Microplastic accumulation in benthic invertebrates in Terra Nova Bay (Ross Sea, Antarctica)

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A R T I C L E   I N F O

Handling Editor: Da Chen

Keywords:
Antarctica
Benthos
Microplastics
Food web
FTIR
Nile red

A B S T R A C T

Microplastic contamination of the benthic invertebrate fauna in Terra Nova Bay (Ross Sea, Antarctica) was determined. Twelve macrobenthic species, characterized by different feeding strategies, were selected at 3 sampling sites at increasing distance from the Italian Scientific Base (Mario Zucchelli, Camp Icarus, Adelie Cove). The 83\% of the analyzed macrobenthic species contained microplastics (0.01–3.29 items mg\textsuperscript{-1}). The size of the particles, measured by Feret diameter, ranged from 33 to 1000 \(\mu\text{m}\) with the highest relative abundance between 50 and 100 \(\mu\text{m}\). Filter-feeders and grazers displayed values of microplastic contamination from 3 to 5 times higher than omnivores and predators, leading to the hypothesis that there is no evident bioaccumulation through the food web. The prevalent polymers identified by micro-FTIR were nylon (86\%) and polyethylene (5\%); other polymers identified in Antarctic benthos were polytetrafluoroethylene, polyoxymethylene, phenolic resin, polypropylene, polystyrene resin and XT polymer.

1. Introduction

The world plastic production from the year 1950 has increased from 1.7 to 348 million tons in 2017, with a proportional increase in the production of plastic waste (PlasticsEurope, 2008, 2018). Part of this waste get discharged into the environment, a problem exacerbated by the common use of throw-away “user” plastic products, that when inappropriately managed and discarded, ultimately reach the sea producing damage to marine life (Cole et al., 2011; Thompson et al., 2009). Plastic waste has been subdivided into 5 dimensional classes (Andrady, 2017): macroplastics (> 200 mm), mesoplastics (200–5.01 mm); large microplastics (5–1.01 mm); small microplastics (1.00 mm-1 \(\mu\text{m}\)) and nanoplastics (<1 \(\mu\text{m}\)). The origin of microplastics can be due to direct input of particles already included in a dimensional range between 5 mm and 1 \(\mu\text{m}\). These are reported as primary microplastics, and are introduced into the environment by discharge of many “open use” products such as glitter, face scrub, syntetic fibers from washed wears and many other goods (Napper and Thompson, 2016). Secondary microplastics are produced instead by the interaction of atmospheric agents, waves, ultraviolet rays and biological agents with macroscopic plastic pieces leading to their progressive fragmentation (Artham et al., 2009; Muthukumar et al., 2011). Eventually the action of fouling (Fazey and Ryan, 2016; Galloway et al., 2017) increase the density of these particles and favors their aggregation in marine snow that sink onto the seabed becoming potentially accessible for benthonic organisms (Porter et al., 2018).

One of the primary environmental risks associated with microplastics is their bioavailability for marine organisms, since they mimic the appearance of food, possibly obstructing and compromising the functionality of the digestive system (Gall and Thompson, 2015). Moreover microplastics can act as a source and vector of toxic plastic additives (Hermabessiere et al., 2017; Hahladakis et al., 2018). Microplastics can be ingested by marine invertebrates with different feeding methods, as the particles are in the size range of plankton: mussels (filter feeders), lugworms (deposit feeders) and sea cucumbers (detritivores) were found to ingest microplastics (Naji et al., 2018; Lusher et al., 2017; Bonanno and Orlando-bonaca, 2018; Browne et al., 2008; Graham and Thompson, 2009). There is growing evidence that microplastics can get transferred in the food chain (Farrell and Nelson, 2013; Nelms et al., 2018), rising concern about detrimental

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https://doi.org/10.1016/j.envint.2020.105587
Received 27 September 2019; Received in revised form 27 January 2020; Accepted 16 February 2020
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implications for bioaccumulation from one trophic level to the next.

