1.6.6.1 OSPAR request on development of a common monitoring protocol for plastic particles in fish stomachs and selected shellfish on the basis of existing fish disease surveys

Advice summary

ICES provides a preliminary protocol for monitoring of plastics in fish stomachs in the OSPAR maritime area. There has been no such monitoring of plastics previously, so it is recommended that, if adopted, this protocol is reviewed at regular intervals and improved on the basis of experience. Integration with fish disease and fish stock surveys will be cost-effective, and the possibility of using samples from commercial vessels should be explored. Certain plastics are better monitored through other ways than the examination of fish stomachs.

Request

ICES is requested to define an appropriate common monitoring protocol [for plastic particles in fish stomachs and selected shellfish] *to be applied across the OSPAR maritime area (taking into account work carried out by pilot projects, e.g. in Germany) as well as clearly articulating and defining the other steps that would be required in the practical work.*

Elaboration on the advice

Protocol

1. Selection and recording of gear

Sampling gear characteristics (mesh size, material, mesh size at codend, presence of plastics) should be recorded. This will enable any contamination from the gear to be identified. Codend mesh sizes larger than 5 mm are recommended to avoid that microplastics from the water column or from the seafloor are included in the samples.

Gear should be clean prior to fishing operations, ensuring that no obvious plastic contamination is present from previous hauls. Following the haul, the litter composition in the gear should be recorded. Additional sampling data for marine litter may be included, such as coupling nets for microplastic identification to the trawl nets, as well as sampling microplastic in the sea surface using manta trawls or plankton pumps.

2. Selection of fish or shellfish species for monitoring of plastic (with consideration of integration of monitoring of fish disease)

Fish species selected for plastic monitoring should ideally be representative for the whole OSPAR area. However, since no one species occurs throughout the entire area, species should be selected that occur widely. For the pelagic zone, mackerel *Scomber scombrus* and herring *Clupea harengus* are recommended, for the benthic zone cod *Gadus morhua* and dab *Limanda limanda* (which is also used for contaminant monitoring under the OSPAR Coordinated Environmental Monitoring Programme (CEMP) and for disease monitoring) are recommended. In addition, flounder *Platichthys flesus*, has been studied in coastal waters for fish disease and is relatively widespread. On coastlines and in estuaries, shellfish such as the mussels *Mytilus edulis* or *M. galloprovincialis* are relatively easy to obtain and can be used for the smaller plastics (and are presently used for contaminant monitoring). Further species would be required in more southern or deeper waters of the OSPAR area (see Suggestions below).

3. Sample size

Sample sizes and sampling stratification depend very much on policy choices associated with the monitoring requirement. ICES suggests that initial monitoring might be on the basis of ICES divisions and subdivisions. These divisions, for example, break the North Sea into three parts. A suitable number of samples per division would be 50 individuals per species, preferably taken randomly from hauls distributed across each division. An alternative, should more funding be available, would be to take one or two samples per haul within each division – assuming the study is undertaken from vessels engaged in fish stock surveys. The cost of a monitoring programme for plastics is related directly to the number of samples processed.

4. Preservation protocol

It is recommended to store biota samples onboard (using aluminium foil for freezing at −20°C or preservation in ethanol using glass receptacles) for subsequent plastics examination in the laboratory. Small fish specimens may be frozen whole or preserved in ethanol; in the case of larger fish, these can be dissected so that the gastrointestinal tracts can be removed intact and frozen individually. If the fish is dissected in open air the probability of contamination from small airborne particles (mainly synthetic fibres) is very high. Possible sample contamination needs to be avoided; if possible fish should be dissected in a clean-air cabinet. If it is not possible to ensure that such contamination cannot occur, ICES recommends not using these samples to monitor fibre contamination.

5. Recording the basic condition of the fish

For fish examined for plastic contamination, sex and total length (rounded down to the nearest cm) should be recorded as a minimum requirement. If equipment onboard research vessels and resources permit, recording should also include age (subsequent reading of otoliths), total and gutted wet weight, as well as the weight of the gastrointestinal tract and the stage of sexual maturation. If onboard measurements are not possible, representative samples of fish can be frozen and processed later. Data generated can be used to assess general body fitness (condition factor, organosomatic indices) and reproductive parameters (gonadosomatic index, maturation index).

