Quantification of microplastics in wild European flat oysters from the Solent region.

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Declaration

I, Katherine Bawden, declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research. I confirm that:

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3. Where I have consulted the published work of others, this is always clearly attributed;
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Abbreviations

Langstone – Langstone Harbour Royal Society for the Protection of Birds Reserve

POPs – persistent organic pollutants

Weston – Weston Shore

WWTWs – waste water treatment works

Abstract

Microplastics are considered the most abundant type of debris in the marine environment. Their persistence and ubiquitous distribution in the oceans, propensity to absorb persistent organic pollutants and potential to be ingested by marine fauna, renders them a threat to aquatic ecosystems. Previous studies have found filter feeders particularly susceptible to microplastic uptake and have suffered problems such as digestive tract blockages and translocation into tissue, ultimately threatening their ability to survive and reproduce. This study is, to our knowledge, the first to investigate the presence of microplastics in wild populations of the European flat oyster (*Ostrea.edulis*), a species of commercial, historical and ecological significance. The study aims to quantify microplastics in the gill and digestive tissues of *O.edulis* from the Solent region, in order to evaluate their potential as biomonitoring organisms, as well as to determine whether differences in abundance, type and size of microplastic exist between locations. Several microplastic studies have highlighted the need for a standardised methodology to allow for comparability of results. This study attempts to address this research gap by developing a novel methodology for the digestion of oyster tissue using the enzyme, Proteinase-K, and a procedure for the extraction and quantification of microplastics. Microplastics were identified in every oyster sampled. The Langstone oysters were found to have the greatest abundance of microplastics overall, followed by Weston and Calshot. Fibres were found to be the most prevalent type of microplastic and size ranges varied across all three locations. These findings were considered attributable to the differences in number and type of pollutant sources, as well as varying population densities and hydrodynamic characteristics. The presence of microplastics in wild *O.edulis* could be an additional threat to the survival of an already threatened species and may pose health risks for predatory species and human consumers of seafood. The use of *O.edulis* as a biomonitoring species for marine microplastic pollution could help determine the extent, distribution and sources of microplastics, potentially informing management measures to reduce their discharge to the marine environment.

Contents

1.0 Introduction

1.1 *Plastics in the marine environment*

Worldwide plastic production has increased considerably over the last 50 years (Bouwmeester et al., 2015) from 1.5 million tonnes in 1950 to 322 million tonnes in 2015 (Plastics Europe, 2016). An estimated 5 – 10% of produced plastic ultimately enters the marine environment (Jambeck et al., 2015). The mismanagement of plastic waste is considered the most significant source of plastic pollution globally (Boucher and Friot, 2017), entering the marine environment via industrial (Ng and Obard, 2006) and wastewater discharges as well as terrestrial runoff (Andrady, 2011; Browne et al., 2011; Avio et al., 2016). The long degradation times of plastics enable them to persist for decades, and has resulted in their ubiquitous distribution in the marine environment (von Moos et al., 2012). Considering the significant predictions for global population growth (United Nations, 2015), plastic production is only expected to continue on a positive trend (Plastics Europe, 2016; Browne et al., 2011). Plastics in the marine environment are considered by a number of scientists to be a hazardous waste and a threat to aquatic ecosystems (Rochman et al., 2013; Green, 2016). Recognised as the most abundant type of plastic waste, microplastics; fragments or particles of plastic which are typically <5mm (von Moos et al., 2012; Vandersmeersch et al., 2015; Cole et al., 2013), are considered to be a particular cause for concern (Green, 2016).

1.2 *Primary and secondary microplastics*

Microplastics can be classified into two main types; primary and secondary (Tanaka and Takada, 2016). Produced at a microscopic size (Mathalon et al., 2014), primary microplastics such as microbeads (Murphy et al., 2016) are used in a wide range of personal care products such as facial cleansers (Fendall and Sewell, 2009), cleaning products (Law and Thompson, 2014) and industrial and medicinal products (Cole et al., 2013; Bayo et al., 2016). These microplastics are discharged directly into the marine environment via pathways such as wastewater treatment work (WWTWs) discharges (Mahon et al., 2017). Secondary microplastics are the result of the degradation and subsequent fragmentation of larger plastics (Mathalon et al., 2014; Cozar et al., 2014; Bayo et al., 2016) such as synthetic textiles and tyres, which account for two-thirds of plastic releases to oceans worldwide (Boucher and Friot, 2017). Furthermore, thousands of microplastic fibres are released in sewage discharges due to the laundering of clothes manufactured from materials such as polyester and polyamide (Habib et al., 1998; Browne et al., 2011).