Microplastics contamination has been reported for many remote and isolated areas of the globe, including Antarctica (Cincinelli et al., 2017; Isobe et al., 2017; Munari et al., 2017). The Antarctic continent has been affected by direct and indirect human activity for about two centuries (Stark et al., 2019). Moreover, the intense industrialization of the world brought new compounds and new hazards to ecosystems worldwide, and Antarctica was not an exception (Stark et al., 2019). In most parts of the continent the effects of the scientific activities and tourism resulted in different types of pollution, such as organic enrichment (Conlan et al., 2004), chemicals contamination, especially by hydrocarbons (Lenihan and Oliver, 1995), metals (Guerra et al., 2011), and microplastics (Cincinelli et al., 2017; Isobe et al., 2017; Munari et al., 2017), with mostly unknown ecological effects. Moreover, terrestrial and marine habitats adjacent to current or abandoned Antarctic scientific bases are affected by localized contamination (Bargagli, 2008).

In the present study, we analyzed 12 invertebrate species from Terra Nova Bay (Ross Sea, Antarctica), with the aims (i) to determine whether the benthos of that remote area exhibit microplastic contamination, (ii) to evaluate differences of microplastic contamination among different species characterized by different feeding strategies, (iii) to characterize polymer type, and finally (iv) to determine whether microplastic presence can be attributed to trophic transfer. This is the first time that such a comprehensive study on microplastic contamination has been carried out on the Antarctic benthos.

2. Materials and methods

2.1. Study area and sampling

The Ross Sea (Southern Ocean) is located between Victoria Land and Marie Byrd Land and is the largest continental shelf ecosystem south of the Antarctic Polar Front. Terra Nova Bay (Ross Sea) is a coastal marine area encompassing 29.4 km$^2$ between Adélie Cove and the coastal marine area encompassing 29.4 km$^2$ between Adélie Cove and south of the Antarctic Polar Front. Terra Nova Bay (Ross Sea) is a coastal marine area encompassing 29.4 km$^2$ between Adélie Cove and Marie Byrd Land and is the largest continental shelf ecosystem south of the Antarctic Polar Front. Terra Nova Bay (Ross Sea) is a coastal marine area encompassing 29.4 km$^2$ between Adélie Cove and Marie Byrd Land and is the largest continental shelf ecosystem south of the Antarctic Polar Front. Terra Nova Bay (Ross Sea) is a coastal marine area encompassing 29.4 km$^2$ between Adélie Cove and Marie Byrd Land and is the largest continental shelf ecosystem south of the Antarctic Polar Front. Terra Nova Bay (Ross Sea) is a coastal marine area encompassing 29.4 km$^2$ between Adélie Cove and Marie Byrd Land and is the largest continental shelf ecosystem south of the Antarctic Polar Front. Terra Nova Bay (Ross Sea) is a coastal marine area encompassing 29.4 km$^2$ between Adélie Cove and Marie Byrd Land and is the largest continental shelf ecosystem south of the Antarctic Polar Front.

Fig. 1. Map of the sampling areas.
digestion method efficiency was performed in triplicate on dried samples of 
Ruditapes philippinarum (Adams and Reeve, 1950) soft tissue and 
Gammarus pulex (Linnaeus, 1758), two representative organisms for soft 
animals and chitinous ones. The digestion efficiency was evaluated as 
percentage of dissolved organism and was obtained as dry weight dif-
ference after filtration of the digest on pre-weighted oven dried 
(105 °C × 1 h) GF-F fiberglass filters (0.7 µm).

