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Monitoring the occurrence of microplastic ingestion in Otariids along the Peruvian and Chilean coasts

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ABSTRACT

Repeated reports of microplastic pollution in the marine pinniped diet have emerged in the last years. However, only few studies address the drivers of microplastics presence and the potential implications for monitoring microplastic pollution in the ocean. This study monitored their in the scats (N = 205) of four pinniped species/subspecies at five different locations in the southern Pacific Ocean (Peru and Chile). Samples from all rookeries contained microplastics, and overall, 68% of the examined scats contained fragments/fibers, mostly blue colored. We confirmed that 81.5% of the fragments/fibers were anthropogenic in origin , but only 30% were polymers. Scats from Juan Fernández Archipelago presented higher microplastic concentrations than continental rookeries. Also, the common diet in each location may influence the levels found in the samples. This study presents a useful non-invasive technique to track plastic pollution in top predator diets as bioindicators for future surveillance/management plans applied to different location.

1. Introduction

Studies have found microplastics in marine environments in two different ways: as primary microplastics, such as microplastic pellets or microspheres, and as secondary microplastics originating from the degradation of larger fragments, the most common (Barnes et al., 2009; Derraik, 2002; Andrady, 2017). An increasing number of studies report the presence of microplastics (< 5 mm) in marine environments, in particular, microfibers (< 1 mm), with several authors pointing out that their concentrations might be seriously underestimated (Thompson et al., 2009; Cózar et al., 2014; Eriksen et al., 2014; Lusher, 2015; Andrady, 2017; de Sá et al., 2018; Thiel et al., 2018; Perez-Venegas et al., 2018; Nelms et al., 2019a). At the biological scale, researchers have found microfragments and microfibers in several species from different trophic levels, ranging from zooplankton (Setälä et al., 2014; Frias et al., 2014), mussels (Farrell and Nelson, 2013), predatory fish (Ferreira et al., 2018; Rochman et al., 2015; Mizraji et al., 2017; Ory et al., 2017; Markic et al., 2019), to marine top predators, such as seabirds (Van Franeker et al., 2011; Kühn and van Franeker, 2012), cetaceans (Lusher et al., 2015), and pinnipeds (Rebolledo et al., 2013;

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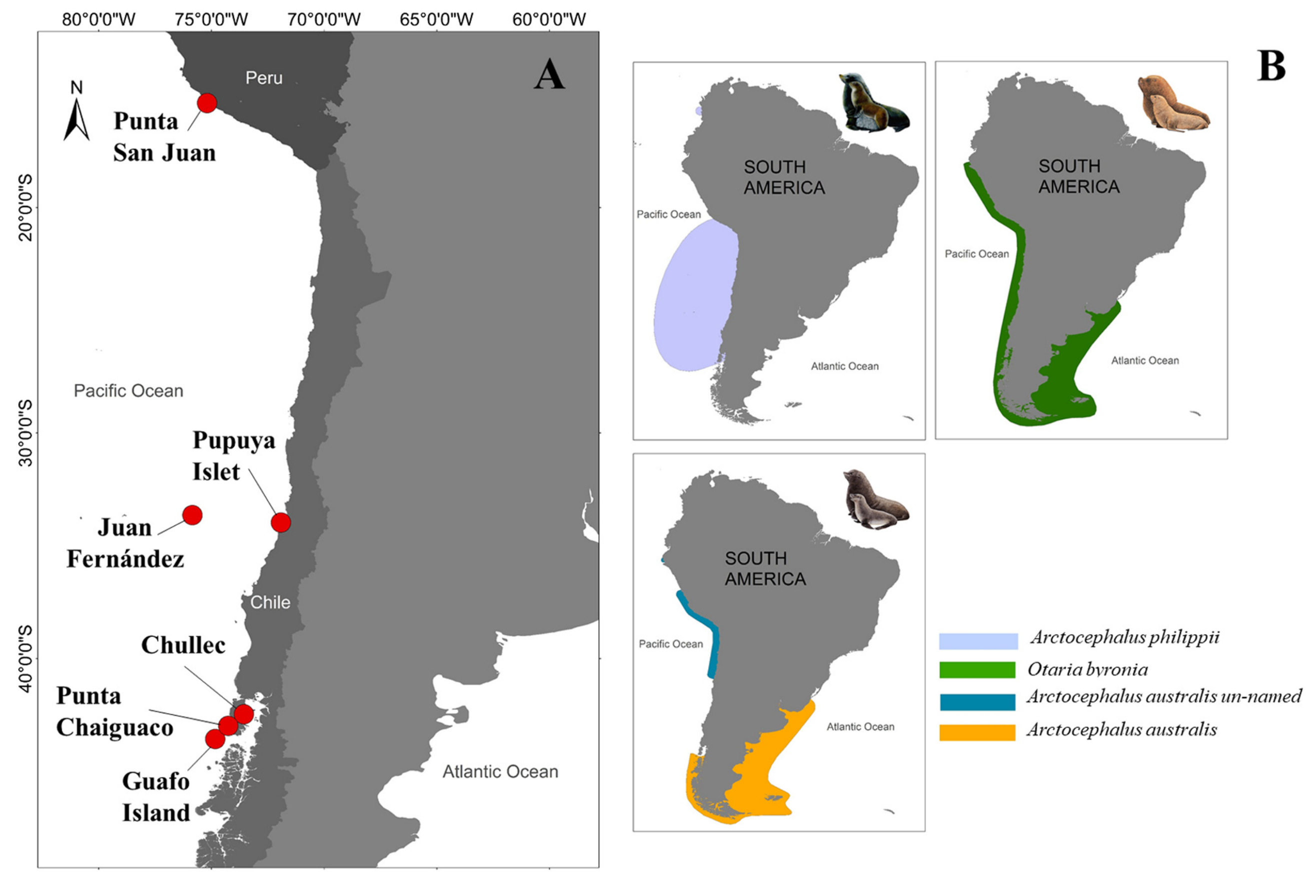