1.3 *Properties of microplastics and their effects on the marine environment*

Many studies have documented the impacts of macroplastic debris, such as entanglement and ingestion (Gregory et al., 2009; Green, 2016) on a wide range of marine organisms (Browne et al., 2008); including marine mammals, cetaceans, seabirds (Eriksen et al., 2014; Andrady, 2011) and turtles (Andrady, 2011). Despite considerable evidence for the presence of microplastics in marine organisms (Hidalgo-Ruz et al., 2012); over 220 species are reported to consume microplastics (Lusher, 2015); knowledge concerning the effects of microplastics is limited (Hidalgo-Ruz et al., 2012; Lusher, 2015), highlighting the need for further studies.

Due to their smaller sizes, microplastics may have different impacts on organisms in comparison to larger plastic debris (Law and Thompson, 2014). For instance, they can be ingested by a wider range of organisms while feeding (Law and Thompson, 2014), including marine fauna at lower trophic levels such as planktonic organisms (Van Cauwenberghe and Janssen, 2014) and other invertebrates, such as echninoderms, polychaetes and bivalves (Van Cauwenberghe and Janssen, 2014; Wright et al., 2013). Microplastic ingestion can cause a range of problems for marine organisms, including limited food uptake as a result of digestive tract blockages (Cole et al., 2013; Murray and Cowie, 2011), translocation into tissue (Browne et al., 2008) and accumulation; all of which threaten organisms' ability to survive, grow and reproduce (Browne et al., 2008; Wright et al., 2013).

Microplastics' larger surface-area-to-volume ratios also render them more susceptible to the absorption of heavy metals and persistent organic pollutants (POPs) (Bouwmeester et al., 2015) such as polychlorinated biphenyls (PCBs) from the marine environment (Wright et al., 2013; Koelmans et al., 2013; Ivar do Sul and Costa, 2014; Bayo et al., 2016). This is a particular cause for concern as the harmful contaminants can be translocated to marine fauna (Teuten et al., 2009) and can potentially affect ecosystems and human health as a result of entering the food chain (Mahon et al., 2017; Bayo et al., 2016). Studies have already found plastics present in fish and bivalve species sold for human consumption (Rochman et al., 2015).

Despite emerging legislation and voluntary action to moderate primary microplastic applications in the cosmetics industry (Bergmann et al., 2015; Bayo et al., 2016), the presence of microplastics in the marine environment is a problem which is only likely to increase in the future; due to the fragmentation of existing plastics in the oceans (Bergmann et al., 2015). Furthermore, these sources only account for a small proportion of primary microplastics discharged to the oceans. Other major sources including tyres, synthetic textiles, plastic pellets, city dust and marine coatings exist (Boucher and Fridot, 2017) but remain largely unregulated.

1.4 *Microplastics and bivalves*

Filter feeding marine organisms such as bivalves are deemed to be particularly vulnerable to the ingestion of microplastics; partially attributable to the feeding and ventilation mechanisms of the gills (Wright et al., 2015, Moore et al., 2001; Avio et al, 2016). Previous studies have shown that

microplastics ingested by wild and farmed mussels can affect their tissues and cells (Li et al., 2015; Browne et al., 2008). Other research has shown that microplastics can accumulate in oysters (Li et al., 2015) potentially affecting their health and biological activity (Green, 2016).

1.5 *Ostrea edulis*

In this study, wild populations of the European flat oyster (*Ostrea edulis*) in the Solent region were examined to determine the quantities of microplastics within individual oysters. *O.edulis* was chosen as the study species because, as of yet, there has been no research investigating the presence of microplastics in *O.edulis* in the wild. The species also has a wide geographic distribution around the UK and across Europe in the Norwegian, North and Mediterranean Seas and off the western coast of Morocco (Gosling, 2004; Jackson, 2007). Therefore *O.edulis* could be considered a useful and appropriate species in terms of future comparability studies.

O.edulis has been harvested for over 6000 years (Lapegue et al., 2007) and was deemed an important source of food for European coastal communities since prehistoric times (Gercken and Schmidt, 2014). However, over a span of 25 years the population *O.edulis* in the UK declined by half, prompting the UK government to classify it as a priority species in the UK's Biodiversity Action Plan (Harding et al., 2016). Since 2003, it has also been recognized at European level as one of the most threatened species (OSPAR, 2009); acknowledging the need for conservation action to prevent its further decline (Harding et al., 2016).

