CleanSea

Towards a Clean Litter-Free European
Marine Environment through Scientific Evidence
Innovative Tools and Good Governance

Testing indicators for biological impacts of microplastics

The EU is still far from its goal of achieving healthy seas and part of the problem is due to marine litter (European Commission, 2014). Plastic materials invariably make up the dominant fraction of marine litter and there are particular concerns regarding the impacts of plastic microlitter (plastic particles in the range of a few nanometers up to 5 mm). The small size, persistence and ubiquity of these 'microplastics' in both pelagic and benthic ecosystems means they have the potential to be ingested, along with naturally occurring particulate matter, by a wide array of marine biota with unknown consequences for Darwinian fitness parameters (growth, survival, performance, reproduction). Due to the varying size, buoyancy and composition of marine litter, ingestion will vary for litter types between feeding guilds; planktivores and filter feeders will encounter low-density litter fragments suspended in the upper water column whereas high density litter fragments are more likely to be available to deposit feeders and detrivores.

Important research gaps remain for the implementation of the Marine Strategy Framework Directive (MSFD) over the suitability of different species for evaluating microplastics impacts across the four main seas regions of Europe. These gaps include the composition of ingested litter, its propensity for retention within the gut, leaching of associated chemicals, translocation within body tissues and transfer through generations and/or the food web.

One of the aims of the CleanSea project task is to provide fundamental scientific knowledge on the scale and nature of the physical and chemical impacts of marine litter, and in particular microplastics, on exemplar marine organisms and the predicted consequences for populations and communities. We aim to determine the impact of this type of marine litter on population-relevant fitness parameters and energy budgets in key species, with a focus on the base of the marine food web. In CleanSea, the main species under study include algae, bacteria, invertebrates such as zooplankton, sponges, echinoderms, bivalves and crustaceans, as well as fish and birds. Here we present a summary update on progress on determining the ecological harm of microplastics and discuss important aspects of indicator species selection that Member States will be confronted with when implementing the Marine Strategy Framework Directive (MSFD).

What makes a good indicator species?

An indicator species, also known as a sentinel organism or bioindicator is an organism (or part of an organism) that is used to assess an anthropogenic pressure in the environment. An indicator species can be used for biomonitoring – a long term set of observations of the same parameter in the same indicator species in field. A famous example is the Mussel Watch Programme in which environmental contaminants are measured in a field-exposed marine bivalve species, creating a database of contamination level in space and time.

A bivalve mollusc was selected as the indicator species for the Mussel Watch contaminant monitoring program for a variety of reasons (Lauenstein & Cantillo, 1993), including:

i. 'Cosmopolitan' species, so can be used on sites at vastly different geographical locations

ii. Sessile, so the body residues reflect the exposure at the location at which they are collected (or deployed);

iii. Body residues are expected to reflect contaminant concentration of surrounding water;

iv. Not too sensitive, so they can survive exposure and other adverse conditions (as opposed to some species of fish and crustaceans that are more sensitive and will die before their body residues can be measured);

v. Bioconcentration observed for many contaminants, i.e. contaminant uptake from water column to organism is high (facilitates detection);

vi. Unable to biotransform and excrete many key toxicants (facilitates detection; gives a worst case scenario for organisms which lack metabolic abilities);

vii. Abundance of individuals existing in relatively stable populations, facilitating repeated sampling;

viii. Commercially important seafood species with public health implications.

These considerations are a good point of departure when selecting biological indicators for Descriptor 10. In the case of plastic litter, the indicator organism is sampled in the field and the internal exposure (see Escher & Hermens, 2004) to plastic particles is assessed by measurement of the concentration of plastic materials in the body of the organism (either the whole body residue or the residue in part of the body). The organisms are collected in the field in order to reflect field exposure in a particular area, so this metric functions as an indicator of plastic exposure levels in the environment.

In addition to the above-mentioned points, when selecting indicator species it is advisable to consider additional aspects such as:

i. Region-specific indicator species, as developed jointly within the framework of Regional Seas Conventions (RSCs);

ii. Species that are not threatened or protected;

iii. Species that can be kept in cages for easy field deployment and collection (such as in Mussel Watch);

iv. Invertebrate species, which require less staff training (cost-effective) for handling than vertebrate species;

v. Perform sampling in a cost-effective manner by synergies with pre-existing programs;

vi. Species (and parameters in species) which when measured are directly linkable to impact and effects (and Good Environmental Status, GES), although this will be difficult;

vii. Species and (parameters in species) that are directly linked to measures and could be used to evaluate progress towards targets and effectiveness of mitigation activities, although this will be difficult to link in most cases.