The microplastics were collected from the digests by density-
floating method. An addition of 3.6 g of NaCl (calculated at 700 °C for 
3 h to remove any trace of microplastics) was dissolved by vortexing for 
30 s, the test tube was quickly degassed by sonication for three seconds 
and let to settle for 1 h. The test tube was put into 100 mL cylinders and 
a saturate filtered NaCl solution (1.22 g cm⁻³) was slowly poured to 
overflow and collect the low density microplastics into the cylinder, this 
operation was repeated three times waiting 1 min between each over-
flow. The microplastics were filtered on GF-F fiberglass filters (0.7 µm) 
and the filters were rinsed with 5 mL of milli-Ro water and 5 mL of 
hexane. The microplastics were stained by 1 mL of Nile red solution 
(10 µg mL⁻¹ in hexane) in 5 cm closed Petri plates for 30 min as in 
Maes et al. (2017) and the excess dye was removed filtering 5 mL of 
hexane to remove background staining. The microplastics were counted 
by epi
fluorescence microscopy (equipped with a 10 W blue 
LED and an orange photo filter). The images were recorded with a di-
gital camera and processed by ImageJ to identify size (Feret diameter) 
in the range 30 µm–5 mm and particle circularity. The number of mi-
croplastics was normalized both on the dry biomass digested and on the 
number of organisms pooled and digested together. The values were 
expressed as number of microplastics per dry weight (items mg⁻¹) for 
station comparison and as number of microplastics per individual 
(items individual⁻¹) for species comparison. The coefficient of varia-
tion was estimated on a composite sample in which R. philippinarum 
was ground and sprinkled with polystyrene microplastics produced by a 
mincer, followed by repartition in three replicates and analysis. A spike 
recovery test was performed by addition of 100 polystyrene micro-
plastics (with approximate diameter size 500 µm) to samples of R. 
philippinarum and G. pulex to estimate the microplastic yield of the 
method for low density microplastics.

2.4. FTIR identification

The two most contaminated benthic species (both per weight and 
per individual) identified by Nile red staining at the stations Mario 
Zucchelli and Adelie Cove were processed for a microFTIR qualitative 
identification of the particles. The organisms were digested in glass 
bottles with 5 mL of hydrogen peroxide (30% w/w in H₂O, contains 
several stabilizer, Sigma Aldrich, purchased from Merck Darmstadt, Germany) 
as performed by Nuelle et al. (2015) on biogenic materials and were 
shaken on an orbital shaker for at least 72 h at room temperature. Di-
gested samples were then filtered on ANODISCs (Anopore Inorganic 
Membrane, 0.2 µ, 47 mm, Whatman™, purchased from Merck, Darm-
stadt, Germany). Filters were rinsed before and soon after the filtration 
with ethanol and stored in glass Petri dishes previously decontaminated 
and dried for at least 72 h, before analysis. All these operations were 
performed in a plastic free clean room ISO 7. In order to qualitatively 
identify polymers, plastic particles were analyzed by micro-FTIR. A 
 Nicolet iN10 infrared microscope (Thermo Fisher Scientific, Madison, WI, USA) with a liquid nitrogen-cooled MCT detector and motorized 
stage was employed; filters were analyzed in transmission mode with the WIZARD section of the OmnicTM PictaTM software and the 
collected spectra from 5 optical fields were then compared with specific reference library databases.

2.5. Statistical analysis

Data analysis was performed on microplastic contents and dry or-
ganisms using R, program version 3.5.1. The model that best fits the 
experimental data was obtained after removing existing outliers and 
examining the kernel density of all samples. As a result a linear re-
gression was firstly applied to all data (Crawley, 2012) by mean of 
package “stats” in R (Rothorn and Everitt, 2009). Afterwards, due to 
lack of fit of linear model (O’Brien et al., 2009), we applied a one step 
non linear regression to samples by means of package “nlstools” in R 
(Baty et al., 2015). The goodness of fit was then assessed through the 
examination of the regression analysis of residuals (Box et al., 2005) 
by plotting the distribution of fitted values versus residuals (Montgomery 
et al., 2012). The model, which distribution of residuals showed no 
visual tendency (so randomly distributed around zero), was selected as 
the most adequate one (Tsai et al., 1998). The parameters were then 
estimated by Maximum Likelihood Estimation (MLE) method (Taboga, 
2012).

