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REPLY TO LENZ ET AL.:

Quantifying the smallest microplastics is the challenge for a comprehensive view of their environmental impacts

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Studies on impacts of emerging contaminants are challenging, as is the case for studying the smallest sizes (<100 μm) of microplastics, mainly because there is no clear view of their actual concentration and characteristics in the natural environment (1). Major developments are required to establish standardized procedures for collecting, fractionating, characterizing, and quantifying polymer particles; probably, the best promising method is in a liquid matrix. In our recent article on impacts of microplastics in oysters (2), the microplastic size tested was of 2 and 6 μm , the size range preferentially ingested by filter feeders, which is far below the size robustly characterized and quantified at sea (i.e., >330 μm) (see, for example, ref. 3). These emerging contaminants need a starting point in assessing their toxicity, even when natural concentrations and behavior (speciation, complexation, and aggregation) are unknown, which is the case for most nanomaterials (4). The use of high concentrations in ecotoxicological studies can be viewed as a proof-of-concept, producing ground-breaking data for assessing the potential risk of a new class of contaminants, such as microplastics, and helping to define biomarkers and phenotypic impairment. Ultimately, exposure studies should be as close to environmentally realistic concentrations as possible to avoid overreaction or misinterpretation of nonenvironmentally realistic data, as Lenz et al. (5) mention.

The realization of dose–response exposure experiments could be recommended as appropriate to assess the extent of microplastic toxicity, tackling the high

spatiotemporal variability of microplastic concentration expected in coastal ecosystems (1) and possible rare accidental events, until methodological barriers preventing the assessment of small microplastic concentrations in natural environments can be knocked down. Once the knockdown of these barriers is achieved, mimicking environment in microcosms and mesocosms will still be challenging. First, we need to statistically consider the probability of encountering microplastics for marine organisms to achieve reproducibility and repeatability in estimating impact in marine life. Second, other criteria are of high relevance for defining an environmental scenario, such as shape, complexion, interaction with organic matter, and biological and chemical loads of microplastics. It is noteworthy that these last criteria, mainly for the adsorbed persistent organic pollutants, were recently debated as possibly negligible (6). As an example of the importance of those criteria, the limited impact of microplastics on fish larvae warrants caution because the smooth and spherical characteristics of exposed polyethylene beads are different from plastic debris found in the environment (7). Authors have hypothesized that the ruggedness of particles of irregular shape could have a longer retention time in the guts of animals and fish, potentially causing inflammation and increasing biological impacts. In many cases, such as these, there is now a need to extend the range of particle types, complexations, and concentrations used in laboratory exposure experiments to recreate environmentally realistic scenarios.

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- 2 Sussarellu R, et al. (2016) Oyster reproduction is affected by exposure to polystyrene microplastics. *Proc Natl Acad Sci USA* 113(9): 2430–2435.
- 3 Gottschalk F, Sun T, Nowack B (2013) Environmental concentrations of engineered nanomaterials: Review of modeling and analytical studies. *Environ Pollut* 181:287–300.

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