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Protocol Article

A microplastic size classification scheme aligned with universal plankton survey methods

J.R. Bermúdez[a,b,c,*], P.W. Swarzenski[a,d]

[a] International Atomic Energy Agency, Principality of Monaco, Monaco
[b] Facultad de Ingeniería Marítima y Ciencias del Mar, Escuela Superior Politécnica del Litoral ESPOL, Campus Gustavo Galindo, Guayaquil, Ecuador
[c] Galapagos Marine Research and Exploration, GMaRE, Joint ESPOL-CDF program, Charles Darwin Research Station, Galapagos Islands, Ecuador
[d] USGS Pacific Coastal and Marine Science Center, Santa Cruz, United States

ABSTRACT

Microplastics (MP) are a pollutant that can be found in all marine ecosystems. Currently one of the most used forms to classify them is through their size. However, the current size categories in use cover an extremely wide range of sizes and are not based on a biological or physical basis. Thus, here we propose to harmonize the MP size categories with the ones already in used on plankton research for more than 120 years. This will allow the implementation of more refined MP size classes that are connected to a biological reality and will also enable the comparison of a myriad of literature on plankton research with the new work on MP.

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* Corresponding author.
E-mail addresses: jrbermud@espol.edu.ec, j.r.bermudez-monsalve@iaea.org (J.R. Bermúdez).

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Reagents/tools:	*Non applicable.*
Experimental design:	*Non applicable.*
Trial registration:	*Non applicable*
Ethics:	*Non applicable*
Value of the Protocol:	• *This piece addresses the current situation in the nomenclature used for the classification of microplastics (MP) based on their size and propose a better system.* • *The current size categories cover an extremely wide range of size classes, which are not based on an underlying biological or physical basis. Thus, we propose to harmonize the current MP size categories with the classification scheme that is widely used for all plankton research and that dates back more than 120 years.* • *We hope that this revised classification scheme will facilitate a more realistic MP delineation that is also aligned with existing marine biological sampling protocols and will also enable intercomparisons of existing literature on plankton research with current research on marine MP.*

Description of protocol

Microplastics (MP) are well-known pollutants of global concern in aquatic ecosystems [1]. MP can be defined as "any synthetic solid particle or polymeric matrix, with regular or irregular shape and with size ranging from 1 μm to 5 mm, of either primary or secondary manufacturing origin, which are insoluble in water" [2]. MP particle characterization refers to the principal chemical component of the polymer (i.e., polyethylene vs polystyrene vs polyvinyl chloride, etc.). While primary vs secondary manufacturing origin refers to 'off-the-shelf' vs "weathered" particles [1]; primary MP are produced, for example, for cosmetics [1], while secondary MP are the product of continuous chemical and physical weathering processes of larger particles in the ocean, such as fibers from textiles of from discarded fishing gear [1].

MP particles disintegrate in seawater according to a power function and thus exhibit a size range distribution spanning many orders of magnitude [3,4]. As a consequence, the progressive fragmentation of MP in the ocean into smaller and smaller fragments will yield a size spectrum that favors very fine particle sizes. A recent review on the MP size distribution and relative abundance from 11 studies confirm this consistent increase in abundance towards smaller-sized particles. However, the lower limit in particle size that was reported by each study varied considerably, with a few even ranging as low as 10 µm (Table 1). According to these authors, the smallest particles are most often overlooked due to technical observational limitations, leading to a potential artificial bias on the presence of small size classes [5]. With improvements in equipment and methods, (e.g. Fourier Transform Infrared microscopy, and Raman microscopy) more studies are now

Table 1
Lower size limit of microplastics on several studies reporting microplastic abundance in the environment reported by Kooi & Koelmans (2019).

Lower size limited reported (µm)	Number of studies
10	3
25	1
30	1
35	1
45	1
250	1
>300	1
500	1

confirming that the smaller size fractions are indeed more prevalent [6]. Therefore, the marine plastics research community should urgently adopt harmonized techniques and definitions to properly describe and quantify these smaller-sized MP particles. This is particularly relevant from an ecological and toxicological perspective, as evidence clearly supports that these smaller-sized MP particles are preferentially ingested by zooplankton and other lower trophic marine organisms [7,8] and may translocate across a cell membrane [9,10].