3. Results

3.1. Method validation

The coefficient of variation of the analysis estimated from a com-
posite sample was within 9%. The blank values were performed before 
and after the analysis and were considered acceptable within 3 items 
per filter. The microplastic spike recovery test yielded from 91 to 97% 
of the low density microplastics added to the samples on both soft and 
chitinous animals. The digestion efficiency was almost complete 
reaching 96% for soft tissues from R. philippinarum, but lower digestion 
efficencies were found for G. pulex (73–86%) due to the abundance of 
isoluble chitin, though the NaOH solution alters the chitin preventing 
a subsequent staining with the Nile red dye that would otherwise in-
terfere creating false positives. Additional information on the results of 
the tests were provided in supplementary material (Tables S2 and S3). 
The NaOH is known to produce the decacytation of chitin and the 
conversion to chitosan (Elieh-Ali-Komi and Hamblin, 2016). This led us 
to assume that Nile red could interact with the acetylated groups that 
are cleaved during the NaOH digestion or the reaction could favor 
hydration and swelling of the chitin reducing the subsequent interac-
tion with the non-polar staining solution. The microFTIR digestion 
method by H₂O₂ proved to be poorly compatible with the Nile red count 
method in this form and viceversa. The H₂O₂ digestion of the samples if 
used for Nile red count in epifluorescence do not remove the inter-
ference of chitin that is stained by Nile red, after the digestion, and 
became strongly fluorescent creating false positives in chitinous sam-

dles. Conversely, NaOH digestion prevents misidentification of chitin 
by epifluorescence but tends to dissolve and clog the ANODISC filters 
used for microFTIR identification (these filters had the lowest back-
ground signal in comparison with GF-F fiberglass filters), moreover 
NaOH digestion produce a dirty background that compromise the ef-
ficentness of the microFTIR identification. This led the choice of the 
use of two different extraction methods for the two analysis.

3.2. Microplastic abundance

The 12 most abundant benthic taxa were considered in the 3 in-
vestigated areas (a total of 35 samples, due to the absence of Eatoniella 
sp. at Adelie cove). The 83% of the biological samples contained mi-
croplastics ranging from 0.01 to 3.29 items mg⁻¹. The size of the 
particles recovered from the environmental samples, measured by Feret 
diameter, ranged from 33 to 1000 µm with the 95% of the particles 
within 500 µm, the 70% within 200 µm and the highest relative 
abundance between 50 and 100 µm. The circularity (0/elongated shape 
particles = 1/circular shape particles) for the 80% of the particles was 
higher than 0.8 approximating the majority of the microplastics to a 
circular shape and only 2% displayed circularity values lower than 0.5 
with elongated shapes similar to fibers (Fig. 2).

The average microplastic content for all species and areas was 0.7 
items mg⁻¹, with relevant differences between different species. H.
similis, Eatoniella sp., Oweniidae sp., and T. debilis displayed microplastic contents higher than 1.0 items mg$^{-1}$ but for the first two organisms values as high as 3.2 items mg$^{-1}$ were recorded at Adelie Cove and Mario Zucchelli, respectively (Fig. 3). Given the widespread presence of microplastics in all stations and species, if we assess the degree of contamination in different stations by the number of samples which were microplastic free (a minority of 17%) then no microplastic-free samples were found at Mario Zucchelli, which appears to contain a more widespread contamination between all the species. Microplastic-free samples were found for Y. antarctica, O. franklini, A. macroura, and E. meridionalis in the other two sites. Eventually Camp Icarus was the station where the highest number of microplastic free samples was found and probably the least contaminated station. A global statistical analysis of average individual weights and relative microplastic contents showed a non-linear model as the best fit (Fig. 4a), particularly the equilateral hyperbole function, as confirmed by fitted values vs residuals plot (Fig. 4b). Additional information on the test results were provided in supplementary material. This hyperbolic relation can be summarized by the smaller the organism (as organism weight), the higher the microplastic content. These microplastics should be considered as sum of both ingested and associated particles because the organisms were digested whole during the analysis and the washing step is not enough to ensure the complete removal of adsorbed particles.

In order to compare organisms that are taxonomically different and with very different average sizes per taxon, we have chosen to unbind the number of plastics from biomass by normalizing the microplastics on the number of individuals analyzed per pool; in such a way the taxa become comparable without influences related to the size of the organism. This operation highlights that the microplastic content per individual is on average the highest in the bivalves (1.9 items individual$^{-1}$), follows with a decreasing trend the gastropod Eatoniella sp. (1.2 items individual$^{-1}$) then polychaetes, amphipods and cnidarians (Fig. 5). Globally, the mean microplastic content for all species was 1.0 items individual$^{-1}$. Filter feeders and grazers displayed on average values from 3 to 5 times higher than omnivore and predators, such as A. macroura, E. meridionalis and H. similis (average value of 0.4 items individual$^{-1}$) and this seems to exclude a trophic chain accumulation of particles toward predators among the identified organisms of the benthic communities.

