Microplastics In Ringed Seals From The Baltic Sea

Method development regarding the detection of microplastics in the digestive tract contents of marine mammals

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Abstract

Plastic pollution is a severe global problem and every year approximately 368 million tons of plastic is produced. Both terrestrial and marine environments are impacted and hundreds of species affected. Marine plastic debris accounts for a weight of 268 940 tons and affects hundreds of marine species through habitat degradation, entanglement and ingestion. Microplastics (MPs) are a type of plastic which have gained traction during the last few decades and are defined as plastic particles with a diameter of 1 μm to 500 μm. They can be of either a primary or secondary nature and come in a wide range of shapes and sizes e.g. fibres, fragments and spheres. Their potential harmful effects if ingested by organisms is not yet fully understood. However, it is known that their biochemical qualities enable them to bind to chemicals which can have harmful effects if ingested, for example endocrine disruptive effects. This study investigated the digestive tract contents of five ringed seals (*Pusa hispida*) from the Baltic Sea, in order to both see if they contained any MPs and to develop an efficient method for detecting MPs. In total, 202 MPs were discovered whereof 143 were fibres and 59 were fragments. The method of using a solution of 30% hydrogen peroxide to dissolve the biogenic matter in the digestive tract contents was successful. Additionally, the study also identified several adjustments which need to be made to the method and gained new knowledge regarding the contamination risk, which needs to be taken into consideration for similar future studies. The fact that MPs were detected in all samples indicates that MPs are a widespread problem and shows the need for further research regarding their potential effects.

Keywords: microplastics, plastic pollution, hydrogen peroxide, ringed seals, the Baltic Sea.

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1.0 Introduction

1.1 Plastic pollution

In 2014 it was estimated that 5,25 trillion plastic particles, weighing 268 940 tons, existed in the world's oceans (Eriksen et al., 2014). That is a greater number than the approximately 100 billion stars in the Milky Way galaxy (The European Space Agency, 2016). In 2019 alone, 368 million tons of plastic was produced globally according to Tiseo (2021) and 4,8-12,7 million tons end up in the oceans annually (Hale et al., 2020). Some marine debris is visible on the ocean's surface, however, much of this plastic is not visible from above (Andrady, 2011). This is either due to the fact that some plastics have a higher density than that of sea water, contain other heavier materials such as metals, or because it has been ingested by marine organisms, meaning it is harder to detect (Andrady, 2011).

Plastic pollution in the world's oceans, freshwater bodies and along shorelines is one of the most severe forms of pollution, according to Li et al. (2016). The use of plastic bags, disposal of solid waste and littering contribute massively to plastic reaching the environment and approximately 80% of plastic marine debris comes from land-based sources (Li et al., 2016). However, waste from aquaculture facilities and fishing grounds are also big contributors to the accumulation of plastic in aquatic environments (Lee et al., 2013).

At the same time, plastics do have many great qualities which have allowed society to achieve a plethora of things (Almroth & Eggert, 2019). They provide light materials for cars and airplanes which saves fuel and thereby reduces CO_2 emissions, they are essential components of a lot of life saving medical equipment and they have improved hygiene when it comes to food safety (Almroth & Eggert, 2019). The development of biodegradable plastics has therefore been on the rise during recent years but these are often not degradable in marine environments as they require the presence of suitable microorganisms and specific oxygen levels, temperatures etc. to degrade efficiently (Almroth & Eggert, 2016; Jefferson et al., 2009).

Plastic consists of synthetic polymers which can take hundreds of years to decompose (Hale et al., 2020). Degradation of plastic is commonly caused by photo-oxidative degradation which occurs when plastic, exposed in air or lying on beach surfaces, is exposed to UV-B radiation from the sun (Andrady, 2011). Photo-degradation of plastic submerged in water or floating on the surface of the ocean is, however, severely decelerated (Andrady, 2011). A common cause of plastic degradation in marine environments is weathering (Li et al., 2016).

1.2 Marine debris & dangers

During the last few decades, research regarding the amount of animals affected by marine debris has risen enormously and today it is known that at least 693 marine species have

encountered some form of debris (Gall & Thompson, 2015). Out of these encounters, 92% were reported to involve plastic debris (Gall & Thompson, 2015). Most cases of encounters between marine debris and marine organisms are due to either entanglement, habitat degradation or ingestion (Gall & Thompson, 2015). The impact microplastics have on humans is not as widely studied, however, a recent study by The University of Newcastle in Australia concluded that the average human ingests approximately five grams of microplastics a week (de Wit & Bigaud, 2019). That is the equivalent to the amount of plastic in a credit card. A few common sources causing plastic consumption, when it comes to humans, are tap and bottled water, shellfish, salt, sugar, fish, honey and beer (Kosuth et al., 2018; Senathirajah et al., 2021).

Due to the large molecular size of plastics which prohibits them from penetrating cell membranes, they are not believed to interact with the endocrine system (Teuten et al., 2009). However, due to their biochemical properties, they have the ability to bind to chemicals of smaller molecular size, which in turn can enter cell membranes (Teuten et al., 2009). If these chemicals interact with biological molecules within cells, considerable damage could be caused such as the disruption of the endocrine system (Teuten et al., 2009). Examples of potentially harmful chemicals include alkylphenols, organotin compounds and plasticizers, which are commonly used additives in polymers (Teuten et al., 2009). The chemical compounds that are added to a lot of plastic products might also be able to cause harm (Banerjee & Shelver, 2021). These compounds include chemicals such as stabilising agents, flame retardants and pigments which can loosen from ingested plastic polymers and have the potential to penetrate cell membranes (Banerjee & Shelver, 2021; Hahladakis et al., 2018). For example, a study by Mattsson et al. (2017) discovered that fish exposed to NPs can develop brain damage and behavioural disorders. It is therefore important to understand the implications of plastic pollution to be able to mitigate its effects.

1.3 Microplastics

Fragmentation of plastic debris can lead to the creation of microplastics (MPs), which is yet another type of plastic that is hard to quantify (Hollerová et al., 2021). Microplastics derived through fragmentation are more specifically referred to as secondary microplastics, while plastic that is purposefully manufactured to be tiny is referred to as primary microplastic (Jaikumar et al., 2019). Primary MPs are commonly used in medical products or as exfoliators in personal care products (Duis & Coors, 2016). Many facial cleaners, hand cleaners and toothpastes contain primary MPs which can end up in the environment via wastewater (Duis & Coors, 2016). Other sources of primary MPs include so-called “pre production” plastics such as plastic resin pellets, plastic powder and plastic fluff (Duis & Coors, 2016). These can reach the environment via run-off from different facilities or accidentally get lost during transport (Duis & Coors, 2016). Gas exploration abrasives and oil drilling fluids are also products which contain primary MPs and reach the environment if they are not disposed of properly or if they are not used in closed systems (Duis & Coors, 2016).

What counts as microplastic is highly debated, however, an increasingly accepted definition is any plastic particle ranging from 1 μm to 500 μm in diameter, i.e. 0,001 mm to 5 mm (Frias & Nash, 2019). This definition, proposed by Frias and Nash (2019), is partly based on the range of sizes that micro-Fourier Transformed Infrared Spectroscopy (μ-FTIR), a commonly used method for identifying MPs, is limited to detecting. Plastic particles smaller than 1 μm are in turn referred to as nanoplastics, (NPs) (Frias & Nash, 2019).

According to Hollerová et al. (2021) the most important types of microplastics are polyethylene (PE), polyvinyl chloride (PVC), polypropylene (PP), polyamide (PA), polystyrene (PS) and polyethylene terephthalate (PET). Depending on their density, they behave differently in water (Hollerová et al., 2021). This both affects the position they take in the water column and how they interact with organisms (Hollerová et al., 2021).

1.4 Seals

Phocids (true seals) are part of the pinniped family, also including Otariidae (fur seals and sea lions) and Odobenidae (walruses) who all live a dual terrestrial-aquatic lifestyle (Kienle & Berta, 2016). However, foraging for food occurs only underwater (Kienle & Berta, 2016). The ringed seal (*Pusa hispida*) is a circumpolar species and indicator species in Arctic marine ecosystems (Reimer et al., 2019). As an ice-obligate species, they are reliant on sea ice for molting, pupping and nursing (Reimer et al., 2019). They have the ability to create breathing holes through sea ice, thanks to their unique sharp claws, allowing them to reside in areas where other seals cannot (Kovacs & Lydersen, 2009). They maintain cylindrical holes in landfast ice as it continues to form and thicken, enabling them to occupy areas further away from the edge of the sea ice (National Geographic, n.d.). Due to the fact that they create snow dens and lairs where they spend most of their time, their population size is hard to quantify (Kovacs & Lydersen, 2009). However, tens of thousands of them are estimated to be hunted annually by peoples of the Arctic Basin (Kovacs & Lydersen, 2009).

Their diet consists mainly of fish such as rainbow smelt (*Osmerus mordax*), Arctic cod (*Boreogadus saida*) and Pacific herring (*Clupea pallasi*) and invertebrates such as mysids (*Mysida*), amphipods (*Amphipoda*) and shrimp (*Caridea*) (Crawford et al., 2015). They consume their prey through pierce feeding where their teeth are solely used to catch and hold the prey, to later swallow it whole (Churchill & Clementz, 2014). It is assumed by both Erikson and Burton (2003) and Panti et al. (2019) that microplastic ingestion by pinnipeds is a result of trophic transfer, i.e. the consumption of their prey who have in turn consumed microplastics. The main predators of ringed seals are polar bears, killer whales, walruses, arctic foxes, ravens and gulls, the three latter predominantly hunting ringed seal pups (Kovacs & Lydersen, 2009).

