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Effective and easy to use extraction method shows low numbers of microplastics in offshore planktivorous fish from the northern Baltic Sea

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ABSTRACT

Although the presence of microplastics in marine biota has been widely recorded, extraction methods, method validation and approaches to monitoring are not standardized. In this study a method for microplastic extraction from fish guts based on a chemical alkaline digestion is presented. The average particle retrieval rate from spiked fish guts, used for method validation, was 84%. The weight and shape of the test particles (PET, PC, HD-PE) were also analysed with no noticeable changes in any particle shapes and only minor weight change in PET (2.63%). Microplastics were found in 1.8% of herrings (n = 164) and in 0.9% of sprat (n = 154). None of the three-spined sticklebacks (n = 355) contained microplastic particles.

1. Introduction

Microplastics are commonly defined as synthetic polymer particles that are smaller than 5 mm with no general agreement of their lower size (Andrady, 2017). The distribution and abundance of microplastics in different marine compartments has been increasingly studied during the past years (Barboza and Gimenez, 2015). These studies show that microplastics are present and widely spread in marine environments. Microplastics are of concern especially because of their size, making them too small to be efficiently removed from the environment with clean-up efforts, and potentially able to enter various food webs (Farrell and Nelson, 2013; Setälä et al., 2014; Setälä et al., 2016b). Microplastics can contain environmentally harmful additives, and act as hot spots for effective adsorption of environmental chemicals from the surrounding environment (Batel et al., 2016; Rochman et al., 2013).

There is already field data on the ingestion of microplastics by invertebrates (Davidson and Dudas, 2016) and different fish species (Boerger et al., 2010; Foekema et al., 2013). These findings are further supported by exposure experiments conducted in laboratory conditions (e.g. Cole et al., 2013; Setälä et al., 2016a).

To obtain reliable overview of microplastics in marine biota, and to compare results across various studies, standardized methods for extracting microplastics from biota should be developed and applied. Methods should also be explained in understandable and explicit way, as mentioned in the Lanzarote Declaration (Baztan et al., 2016).

Based on the present literature, there are various protocols being used for extracting microplastics from soft tissues of marine organisms (Fig. 1). The most simple and straightforward approach for extracting microplastics from fish is by dissecting the fish, and removing the ingested material under a microscope, and visually observing it (Boerger et al., 2010; Lusher et al., 2013; Neves et al., 2015). Stomach contents can further be categorized by size (Rummel et al., 2016), and larger particles be observed by naked eye (Romeo et al., 2015). The drawback of this approach is the possibility of a low (ca 60%) retrieval rate (Avio et al., 2015). Different staining approaches have also been applied to improve the visual isolation, with the concept of either staining the organic material (Davison and Asch, 2011) or fluorescence staining of the plastic for easier identification and counting (Maes et al., 2017).

In order to improve the retrieval efficiency of microplastics from fish, different methods aiming to digest soft tissues of marine organisms have been developed and applied. The basic idea in such methods is to extract the microplastics from either fish soft tissues, guts and its content or the whole fish by digesting the organic material and preserving the plastic particles. The methods presently used are based on alkaline, acid or oxidative reactions and the procedure can be further improved with additional digestions steps with different chemicals (Wesch et al., 2016b; Vandermeersch et al., 2015). Potassium hydroxide (KOH) and sodium hydroxide (NaOH) have been used for alkaline digestion of biota samples. KOH digestion has been commonly used for microplastic extraction fish, however if used in room temperature it can take up to 3 weeks for the digestion to finish (Foekema et al., 2013). NaOH has been used for biota digestion protocols (Catarino et al., 2017) and also in plankton rich water samples (Cole et al., 2014). NaOH has been shown to damage the polymer particles in the concentration of 10 M and temperature of 60 °C (Dehaut et al., 2016).

Nitric acid (HNO_3) was used for microplastic extraction from

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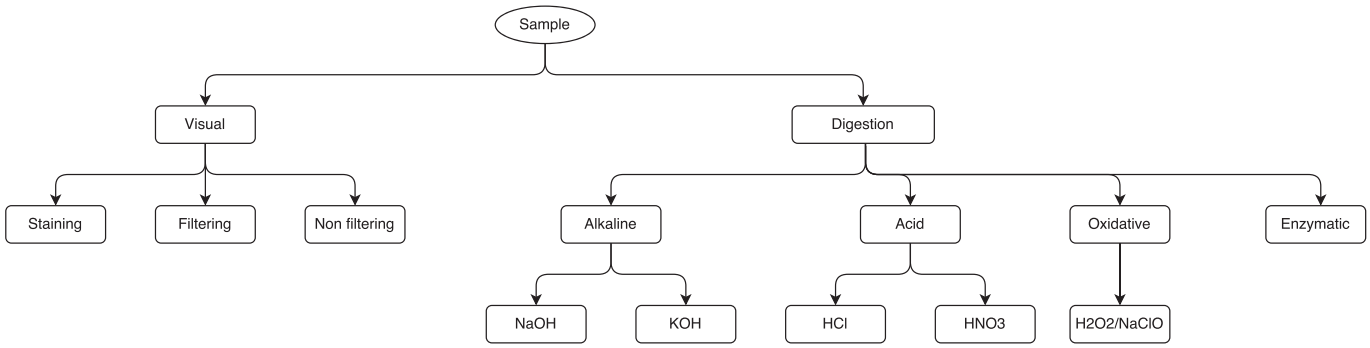

Fig. 1. A diagram showing presently used approaches to examine and extract microplastics from biological material. These approaches can be used either as single methods or as in combination, as in the case of this study.