Eventually in Fig. 6 data were plotted for all species and all areas about individual microplastic content and number of individuals counted per grab. The graph highlights a trend in which the numbers of microplastics per individual decrease at increasing numbers of

Fig. 2. Histograms of the relative abundance of microplastic for size range measured by Feret diameter – inset – Pie chart with the relative abundance of particles for circularity range (0/elongated shape-1/circular shape).

Fig. 3. Histogram of microplastic content per weight in different taxa.

Fig. 4a. Plot of individual weights and relative microplastic contents with best fitting curve.

Fig. 4b. Fitted values vs residuals plot.

Fig. 5. Box-plot of microplastic content per individual in different taxa (combined data from all stations) with mean values (black dots). – inset- Box-plot of microplastic content per individual grouped at higher taxonomical orders.

similis, Eatoniella sp., Oweniidae sp., and T. debilis displayed microplastic contents higher than 1.0 items mg$^{-1}$ but for the first two organisms values as high as 3.2 items mg$^{-1}$ were recorded at Adelie Cove and Mario Zucchelli, respectively (Fig. 3). Given the widespread presence of microplastics in all stations and species, if we assess the degree of contamination in different stations by the number of samples which were microplastic free (a minority of 17%) then no microplastic-free samples were found at Mario Zucchelli, which appears to contain a more widespread contamination between all the species. Microplastic-free samples were found for Y. antarctica, O. franklini, A. macroura and E. meridionalis in the other two sites. Eventually Camp Icarus was the station where the highest number of microplastic free samples was
Table 1
List of identified polymers and relative abundances at Mario Zucchelli, Adelie Cove and global contamination.

<table>
<thead>
<tr>
<th>POLYMER</th>
<th>Mario Zucchelli</th>
<th>Adelie Cove</th>
<th>Global</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyphthalamide-Aromatic (PA) (Nylon)</td>
<td>45%</td>
<td>73%</td>
<td>84%</td>
</tr>
<tr>
<td>Polyarylamide (PARA)</td>
<td>22%</td>
<td></td>
<td>3%</td>
</tr>
<tr>
<td>Polyphthalamide</td>
<td>6%</td>
<td></td>
<td>4%</td>
</tr>
<tr>
<td>Polymide (PA)</td>
<td>0%</td>
<td></td>
<td>2%</td>
</tr>
<tr>
<td>Polyethylene type F (Polyethylene-PE)</td>
<td>4%</td>
<td>6%</td>
<td>2%</td>
</tr>
<tr>
<td>Ethene homopolimer</td>
<td>0%</td>
<td></td>
<td>1%</td>
</tr>
<tr>
<td>Polytetrafluoroethylene (PTFE)</td>
<td>14%</td>
<td></td>
<td>0%</td>
</tr>
<tr>
<td>Polyoxymethylene</td>
<td>0%</td>
<td></td>
<td>3%</td>
</tr>
<tr>
<td>Phenolic resin</td>
<td>2%</td>
<td></td>
<td>0%</td>
</tr>
<tr>
<td>Polypropylene (PP)</td>
<td>2%</td>
<td></td>
<td>0%</td>
</tr>
<tr>
<td>Polystyrene resin (PS)</td>
<td>2%</td>
<td></td>
<td>0%</td>
</tr>
<tr>
<td>XT Polymer (375-000-301)</td>
<td>2%</td>
<td></td>
<td>0%</td>
</tr>
</tbody>
</table>
Following the conceptual model of the “boomerang effect” proposed by Liubartseva et al. (2018) the microplastic source may be local for about 50% of the polymers, probably finding in Mario Zucchelli its main source, however we must emphasize that a polymer composition similar to ours was reported by Fang et al. (2018) for the Arctic and sub-Arctic benthic organisms on the opposite side of the globe, in which the three dominant polymers were: polyamide (nylon – 46%), polyethylene (23%) and polyester (18%). The main polymers reported for the Arctic sediments in Peng et al. (2018) were polyethylene, polyamide and polypropylene that were reported to account for 76% of the plastic polymers. Therefore especially nylon and polyethylene microplastics could have a global diffusion in benthic habitats.