Fig. 1. A)Georeferenced location for each study rookery (red dots) and B) information about species distribution.

Eriksson and Burton, 2003; Denuncio et al., 2017; Perez-Venegas et al., 2018; Donohue et al., 2019; Nelms et al., 2019a, 2019b; Hudak and Sette, 2019; Hernandez-Milian et al., 2019). It has been demonstrated that microplastics cause a wide range of adverse effects on marine organisms, for example, malnutrition (Watts et al., 2015) and impaired reproduction (Galloway et al., 2017). Other known negative impacts include their role as vectors of toxins or pathogens, which in conjunction with bioaccumulation and biomagnification processes make microplastics a potential threat for the health of marine organisms (Galloway and Lewis, 2016; Provencher et al., 2017; de Sá et al., 2018). However, in top predators, the health impact of microplastics is not well understood, and data are scarce regarding the occurrence of these pollutants in pinniped species.

Researchers face challenges when evaluating the ingestion of microplastics in living, wild, free-ranging marine organisms, especially at higher trophic levels such as marine mammals (Nelms et al., 2019a; Rochman et al., 2019). The occurrence of macro and microplastics in marine mammals appears to be related or influenced by their diet (Rebolledo et al., 2013; Denuncio et al., 2017; Nelms et al., 2019a, 2019b). For instance, McMahon et al. (1999) reported small plastic fragments in New Zealand sea lion (*Phocarctos hookeri*) scats, suggesting the presence of plastics in their prey. Subsequently, it has been shown that these marine invertebrates and fishes mistake microplastic particles for food items when they select their food by color (Mizraji et al., 2017; Ory et al., 2017). Also analyzing scats, Eriksson and Burton (2003) reported the occurrence of microplastics in fur seals (*Arctocephalus spp.*). Nevertheless, both studies only reported plastic particles larger than 1 mm, with no information regarding microfibers. Recently, microfibers and microfragments smaller than 1 mm have been found in fur seal scats (Perez-Venegas et al., 2018; Donohue et al., 2019; Nelms et al., 2019b), opening up the opportunity to further assess trends in the distribution of these pollutants with non-invasive techniques.