This should be seen as a list of considerations to take into account, however it is unlikely that indicator species can be selected optimizing every aspect on the complete list. That being the case, suitable indicators can nevertheless be identified that are fit-for-purpose and effective.

Indicator species for Regional Sea Conventions and EU

In various regional seas, progress towards selecting indicator species is being made already, also with the support of the EU Technical Subgroup on Marine Litter. The indicators are currently being discussed within HELCOM contracting parties (including the Baltic Sea Member States) and MEDPOL (Mediterranean Contracting Parties), with somewhat less attention going to marine litter indicators in the Black Sea region (only two EU Member States, Bulgaria and Romania). In the North East Atlantic (OSPAR) region, litter in the stomach contents of Northern Fulmar seabirds is being monitored as an ecological quality indicator for MSFD indicator 10.2.1 “Litter ingested by animals” (OSPAR Commission, 2008). Some Member States in the OSPAR region have already stated that they intend to include this parameter in their monitoring programmes. The parameter measured is the mass of plastic in the stomach contents, and the target is: “There should be less than 10% of Northern Fulmars (*Fulmarus glacialis*) having more than 0.1 g plastic particles in the stomach.”

A review of the national initial assessments has shown that there is room for improvement in MSFD implementation in several identified areas (European Commission, 2014). Without baselines, it’s also difficult to assess how far a Member State has to go to achieve a future target (be it aspirational or binding). There has been little coherence within the EU and even between neighbouring countries, even though regional cooperation is supposed to be 'at the very heart of MSFD implementation'. The recommendation is that the upcoming monitoring programmes (and also programmes of measures) should address some of the gaps and problems identified in the review of the initial assessment.

Effective application of biological indicators

The Northern Fulmar regularly ingests and retains plastic at detectable contaminant concentrations. The abundance of the contaminant is important because it enables the measurement of a decrease in concentration in time, i.e. concentrations that are lower than the baseline (start of a time series) must also be quantifiable. If the analyte is already at the level of the limit of detection (LOD) or limit of quantification (LOQ), then no decrease in contamination is detectable in time, and also it is impossible to compare contaminant levels between locations when the measurements are <LOQ. In such situations, the indicator is not fit-for-purpose because it does not enable data comparisons in time (at same geographical location) or space (between geographical locations). In the current wording of the MSFD Descriptor 10, the GES indicator to be measured in biota for refers specifically to trends in amounts and composition of ingested litter.

Biological indicators are important for the MSFD implementation for Descriptor 10 Marine Litter in that they can *inter alia* provide a baseline to measure progress towards targets and measure field exposure to litter which is important for determining risk. To be effective, these indicators should be regional in character, and ideally they should be comparable across the EU regions (European Commission, 2014).

For indicator species data to be effective, there also needs to be a good registration system, a user-friendly database of collected measurements and accompanying metadata including quality assurance/quality control (QA/QC) metadata. QA/QC[1] is in fact important not only for sampling and analysis methods, but also throughout the entire monitoring programme design and definition of targets and indicators. The inclusion of metadata is critical for data users to be able to apply the information and knowledge stored in the database. The data management system should aim for effective data sharing with clear intellectual property attribution, and access online via web interfaces (e.g. OSPAR data management systems).

Future development of QA/QC for biological litter indicators should include such aspects as: training programs, proficiency testing, greater validation and more standardization of sampling and analytical protocols and accreditation. As Criddle et al. (2009) previously pointed out, when it comes to quantitative, quality-controlled marine litter data, there is only a limited amount available to date.

[1] QA/QC is an essential aspect of marine litter environmental monitoring. QA/QC includes attention to many things, such as validated methods and procedures, training personnel, appropriate and well-functioning equipment, control charts to assess quality of laboratories over time, blanks, duplicates, standard reference materials, certification programs, interlaboratory studies. Building up a QA/QC system for a new analyte takes time (normally years), appropriate budgets and significant effort by the international research community, standards institutions and proficiency testing providers etc. This is especially true for microplastic. This is a large, complex group of analytes consisting of many size fractions (over 5-6 orders of magnitude) with a wide variety of material properties and content.