As for the content of microplastics in the organisms, taxa specific accumulations are revealed when we consider the content of microplastic fragments per individual, untying it from the average size of the organisms. Both the gastropod Eateniella sp. and the bivalves displayed values averagely higher than 1.0 item individual$^{-1}$ and bivalves displayed on average the highest number of microplastics per individual in accordance with previous reports on bivalves and filter feeders from laboratory experiments and a survey work (Kaposi et al., 2014; Setallü et al., 2016; Waller et al., 2017). Filter feeders, by their feeding mode concentrate food from large volumes of water and usually display the highest amount of microplastics. The average values found for bivalves correspond to half of the lowest microplastic content per individual reported by Li et al. (2015) in bivalves from the Shanghai market (4.3 items individual$^{-1}$) and the values are within the range found by Su et al. (2018) of 0.4–5 items individual$^{-1}$ from the estuarine areas of the Yangtze River. Moreover, the mean abundance of microplastics in all the Antarctica benthic organisms, corresponding to 1.0 items individual$^{-1}$, was slightly higher than the 0.8 items individual$^{-1}$ reported for benthic organisms from Arctic and sub-Arctic regions. This highlights a similar sparse microplastic contamination reaching two poorly populated extremes of the earth further confirming the wide diffusion of this contaminant.

The role that microplastics play in the environment, in many cases mimicking food, is closely linked to the feeding mode and size of the investigated organisms (Wright et al., 2013) and an inverse relationship has been found between microplastic abundance in the organisms by weight and the individual specific weights. This inverse relationship describes higher scores of microplastics associated with smaller organisms. This fact could be explained by the higher specific surface of interaction of small organisms with the surrounding habitat and surface sediments. The debate on the effects of microplastics at different levels of the ecosystem complexity and their role as pollutants or carriers is still open. Concerns has been raised in regard to the transfer toward the sediments and the benthic communities of hydrophobic persistent organic pollutants such as polychlorinated biphenyls (PCB) and polycyclic aromatic hydrocarbons (Batel et al., 2016; Derai, 2002; Mato et al., 2001; Wang et al., 2018a,b). However the evidences on the contribution of microplastics to the trophic transfer of persistent organic pollutants are still scarce and the microplastics do not seem to clearly meet all the criteria (especially bioaccumulation and adverse effects) for being themselves defined as persistent organic pollutants (Koelmans et al., 2016; Ziccardi et al., 2016; Lohmann, 2017). No phenomena of accumulation of microplastics towards predators in the benthic trophic chain was noted among the investigated organisms (all invertebrates) as previously stated by Bour et al. (2018), conversely the lowest levels in the trophic chain, filter feeders and grazers, have shown the highest numbers of particles per individual. However this does not exclude accumulation phenomena toward bigger predators, for which a severe lack of evidence was reported (Au et al., 2017). The microplastic contamination seems to be shared among the organisms of the benthic community highlighting that the structure of the community affect as much as the environmental contamination the repartition of microplastics between benthic organisms. Even if microplastics did not have any direct effects on the organisms by simply being continuously ingested and expelled (Dawson et al., 2018) this could progressively ease the release of organic and inorganic plastic additives for which very few data are currently available (Lohmann, 2017) and that could represent an ecotoxicological risk especially for benthic marine organisms (Hermabessiere et al., 2017).

5. Conclusions

The benthic communities of Terra Nova Bay showed diffuse microplastic contamination in all the areas investigated and at all the levels of the benthic trophic chain. The most abundant polymers identified in the benthic organisms were part of the nylon and polyethylene family. Bivalves and gastropods displayed the highest microplastic contamination among the Antarctica benthic invertebrates, comparable to the values reported for other, less remote areas. No evident accumulation through the food web was detected. It is still not clear if the role of microplastics is that of pollutants or only of contaminants, however, it is necessary to deepen the knowledge on distribution and effects of microplastic and additives at all the levels of the food web to evaluate from a wider viewpoint the effects on marine organisms and ecosystems.

CRediT authorship contribution statement


Declaration of Competing Interest

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Acknowledgements

Three anonymous Reviewers are acknowledged for constructive criticism.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.105587.

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