The study of the occurrence and trend in the marine environment of smaller microplastics, such as microfibers (< 1 mm) has become important because these fibers are composed of natural materials chemically modified by the textile industry, e.g., wood fiber for plastic polymer reinforcement (Bolton, 1994; Saheb and Jog, 1999; Remy et al., 2015; Ma et al., 2019). Thus, these fibers may present a significant impact on ecosystems, especially when incorporated into trophic webs. However, the ultimate health consequences and accurate identification of these pollutants in marine wildlife currently constitute a knowledge gap (Ladewig et al., 2015; Galloway et al., 2017; Compa et al., 2018; de Sá et al., 2018).

Pinnipeds (seals, sea lions, and walruses) are top marine predators, i.e., sentinel species for ocean health, and can act as bioindicators. They are sentinel species, in part, because of their high sensitivity to environmental changes (Suter II et al., 2003), apex position in trophic webs (Kelly et al., 2007; Gerhardt, 2002; Galloway and Lewis, 2016), and long generation cycles (Reddy et al., 2001). Acknowledging these characteristics, researchers have widely used pinnipeds to monitor the state of ecosystems against different types of pollutants (Bossart, 2006; Reddy et al., 2001; Wells et al., 2004; Cipro et al., 2012; Alava and Ross, 2018). Similarly, they could be useful to understand the impact and distribution of microplastics in marine environments (Gerhardt, 2002; Reddy et al., 2001).

This study evaluates the concentration of microplastics (microfragments and microfibers) in scats of *Otaria byronia, Arctocephalus phillippii*, *Arctocephalus australis* and *A. australis un-named* [Oliveira et al., 2008]) from five different locations across the southeastern Pacific Ocean. Additionally, we report the differences in pollution levels between locations, assessing these variations according to diet composition, species, or study areas. Our research covered a high latitudinal range of approximately 3000 km in rookeries distributed from 15°S to 43°S, exemplifying how the use of non-invasive techniques in top marine predators can yield vital information for the evaluation of microplastic pollution in the marine environment.

2. Methodology

2.1. Fieldwork/sample collection

Scats from three different species of otariids were collected from six rookeries along the coastline of Peru and Chile (Fig. 1A): Punta San Juan (15°22′00″S; 75°11′28″W), Juan Fernández Archipelago (33°38′29″S; 78°50′28″W), Pupuya Islet (33°58′00″S; 71°55′00″W), Chullec/Chiloé Island (42°28′16″S; 73°33′40″W), Punta Chaiguaco/Chiloé Island (42°59′10″ S; 74°15′14″W), and Guafo Island (43°33′48″S; 74°49′58″W) (Tables 1 and S1). Samples were collected following the methodology given in Perez-Venegas et al. (2018). Briefly, samples were collected by wrapping them in aluminum foil (avoiding cross contamination) and then frozen at -4 °C until transport to the laboratory, where samples were thawed inside the foil envelopes for downstream processing.

2.2. Microplastic extraction

Microplastics were extracted following the methodology used by Perez-Venegas et al. (2018), modified from Foekema et al. (2013). Samples were digested using KOH 20% adding 20 mL g^{-1} wet weight for seven days in a glass container. The solution used has no impact on any hard structure, as otoliths, rocks and plastics, allowing us to extract them without altering the actual concentrations of this pollutant. The extracts were filtered using a GF/F filter (47 mm Ø and 0.7 μm pore size) in a vacuum pump. Filters were then introduced into 50 mm Ø covered glass Petri dishes to avoid contamination and stored at room temperature. Samples were examined using a microscope and classified by type (microfragment or microfiber of plastic) and color (blue, red, black, white, or undetermined). For plastic particle classification, we applied the criteria used by Norén (2007), used in several studies (e.g. Hidalgo-Ruz et al., 2012; Wright et al., 2013; Cole et al., 2015; Perez-Venegas et al., 2018; Carrasco et al., 2019), as follows: i) no cellular or organic structures visible in the plastic particle/fiber, ii) if the particle is a fiber, it should be equally thick and have a three-dimensional shape (not entirely straight fibers which indicates a biological origin and not tapered toward the ends); and, iii) be clear and a homogeneously colored particle/fiber. During the handling of samples, strict measures were taken to avoid potential cross-contamination, as recommended in previous studies (Mizraji et al., 2017; Perez-Venegas et al., 2018). Microplastic counts were standardized as the number of fragments/fibers by sample weight (g), to compare species and zones.