1.5 Current threats facing ringed seals

Pinnipeds face a variety of threats ranging from climate change and pollution to fisheries interactions and hunting (Kovacs et al., 2012). Ringed seals are especially susceptible to rapid environmental changes such as changes in ice phenology, ice quality and snow abundance (Reimer et al., 2019). Climate change is therefore a serious threat to the world's remaining ringed seals (Kovacs & Lydersen, 2009). As the global average temperature rises causing arctic ice to melt earlier each year, pups are separated from their mothers prematurely giving them a lesser chance of survival (Kovacs & Lydersen, 2009).

With ocean temperatures rising, ringed seal pathogens and parasites are also granted more favourable conditions meaning the seal population is at greater risk of contracting diseases (Kovacs & Lydersen, 2009). The hydrophobic surface of plastic has also proven to be a suitable environment for microbial growth (Zettler et al., 2013). A study by Zettler et al. (2013) discovered a rich bacterial and eukaryotic microbiota living on PE and PP fragments collected from the North Atlantic. The only study yet exploring the potential correlation between pathogen aggregation and MP concentration in any species of seal, was executed by Panti et al. (2019) on grey seals. No significant correlation between MP and pathogen aggregation could be identified, however, this field needs further research (Panti et al., 2019).

1.6 Previous studies

Macroplastics have been reported to be ingested by a wide range of marine mammals such as baleen whales (*Mysticeti*), beaked whales (*Hyperoodontidae*), seals (*Phocidae*), dolphins (*Delphinidae*) and porpoises (*Phocoenidae*) (Panti et al., 2019). Microplastics were first studied in the 1970s and have since been reported in many marine environments such as in surface waters, enclosed seas, water columns and deep sea floor as well as in marine organisms (Panti et al., 2019). Studies aiming to identify the MP content in other marine species and environments include that of MPs in crayfish (Zhang et al., 2021), bivalves (Ding et al., 2021; Jahromi et al., 2021), sediment samples (Zhang et al., 2021; Jahromi et al., 2021), water (Zhang et al., 2021) and a variety of fish species (Cabansag et al., 2021; Rummel et al., 2016), only to name a few.

No previous studies on the MP content in the digestive tract contents of ringed seals from the Baltic Sea have previously been performed. However, there are studies on both the MP content in faecal samples and organs from other species of seals, on the efficiency of various MP identification methods and on MPs in other seal species from the Baltic Sea (Philipp et al., 2020; Zhang et al., 2021; Jahromi et al., 2021; Wang et al., 2021; Panti et al., 2019; Donohue et al., 2019). The only available study on ringed seals was a study by Bourdages et al. (2020) who found no evidence of macroplastics in the stomach contents of 135 ringed seals from the Eastern Canadian Arctic (Bourdages et al., 2020). A similar study by Panti et al. (2019) on grey seals did not identify any macroplastics in the seal intestines examined. They did however find microplastics in all seal intestines, all of which had been caught as

bycatch. A study by Philipp et al. (2020), investigating the gastrointestinal tracts of grey and harbour seals from German Waters and the Baltic Sea, detected a total of 255 MPs from nine faecal and ten seal intestine samples.

The only study found regarding MPs in the Gulf of Bothnia investigated the impacts of MPs within Arctic sea ice (Geilfus et al., 2019). Their field studies found a range of 8 to 41 particles per litre of melted sea ice and they were able to confirm that ice concentrates MPs within its structure. MPs do not affect the growth rate of sea ice according to Geilfus et al. (2019). However, they did detect a connection between sea ice albedo and the light-absorption of MPs within ice, concluding that a higher concentration of MPs within sea ice leads to a faster rate of ice melt (Geilfus et al., 2019).

1.7 Previous methods

Panti et al. (2019) used a solution of 10% KOH to dissolve the organic material in their seal intestine samples and filtered them using microfiber filter paper. The isolated MPs were analysed visually using an Olympus SZX10 microscope (Panti et al., 2019). Wang et al. (2021) also used a solution of 10% KOH to dissolve the biogenic materials in the stomachs, small and large intestines of spotted seal cubs. To accurately identify the MP types they detected, infrared spectrums of each detected MP particle was obtained. Thanks to this, they could later use a "reference database in the Bio-Rad KnowItAll® Informatics System 2018 (64-bit)-IR Spectral Library (Bio-Rad Laboratories, USA)." which they could compare their IR spectrums to in order to identify the polymer type of each particle (Wang et al., 2021).

Philipp et al. (2020) used washing sachets to wash their seal intestine samples in an enzyme-based washing powder. A fluorescence microscope was used to visually identify the MPs and transferred to a µRaman spectroscope for further analysis. Yet another tested method is the use of a solution of 30% H_2O_2 to dissolve the organic matter in seal faecal samples (Donohue et al., 2019). Donohue et al. (2019) added hydrogen peroxide to faecal samples at 75 °C and simultaneously placed their samples in a saline solution, which in turn was placed in a density separator. This, in order to allow higher density material to sink and lighter material to rise. The lower density material was filtered using a sieve with a pore size of 330µm to later be visually analysed using an Olympus C0111 dissecting microscope. In the 44 examined faeces, 398 MP fragments were identified as well as 186 MP fibres (Donohue et al., 2019).

1.8 Purpose & research questions

The purpose of this study is to evaluate the efficiency of using hydrogen peroxide, micrometer fine sieves and a microscope to detect microplastics in the faecal samples of marine mammals as well as determine if microplastics are present in ringed seals from the Baltic Sea (figure 1). This study will work as a pilot study for a larger project that aims to

examine the ability of MPs to enter different organs in marine mammals e.g. lungs, liver, brain and blood. However, for this study on ringed seals from the Gulf of Bothnia, the two following research questions will be explored:

1. Is it possible to detect microplastics in the digestive tract contents of five ringed seals from the Baltic Sea?
2. How does the used method need to be altered to improve its efficiency and accuracy for future studies?

Figure 1. A map of Europe showing the following locations; Sweden, the Baltic Sea, the Gulf of Bothnia, Örnsköldsvik (1), Gussöfjärden (2) and Tistersöarna (3). The latter three locations are where the five ringed seals, used in this study, were collected from. An illustration of a ringed seal is also visible in the top left corner. Illustration: Nicola Parfitt.

2.0 Method

To prevent microplastic contamination of the samples, almost the whole process was executed within a fume cupboard. Laboratory material containing plastic was also avoided as much as possible. All beakers and droppers were made out of glass, the sieves were made out of stainless steel and the hydrogen peroxide was ordered in a glass bottle. Cotton laboratory coats and non-plastic gloves (latex) were used. However, the petri dishes were made out of plastic and single-use face masks were used due to COVID-19 regulations. All rinsing of laboratory equipment was done with tap water instead of MilliQ water due to there being a larger amount of MPs in MilliQ water than in tap water (ALS Scandinavia AB, 2020).

2.1 Sample collection

The Swedish Natural History Museum (NRM) and Sweden's National Veterinary Institute (SVA) regularly receive marine mammals which have either been caught as bycatch, shot as part of so called "protection hunting", died of natural causes such as age, disease/sickness or as a result of being stranded (SVA, 2021). The marine mammals, predominantly porpoises and seals, are all found along the Swedish coast and are autopsied as part of The Swedish Agency for Marine and Water Management's (SwAM) environmental monitoring program (SVA, 2021). The program aims to monitor the viability of the seal and porpoise populations through identifying their causes of death and eventual diseases they carry to get a better understanding of the health of the oceans (SVA, 2021).

As an extension of the NRM's and SVA's collaboration, Lund University and Simrishamn's Marine Centre were given the task of developing an effective method for analysing the possible microplastic levels in deceased marine mammals. The extent of this first project will only cover the development of a method to detect MPs in the faeces of marine mammals. However, further studies are planned to hopefully be able to develop an effective method for analysing MP levels in other organs such as the lungs, liver, blood and possibly the brain.

For this first part of the project, the visceral contents from the intestines of five ringed seals and six porpoises were examined. All eleven samples were put through the same process and kept in the same fume cupboard. However, this paper will only cover the seal samples and my fellow lab partner, Thea Eriksson, will be covering the porpoise samples in a separate thesis.

Figure 2. The frozen gastrointestinal tract of a ringed seal from the Baltic Sea, in a plastic bag.

After the autopsies, performed by SVA, of five ringed seals named 05277, 05275, 05273, 05274 and 05290 (table 1), the intestines from each individual were placed in separate plastic bags and sealed (figure 2). They were then stored in freezers (-18°C) until day 1 of the study.

Table 1. Information, from Sweden's National Veterinary Institute, on each ringed seal used for the study.

Individual	Weight (kg)	Length (cm)	Sex	Age	Place of discovery	Sea District	Condition	Cause of death
05277	unknown	135	Female	Adult	Gussöfjärden	The Gulf of Bothnia	Cadaverous	Shot
05275	unknown	138	Male	Adult	Tistersöarna	The Gulf of Bothnia	Cadaverous	Shot
05273	unknown	133	Female	Adult	Tistersöarna	The Gulf of Bothnia	Cadaverous	Shot
05274	114,7	156	Male	Adult	Tistersöarna	The Gulf of Bothnia	Cadaverous	Shot
05290	unknown	unknown	Male	Juvenil	Örnsköldsvik	The Gulf of Bothnia	Cadaverous	Bycatch

2.2 Sample preparation

After defrosting the intestines at room temperature for about 24 hours, still sealed off in their plastic bags, one bag at a time was opened and examined. Wearing latex gloves, an approximately 30 cm long piece of the intestine was cut off using regular scissors and separated from the rest of the gastrointestinal tract. The piece was held vertically over a newly cleaned glass beaker and with the help of massaging hand motions, the faecal content of the intestine was squeezed into it (Figure 3A). Depending on the amount of faecal content from each cut piece, this was repeated until the needed sample size was produced. This was not executed in a fume cupboard.

