Using monk seal faeces as a non-invasive technique to monitor the incidence of microdebris

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Abstract

Marine litter monitoring and assessment is required under the EU Marine Strategy Framework Directive to prevent any harm on marine ecosystems and their biota. Sampling to evaluate effects of marine litter, including microdebris, in top predators is difficult. Usually, microdebris is examined in the gastro-intestinal tract of stranded dead animals. However, the population of the endangered Mediterranean monk seal is too small for obtaining sufficient samples. The present study implemented for the first time a non-invasive technique for collecting monk seal samples to assess the microdebris ingestion in a systematic manner. A total of 12 samples of monk seal faeces were collected from marine caves in Zakynthos Island, Greece (INTERREG MED project "Plastic Busters MPAs"). A total of 166 particles were identified; 77% of the particles were smaller than 3 mm but a piece of net larger than 5 cm was also found. The majority of particles were filaments (84%), and only one sphere was found. Faeces contained on average 14 particles per sample, half of the average in previous studies using the whole digestive tract. The use of faeces represents an effective non-invasive tool to assess the incidence of microdebris and the trophic transfer of these pollutants.

Keywords

Mediterranean monk seal · Microdebris ·Non-invasive technique · Zakynthos · Greece

Introduction

Anthropogenic debris, including plastics, has recently been identified as a major threat for marine mammals [1]. The impacts of macrodebris on these species can be easily detected [2], however the effects of particles smaller than 5mm (called microparticles) in these top predators are still unknown. To understand the effects of this pollutant, microdebris incidence within the digestive tracts of marine mammals found dead ashore are being regularly investigated [3, 4, 5].

The four-year project "Plastic Busters MPAs"[1], co-funded by the programme INTERREG Mediterranean of the EU, was launched in 2018, and aims to contribute to maintaining biodiversity and preserving natural ecosystems in marine protected areas (MPAs) by defining and implementing a harmonized approach against marine litter. One of the project's study areas is the National Marine Park of Zakynthos (NMPZ) and its surrounding area in Zakynthos Island, Ionian Sea, Greece (Fig. 1). Within the above project, the Mediterranean monk seal *(Monachus monachus*) was selected as a top predator species indicator for microdebris ingestion. This is one of the rarest seal species worldwide, listed as endangered species according to the IUCN [6].

[1] https://plasticbustersmpas.interreg-med.eu/

The best estimation of population for the study area is 17-20 seals of all age classes [7, 8]. The current work examines the possibility to study the microdebris in faeces collected at their resting areas as a non-invasive technique to assess the incidence and trophic transfer of this pollutant, after a promising pre-investigation [9].

Methods

Monk seal faeces were collected at their resting areas in Zakynthos Island in June 2019. All potential external contamination was noted during the survey (e.g., debris in the caves, researchers' clothes and material). Samples were collected in one-use bags and were transported and stored at 4 ºC until processed. During the lab work in the Hellenic Centre for Marine Research (HCMR) precautions were taken for avoiding airborne contamination: two or three blanks were always set around the working area, all chemicals and water was pre-filtered, the room was closed to avoid currents and only one researcher was allowed to be working. The procedure followed the standardized protocol [10].

Faeces were washed through a set of three sieves with different mesh sizes, where the smallest mesh size was at the bottom. Macrodebris, prey items and parasites were collected from the first two top sieves and stored for future analysis. The sample at the bottom sieve was transferred to a clean glass jar with 10% KOH for 3 weeks at 30-35ºC. After that period, a density separation process with NaCl and NaI was carried out as faeces contained an important amount of non-digested material. Solutions were filtrated with a Buchner Filter with a vacuum pump using glass fibre filters (47mm Whatman). The filtration was carried out within a hermetic plastic pyramidal structure used by the HCMR team for microplastics analysis.