State of the art

Currently there is a lack of consensus in the classification of plastic debris, which can contribute to further uncertainties [11]. While several factors may be considered in classifying MP particles, such as, chemical composition, state, solubility, shape and structure, color and origin [2,11], the most widely used and reported nomenclature is based on MP size [11,12]. Currently it has been suggested only four size categories: nanoplastics: 1–1000 nm (subdivided in nanoplastics 1–100 nm and sub-microplastics 100–1000 nm), microplastics: 1–1000 µm, mesoplastics: 1–10 mm and macroplastics: >1 cm [11]. Each of these categories covers a wide range of sizes and the proposed cutoffs is based on an operational compromise to accommodate current literature [11], but does not correspond to a defined biological or physical attribute. Now that ecological and toxicological studies on plastic pollution are becoming widespread [13] and quantification methods are much more precise for smaller sizes, a revised MP classification scheme that is aligned with universal plankton sampling strategies is proposed.

Particle size has a major ecological relevance as it is potentially the most important factor determining the MP interaction with biota and its environmental fate [7,8,14–16]. Experimental laboratory studies have demonstrated that zooplankton have the capacity to readily ingest suitably-sized MP particles [7,8]. A field study of mysid shrimps, copepods, cladocerans, rotifers, polychaete larvae and ciliates all showed that MP was directly ingested [7], and size is a crucial factor influencing the numbers of plastics ingested [17]. This can be expected, as it has been shown on plankton that there is a distinct size relation between predator and prey. Size selectivity spectra of 28 planktonic predators from 18 studies shows that there is a linear size ratio between predators and their optimal prey [18]. This relation is 1:1 for dinoflagellates, 3:1 for other flagellates, 8:1 for ciliates, 18:1 for rotifers and copepods, and ∼ 50:1 for cladocerans and meroplankton larvae [18,19]. Also there is a difference in prey selectivity between filter feeders and raptorial-interception feeders, preferring relatively smaller and larger prey, respectively [18,19]. Thus, there is an expected size range of MP that can be ingested by different zooplanktonic organisms, and for most species with available information this range is between ∼ 32 to ∼2 µm [18,19] (Fig. 1). Thus, size classification of MP should be expanded to include categories on aquatic ecosystem function and interactions as has been already achieved with the universally accepted plankton sampling methods. Plankton - plastic size class interactions are currently hard to investigate, due to a potential mismatch in size class definitions, which hinders the comparison of previous literature on plankton research with the new research on MP.

Proposed new classification

Standardized MP size class definitions would greatly benefit of harmonization with the nomenclature used in marine plankton studies for more than 125 years, and upon which most of the plankton research is based. Indeed, Schütt, (1892) was the first to introduce such size categories [20], and first applied the terms ‘micro’, ‘*meso*’ and ‘macro’ in this capacity; subsequently Lohmann (1911) added the categories ‘nano’ and ‘mega‘; and Sieburth, Smetacek & Lenz (1978) proposed the terms “pico” and “femto”, to further define the size spectrum. These authors have argued that these size classes are based on the International System of Measurement (SI) (e.g., micro:10^{-6}, nano:10^{-9}, pico: 10^{-12}, and femto: 10^{-15}. They also demonstrated that such size class separations are all the more applicable if the plankton organisms are regarded on the basis of volume (three-dimensional) rather than length (one-dimensional).