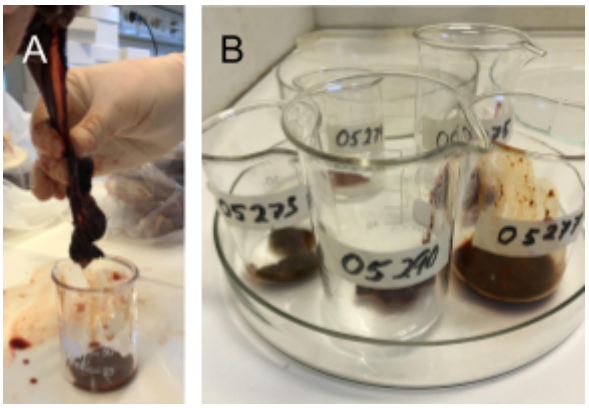

Figure 3. [A] pictures the process of squeezing out the digestive tract contents from the intestines of a ringed seal from the Baltic Sea. [B] pictures beakers filled with three grams of digestive tract contents from five ringed seals.

Another newly cleaned glass beaker was marked with the corresponding seal name and placed on a scale. Three grams of the seal faecal matter was weighed out and transferred from the previous beaker, using a metal spoon, to the one on the scale. The beaker containing the three grams of faecal matter was then placed in a fume cupboard. The same process was repeated with each intestine (Figure 3B). The amounts measured ranged from 2,996 grams to 3,043 grams.

2.3 Hydrogen peroxide treatment

50 ml of 30% H_2O_2 was added to each beaker, based on a previously established method performed on i.e. seals, crayfish, sediment samples and horse stool (Zhang et al., 2021; Donohue et al. 2019; Lind et al., 2020). A solution of 30% H_2O_2 has been found to be ideal for dissolving up to 50% of biogenic organic matter in samples of marine sediments whilst damaging the plastic polymers as little as possible (Nuelle et al., 2014). The role of the hydrogen peroxide is to digest any organic material within the samples, through oxidation, leaving only inorganic materials such as plastics, metals and minerals, in the solution (Kitis & Kaplan, 2007). As hydrogen peroxide is caustic and may react powerfully with the organic matter, only a few millilitres at a time was added (Grzegorz et al, 2019). A metal spoon was used to stir the samples.

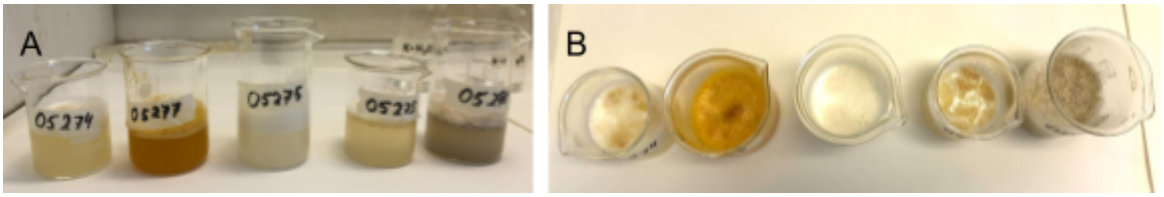

Figure 4. [A] pictures the process of squeezing out the digestive tract contents from the intestines of a ringed seal from the Baltic Sea. [B] pictures beakers filled with three grams of digestive tract contents from five ringed seals.

Each sample showed a different colour, as seen in figure 4 (A & B). The beakers were given glass lids (not airtight) and placed randomly amongst Thea Eriksson's porpoise samples, as well as with the controls. This to ensure that possible differences in light, temperature and draft in the fume cupboard, would not affect the results. This was performed in a fume cupboard which was kept at room temperature (approximately 21°C).

2.4 Contamination controls

In order to evaluate any potential contamination throughout the study, two blank controls were prepared (Figure 5). One control was created by adding three grams of tap water, instead of faecal matter, to a newly cleaned glass beaker, along with 50 ml of 30% H_2O_2. The second control was created by adding three grams of tap water, instead of faecal matter, to a newly cleaned glass beaker, along with 50 ml of tap water. The hydrogen peroxide solution and 50 ml of tap water were both measured using a graded glass cylinder. The controls were then placed in the same fume cupboard as the beakers containing the faecal samples and treated the same way as the faecal samples throughout the rest of the study.

Figure 5. (Left) A contamination control sample containing water and hydrogen peroxide. (Right) A contamination control sample containing water.

2.5 Intermediary steps

2.5.1 Stirring

During the first two weeks, the samples were stirred every two to three days using a metal spoon. However, due to COVID-19, access to the laboratory was restricted which led to an irregular number of days in between stirring the beakers. The beakers were stirred during the following days shown in table 2.

Table 2. Timetable over all steps performed during the study on microplastics in ringed seals from the Baltic Sea.

Day	Summary of steps
1	Weighed out 3 grams of each sample and added 50ml of 30% H_2O_2 to all samples and the H_2O_2 control (50ml of tap water to the H_2O control). Stirred all samples.
3	Stirred all samples.
5	Stirred all samples.
9	Stirred all samples.
11	Heated, stirred and added 25ml of 30% H_2O_2 to all samples (25 ml of tap water to the H_2O control)
12	Stirred all samples.
13	Stirred all samples.
19	Filtered sample 05277. Stirred all samples and added 25ml of 30% H_2O_2 to the remaining samples and the H_2O_2 control (25ml of tap water to the H_2O control).
32	Filtered both controls and sample 05275. Stirred all remaining samples.
37	Filtered samples 05273, 05274 and 05290.

The foam, which developed in each beaker after adding the hydrogen peroxide, eventually disappeared in all five samples (figure 6A). There were, however, still visible lumps of undigested matter in all samples on day 9 of the study (figure 6B).

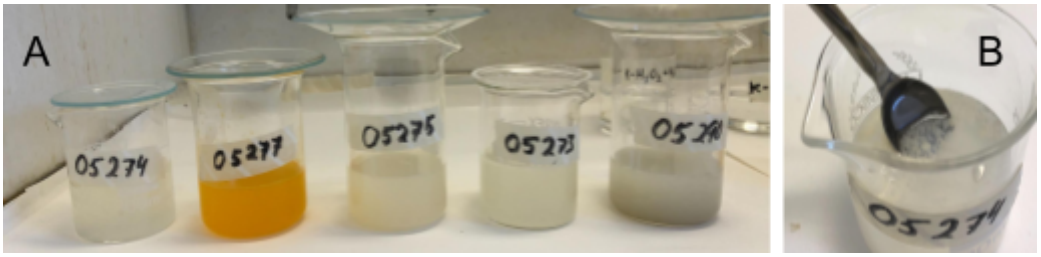

Figure 6. [A] Pictures of all five seal samples from day 9 of the study. [B] Undigested matter in sample 05274.

2.5.2 Heating

Starting with only 50 ml of 30% H_2O_2 in the seal faecal samples, it was noted while stirring that there was still a lot of organic material in the beakers. Therefore, all beakers were held in a water bath heated to approximately 80°C (figure 7), for a few minutes each, in order to expedite the efficiency of the hydrogen peroxide (Chu et al, 2012). This was executed on day 11 of the study.

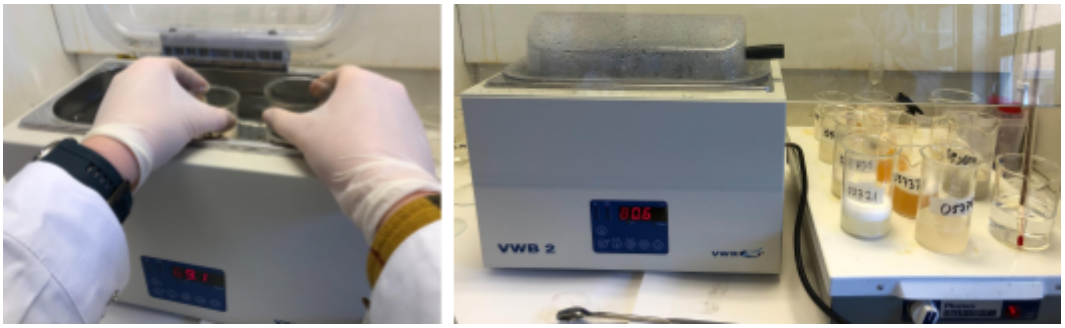

Figure 7. Using a water bath to heat up beakers, containing the digestive tract contents of ringed seals and hydrogen peroxide (as well as the two controls), to 80 °C.

2.5.3 Adding more H_2O_2

On day 11, consecutively to heating the beakers, another 25 ml of 30% H_2O_2 was added to all beakers (figure 8A), including the H_2O_2 control. Instead of hydrogen peroxide, the H_2O control was supplemented with 25 ml of tap water. All samples were stirred and placed back into the fume cupboard with their glass lids on. The five seal, six porpoise and two control samples were placed randomly in the fume cupboard (figure 8B).

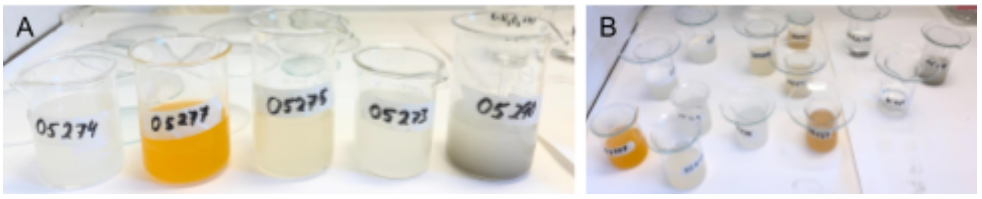

Figure 8. [A] The digestive tract contents of ringed seals from the Baltic Sea after 11 days in hydrogen peroxide. Samples pictured from left to right; 05274, 05277, 05275, 05273 and 05290. [B] Five seal samples, six porpoise samples and two control samples placed randomly within a fume cupboard.

2.6 Filtration of seal sample 05277 and adding H_2O_2 to the remaining samples

On day 19, the samples were stirred within the fume cupboard (figure 9). The filtration set up was assembled and consisted of three stainless steel test sieves placed on top of each other. Each sieve contained a grid with different sized pores ranging from the biggest at 500 µm (5 mm) to the smallest at 40 µm (0,04 mm) (Figure 10B). To improve the probability of MPs being intercepted by the filtration set up, the sieves were stacked from smallest to largest from the bottom up (Figure 10A). The stack was placed on top of a glass beaker to collect the liquid passing through the sieves during the filtration.