The Solent, once the largest *O.edulis* fishery in Europe (Blue Marine Foundation, 2015; Grecken and Schmidt, 2014), has suffered a long-term reduction in oyster stocks since the 19th century mainly attributable to overfishing (Harding et al., 2016). The Solent native oyster fishery collapsed and closed in 2013 (Harding et al., 2016). More recently, a stock report conducted in 2015 found that out of a total of 13 different sites sampled in the Solent, oysters were absent from hauls taken at five of the sites, and low in numbers elsewhere (Southern IFCA, 2015). The loss of habitat, exposure to water pollution, invasive species and disease are among the other environmental issues influencing the decreasing *O.edulis* populations in the Solent (Harding et al., 2016). This decline is a cause for concern for numerous reasons. *O.edulis* is considered a keystone species (Harding et al., 2016; Smyth and Roberts, 2010) and an ecosystem engineer, creating biogenic reefs (Barbier et al., 2011) and supporting other marine species of commercial importance (Grabowski and Peterson, 2014; Smyth and Roberts, 2010). They provide a food source for a wide variety of organisms (Dame, 1996), support nutrient cycling processes and increase biodiversity of marine ecosystems (Harding et al., 2016). As a result of the failing oyster populations in the Solent, these ecosystem services have also decreased (Harding et al., 2016) and can have a negative impact on the economic value and biodiversity of the Solent ecosystems (Smyth and Roberts, 2010).

1.6 *Study area*

The Solent is located between the south coast of the UK and the Isle of Wight (Fig.1) (Quinn et al., 2012) and is one of the most densely-populated areas in the South East (Hampshire County Council, 2013). The Solent estuarine system encompasses Southampton Water, the West Solent, East Solent and Spithead, as well as Portsmouth, Langstone and Chichester Harbour (Harding et al., 2016). It also possesses two of the largest shipping ports in the UK (Quinn et al., 2012) as well as 12 other estuaries and harbours (Fletcher et al., 2007). The current threats to the existing *O.edulis* populations in the Solent are likely to be exacerbated by the pollutant inputs from the associated industrial and commercial fishing activities in the surrounding area (Harding et al., 2016). As sinks for pollution, the estuaries within the Solent region are also likely to accumulate microplastics (Costanza et al., 1997; Vermeiren et al., 2016) and have been recently discovered in the water column (Gallagher et al., 2016). The numerous WWTWs, debris from roads, shipping and port activity along major tributary rivers to Southampton Water (Itchen, Test and Hamble) are all considered potential microplastic sources (Gallagher et al., 2016). As it is estimated that an individual oyster can filter 200 litres of seawater every day (Harding et al., 2016), oyster species are considered especially susceptible to exposure and ingestion of microplastic pollution (Wright et al., 2013).

1.7 *Study rationale*

This study therefore focuses on intertidal areas within the Solent region, to determine whether there are microplastics present in the remaining wild *O.edulis* populations. It may also indicate whether there are potential health risks regarding the human consumption of wild oysters. A previous study suggests that European shellfish consumers may ingest approximately 11,000 plastics per annum (Van Cauwenberghe and Janssen, 2014).

The study may provide useful information to aid management and mitigation strategies; to protect *O.edulis* as a threatened and declining species, its associated ecosystem services and ultimately human health. There has been a greater emphasis on the use of biomonitoring in recent years to improve understanding of the relationship between human health and exposure to environmental contaminants (Yarsan and Yipel, 2013). There is scope to use *O.edulis* as an indicator species for biomonitoring microplastic pollution. By monitoring the uptake of microplastics into this species and its resulting effects, it could act as a gauge for other marine invertebrate species and provide useful information concerning the levels of microplastic pollution in the UK and potentially across Europe. Its wide geographic range (Gosling, 2004), sessile nature (Smyth and Roberts, 2010) and accessible habitat in shallow estuarine waters (Gosling, 2004) make it a suitable candidate for this purpose.