2.3. Micro-fourier transform infrared (FTIR) analysis

The content extracted from six scats, containing fibers and fragments were randomly selected, for each seal population and sent to the Laboratorio de Fisiologia Animal Comparada at Universidad de Concepcion, for Micro-Ftir Analysis. Filters were opened in the laboratory under a stereomicroscope (Nikon SMZ18). The infrared spectrums of each fiber were obtained on a Spotlight 400 FTIR Imaging System, and then compared against a polymer library, to identify if they were polymers and its type.

2.4. Statistical analysis

An exploratory analysis showed a non-normal distribution of microplastic concentration (Shapiro-Wilk Normality Test; $p < 0.05$). Consequently, a non-parametric Kruskal-Wallis analysis was conducted to analyze microplastic concentration (item g^{-1}) differences among sampling locations. A *post-hoc* Tukey test was applied to determine which rookeries differed in plastic concentrations. We discarded scats without microplastics from the analyses since the technique used here does not discriminate real absences (Perez-Venegas et al., 2018).

3. Results

We found fragments or microplastic fibers in all of the studied rookeries (see Table S1). The percentages of detection of microplastics in samples varied among the surveyed rookeries and latitudinally. For Punta San Juan, 64% and 14% of the samples contained fibers and fragments, respectively. Similarly, scats from Juan Fernández Archipelago had 63% and 8% in fibers and fragments, respectively. For Pupuya Islet, 71% of scats contained fibers, and only one sample contained fragments. For the southernmost sampling locations, the scenario appeared to be consistent among rookeries: Guafo Island, 66% contained fibers and only one sample contained fragments; Chullec, 92% contained fibers, and only two samples contained fragments; and Punta Chaiguaco, 100% contained fibers, and only one sample contained fragments (See Table 1). The most common type of plastic was microfiber, especially blue-colored, followed by white- and red-colored fibers (Tables 1 and S2). These results suggest that fibers are more abundant compared to fragments. We did not conduct statistical analyses since the occurrence of microfragments was very low. The results form Micro-FTIR analysis, showed that 81.5% of the fragments/fibers were confirmed of anthropogenic origin (Fig. 3), with 51.5% were concordant with Cotton and the remaining 30% as polymers (PET and Nylon). The remaining 18.5% did not matched well with any standard of the library, and was classified as cellulose/non identified as the spectrums presented some bands typical from cellulose but results were not conclusive (Table 2).

When standardizing the values by microplastics units per scat weight, as the concentration of microplastics [item g^{-1}] (Table 1 and Fig. 2), the microplastic concentration by rookery showed significant differences (X^2: 29.712; $p \ll 0.05$). Juan Fernández Archipelago showed the highest concentration, with a mean ± S.D. of 3.7 ± 4.7 fibers g^{-1} and 3.1 ± 3.3 fragments g^{-1}, with the lowest concentration in Punta Chaiguaco, with a mean ± S.D. of 0.4 ± 0.3 fibers g^{-1}, and 0.1 fragments g^{-1} in one sample. Juan Fernández Archipelago showed significant differences against Chullec and Punta Chaiguaco but did not differ from Punta San Juan, Pupuya islet and Guafo Island. However, Chullec and Punta Chaiguaco showed significant differences against all rookeries, but not between each other (Fig. 2A). Scats from the Juan Fernández rookery had the highest values of microfibers, followed by those from Pupuya Islet, Guafo Island, Punta San Juan, Chullec and Punta Chaiguaco, therefore, we observed a decreasing trend of microplastic pollution from North-West to South-East (Fig. 2A). In the case of microfragments, no differences were found ($p > 0.05$) among studied rookeries (Fig. 2B).

4. Discussion

Microfibers were the most abundant type of microplastics identified in the present study, with higher abundances in oceanic rookeries compared to coastal rookeries, similar to the observed trends of small plastic items on sandy beaches across Chile (Hidalgo-Ruz and Thiel, 2013), where oceanic islands had higher concentrations due to the influence of currents (e.g., Eriksen et al., 2013, 2014, 2018). Microfragments were less abundant than microfibers, but they showed a trend similar to microfibers with higher concentrations in oceanic rookeries. This difference in proportions between microfibers and microfragments is commonly reported for marine mammals (Wright et al., 2013; Woodall et al., 2014; de Sá et al., 2018; Nelms et al., 2019a), suggesting that microfibers could be more bioavailable and preferred for preys (Watts et al., 2015).