Microdebris particles were firstly identifying visually through a stereomicroscope (Olympus SXE) with a camera attached (Infinity 1), classified into fibres, fragments and beads/microspheres, measured for size and photographed. Anthropogenic items found in blanks were also measured and photographed. Chemical characterization for polymer confirmation of each anthropogenic item was carried out using Fourier Transform Infrared Spectroscopy (FT-IR).

Results

A total of 12 samples of monk seal faeces were collected from 4 marine caves. One trammel net was found on the beach of one cave, 5 items of plastic in a second cave and 20 pieces of plastic in the 3rd cave. Faeces weighted between 11.8g to 751.4g, with an average weight of 225.6g. A total of 165 microparticles were identified and a piece of trammel net. The number of particles per sample ranged from 6 to 24 with an average of 14 particles per sample. Most of the particles were filaments (n=140, 84.9%) followed by fragments (n=24, 14.6%) while only one sphere was recorded (0.6%). The length of filaments ranged from 114.03μm to 28.76mm with an average of 2.78mm. Fragments were smaller ranging from 45.15μm to 5.06mm and averaging 509.61μm. The sphere size was 132.74μm. Most of the particles were transparent (n=57, 34.5%), comprising 52 filaments, 4 fragments and the sphere, and most of them were of blue colour (n=65, 39.16%) followed by transparent/translucid (n=57, 34.34%), red (n=13, 7.83%), green and yellow (including the trammel net) (n=10, 6.02%), while the remaining 5% were of several other colours. The polymer composition of 37.35% of particles were correctly identified (level of certainty >70%), where polyacrylamide was the most common polymer (n=45, 27.11%), followed polycarbonate (n=9, 5.4%). The incidence of the rest of the polymers was below 5%.

Discussion

Marine litter, including microdebris, has been detected in all compartments of the marine environment [11]. Highly mobile top predators may ingest marine litter far away from the place they are found stranded; in addition, strandings are rather rare events. On the other hand, useful information on the trophic transfer of microdebris can be obtained when investigating such species.

The European Commission established the Marine Strategy Framework Directive (MSFD) as a mandatory framework to achieve the good environmental status of all member states' marine waters (Directive 2008/56/EC). Among the indicators to tackle is marine litter ingestion and effects on marine biota. In our study, we used monk seal faeces to investigate the incidence of microdebris in top predators. Faeces contained on average 14 particles per sample, half of the average in studies using the whole digestive tract

[8, 12]. We considered all potential external contamination and we took all precautions and measures to avoid it. Despite that airborne contamination during most of the process was controlled, potential contamination at the time of collecting the samples in the field is still something to take care of. The idiosyncrasy of this species of using beaches within caves, suggests that the airborne contamination of their faeces in the field might be lower than those pinniped species resting at haul out areas exposed to wind. We believe that this type of samples can be used as a non-invasive technique for monitoring the incidence of this pollutant in top predators and could be useful to design indicators within the monitoring programmes for the marine litter descriptor of the MSFD.

Figure 1. Map of Zakynthos Island location (37°46'50.12" N, 20°49'01.03" area comprises the National Marine Park of Zakynthos.

Table 1. Anthropogenic debris detected in 12 faeces samples of monk seals collected in four caves (F, S, A and M), Zakynthos island (Greece).

Sample	Num of particles	Type of particles	Size range of particles (µm)
F1	8	Fibres, fragments, sphere	132.7-7571.4
F2	13	Fibres, fragments	171.5-5038.6
F3	9	Fibres, fragments	114.0-714.2
F4	9	Fibres, fragments	94.2-3100.2
S1	14	Fibres, fragments	539.1-13871.7
S2	24	Fibres, fragments	54.0-5950.7
A1	15	Fibres, fragments	143.6-6248.8
A2	15	Fibres	848.7-28763.2
A3	18	Fibres	695.5-6018.6
M1	6	Fibres	100-2831.2
M2	17	Fibres, fragments	45.2-13757.8
M3	18*	Fibres, fragments Trammel net	201.2-5000
Average	14		2448.2

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