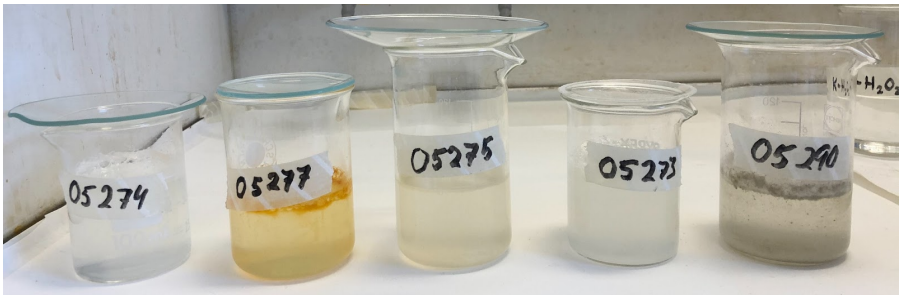

Figure 9. The digestive tract contents of ringed seals from the Baltic Sea after 19 days in hydrogen peroxide. Samples pictured from left to right; 05274, 05277, 05275, 05273 and 05290.

The beaker containing sample 02577 was stirred one last time and then poured through the filtration set up (Figure 10A). As it was noticeable that some particles had stuck to the sides of the beaker, a glass dropper was used to squirt tap water along the beaker's inside and swirled around. This was also poured through the filtration setup. When almost no liquid had accumulated in the beaker below the sieves, we discovered that a vacuum had been created in between each filter, trapping some of the liquid. Each sieve was therefore lifted slightly, one by one, letting the liquid pass through.

They were placed back on top of one another and stood for another few minutes. To make sure the filters were not removed too quickly, compared to the passing time of the liquid, only the top 500 µm filter was removed in order to be analysed. The 100 µm and 40 µm filters were left stacked on top of the beaker until the 500 µm filter had been analysed.

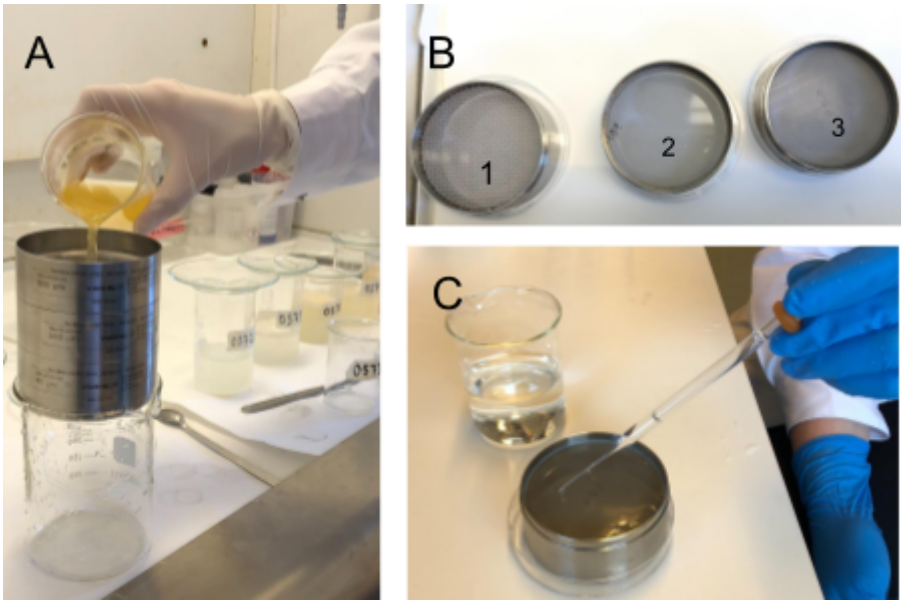

Figure 10. The filtration process to isolate potential microplastics from the digestive tract contents of ringed seals from the Baltic Sea. [A] pictures the filtration of a sample. [B] pictures the three different sieves used for the filtration. (1) is the 500 µm sieve, (2) is the 100 µm sieve and (3) is the 40 µm sieve. [C] pictures the process of squirting water at a sieve to remove any stuck particles onto a petri dish.

The 500 µm filter was placed upside down in a newly rinsed plastic petri dish (figure 10C). A new beaker was filled with tap water and a glass dropper was used to squirt water through the filter, reversed to how the sample solution had been poured in, in order for the particles caught by the filter to loosen and drop into the petri dish (figure 10C). In order for some of the particles to loosen, the water had to be squirted with quite some force onto the filter. The filter was placed into a separate petri dish to be able to double check for possible remaining particles on the grid. The same process was repeated with the two remaining sieves.

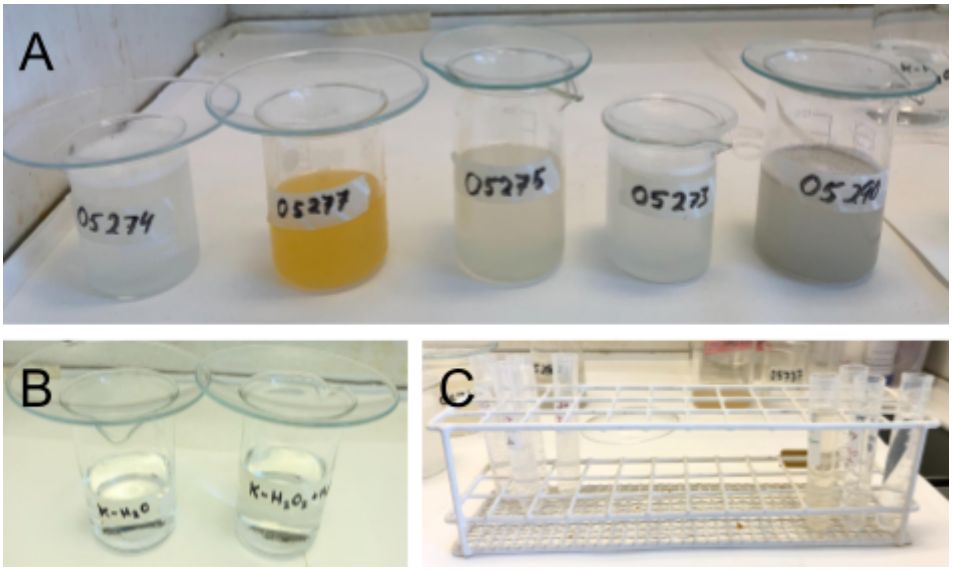

Figure 11. [A] The ringed seal samples on day 19 of the study. Pictured from left to right; 05274, 05277, 05275, 05273 and 05290. [B] The two controls on day 19 of the study. [C] The contents from the petri dishes from filtering sample 05277, transferred into three different plastic test tubes marked after the respective sieve size and seal sample number (ex. 05277 - 500 µm).

The four remaining samples and two controls were stirred and 25 ml of 30% H_2O_2 was added to all beakers, except for the H_2O control where 25 ml tap water was added (figure 11A & 11B). The remaining liquid after the filtration of 05277 was added back to the beaker to be kept for further analysis in future projects. No extra 30% H_2O_2 was added to the remaining liquid of sample 05277. The contents of the 05277 petri dishes were transferred into three different plastic test tubes marked after the respective sieve size and seal sample number (figure 11C).

2.7 MP identification method and seal sample 05277

Visual observation was used to identify all potential MPs in the seal faecal samples as well as in the two control samples. Beforehand, photographs of MPs and other particles detected in similar studies were studied and used as reference material to enable identification of potential MPs. Photographs from a fluorescence microscope from a study by Philipp et al. (2020) were particularly useful in identifying non-plastic particles such as potential fish vertebrae and fish bones. Another study by Li et al. (2015) was, in turn, particularly useful in identifying plastic particles such as fibres and fragments.

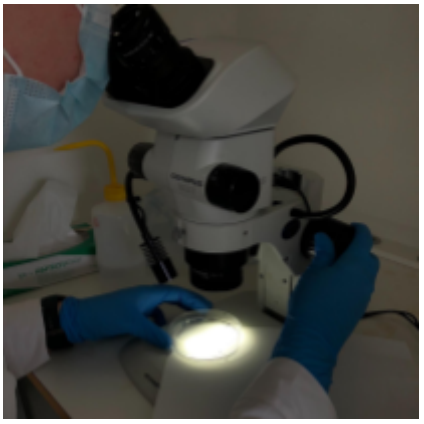

Figure 12. Using an Olympus SZX7 stereomicroscope to visually detect potential MPs in the digestive tract contents of ringed seals from the Baltic Sea.

Sample 05277 was visually analysed using an Olympus SZX7 stereomicroscope, as all samples later were (figure 12). Potential MP particles were noted and photographed through one of the objective lenses, using an iphone X. Notes included information regarding both the colour of each identified particle and type of MP; fibre, fragment or sphere. During the process of analysing the 500 µm sieve from seal sample 05277, through the microscope, it was visible that some tissue/fat was stuck to the grid. However, no possible MP particles could be identified on the grid so it seemed as if most of the particles had managed to drop onto the other petri dish. Still, as an attempt to completely hinder particles from sticking to the filter, a few adjustments to the filtration process were made for the remaining samples.