The observed particles were mainly blue-colored, as reported for other species of pinnipeds (Perez-Venegas et al., 2018; Donohue et al., 2019; Nelms et al., 2019a, 2019b), being the most common fiber color

Table 1
Details of sample collection across the Peru-Chile coastline.

Location	Country	Coordinates	N	Microfragments occurrence (%)	Microfibers occurrence (%)	Specie	Sample Dates
Punta San Juan	Peru	15°22′00″S; 75°11′28″W	50	14	64	Arctocephalus australis un-named	06/2017 to 07/2017
Juan Fernández Archipelago	Chile	33°38′29″S; 78°50′28″W	40	8	63	Arctocephalus phillippii	12/2016–03/2017
Pupuya Islet	Chile	33°58′00″S; 71°55′00″W	14	1	71	Otaria byronia	08/2017 to 09/2018
Guafo Island	Chile	43°33′48″S; 74°49′58″W	79	3	100	Arctocephalus australis	02/2016 and 02/2017
Chullec	Chile	42°28′16″ S; 73°33′40″W	12	8	66	Otaria byronia	03/2016
Punta Chaiguaco	Chile	42°59′10″ S; 74°15′14″W	10	10	92	Otaria byronia	03/2016

found in lower trophic levels in the Pacific Ocean (Ory et al., 2017; Thiel et al., 2018). As previously reported and due to their size, the uptake of microplastics in marine mammals is likely to occur through trophic transfer than by direct consumption (McMahon et al., 1999; Eriksson and Burton, 2003; Perez-Venegas et al., 2018; Donohue et al., 2019; Nelms et al., 2018, 2019a, 2019b). Moreover, as previously reported in Nelms et al. (2018), the stomach of marine mammals could act as a trap for small plastic particles. To evaluate the influence of prey contamination input in the study rookeries, we carried out a literature search to determine the diet of each species/subspecies of otariids in this study and the differences among them (Table S2). Showing some overlap in diet composition by rookeries (as *Merluccius australis; Engraulis ringens*), but most differences occurred at the species level. *A. philipii* on Juan Fernández Archipelago has an entirely different diet compared to other species of otariids in the South Pacific (Acuña and Francis, 1995), such as *A. australis* at Isla Guafo that feeds mainly on crustaceans and fish, *Merluccius australis*, depending on seasonal availability (Vargas, 2012) (Table S2). The different diets suggest that differences in feeding areas and preferences, related to home ranges (see Fig. 1B) might explain the differences between Oceanic rookeries and Continental shelf rookeries found in this study. However, without a simultaneous sampling of predators, prey items, and the surrounding environment, it is hard to demonstrate a direct link between diet and microplastic ingestion. Nevertheless, recently was reported in Chile the microplastic ingestion in the same family and genus that the otariids' prey from this study (Ory et al., 2018; Pozo et al., 2019), suggesting an indirect contamination by prey ingestion (as Nelms et al., 2018). Since the stomachs of marine mammals could act as an entrapment site for microplastics delaying the output of them in fecal samples (Nelms et al., 2019a), otariids could be bioaccumulating microplastics from trophic webs and environments where prey items feed, thus suggesting that microplastics may be polluting the associated ecosystems.