There is also a need, widely acknowledged in the literature, to develop a standard methodology for the digestion of biological samples and the subsequent identification and enumeration of

microplastics (Hidalgo-Ruz et al., 2016, Karlsson, 2014, Lusher et al., 2017; Catarino et al., 2017). There are currently a wide range of different techniques used for this purpose and as a result, comparability of data is not easily achievable (Karlsson 2014; Lusher et al., 2017). This study attempts to address this research gap by developing a methodology for the digestion of soft tissue, specifically oyster tissue, and a procedure for the extraction and quantification of microplastics.

1.8 *Aim and objectives of study*

Aim

To determine the quantities of microplastics in gill and digestive tract tissues of wild *O.edulis* from sampling locations with different pollution loads within the Solent region, in order to evaluate their potential as biomonitoring organisms.

Objectives

- To develop a methodology for the digestion of *O.edulis* tissue and extraction of microplastics.
- To determine the quantities of microplastics in gill and digestive tissues of wild *O.edulis* from sampling locations within the Solent estuarine complex.
- To determine whether differences in microplastic type, size and quantities exist between two different tissue types and between sampling locations.

2.0 Methodology

As stated, this study examines the quantities of microplastics found in the gills and digestive tissues of *O.edulis*, with a focus on the development of methods for tissue extraction and microscopy analysis.

2.1 *Field sampling*

Samples of *O.edulis* were collected in July 2016 from the following intertidal locations within the Solent region; Weston Shore (50.886546, -1.375198), Calshot (50.816272, -1.306495) and an intertidal shingle bar at Langstone Royal Society for the Protection of Birds (RSPB) Reserve in Portsmouth (50.817642, -1.008355) (Solent Forum, 2017) (Fig.1).

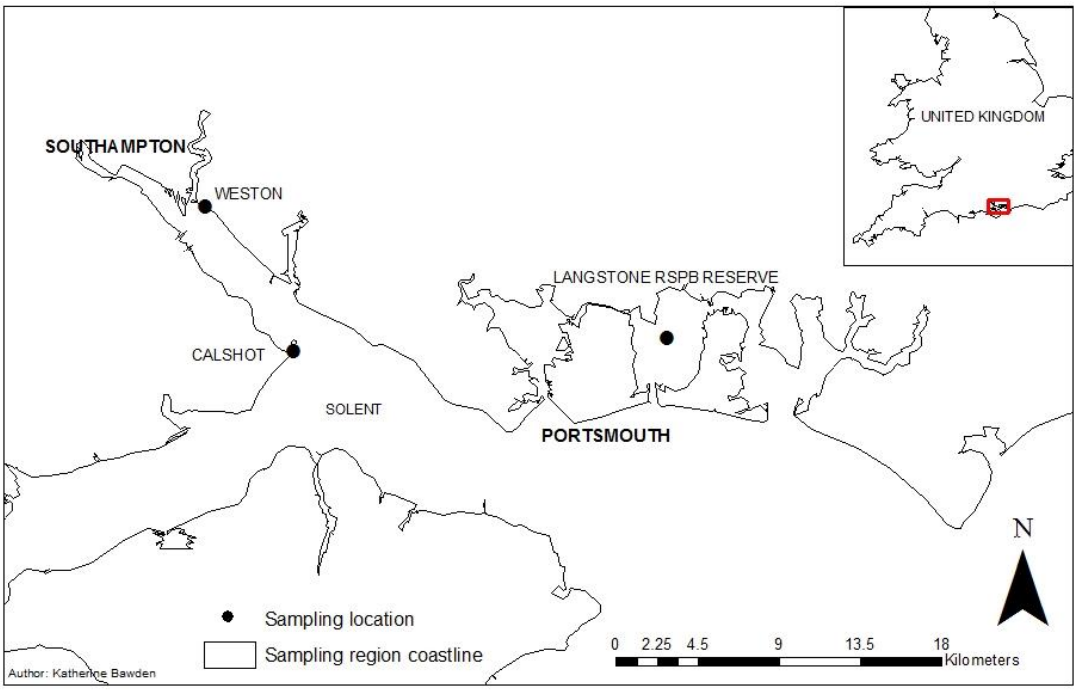

Figure 1. *O.edulis* sampling locations in the Solent region.

Calshot was selected as the area was perceived to have relatively low levels of pollution. In the Environment Agency's assessment of visual pollution (2013-2016) no sewage debris was observed and the presence of litter was negligible (Environment Agency, 2016). Furthermore, the geographic position of Calshot, near the mouth of the Solent estuary (Williams and Muxagata, 2006) means it is more exposed and experiences greater tidal mixing. Therefore, it is likely that the high flushing rate limits the accumulation of pollutants (Levasseur, 2008).