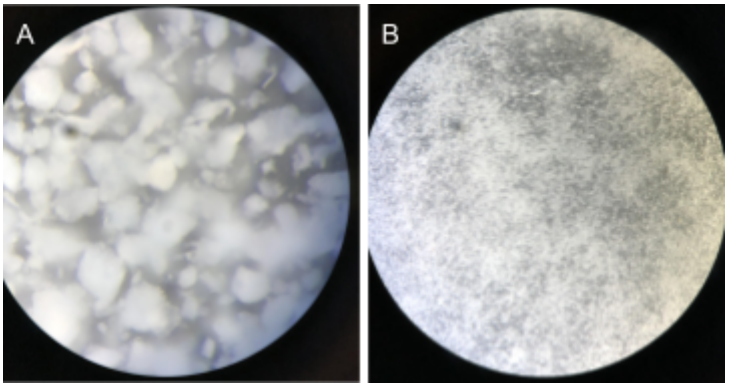

Figure 13. Fatty tissue from the digestive tract contents of a ringed seal, after being soaked in a solution of hydrogen peroxide for 37 days and filtered through a sieve set up consisting of micrometer fine sieves. Photo [A] shows the fatty tissue caught from a sieve with a pore size of 40 µm. Photo [B] shows the fatty tissue caught from a sieve with the pore size of 100 µm.

Many transparent particles detected in sample 05277 were quite hard to distinguish from the remaining white fat/tissue particles (figure 13) and due to the faint contrast between the particles and the white background. Therefore, different coloured backgrounds were tested as a stage plate. The stage plate's black side was helpful in identifying transparent and whitish particles (figure 14B). Green and blue paper was also used as a stage plate (figure 14C). However, neither improved the efficiency of identifying particles. The white side of the stage plate proved to be most efficient for the identification of coloured particles (figure 14A).

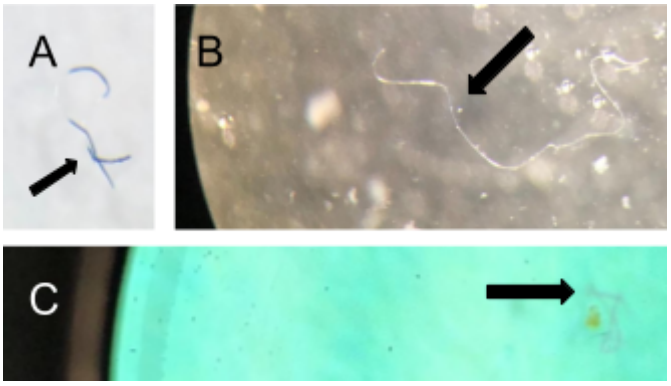

Figure 14. MP fibres from the digestive tract contents of ringed seals from the Baltic Sea. [A] pictures a fibre with a white stage plate. [B] pictures a fibre with a black stage plate. [C] pictures a fibre with a green stage plate.

2.8 Filtration and MP identification of controls

Both controls had been exposed to 30% H_2O_2 for 32 days before being filtered and analysed. The 500 µm, 100 µm and 40 µm sieves were set up in the same order as for analysing sample 05277, i.e. the 40 µm sieve at the bottom, the 100 µm sieve in the middle and the 500 µm on top. After pouring the H_2O control through the filter set up, turning each filter upside down, placing them on petri dishes and squirting tap water at them to loosen any particles, the microscopic analysis of all three samples showed a higher level of contamination than expected.

When analysing the sieves through the microscope, all three showed there was fat/tissue residue left on the grids. As the controls did not contain any faecal matter, it was concluded that the residue was from the previous filtration of seal sample 05277. There could therefore potentially even have been potential MP particles left on the sieves which then loosened from the grid during the filtration of the H_2O control, leading to contamination. Due to the delicacy of the micrometer sized steel grids, they were difficult to wash as any touch to their surface could lead to a change in pore size. However, before filtering the next control, the H_2O_2 control, each sieve was thoroughly washed as best as possible without damaging the grids. Instead of only using high pressure water to flush through the sieves from both sides, a

stream of high pressure air was used consecutively. The sieves were analysed using the microscope to ensure they were residue free (to the eye). The H_2O_2 control was then filtered and analysed.

Another important discovery was that some particles believed to be potential MPs were not movable when poked at with tweezers. Even though the resemblance was pretty much indistinguishable to that of MP particles, they most likely belonged to the stage plate, the bottom of the petri dish or were loose particles that had been stuck in between the petri dish and the stage plate. From there on, every potential MP particle which was identified was poked at. It was only noted as an MP if it was movable. A kimwipe was also used to wipe the stage plate and bottom of the petri dish. Another two controls were also analysed; one consisting of tap water and one consisting of tap water and scrapings from a face-mask.

2.8.1 Filtration and MP identification of seal sample 05275

After 32 days of being exposed to 30% H_2O_2, sample 05275 was filtered and analysed. The 500 µm, 100 µm and 40 µm sieves were set up in the same order as for analysing sample 05277 and the two controls, i.e. the 40 µm sieve at the bottom, the 100 µm sieve in the middle and the 500 µm on top. However, each sieve was washed thoroughly and checked under a microscope for any potential residue, before filtering seal sample 05275. A dropper was used to rinse the inside of the beaker with tap water in order to ensure all particles were poured through the filter.

Instead of removing the top 500 µm sieve and taking it to be analysed, every sieve was removed from the filter set up directly and placed on a petri dish and squirted with tap water to loosen all particles. This as visually identifying potential MP particles through the microscope could take anywhere from 30 minutes to two hours. During this time, the liquid on the grid of the remaining sieves, still left in the set up, had time to start evaporating. This would, theoretically, complicate loosening any particles from the grid when squirting tap water at it as the particles would have had a chance to dry firmly to the grid. The petri dishes were marked with their corresponding sieve size and lids were placed on each dish to ensure they were not contaminated while waiting to be analysed. The filtered samples were bottled into test tubes to be saved for future projects.

The three remaining samples, 05273, 05274 and 05290 were stirred and left in the fume cupboard. No further hydrogen peroxide was added to the remaining samples.

2.8.2 Filtration and MP identification of seal samples 05273, 05274 and 05290

Having left seal samples 05273, 05274 and 05290 in hydrogen peroxide for 37 days, they were stirred one last time and filtered. The filtration setup was put in place with the same order of smallest to largest from top to bottom. Sample 05273 was poured through the setup and each sieve was loosened slightly to remove the vacuum. Three completely new petri

dishes were marked with 500 µm, 100 µm and 40 µm respectively and each sieve was removed from the setup simultaneously and placed upside down onto each petri dish. Tap water was squirted at the sieves using a glass dropper and lids were placed on each dish.

The three petri dishes were taken to the microscope and analysed. All surfaces were wiped using a kimwipe and only moveable particles were noted as potential MPs. This same procedure was repeated with the two remaining samples; 05274 and 05290. Afterwards, the filtered samples were bottled up in test tubes and saved for future projects.

3.0 Results

3.1 Abundance of MPs in samples

A total of 202 potential MPs were found in the digestive tract content samples of five ringed seals from the Baltic Sea (table 3). In total, the two controls contained 27 potential MPs, indicating contamination (table 3). The highest amount of particles were identified from the 500µm sieve capturing 77 potential MPs in total (table 3). The least amount of potential MPs were caught using the 40 µm sieve with a total of 54 MPs (table 3).

Table 3. Microplastic abundance in digestive tract content samples from five ringed seals from the Baltic Sea and two control samples, one consisting of tap water and the other of tap water and hydrogen peroxide. Each sample was filtered through three sieves of varying sizes; 500 µm, 100 µm and 40 µm after having been emerged in 30% H202 for 19-37 days. All particles were identified through visual observation through an Olympus SZX7 stereomicroscope.

Seal Sample Nr.	500 µm	100 µm	40 µm
05277	19	20	9
05275	9	18	23
05273	8	11	8
05274	16	15	9
05290	25	7	5
H_2O control	6	3	8
H_2O_2 control	4	4	2

The highest number of MPs was detected in sample 05275 from an adult ringed seal male shot as part of protection hunting at Tistersöarna in the Gulf of Bothnia (figure 15). The least

amount of MPs was detected in sample 05273 from adult ringed seal female also shot as part of protection hunting at Tistersöarna (figure 15).

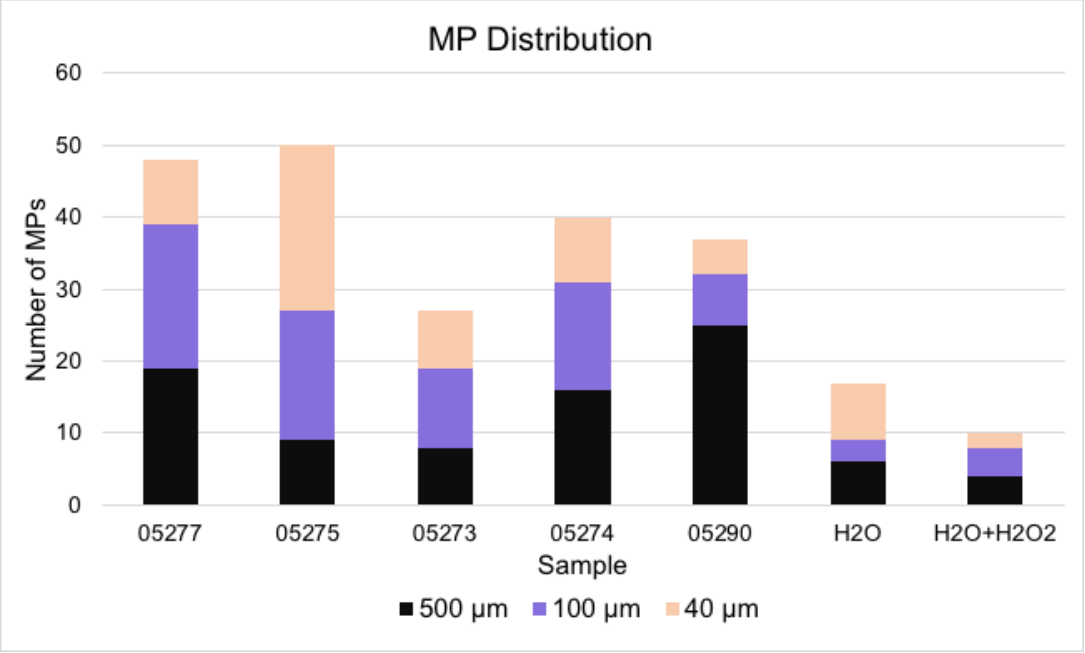

Figure 15. Number of microplastics found in the digestive tract contents of five ringed seals from the Baltic Sea and two control samples. Black shows the number of particles caught by a 500 µm sieve, purple shows the number of particles caught by a 100 µm sieve and pink shows the number of particles caught by a 40 µm sieve.