If plastic monitoring is included as part of an integrated fish disease, biological, and chemical monitoring programme, additional supporting parameters may be measured, such as other biomarkers and contaminant concentrations in fish. These data are of course also valuable, since they may show more subtle biological effects and the role of contaminants. This would possibly imply measuring more substances than are analysed presently as part of the OSPAR/HELCOM/EU reporting requirements.

Data on marine litter in fish should be generated in such a way that they can be reported to the ICES Environmental Database (DOME), preferably together with the fish disease data.

6. Examination of (micro)plastic content in laboratories

The examination protocol depends on the type and size of plastic to be monitored. Airborne contamination by synthetic microfibres is an important problem in plastic assessment and the number of quantification methods described in literature is limited and has not been harmonized. If monitoring of only mesoplastic (5 mm to 25 mm) or macroplastic (>25 mm) is required, visual inspection of the stomach content will be sufficient for quantification, and this could be carried out on board. However, the standard laboratory method recommended by ICES below will allow reliable and reproducible assessment of microplastics >5 µm in shellfish and >20 µm in the gastrointestinal tract of fish specimens. Assessment of the quantities of fibre under 5 mm in length is particularly susceptible to airborne contamination and is therefore not considered reliable.

- *Airborne contamination, particularly by microscopic fibres less than 5 mm, must be avoided*

Due to great difficulty in controlling airborne contamination, ICES does not recommend examining for fibres under 5 mm in length. However, rigorous precautions must be adopted while handling and processing the samples to avoid airborne and solvent contamination with microplastic, particularly of longer fibres. For example, all required solutions must be filtered with a qualitative filter before starting the protocol, all laboratory glassware must be cleaned with acetone and filtered type 1 ultrapure water before use, a 100% cotton lab coat should be worn at all times, and all sample preparation must be performed in a clean-air cabinet.

- *Microplastic examination is based on the digestion of tissues*

Diverse digestion methods are described in literature, but ICES considers that the acid destruction method is the best available as it not only digests tissues, but also removes all other organic material, leaving only silica (e.g. sand particles) and plastic particles. The extraction of microplastics from the gastrointestinal tract (or complete, non-depurated, shellfish soft tissue) is performed using a technical grade acid mixture HNO_3:$HClO_4$ (4:1 v:v). Acids such as H_2SO_4 and other acids that attack plastics should not be used. Further studies are required on the use of the HNO_3:$HClO_4$ mixture as there are reports of degradation of microfibres of nylon. For optimal digestion of the tissues and organic matter, 500 ml acid solution should be used per 100 g tissue. Digestion is executed in an acid-proof closed fume hood for 1 h or more (up to 5 h for large samples). It

is important to protect the sample from airborne contamination while in the fume hood (e.g. through the use of a clock glass). The digest is boiled (> 80°C) for 10 min, followed by a dilution of the digest with heated and filtered type 1 ultrapure water. If needed, the solution is boiled a second time until the tissue is completely digested as observed by visual inspection, followed by a cooling period of 30 min. The acid digest is filtered using a cellulose-nitrate qualitative filter and the filter is transferred on a glass Petri dish for transport and visual inspection for microplastics under a microscope (200x is recommended). The particle retention of the filter will determine the observed microplastic size range and ICES recommends evaluation of microplastics >20 µm (or >5 µm for mussels). Observed microplastics can be classified by colour, size, and category (fibre, film, spherule, fragment). A hot point test (hot needle held with tweezers) can be done on suspected particles. The hot point will make the plastic sticky and leave a mark. If wanted, plastic particles may be further identified by polymer type using FT–IR or Raman spectroscopy.

- *The assessment should include procedural blanks*

In order to account for any possible contamination, procedural blanks should be included for each batch of samples being destructed by acid. Typically three blanks have been used per batch. The procedural blanks are performed without tissues in parallel with samples containing the digest of the gastrointestinal tract. Any particles detected in these blanks are characterized according to type, size, and colour. Any particles or fibres matching these characteristics are omitted from analysis in samples containing tissue. As airborne contamination by fibres is possible (in the lab or on the research vessel), synthetic fibres smaller than 5 mm in length are excluded from this monitoring protocol.

- *Recording plastic contamination*

Data on plastic contamination in fish or shellfish should be generated in such a way that they can be reported to the ICES Environmental Database (DOME). Plastic data should include the number of plastic items, size, colour, shape (according to the JRC-EU guidance for marine litter; EU, 2013), plastic weight (if possible), and polymer type (if possible), and must be linked to supporting parameters of the assessed fish/shellfish and sampling information.