The area surrounding Weston is characterised by residential properties and park-land (Mouchel, 2011). The shoreline consists of shingle and intertidal mudflats and is designated as a Site of Special Scientific Interest (SSSI) (Mouchel, 2011). Thus, the location was considered to have relatively low levels of pollution from terrestrial inputs. However, its proximity to the pollutant inputs from around Southampton City Centre such as from storm-water discharges, Woolston

WWTW (Yates, 2015) and other industrial contaminants from the River Itchen; including two further WWTWs, shipping and port activity, road debris and a polythene bag and sheet wrapping organisation (Gallagher et al., 2016) mean that the site is still likely to be exposed to a degree of pollution from these sources.

Langstone RSPB Reserve is situated in Langstone Harbour in Portsmouth (Hirons and Thomas, 1993). It was selected for the study on the basis that it was considered a polluted site in which the oysters would have a greater exposure to microplastics. It receives a large discharge of sewage effluent (Soulsby et al., 1978) and storm-water discharges are an ongoing, regular occurrence, especially at the Southern Water treatment plant at Budds Farm (Langstone Harbour Board, 2017). As sewage sludge is recognised as a source of microplastics (Habib et al., 1998; Mahon et al., 2017), Langstone was expected to have a relatively high exposure to microplastic pollutants. Furthermore, as a semi-enclosed tidal inlet (Soulsby et al., 1978), the area is poorly flushed, leading to contaminants remaining in close proximity to the source and potentially posing a long-term risk to the oysters (Dassenakis et al., 2003; Oaten et al., 2017).

Authorisation to fish for scientific purposes in these locations was gained from Southern Inshore Fisheries and Conservation Authority (IFCA) (Appendix A). Five oysters were collected from each site and transported from field to laboratory in an isothermic container, a method used by Oaten et al., (2015) for the collection of *Mytilus edulis*. The samples were subsequently frozen at -20 °C and stored in the laboratory until dissection.

2.2 *Preparation of samples for digestion*

The digestion protocol was developed following several preliminary investigations to determine the most effective procedure for microplastic recovery. The oysters were removed from the freezer and thawed out at room temperature (Qiu et al., 2016). The length, height and width of each oyster shell was measured using digital calipers (Fig.2).

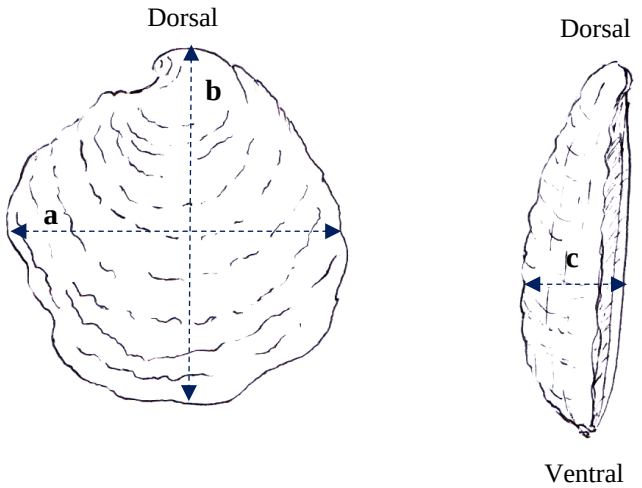

Figure 2. Diagram showing the areas measured to obtain the length (a), height (b) and width (c) of *O.edulis* shells (Katherine Bawden, 2017).

Upon opening the oysters and draining excess water, a mass of 0.2g of gill and digestive tract tissue was dissected (Fig.3) and weighed on aluminium foil to minimise contact with plastic materials.

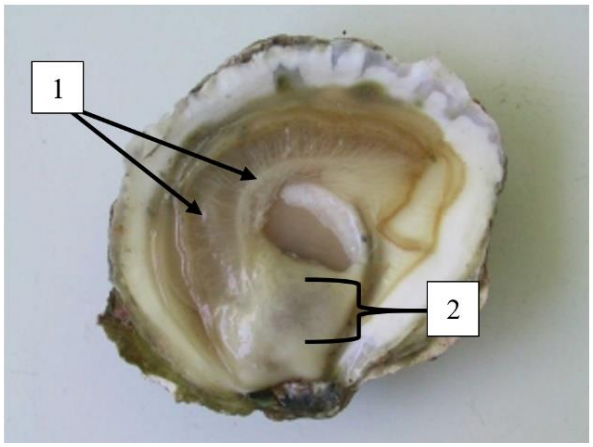

Figure 3. The areas dissected to obtain gill (1) and digestive tissue (2) from *O.edulis.* (Image adapted from FAO, 2004).