As the exact number of MPs found was not the main focus of the study, the method used for counting was not optimal and may include some double-counts of MPs. Therefore, grouping the amounts of MPs found in each sieve into intervals was thought to give a more accurate representation of the distribution of MPs. An interval consisting of the following categories; 0-5, 6-10, 11-15, 16-20 and 21-25, was used (figure 16). For example, 19 MPs were detected from sieve 500 µm in seal sample 05277 (table 3), and therefore assigned to the 16-20 category. This allows for a few double counts, or uncounted MPs, to be taken into account and does not imply that the number of detected MPs should be seen as exact.

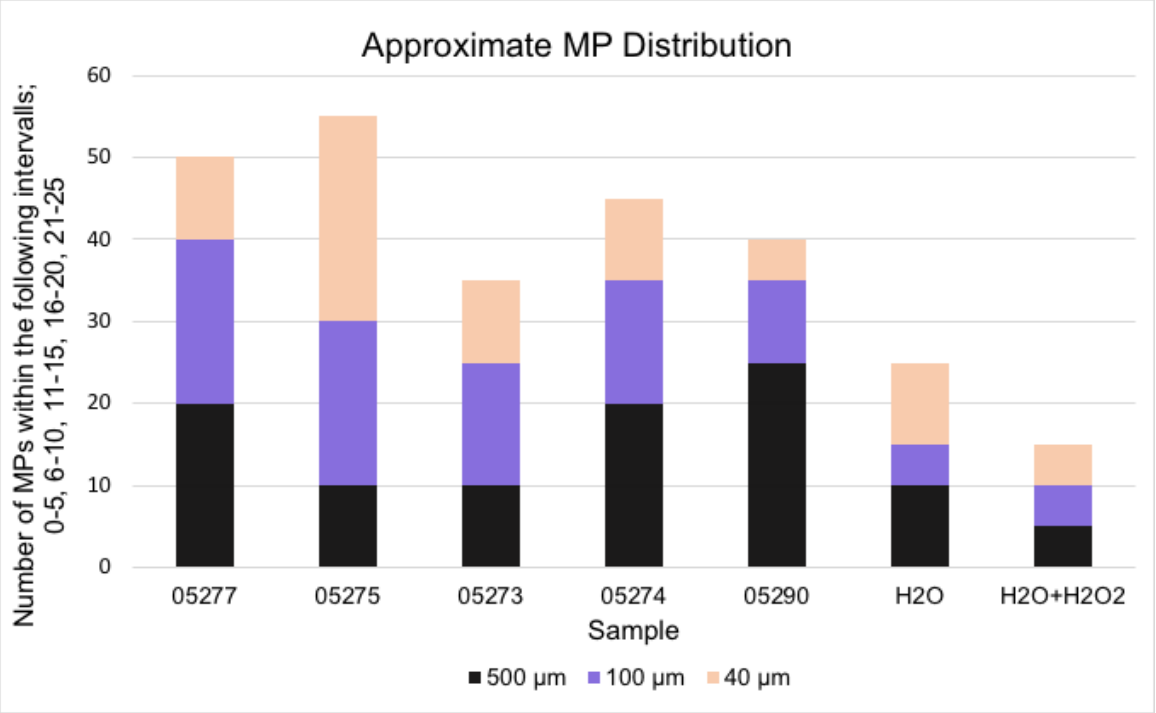

Figure 16. Approximate number of microplastics found in the digestive tract contents of five ringed seals from the Baltic Sea and two control samples. Black shows the approximate number of particles caught by a 500 µm sieve, purple shows the approximate number of particles caught by a 100 µm sieve and pink shows the approximate number of particles caught by a 40 µm sieve.

3.2 Types of MPs in samples

All detected plastic in the seal samples consisted of secondary MPs in the form of fibres and fragments (figure 17). The most abundant MP type detected was fibres (143) and thereafter fragments (59). Only one primary MP was detected and came from the H_2O control (Figure 22).

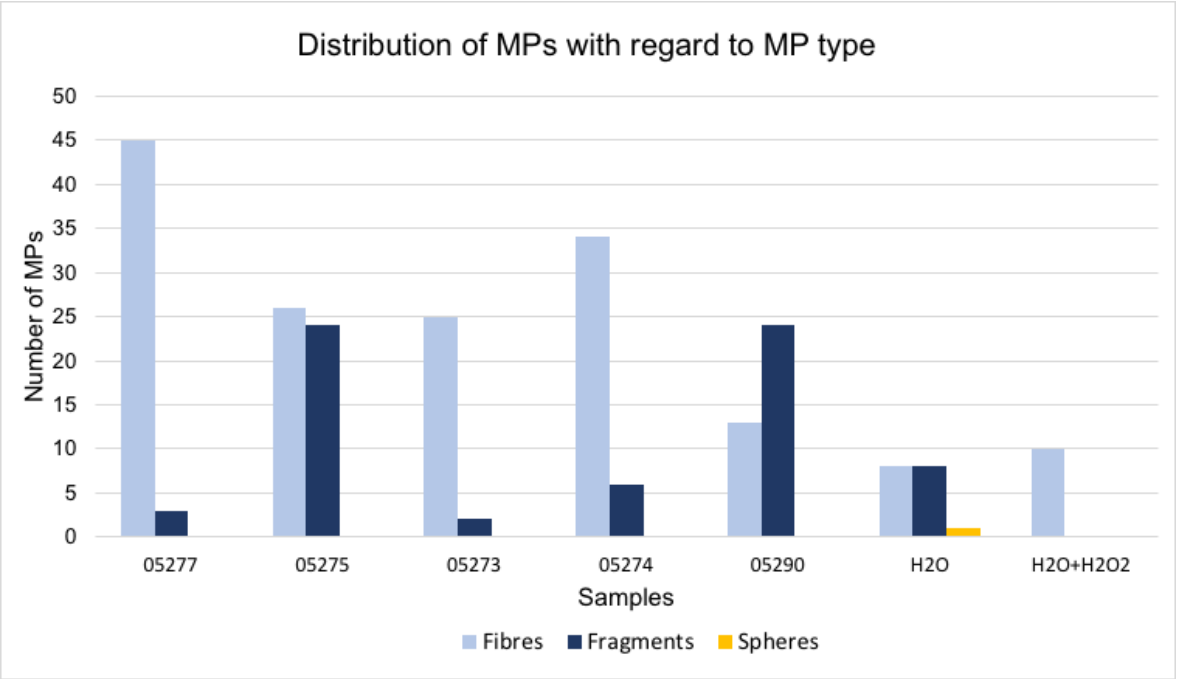

Figure 17. The composition of different types of microplastics in five seal faecal samples from the Baltic Sea and two control samples consisting of water and hydrogen peroxide. Light blue represents MP fibres, dark blue represents MP fragments and yellow represents MP spheres.

Similarly to the reasoning behind the creation of figure 16, each number of counted MPs were placed in an interval consisting of nine categories; 0-5, 6-10, 11-15, 16-20, 21-25, 26-30, 31-35, 36-40 and 41-45, to represent the approximate abundance of each MP type. Light blue represents MP fibres, dark blue represents MP fragments and yellow represents MP spheres (figure 18).

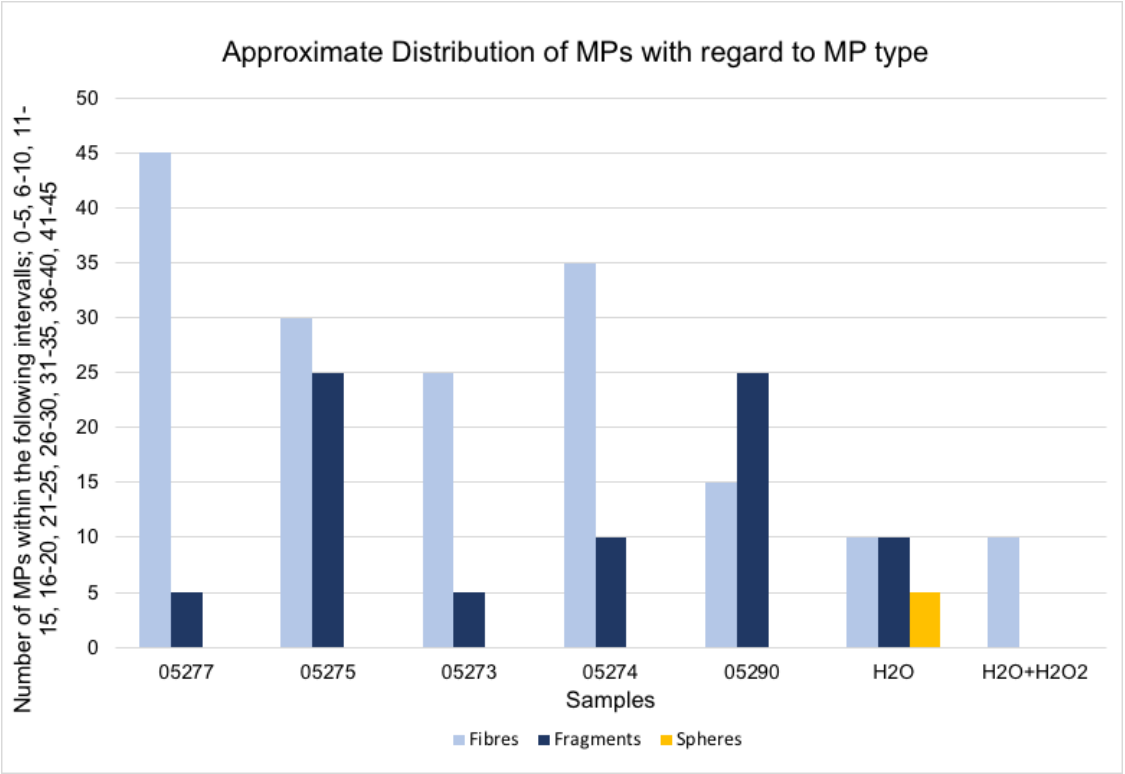

Figure 18. The approximate composition of different types of microplastics in five digestive tract content samples from ringed seals from the Baltic Sea, as well as in two control samples consisting of water and hydrogen peroxide. Light blue represents MP fibres, dark blue represents MP fragments and yellow represents MP spheres.