7. Quality assurance

The status of any new indicator should only progress towards an operational (common) indicator if quality assurance procedures are in place. This is especially important if the indicator is going to be used in (sub-)regional assessments, across countries. In order to achieve coherent monitoring for new thematic areas, such as litter, the OSPAR Coordinated Environmental Monitoring Programme (CEMP) is in place. ICES notes the work of the CEMP Task Group (cf. ICG ML(12) 14/4/2; OSPAR, 2014) to develop a new template for CEMP requirements.

In the current advice it is assumed that sampling will use existing surveys, where possible fish disease surveys, or surveys conducted for fish stock assessments. These surveys use sampling strategies and protocols as well as reporting and validation of data submitted to the ICES Environmental Data Centre, which is done according to ICES standard quality assurance procedures.

There are no quality assurance protocols in place for monitoring of plastics in fish stomachs, and the current advice is the first attempt to develop any protocol. ICES can only start completing the proposed new CEMP appendix if a decision is made to start monitoring.

Suggestions

A number of aspects of the occurrence of plastics in fish and shellfish would not be covered by the recommended protocol. This is mainly due to inadequate scientific knowledge on which to base recommendations and advice. If monitoring of these aspects is to be considered, initial scientific studies are required as a minimum.

Crustaceans take up some plastics and studies both in brown shimp *Crangon crangon* and in *Nephrops norvegicus* have shown the presence of plastics. The former species may be suitable to monitor for microplastics in nearshore waters, while the latter may be suitable to monitor some of the sediment sink areas of northern OSPAR waters. There have been no studies of deep-water species in the OSPAR area; some of these may be expected also to consume plastics in those waters. There

may be an issue with the evacuation of stomach contents in fish brought up from depth; this would need to be examined. It is advised to conduct further studies of the suitability of nearshore shellfish as monitors of microplastics, particularly if these studies were to look at ways of integrating sampling carried out for, e.g. shellfish quality or water framework directive purposes. In the southern part of the OSPAR maritime area, species such as anchovies *Engraulis encrasicolus*, hake *Merluccius merluccius*, and megrim *Lepidorhombus whiffiagonis* may need to be chosen; however, there has been no study of the suitability of these (or other) species to date.

If surveys of ingested plastic in fish commence using ships that are engaged in existing fish (e.g. the ICES International Beam Trawl Surveys [IBTS]) and environmental monitoring surveys, consideration should be given to expanding the fish disease monitoring programmes to cover the OSPAR maritime area. This may inform future assessments of the effects of plastic ingestion and would save ship time. The disease monitoring programmes should be based on standardized ICES/OSPAR protocols and guidelines. An approach explaining how an integration of monitoring of fish diseases and ingested plastic can be achieved is shown in the Annex.

Basis of the advice

Background

The surveys for fish and shellfish diseases aim to gain data on the prevalence of diseases in wild fish submitted by ICES Member Countries conducting this kind of monitoring. All steps involved in the practical work during fish disease surveys (sampling strategies, inspection of fish for target diseases, disease diagnosis) as well as reporting and validation of data submitted to the ICES Environmental Data Centre are done according to ICES standard quality assurance procedures. It is feasible, via these surveys, to collect sampling material in sufficient amounts in order to conduct regular monitoring of plastic particles in fish stomachs and selected shellfish such as the common mussels.

There are a number of reasons to monitor plastics in the environment. These may be divided into (a) gaining knowledge of the occurrence, distribution, and trends through time of plastics in the marine environment, and (b) gaining knowledge of the effects of these plastics on biota. There are a number of advantages to using fish species for the monitoring of plastics: fish can integrate the occurrence of plastics over a wide area and at a variety of depths in the water column and on the seabed. Fish also eat other biota and might thus accumulate and magnify amounts of plastic from the foodchain. Fish are also already sampled in a number of existing surveys, including those for fish disease. In contrast to the statement in the background to the request (above), ICES does not have information on shellfish monitoring and there have been only limited studies on some crustaceans.