Tissue extracts were then placed in 100ml glass containers and sealed immediately with metal screw top lids to minimise the risk of contamination from airborne microplastics. Two replicates for each tissue type were obtained and placed in separate containers. Care was taken to prevent contamination of samples; an issue acknowledged in other microplastic studies (Claessens et al., 2013, Cole et al., 2013; Woodall et al., 2015). Dissection tools (razor blade and dissection tweezers) were washed thoroughly with ultrapure, deionised water (MilliQ) between replicates and samples to prevent cross-contamination.

15ml homogenisation buffer (400mM Trizma-HCL, 60mM EDTA, 150 mM NaCl, 1% SDS, pH 8) was prepared, and added to the tissue samples, based on the reagent quantities recommended by Karlsson (2014). A glass homogeniser was used to manually break down the tissue. However, the preliminary investigations suggested this method was not always effective in aiding the full digestion of the soft tissue. This was attributable to the incompatible shapes of the homogeniser and the flat-bottomed 100ml container; making it difficult to break down the tissue to increase the surface area on which the digestion reagents could act. It was necessary to use the dissection blade to cut the tissue into smaller pieces before using the glass homogeniser. After the introduction of this stage into the procedure, the issue of incomplete tissue digestion was reduced; tissue fragments <4mm were found on only a few filters.

Following the addition of the homogenisation buffer, the samples were incubated at 50°C for a period of 15 minutes. An enzymatic digestion protocol using Proteinase-K was selected as the most appropriate method for this study. Previous studies involving chemical digestion of tissue for microplastic analysis have used alkaline, acid and enzyme digestion techniques (Claessens et al., 2013; Cole et al., 2013; Van Cauwenberghe and Janssen, 2014). However, there has been

evidence to suggest that methods using acid and alkalis can destroy common marine plastics such as polyamides and polyethylene (Cole et al., 2013; Catarino et al., 2017), limiting recovery of microplastic particles (Claessens et al., 2013; Cole et al., 2013; Catarino et al., 2017). These digestion methods were therefore not considered appropriate for the extraction and quantification of microplastics in this study. Previous studies have found that Proteinase-K has the greatest soft-tissue digestion efficacy (Cole et al., 2013; Catarino et al., 2017) and can achieve greater than 97% efficacy where temperatures are sustained at 50°C for an incubation period of 2 hours (Cole et al., 2013). Proteinase-K is not destructive to microplastics and has the highest recovery rate in comparison to the other digestion protocols (Catarino et al., 2017). For these reasons, it was considered the most suitable reagent for determining microplastic quantities in oyster tissue. Table 1 provides a summary of digestion methods used in other microplastic studies.

Table 1. Digestion methods used in previous microplastic studies and their effectiveness in microplastic recovery.

Extraction protocol	**Effectiveness**	**References**
Acid digestion. Nitric acid (HNO_3)	High efficiency for fishing line fibre recovery but destruction of nylon fibres.	Van Cauwenberghe and Janssen, 2014; Claessens et al., 2013
Acid digestion. Hydrochloric acid (HCL)	Damage to microplastics and reduced recovery rate.	Cole et al., 2013
Acid digestion.	The use of strong acids can result in melding and or damage of microplastics.	Catarino et al., 2017
Enzyme digestion. Proteinase-K	Digestion efficacy of 97% and no damage, loss, degradation or surface change to microplastics.	Cole et al., 2013; Lusher et al., 2017
Alkaline digestion. Sodium hydroxide (NaOH)	Partial destruction of nylon fibres, melding of polyethylene fragments, yellowing of uPVC granules. Loss of several polyester fibres. Overall reduced recovery rate.	Cole et al., 2013
Acid digestion. Hydrogen peroxide (H_2O_2)	Lower microplastic recovery from samples due to production of foam and incomplete soft tissue digestion.	Claessens et al., 2013

After the addition of the homogenisation buffer and a 15-minute incubation period; 8mg of Proteinase-K was added to each container and incubated for 2 hours at 50°C. Sodium perchlorate (375 µl) was then added to aid deproteinisation (Wilcockson, 1973). The samples were placed on a shaking table at room temperature for 20 minutes before being incubated at 60°C for a further 20 minutes. Each digested tissue sample was then vacuum filtered over a nominally 1.2µm pore size, 47 mm diameter glass fibre filter (Whatman GF/C) and rinsed with >100ml MilliQ water. The filters were then placed in an aluminium foil lined and covered tray, and dried in the oven at 60°C overnight. Once dry filters were stored in aluminium lined petri dishes.