All digestive tract contents of the ringed seal samples contained both MP fibres and MP fragments. However, none of the samples contained any primary MPs, such as beads. Only secondary MPs were detected. Photographs of MPs from studies by Philipp et al. (2020), Li et al. (2015) and Nelms et al. (2019) were used as reference photos to identify polymer types of some fibres (figure 19) and fragments (figure 20).

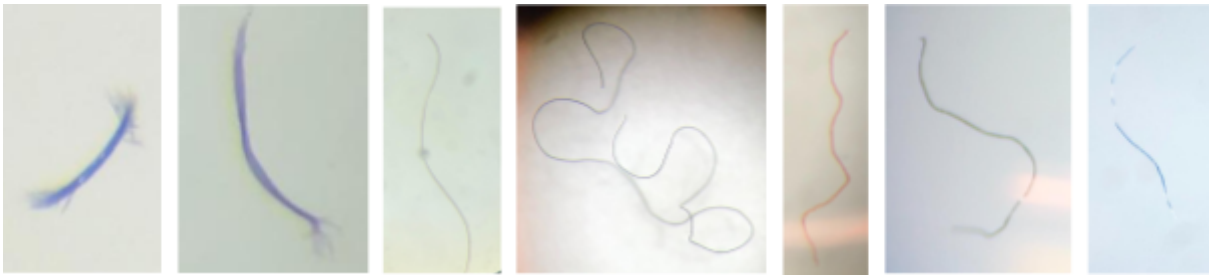

Figure 19. Examples of either nylon and/or polyethylene terephthalate ((PET)-fibres), from the digestive tract contents of ringed seals from the Baltic Sea.

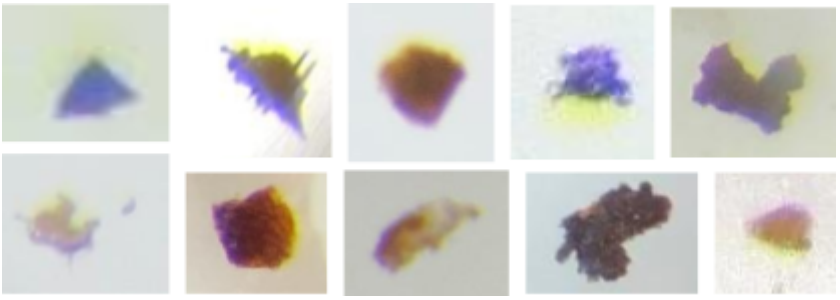

Figure 20. Examples of microplastic fragments from the digestive tract contents of ringed seals from the Baltic Sea.

Some fibres and fragments were found embedded in remaining fatty tissue which had not been digested by the hydrogen peroxide (figure 21).

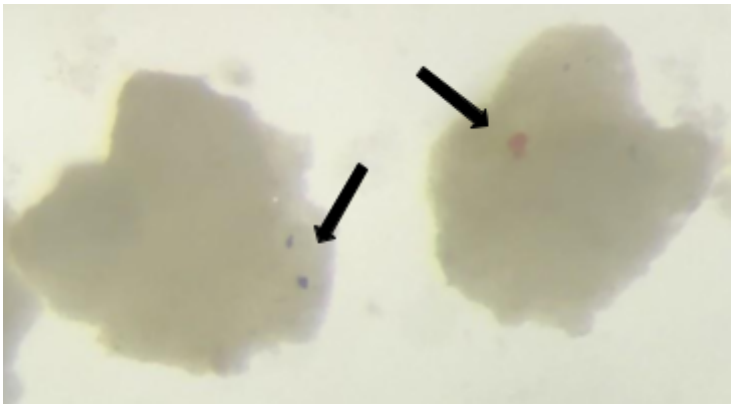

Figure 21. Three microplastic fragments entangled in fatty tissue from the digestive tract contents of a ringed seal from the Baltic Sea.

3.3 Non-plastic particles in sample 05290

The supplementary material from Philipp et al.'s (2020) study on MPs in seals from German waters was used to identify a few non-plastic particles found in seal sample 05290 (figure 22).

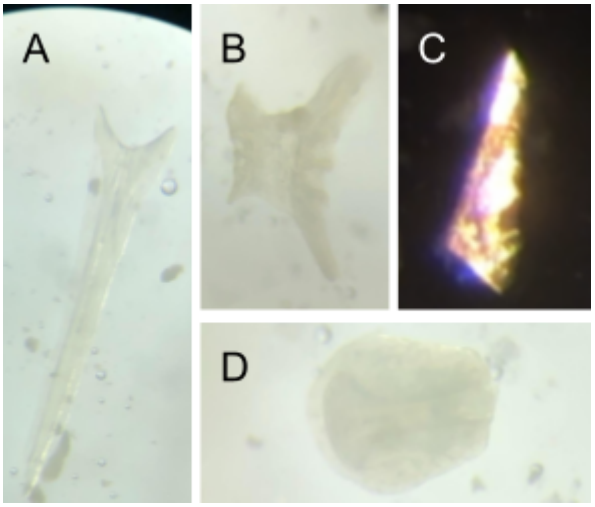

Figure 22. Non-plastic particles from the digestive tract contents of a ringed seal from the Baltic Sea. [A] is believed to be a fish bone, [B] is believed to be a fish vertebrae, [C] is believed to be a mineral of sediment origin and [D] remains unknown.

3.4 MPs in contamination control samples

The two procedural controls analysed to evaluate possible contamination throughout the method showed a share ranging from two to eight particles per filter. The H_2O control showed a total of 17 potential MPs whereof six were retrieved from the 500 µm filter, all coloured. These six MPs consisted of 4 fibres (3 blue fibres, 1 orange fibre) and 2 fragments (1 grey slightly see through flat fragment and 1 brownish purple flat fragment). The 100 µm filter retrieved three MPs whereof two were fibres (1 transparent fibre, 1 blue fibre) and one brownish/purple flat fragment. The 40 µm filter retrieved eight MPs whereof two were fibres (1 yellow, 1 blue), five were fragments (4 brownish/purple, 1 rectangular yellow) and one brownish/purple sphere (figure 23).

The H_2O control was analysed after analysing seal fecal sample 05277 which is believed to be one of the reasons for the number of MPs found. The experienced difficulties cleaning the filters can have led to contamination from residue.

Figure 23. A primary microplastic particle from the digestive tract contents of a ringed seal from the Baltic Sea.

The H_2O_2 control showed a total of ten potential MPs whereof four were retrieved by the 500 µm filter. All four MPs were fibres (3 blue, 1 see through). The 100 µm filter likewise retrieved four MPs which were all blue fibres. The 40 µm filter retrieved two MPs consisting of one blue fibre and one transparent fibre.

3.5 Extra procedural blanks

Another two controls were analysed to detect any potential contamination from tap water and/or the used face masks. No MPs were detected in the tap water. However, three fibres were found after scraping tweezers against a surgical face mask whilst held over a petri dish filled with tap water (figure 24).

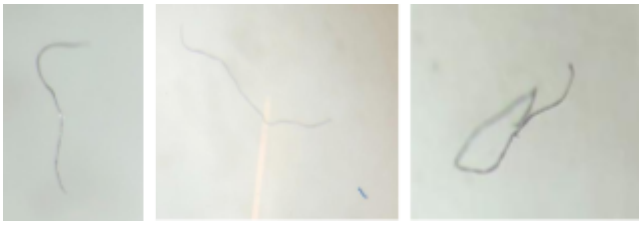

Figure 24. Microplastic fibres from a surgical face mask.

4.0 Discussion

4.1 Summary

This is the first study to provide evidence of the presence of microplastics in ringed seals from the Baltic Sea and more specifically in ringed seals from the Gulf of Bothnia. To be able to study the potential effects microplastics might have on organisms, such as on seals and ourselves, it is essential to develop a method that is both accurate and efficient at detecting MPs in the different bodily tissues, organs and excrements that wish to be studied.

The aim of this study was to improve one of the methods for detecting MPs in the digestive tract contents of marine mammals as well as assessing the possible presence of MPs in ringed seals from the Baltic Sea. As the main focus of this study was not to quantify the amount of potential MPs or identify the different types of microplastics found, results regarding these factors should be taken with consideration. Despite this, the study does support the current discourse surrounding MPs and their presence in marine environments and organisms (Hollerová et al., 2021).

The main results are those regarding method development, such as the efficiency of using hydrogen peroxide to dissolve the organic matter of digestive tract contents and the crucial steps that need to be taken to avoid secondary contamination. The used method did prove to be effective in dissolving the biogenic material in the digestive tract samples of seals and could, if done in a more plastic free environment, be turned into a widely used method for dissolving biogenic material in GIT and faecal samples from marine mammals. It is also important to take into account that the results from samples 05273, 05274 and 05290 probably give the most accurate representation of MP quantity as a more efficient process of identifying MPs was developed along the way.

The discovery of MPs in ringed seals from the Gulf of Bothnia proves that MP pollution is affecting other species in the same area as well. This as seals most likely ingest MPs through their diet which consists of different fish and crayfish species and due to the fact that seals are the prey of polar bears, walruses, arctic foxes and ravens, among others (Panti et al., 2019). In order for seals to contain MPs, their prey must have contained MPs. In turn, the predators must then also consume MPs as they are consuming seals. There is thus a transfer of MPs between land and marine environments. This also leads to the conclusion that humans are very likely consuming MPs, just as discovered in Schwabl et al.'s (2019) study on MPs in human stool. Especially those who eat seals, such as peoples of the Arctic Basin (Kovacs & Lydersen, 2009), as well as those who eat fish from the Gulf of Bothnia.

