of them highly diverse and responsible for supporting different goods and services (such as food and the biodiversity itself — Martínez et al., 2007), microplastic input and the resulting impact should be considered an important issue to be assessed.

For humans, the direct risks brought by microplastic marine pollution are associated with their bioavailability to food resources, becoming a matter of food safety. A large proportion of fisheries, shellfisheries and aquaculture systems are concentrated either in or near coastal regions, which makes microplastics another worrying contaminant for human health beyond those already well known, such as persistent organic pollutants (POPs) and metals. Recent studies addressed the contamination of commercial organisms in nature (Lusher et al., 2013; Foekema et al., 2013; Van Cauwenberghe and Janssen, 2014; Witte et al., 2014; Mathalon and Hill, 2014; Van Cauwenberghe et al., 2015), approximating microplastic impacts on humans and thus increasing related concerns.

To investigate microplastic contamination in nature, three marine compartments could be used: water column, sediment and biota. However, the abundance (concentration) in water or sediment does not always reflect the quality of the living resources (EPA, US, 2000), which should be considered the major concern for environmental health. The presence of microplastics in seawater and on the sea bottom seem to have a stochastic pattern, influenced by oceanographic biotic and abiotic forces, such as the development of biofilms, bioturbation, flood tide, winds, currents and wave fronts (Turra et al., 2014; Eriksen et al., 2014; GESAMP, 2015; Gallagher et al., 2015). All these factors can temporally influence the microplastics' re-suspension from bottom sediments and their depth distribution between the bottom and sea surface, increasing the variability of microplastics' abundance in these compartments. The composition of microplastics in an environment can vary according to the sampling materials, and the ability to identify them varies with plastic size (GESAMP, 2015). To illustrate that, most studies assessing water column have used plankton nets for collecting samples (Gallagher et al., 2015), which underestimates the abundance of microplastics smaller than their mesh size. Experimental studies on microplastics intake and effects on marine biota use particles with less than 1 μm (Santana et al., submitted for publication-b) up to 80 μm diameter (von Moos et al., 2012) as plastic models, sizes that are not retained by plankton nets. This methodological bias suggests that the current evaluation of abiotic compartments may not be fully supportive of risk assessments, leaving out data relevant to the hazard that microplastics pose to marine biota.

The use of biological indicators, in contrast, relies on the relationship between the organism and the polluted environment (EPA, US, 2000), helping improving our understanding of the realistic risks of the potential biotic impacts observed in laboratory studies. Due to the variety of microplastic types, sizes and shapes, bioassessments allow the understanding of the most threatening plastics for marine biota, for example. Initiatives of evaluating microplastic pollution in marine environments using sedentary invertebrates as bioindicators are just beginning but it calls attention of scientists, especially when bivalves for human consumption were reported contaminated (Van Cauwenberghe and Janssen, 2014). Nevertheless, there are no standardized protocols for assessment of microplastics in organisms as there are for persistent organic pollutants (e.g. Tanabe et al., 1987 and the Mussel Watch Program), highlighting the need for additional methodological developments. One significant problem of biomonitoring microplastic pollution is the lack of efficient and standardized methodologies for extracting and identifying the particles, making it difficult to compare studies and discuss the results.

The goal of this study was to broaden the scope of estimates of microplastic contamination in nature using marine biota as sentinels. We analyzed the presence of microplastics on the filter-feeding mussel *Perna perna* around estuary of Santos (Southeastern Brazil). Santos estuary is an important Brazilian coastal region, strongly influenced by industrial, port and urban activities and the most urbanized coastal area of São Paulo State, Brazil. As a first and rapid method to assess the state of microplastic contamination of the region, we identified the frequency of occurrence of such contamination on six natural mussel beds in the area. The use of this species of bivalve was based on (i) their features commonly appreciated for the purpose of bioassessments (e.g. widespread distribution, sedentary lifestyle, easy sampling and accumulation of chemicals — NOAA. International Mussel Watch Committee, 1995); and (ii) their importance as food resource. In addition, because of the incipient use of bioindicators for microplastic pollution, we also discussed methodological aspects that might be relevant for establishing applicable tools for analyzing biological matrices.

2. Methods

2.1. Assessed area: Santos estuary

The marine environmental health of Santos is of longstanding concern, but not much is known about its level of microplastic pollution. From the beginning of 20th century, this region has been strongly affected by anthropogenic activities (David, 2007); it houses the largest port in South America (Santos Harbor), one of the most important industrial complexes in Brazil (Cubatão industrial complex, Cesar et al., 2007; Fisner et al., 2013a) and has a well-established tourism industry that may attract up to 4.7 million people during the summer (data for 2012; Santos Turism Office, 2014). Considering potential sources of microplastics to coastal regions, all these characteristics can contribute to the microplastic contamination in Santos estuary, as detailed below.

Beside the solid waste produced by vessels that berth in Santos Harbor (including plastic packing ships), virgin plastic pellets (granules with an average diameter of 5 mm, made from different types of polymers, such as polyethylene and polypropylene, EPA, US., 1992), and Emulsion/Microsuspension PVC (small dense microspheres with a size ranging from 0.1 to 1.0 μm diameter, Rodolfo et al., 2006) are among the types of loads handled in this port. Both types of pre-consumption microplastics can potentially be entering the estuary after accidental losses (Pereira, 2014), putting marine biota at risk from their associated impacts. Probably as a consequence of these losses, Santos Bay was already observed to have high quantities of pellets, with a standing stock calculated at 762 million particles (Turra et al., 2014).

Other pollution sources such as landfills and sewage also contribute to the degradation of this estuary; these are important sources for microplastic contamination of coastal environments, especially during tourist periods when waste treatment system reach maximum capacity. For over 30 years, all solid waste from Santos' city was destined for a dumpsite in the neighborhood of *Alemoa*, an area close to the estuarine system. Although currently inactive, previous losses of plastic waste from this dumpsite can still serve as a microplastic input for the marine ecosystems of Santos because of slow degradation and the persistence of plastics in marine environments (David, 2007). Sewage discharges may also be introducing both microplastics used in cosmetic industries (Fendall and Sewell, 2009) and those derived from washing synthetic clothes (e.g. polyester fibers, Browne et al., 2011) because sewage treatment plants are not typically specifically designed to retain microplastic particles (Browne et al., 2011). Large sewage discharges occur clandestinely along the estuary of Santos, without any treatment