Juan Fernández Archipelago is the area most influenced by the South Pacific subtropical gyre (area with high concentration of microplastics) and *A. philipii* has the most oceanic home range (Fig. 1), which might explain the higher concentration of microplastics found in this group (Hidalgo-Ruz and Thiel, 2013; Van Sebille et al., 2015; Eriksen et al., 2013, 2018). On the other hand, *A. australis* in Guafo Island, far away from the gyre, is located close interaction with fisheries (Perez-Venegas et al., 2017) and they have a diet rich in crustaceans, which are reported to accumulate mostly microfibers (Vargas, 2012; Watts et al., 2015; Andrade and Ovando, 2017; Devriese et al., 2017). Furthermore, it is well known that *O. byronia* engage a lot in human activities (i.e., Sepúlveda and Oliva, 2005; Goetz et al., 2008) which may explain the high concentrations of microfibers in Pupuya Islet rookery, closely located to a touristic beach area. However, even with the strong influence of aquaculture activity in Chiloé archipelago (included Chullec and Punta Chaiguao), the concentrations for *O. byronia* in Chullec and Punta Chaiguao were low compared to other groups (Fig. 2). The low number of samples obtained from this area could explain the low concentrations (Table 1). However, we collected scats from Chullec from a rookery located on an abandoned mytiliculture raft close to an operating aquaculture center, as opposed to Punta Chaiguaco where there was no presence of human activity in the area. This could explain the different plastic concentrations found in Punta Chaiguaco and Punta San Juan, compared to the higher values for Chullec. Finally, the microfiber concentrations in Punta San Juan are statistically similar to both Juan Fernández and Chullec, which is probably related to the differences in sampling season, since foraging trips during the non-breeding seasons (Winter) are farther and longer than those during the breeding season (Summer) (Boyd, 1996). This highlights the importance of timing in sample collection for monitoring of microplastic pollution in otariids diets.

The potential for microfiber contamination both in the field and laboratory has become an emerging issue of concern in marine pollution science (Lusher et al., 2017a, 2017b; Nelms et al., 2018, 2019b;

Table 2
Concentration of microplastics (Item g $^{-1}$) from the sampling locations along Peru-Chile Coastline.

Rookery	Microplastics (Items g $^{-1}$)													
	Microfibers												MicroFragments	
	Blue		Red		White		Black		Undetermined		All		All	
	Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd
Punta San Juan	1.3	± 0.9	1.1	± 0.6	1.8	± 1.2	1.1	± 0.7	1.2	± 0.8	1.8	± 1.7	0.9	± 0.5
Juan Fernández Archipelago	2.7	± 4.0	1.1	± 1.1	2.9	± 3.3	1.9	± 0.9	3.6	–	3.7	± 4.7	3.1	± 3.3
Pupuya Islet	3.1	± 2.9	1.3	± 1.0	1.7	± 2.1	–	–	1.8	± 1.3	3.6	± 3.5	0.2	–
Guafo Island	1.4	± 1.4	0.8	± 0.6	2.0	± 2.8	0.9	± 0.5	1.5	± 1.5	2.1	± 2.5	1.4	–
Chullec	0.6	± 0.8	0.3	± 0.3	0.4	± 0.5	–	–		–	0.8	± 1.0	0.04	± 0.006
Punta Chaiguaco	0.2	± 0.2	0.2	± 0.2	0.1	± 0.1	–	–	0.1	± 0.02	0.4	± 0.3	0.1	–

Nuelle et al., 2014; Woodall et al., 2015; Perez-Venegas et al., 2018), creating a challenge to investigate microplastic pollution in living, wild, free-ranging marine mammals. Some authors (Perez-Venegas et al., 2018; Donohue et al., 2019) have detected microfibers in fur seal scats without chemical composition analysis, however this study identified that of the obtained compounds a higher proportion has an anthropogenic origin. Among the fibers a high proportion comes from cotton, which is clearly of anthropogenic origin. However, all these fibers and particles could be potentially harmful to the population. This due the application of as chemicals during the processing and treatment to improve their durability and make them potentially more resistant (Remy et al., 2015; Ma et al., 2019) limiting for example the biodegradation in the environment (Bolton, 1994).

There is an evident increase in the number of reports on microfiber detection around the globe (de Sá et al., 2018). Thus the importance of the evaluation of the presence of microfibers and identifying their origin is a critical point. This is due the potential persistent organic pollutants (POPs) absorption (Jonker, 2008; Tursi et al., 2018) as an emerging pollutant (Lohmann, 2017). Nevertheless, even for microplastic, and specially microfibers, effects on individual and population health in marine mega fauna are still unknown (Nelms et al., 2019a; Rochman et al., 2019).