Fibres were the most abundant MP type detected in the five digestive tract content samples (figure 17 & 18). However, due to only visually analysing the detected particles, it is possible that some fibres counted as MPs were actually cellulose fibres as the two have a similar appearance (Philipp et al., 2020). It was not either possible to identify the MP fibres and fragments down to polymer level which could be an important area to focus on going forth. This in order to see if certain polymers are more commonly found and, depending on the results, be able to conclude which industries are causing significant levels of MP pollution. For studies focusing on the exact MP count and MP types, methods such as μFTIR spectroscopy used by Wang et al. (2021) and μRaman used by Philipp et al. (2020) for the MP analysing phase, seem to be both efficient and accurate, if you have the necessary equipment. However, these methods are expensive and were therefore not within the scope of this project.

4.2 Contamination

Even though many preventive steps were taken, the two control samples still managed to get contaminated (table 3 & figures 15-18). The preventive steps included ordering the solution of 30% hydrogen peroxide in a glass jar, using glass beakers, droppers and graded cylinders, wearing non-plastic gloves and cotton laboratory gowns, using stainless steel sieves for the filtration part of the study and working mostly inside a fume cupboard. As one of the extra procedural blanks consisting of only tap water showed no sign of MPs, the tap water, used for rinsing the beakers and removing the filtered particles from the sieves, is most likely not a source of contamination. The most likely sources of secondary contamination include plastic based surgical masks, plastic petri dishes, airborne plastic particles and plastic based clothing items.

As previous studies have shown, secondary contamination has proven to be problematic in achieving accurate results which was also the case for this study (Philipp et al., 2020). If future studies wish to use this method for similar purposes, there are some crucial adjustments which need to be made. All samples should be prepared, treated, filtered and microscopically analysed in a closed working environment to reduce the risk of contamination. Samples should also be handled in a completely plastic free environment including clothes, laboratory material and safety items such as masks, gloves and glasses. Even the rinsing of laboratory equipment should be done in a fume cupboard, if possible, to prevent contamination from air borne MP particles. Another improvement to reduce, or hopefully completely eliminate, contamination includes using an acrylic box, as done by Phliipp et al. (2020). Their procedural blanks contained only 0-2 particles per sample which is significantly lower than in many other similar studies (Philipp et al., 2020).

4.3 Improved Method

The hydrogen peroxide was efficient at dissolving the biogenic materials in the digestive tract contents of ringed seals. However, due to contamination of the control samples and some realisations along the way, there are a few things that could be altered to make the method of using H_2O_2 for detecting MPs more efficient and accurate. Considering the discoveries that were made during this study, the method would be carried out as follows if redone.

The frozen seal intestines would be thawed and moved directly into a fume cupboard where the remaining steps of the study would take place. A section of the intestine would be cut off and the digestive tract contents would be squeezed into glass beakers, with the capacity of holding at least 500 ml. Three grams of the digestive tract contents would be added to beakers and 50 ml of a solution of 30% H_2O_2 would be added to each sample. This could possibly be doubled to 100 ml, as the hydrogen peroxide and samples did not react as powerfully as first thought. A similar study performed by Li et al. (2015) found that an effective amount of 30% H_2O_2, in order to digest five grams worth of soft tissue from bivalves, was 200 ml. Depending on your specific samples (composition and amount), it

would thus be beneficial using slightly more hydrogen peroxide than in this study. However, it is also important not to use wasteful amounts.

Instead of irregularly stirring the samples, which was due to COVID-19 related issues, they would be stirred every day. This to increase the biogenic matter's exposure to the H_2O_2 and to ensure that the particles stuck in the foam layer on top of the liquid get mixed back in with the hydrogen peroxide allowing it to dissolve the biogenic material. Another 50 ml of 30% H_2O_2 could be added on day 3-5, already, instead of waiting for 11 days. The samples would also be heated more regularly to speed up the degradation of biogenic matter.

The method needs to be altered to accommodate the fact that the samples contained a lot of fatty lumps which the hydrogen peroxide did not manage to dissolve (Figure 13). In order to not remove any MPs that are embedded in the fatty tissue, as seen in figure 21, it is preferable to use a method to dissolve the fatty tissue instead of a method which removes the fat e.g. an extra filtration. To dissolve any fatty tissue, a solution of 0,05 M NaOH could be used as done in a study by Schwab et al. (2019) on MPs in human stool (Pérez-Guevara et al., 2021). The NaOH solution could be added after 3-5 days and once again a few days before it is time to filter the samples.

To eliminate the possibility of counting cellulose fibres as MP fibres, imidazolium salt could be added to the beakers in order to dissolve them (Pérez-Guevara et al., 2021). This might be suitable when most of the biogenic matter has been dissolved to increase the chance of contact between the remaining cellulose fibres and imidazolium salt.

As a sieve set up consisting of a 40 µm sieve at the bottom, a 100 µm sieve in the middle and a 500 µm sieve at the top worked well in this study, the same set up would be used (Figure 10A). However, each sieve would be analysed under a microscope before being used to check for contamination, as done in the latter stages of this study. The samples would be poured through the filtration set up and each sieve would be loosened to enable the liquid to pass through the pores more easily. There is no guarantee that all particles will be filtered out as some might manage to pass through all three sieves. If possible, it would therefore be preferable to filter the liquid which has passed through, once again.

Each sieve would then be removed in tandem and placed upside down onto completely new glass petri dishes. As done in this study, a glass dropper would be used to squirt tap water through the sieves, enabling the caught particles to drop onto the petri dishes fFigure 9C). However, tap water has also been found to contain MPs, such as in Kosuth et al.'s (2018) study. Therefore, any tap water used should preferably be analysed beforehand, using for example µ-FTIR or a microscope, and any detected MPs should be removed. If fatty lumps are still present in the liquid, oil could be added in order to bind to the plastic polymers and create a layer that can be separated from the fatty tissue, simplifying visual observation of any particles (Lind, 2020). When reusing the same set of sieves, it is crucial to clean them properly in between each filtration in order to avoid contamination between samples. Using both highly pressured water and a stream of air to clean the sieves, as done in the latter parts

of this study, would be a useful cleaning method. A non-plastic cloth, that does not damage the sieves by altering the pore sizes, could also be helpful.

If using a microscope to visually detect potential MPs, the microscope should be placed in the fume cupboard so the petri dishes do not have to be moved outside of the closed working environment. Before counting a detected particle as a MP, tweezers would be used to nudge each particle and ensure that it is moveable, as discovered in this study. This to ensure it was not just part of the microscope's stage or bottom of the petri dish. Each potential MP would be moved from the petri dish to another dish in order to eliminate the risk of counting the same MP particle multiple times. This could potentially be done by using an extended glass pasteur pipette to carefully suck up each particle. Another potential method to accurately count the number of detected MPs could be by using a microscope grid. It was also discovered that it was efficient to switch the colour of the stage plate between black and white for each sample. This as the black background made it easier to visually identify transparent MPs and the white background made it easier to discover coloured MPs (Figure 13).

From my observations, somewhere around 19 days to three weeks seems like an optimal period of time to leave the samples in hydrogen peroxide for, as it was considerably easier to detect MPs in the sample which was filtered on day 19 compared to the samples filtered on days 32 and 37. Keeping them in the hydrogen peroxide for longer may bleach the MPs considerably, making it harder to identify the exact type of plastic.

4.4 Assessment and future research

As this study is only meant as a precursor to future studies where µ-FTIR will be used and as my study is focused on developing a method of extracting MPs from digestive tract content samples of marine mammals and not on quantifying the abundance of MPs, µ-FTIR spectroscopy was not used. It is therefore highly probable that there is an error in the number of MPs found. Especially as the organic residue and fatty tissue still left in the filtered samples made some particles hard to identify. It is also important to keep the following point by Li et al. (2015) in mind when reading the result section of this paper as it is highly likely that at least some particles included as MPs are actually non-plastic particles. "Several particles, which were previously identified as microplastics by visual observation under the microscope, were demonstrated to be other materials, rather than microplastics using the µ-FT-IR." (Li et al., 2015).

Except for µ-FTIR, another efficient method used by Philipp et al. (2020) is the use of washing machines and an enzyme-based washing powder to separate the potential MPs from biogenic matter such as tissue, blood and plant matter in the digestive tract contents. According to the authors, this is both more time and cost efficient than using chemicals or enzymatic solutions to dissolve any biogenic materials (Philipp et al., 2020).

An interesting question for future research, would be if there is a connection between the amount of MPs within marine organisms and their cause of death. You could hypothesize that individuals caught as bycatch or who have died as a result of malnutrition due to plastic consumption, might contain higher levels of MPs than individuals who have died of natural causes or as a result of hunting. The individuals caught as bycatch might present higher levels of MPs due to living in fishing territories where there is a lot of plastic marine debris in the form of fishing equipment. Another possible cause of death which could lead to higher MP contents in organisms is protection hunting as those individuals are deemed to be a possible danger due to their close proximity to human settlements. This close proximity might mean they are exposed to plastic litter in higher concentrations, leading to higher plastic ingestion rates. An important topic for future research is also to explore the potential impacts MPs might have on different bodily functions, both in seals, humans and other organisms.

4.5 Conclusion

To conclude, MPs are present in ringed seals from the Baltic Sea and are therefore, most probably, also in other species within their food web. In order to prevent even higher MP levels in marine mammals as well as in all species, more needs to be done to prevent plastic from reaching the environment or from being produced in the first place. The production of primary MPs also needs to be reduced, or completely replaced with other more environmentally sustainable alternatives. This to reduce the amount of microscopic plastics whose overall and potentially harmful effects, still remain unknown.

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