The choice of a monitoring protocol depends very much on the objective of the monitoring. At present, ICES does not consider that there is enough information to be able to monitor trends in the effects plastics have on fish, but if such monitoring is required by OSPAR, then ICES strongly recommends that experimental studies be undertaken to understand the extent to which various plastics (and in what size, form, or composition) adversely affect fish, compared with the influence of other causes of fish disease. Without such information it is not possible to design a suitable monitoring programme of effects (or adapt the fish disease monitoring programme). ICES therefore here advises primarily on the monitoring of the occurrence, distribution, and trends through time of plastics in the marine environment.

Monitoring the OSPAR area

A number of current surveys capture samples of fish that would be suitable for the monitoring of the occurrence of plastics in stomachs. Fish disease surveys cover a relatively small part of the OSPAR area at present, with Belgium, the Netherlands, Germany, and UK sampling in the North Sea and the English Channel, and with UK also sampling in the Irish Sea. Surveys to provide information for fish stock assessment are much more widespread and could be used to gather fish samples. In addition it may be possible to use samples from commercial fisheries (if the fish are handled and stored correctly). In view of this more widespread sampling, ICES advises not confining the monitoring of litter in fish stomachs to fish disease surveys. It is important that inshore waters, particularly those in estuaries, are not overlooked; these are rarely covered in traditional wide area surveys and a combination of surveys may be needed to sample fish in these waters.

The spatial and temporal design of the monitoring programme ultimately depends on the requirements of the assessment of an indicator against its baseline and target. OSPAR has so far made no choices in relation to indicators or monitoring programmes for plastics in fish; therefore, ICES refers to the current Commission Decision (2010/477/EU) of 1 September (EU, 2010) on indicators as guidance on requirements:

"Trends in the amount and composition of litter ingested by marine animals (e.g. stomach analysis) (10.2.1)".

Any programme, therefore, should be able to detect trends with a specified magnitude (e.g. 20%) and precision in a specified period (e.g. one MSFD assessment cycle: six years). The numerical definition of the target and the baseline, i.e. a decline or increase compared to a specific reference, has implications for the design and the costs of the monitoring programme and depends on the heterogeneity of the indicator in space and time.

Since there are no time-series of ingested litter in fish, the heterogeneity within the data is unknown and the only way to develop a programme with sufficient and known statistical power (that is still affordable) is by making initial assumptions and collecting data to better understand the variability of the indicator in time and space. This would allow the monitoring design to be improved in time. There are some existing datasets on incidence of plastics in fish stomachs that could be examined to determine the variability in the data and thus inform the initial assumptions. Once sufficient data are available it may be possible to better stratify the sampling area and thereby reduce the number of samples needed for an assessment. Such strata will in many cases cross national borders, which in turn suggests that countries should cooperate and optimize their monitoring (as happens already with most fish stock surveys organized under ICES).

Which plastics to monitor

There are many types and forms of plastics in the marine environment. Many plastics degrade or fragment, starting large and becoming smaller through time. The size of particles in fish stomachs is related to mouth size and diet. Pelagic species generally ingest smaller food items (mostly plankton) than demersal species (of the same size) that feed on larger food items. The pelagic species may, therefore, take small plastic particles from the water column. The choice of which plastics to monitor in fish stomachs is also related to ease of monitoring elsewhere in the marine environment, as well as technical ability to monitor within fish. Plastic particles are typically described by size category: microplastics (5 microns to 5 mm), mesoplastics (5 mm to 25 mm), and macroplastics (> 25 mm). Particles smaller than 5 microns are difficult to detect reliably in fish, while overall quantities of the larger plastics are better monitored by direct sampling. Plastics can occur in a number of shapes e.g. fibres, films, granules, or beads. Microfibres are particularly difficult to monitor as they are pervasive throughout the environment, including in the atmosphere and in fish nets. It is very difficult to avoid contamination of samples by microfibres when working on ships; for this reason ICES does not recommend monitoring the smallest of these, at least initially. Plastics are also formed from a wide variety of molecules, for example polythene, polystyrene, polypropylene, PVC, and nylon. Each plastic has its own characteristics and the different types vary in their ability to absorb other chemicals, including those that may be toxic to marine life. For these reasons, ICES recommends recording the size, form, and type of plastics found in samples. Further clues to the origin of plastics may be gained from, e.g. the colour of the plastic; however, colour can change through time (or during treatment of samples), so while this information should be recorded it may not necessarily be useful.

Sources and references

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Annex