5. Conclusion

Microplastic and microfiber detection in free-ranging marine mammals is not an easy task (de Sá et al., 2018; Donohue et al., 2019). However, new techniques and studies have helped to develop effective methods to detect small over large area microplastics (Perez-Venegas et al., 2018). The non-invasive collection of scats from remote otariids rookeries used in this study makes this a simple and effective technique to track plastic pollution in top predator diets, and therefore the presence of this new anthropogenic pollutant on marine ecosystems (Reddy et al., 2001; Gerhardt, 2002; Nelms et al., 2019b). This novel monitoring tool and sentinel specie could be incorporated in future monitoring/management plans.

Credit author statement

Diego J. Perez-Venegas: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization, Project administration, Funding acquisition; Constanza Toro-Valdivieso: Investigation, Funding acquisition; Félix Ayala: Investigation;Beatriz Brito: Investigation,; Lunna Iturra: Investigation;

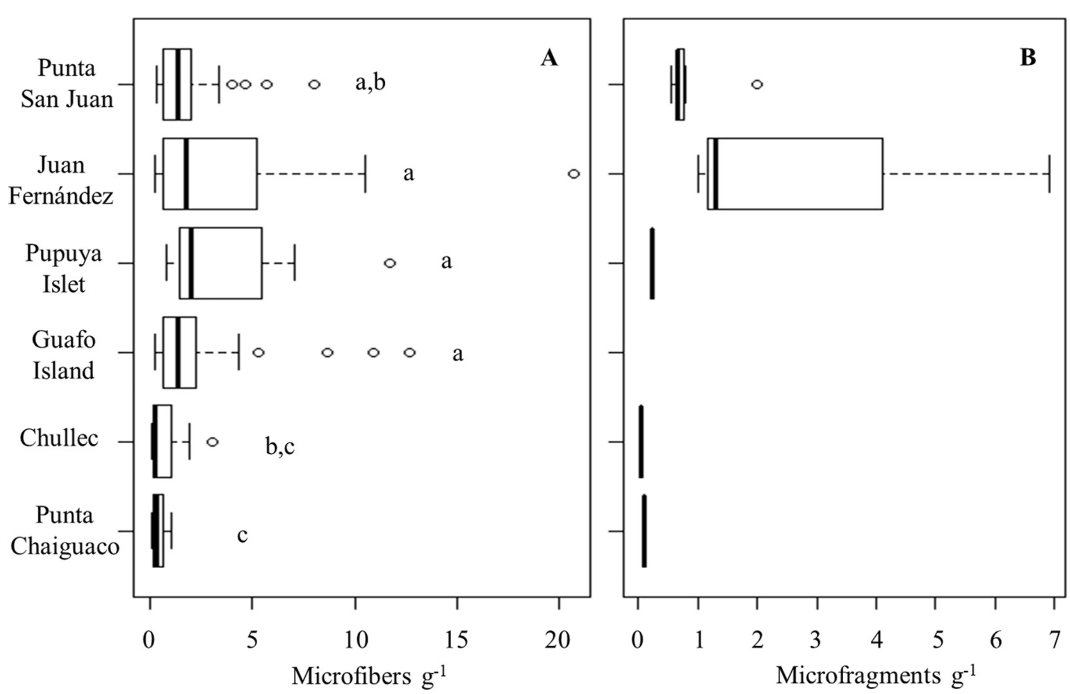

Fig. 2. Microplastic concentration values (Item g $^{-1}$) per rookery. (A) The microfiber concentration and (B) the microfragment concentration. Different letters indicate significant differences ($P < 0.05$).