mussels (Claessens et al., 2013) where the process destroyed some of the fibers. Boiling nitric acid in mixture of perchloric acid HNO_3:$HClO_4$ (4:1 v:v) has been advised by ICES (2015), however the follow up testing has shown the destruction of polymers by the suggested protocol (Enders et al., 2016). Hydrochloric acid (HCl) has been tested for digestion of zooplankton with some success, but less effective than NaOH (Cole et al., 2013). HCl dissolves calcium carbonate ($CaCO_3$) which can be found in many species of molluscs, crustaceans, and echinoderms as a building material of their shells or skeleton. In this study the usage of HCl was tested as a complementary step in digestion of the fish gut content. Combination of different digestion steps and usage of oxidative agents such as hydrogen peroxide (Avio et al., 2015) or sodium hypochlorite (NaClO) (Collard et al., 2015) has also been used to improve digestion and microplastic extraction.

For establishing a new protocol it is crucial to have quality control or method validation. Efficiency of microplastic isolation, the retrieval rate, can be measured using positive controls or spiking the samples (Hermsen et al., 2017; Avio et al., 2015). Effects of isolation method on different polymers, i.e. how will the digestion protocol affect the plastic particles should also be tested (Dehaut et al., 2016).

The aim of this study was to develop a reliable digestion method for extracting microplastics from fish tissues, which would be fast and cost-effective. Presently in European legislation (EC, 2008) monitoring microplastics in marine biota is already recommended, but not yet obligatory. However, if monitoring microplastics in fish becomes necessary, effective and easy-to use methods for processing monitoring samples are needed.

2. Materials and methods

The protocol presented here (Fig. 2) was developed and tested in the laboratory facilities of the Marine Research Centre, Finnish Environment Institute, Helsinki. Preliminary testing was carried out with Baltic herring (*Clupea harengus membras*) purchased from a local supermarket. During the pre-testing phase different concentrations of the chemicals (NaOH, HCl, sodium hypochlorite, hydrogen peroxide, nitric acid) were applied and the effect of incubation temperature (20–60 °C) and digestion time (2 h–7 days) and sample processing steps (centrifuging vs. filtering) examined. Once a satisfactory combination for digestion of soft tissues was achieved, the method was further validated by: i) testing the method effects on the properties of different polymer types (PC, PET and HD-PE) and ii) spiking the fish samples with a common plastic polymer type, high density polyethylene (HD-PE) in two size classes.

2.1. Tissue digestion protocol

The reagents were prepared by dissolving dry NaOH (technical grade) and SDS (sodium dodecyl sulphate, Sigma-Aldrich) to Milli-Q water. All chemicals used in this method were pre-filtered before use to exclude microplastic contamination from the chemicals. In this study 20 μm mesh size was used for pre-filtration, but also smaller mesh size can be used. Sodium dodecyl sulphate SDS is a common detergent that is known to denaturate proteins and because of its destructive characteristics has also recently been used for destructing organic material in another study on marine microplastics (Löder et al., 2017). Ten mL of 1 M NaOH and 5 mL of SDS 0.5% w/v (ca 5 g/L) is added to a glass jar per 1 g of fish tissue (in case the weight is less than 1 g volumes for 1 g are used), kept in the oven at 50 °C for 24 h after which the contents of the jar are gently shaken and let to incubate for another 24 h; (48 h in total). After the incubation the contents of the jar are filtered through a 100 μm mesh size filter. The walls of the incubation jar are consecutively rinsed with 96% ethanol 3 times and filtered. If the fish diet includes animals with calciferous shells (e.g. mussels, gastropods, sea urchins, etc.) hydrochloric acid (HCl, technical grade) can be added to the filter to remove calciferous structures. Following the sample filtration, filters with the residue should be thoroughly rinsed with distilled water whilst vacuum suction on. After the vacuum suction is turned off, and 2 M HCl is added to cover the sample, at least 10 mL. After 5 min the sample is filtered and rinsed with Milli-Q water. This HCl treatment can be repeated if necessary. In the case there are still remains of organic material left on the filter they can be further diminished with hydrogen peroxide (30%) in the same way as HCl.

Sodium hydroxide reacts with hydrochloric acid forming sodium chloride (NaCl) or table salt and water. In such way after the digestion protocol the remaining liquid can be neutralized. It is important that the reaction is equimolar (using exactly the same amount of NaOH and HCl) including the HCl used for destruction of calciferous residue. Throughout the work, standard laboratory safety regulations were used: safety glasses, nitrile gloves, cotton lab coats and the when possible, work was conducted in fume hood.

The developed method does not include a protocol for chitin digestion. However hydrogen peroxide is used in the case of zooplankton remains to increase their transparency. The filters are finally placed in petri dishes, covered with lids, and ready for microscopy.

2.2. Validating the digestion protocol

2.2.1. Effects on polymers

To test the protocol and its possible effects on common polymer types (PlasticsEurope, 2016), 3 plastic particles (ca 5 × 5 mm) of polycarbonate (PC), polyethylene terephthalate (PET) and high-density polyethylene (HD-PE) were cut from a CD, a soft drink bottle and a shampoo bottle respectively. Polymers already known to be resistant for NaOH (Dehaut et al., 2016) were excluded. The particles were treated with the digestion method described above (NaOH and SDS), incubated at 50 °C for a prolonged time of one week, and treated with the HCl and H_2O_2. Each particle was photographed under a stereomicroscope (Leica MZ 9.5) and weighed three times (Precisa EP 225sm) before and after the treatment for the detection of changes in the particle morphology