Key summary points

- Biological indicators are important for the MSFD implementation for Descriptor 10 Marine Litter in that they can provide a baseline to measure progress towards targets and measure field exposure to litter which is important for determining risk. To be effective, these indicators should be regional in character, and ideally they should be comparable across the EU regions.
- Plastics can be ingested by organisms from across trophic levels, including zooplankton, bivalves, fish, birds and marine mammals. Plastic exposure is widespread and practically unavoidable for sea creatures – over 80% of field collected individuals of a variety of marine species and taxonomic groups studied in CleanSea had plastic in their bodies at the time of sampling. Plastic in the sea is colonized by microorganisms, some pathogenic, some invasive species. Preliminary ecosystem modelling work in the North Sea showed that the impact of these contaminant plastic materials could potentially result in a 5-10% reduction of secondary productivity, i.e. production of biomass by consumers (animals) in ecosystems. More input data is needed to improve the accuracy of the initial model outputs, but the message is that significant ecosystem level impacts of microplastics cannot yet be ruled out.
- The overall hazard posed by plastics in the ocean remains to be established, but is predicted to encompass entanglement (macroplastic items), substrate for invasive species and/or pathogens, ingestion, physical damage, particle toxicity, the effects of sorbed and leaching chemicals. Plastic materials in marine litter consist of a mix of polymers and chemical additives and often some residual monomers that are still in the material (e.g. styrene monomers in polystyrene). Preproduction pellets that are ubiquitous on marine beaches are often virgin resins to which additives had not yet been added, so they contain only chemicals sorbed from the air or seawater they are exposed to. It's important to remember that substances of high concern and other toxics are applied in plastic materials, so that a discussion of the impact of marine plastic litter is also a discussion of the chemicals present in the litter items. This polymer-chemical mixture gives rise to the potential for multiple stresses on exposed organisms (e.g. chemical toxicity, particle toxicity, entanglement, physical damage) that can potentially arise from a plastic marine litter item. Chemicals so far identified as being associated with plastics debris include solvents, plasticisers, UV screening compounds and antimicrobials, persistent and priority organic pollutants and metals.
- Microplastics reaching the oceans have been identified from all main polymer groups, with an abundance of polypropylene, polyethylene, polystyrene and polyvinylchloride. Microplastics contaminating the marine environment belong to a potentially large, complex group of analytes consisting of many size fractions (over 5-6 orders of magnitude) with a wide variety of material properties and mixed contents (polymers, copolymers, chemical additives and residual monomers).
- Biological effects that have been studied following ingestion of microplastics include inflammation, oxidative stress, tissue damage, and effects on survival, growth and reproduction. Currently, biological effects on feeding, growth and pathways of energy assimilation may be a potential mechanism by which uptake of microplastics could affect natural populations.
- A systematic approach is being trialled to understand the biological effects of microplastics ingestion in aquatic species. Measurement of biological and physical effects focuses on population-relevant endpoints; growth, survival and reproduction. For primary producers (algae) this includes pulse amplitude modulation (PAM) fluorometry. Histology, confocal and electron microscopy bio-imaging can identify uptake of microplastics into the body, into and across gut and gills and to determine the extent, if any, of translocation to other tissues.
- Methods for identifying plastics in environmental samples include Direct Analysis in Real Time Mass Spectrometry (DART-MS), Fourier Transform Infrared Spectroscopy (FT-IR), Raman spectroscopy and coherent anti-Stokes Raman spectroscopy (CARS). Collectively, these methods can provide information on the identity of plastics present in samples.
- Tissue residue analysis of organic pollutants associated with plastic materials (flame retardants, fluorinated surfactants, bisphenol A, chlorinated paraffins) can be determined by techniques such as mass spectrometry time of flight (MS-TOF). Such techniques enable us to quantify plastic additives in tissues, but also in plastic materials themselves.

References

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CleanSea at glance

- Title: Towards a Clean, Litter-Free European Marine Environment through Scientific Evidence, Innovative Tools and Good Governance.
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