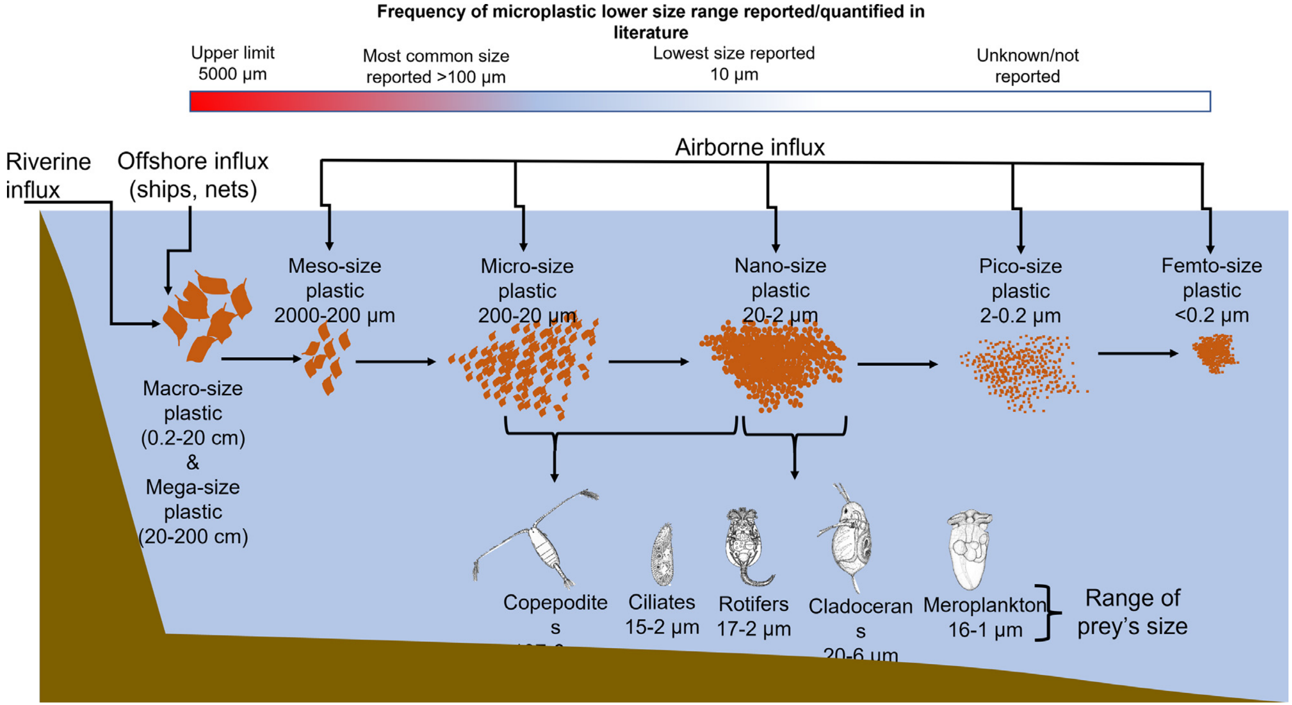

Fig. 1. Microplastic fragmentation and concomitant reduction in size/increase in abundance. There is a gap in the reporting of small microplastic abundance in the environment and the size fractions that different types of zooplanktonic organisms can ingest. It is proposed to denominate microplastic size fraction according to nomenclature already in used by the plankton community in order to make more intuitive relations between predators and potential microplastic "prey". Frequency of reporting of microplastic lower size derived from Kooi & Koelmans, (2019). Prey size for zooplankton groups is taken from Hansen et al., (1994); the upper and lower limits of prey size that can be ingested, in terms of equivalent spherical diameters, are presented in micrometers. Figure not to scale.

Table 2
Current and new proposed size class nomenclature for microplastics, as well as their respective proposed size range, and several organisms of equivalent size in the environment.

Current size categories	Size range	Proposed size categories	Size Range	Organism of equivalent size
Nanoplastic	0.001–1 µm	Femto-size plastics	0.02–0.2 µm	Virus
Microplastic	1–1000 µm	Pico-size plastics	0.2–2 µm	Bacteria
		Nano-size plastics	2–20 µm	Flagellates
		Micro-size plastics	20–200 µm	Diatoms, dinoflagellates, ciliates, daphnids
		Meso-size plastics	200–2000 µm	Amphipods, appendicularians, chetognatos, copepods, thaliaceans
Mesoplastic	1–10 mm			
		Macro-size plastics	0.2–20 cm	Euphausiids, heteropods, jellyfish, larval fish, mysids, pteropods, solitary salps
Macroplastic	> 1 cm			
		Mega-size plastics	20–200 cm	Jellyfish, colonial salps

Thus, resulting equivalent MP particle size class definitions would include femto-size plastics (0.02–0.2 µm), pico-size plastics (0.2–2 µm), nano-size plastics (2–20 µm), micro-size plastics (20–200 µm), meso–size plastics (200–2000 µm), macro-size plastics (0.2–20 cm) and mega-size plastic (20–200 cm) (Table 2, Fig. 1). The subfix “size” is proposed to avoid terminology confusions between the global term microplastic and the micro-size fraction. Such an approach would facilitate intercomparisons by diverse groups studying marine plastics, would allow current research to be placed into 100-yr plankton research records, and will yield a common framework to advance our understanding of this universal pollutant.

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.

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