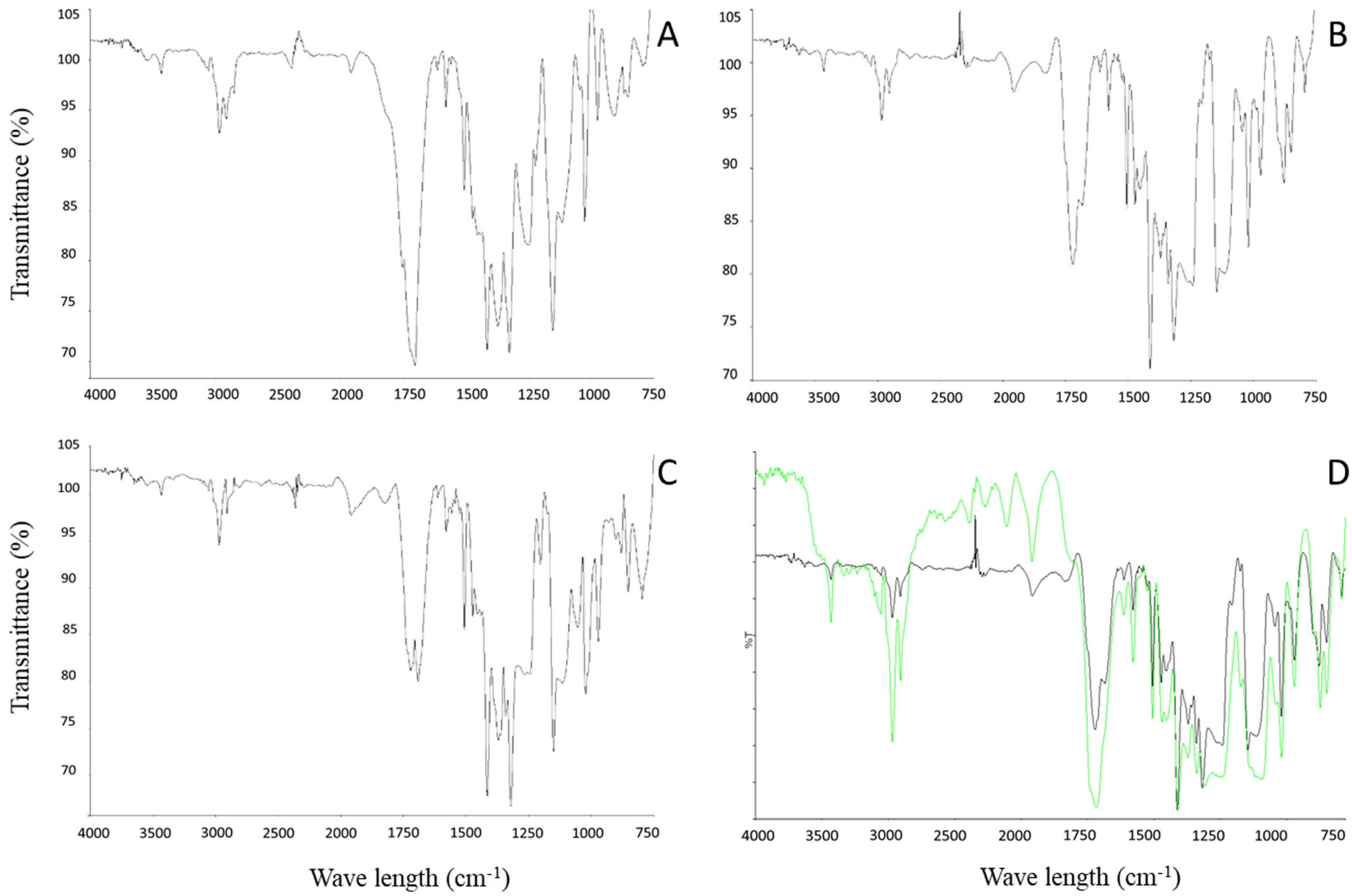

Fig. 3. Examples of spectrum obtained by FTIR found, where (A) is a blue fiber corresponding to Cotton, (B) black fiber that corresponded PET, (C) red fiber corresponding also PET, and (D) black PET fiber (black line) compared with a known Polyester (PET) (Green line). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Maite Arriagada: Investigation; Mauricio Seguel: Investigation, Funding acquisition; Carmen Barrios: Investigation; Maritza Sepúlveda: Investigation, Resources, Review & Editing, Funding acquisition; Doris Oliva: Investigation, Resources, Review & Editing, Funding acquisition; Susana Cárdenas-Alayza: Investigation, Resources, Review & Editing, Funding acquisition; Mauricio A. Urbina: Methodology Formal analysis, Review & Editing, Funding acquisition; Alberto Jorquera: Investigation; Eduardo Castro-Nallar: Investigation, Resources, Review & Editing, Funding acquisition; Cristóbal Galbán-Malagón: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision, Project administration, Funding acquisition.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2020.110966.

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