Initially, during the preliminary investigations, envelopes of aluminium foil were made for the storage of the filters. This was to address the risk of microplastic contamination from the plastic petri dishes due to limited availability of glass petri dishes. However, concerns arose regarding the effectiveness of this method, as there was a risk of dislodging microplastics from the surface of the filter paper. Therefore, lining plastic petri dishes with aluminium foil was considered the most appropriate method.

2.3 *Microscopy and microplastic identification*

The filters were inspected under an Olympus BH2 Polarised Light Microscope at magnification x400, and photographic images were obtained using a Nikon D5000 digital camera. The filter inspection method was adapted from the Marine and Environmental Research Institute (n.d.). The method involved starting at the top of the filter and scanning across from left to right, moving down the filter once reaching the edge and subsequently scanning from right to left. For every microplastic encountered, a photograph was taken and its reference number was recorded. The colour of each particle was also noted. In some cases, many variations of the same colour were observed. For instance, there were a range of different shades of blue. Due to the challenges of differentiating between colour variations on different filters and to avoid error in the overall counts of different observed colours, any variations of the same colour were subsequently grouped and classed as one (Appendix B).

Each particle was also categorised into one of three main types; fibre, irregular or round. Any which were angular, appeared to be fragments of larger plastics or were unlike the other two types were classed as irregular. The microplastic classification method was adapted from previous studies using similar techniques (Kyong Song et al., 2015; Gallagher et al., 2016). The magnification at which each photograph was taken was also noted to accurately determine particle size at a later stage. A subsample was taken of the round microplastics, specifically those with a 3-dimensional, spherical structure. These were considered to have a similar structure to microbeads and so five from each sampling location were selected for further analysis. ImageJ was used to determine the diameter of these particles to allow for comparison between each sub-sampled particle, and the size ranges stated in the literature; to determine whether these were truly microbeads.

2.4 *Measuring microplastic size*

To determine the size of the smallest microplastic on each filter paper, all of the individual microplastic photographs were examined manually. A subsample was then taken; selecting the microplastics appearing the smallest overall. Their longest dimensions were measured using ImageJ software (Ferreira and Rasband, 2015). These dimensions were compared so the smallest microplastic for each filter could be determined. As there were over one hundred photographs for some filters, and photographs had been captured at either x400 or x1000 magnification, it was often necessary to measure several of the particles which appeared very similar in size in order to determine accurately which of them was the smallest. The images of the stage micrometer at x400 and x1000 magnification were used to calibrate these photographs to the correct scale. The same procedure was used for finding the largest microplastic particle on each filter. Each of the different microplastic types required slightly different measurement techniques. The lengths of fibres were measured using the freehand tool due to their coiled or curved shapes. The diameter of round microplastics and the widest point of angular microplastics were measured using the straight-line tool. The smallest and largest microplastic was measured from replicates 1 and 2 of both gills and digestive tissue extracts. The mean was calculated from these two replicates to give a single minimum mean size and a maximum mean size for each tissue type.

The observation of round particles of very similar shape, colour and size in several samples led to a further subsample being taken for comparison. Five round microplastics from each sampling location were selected randomly and their diameters were measured.

2.5 *Statistical analyses*

To determine whether there was a significant difference in microplastic count between the three different sample locations, a one-way analysis of variance (ANOVA) was carried out, followed by a Tukey's HSD post hoc test (Field, 2009). As the data had a non-normal distribution, it was first Log transformed in order to carry out the test. An ANOVA was also used to determine whether there was a significant difference in microplastic count between the gills and the digestive tract tissues. Statistical analysis was also used to determine whether there was a significant difference between the mean counts of the gill and digestive tissue and the different types of microplastics. A normality test indicated the data had a non-normal distribution. Based on this assumption a Kruskal Wallis H test was carried out. For the above statistical tests, a significance level of 0.05 was used.

2.6 *Contamination control measures*

As a result of the preliminary investigation and review of other microplastic studies, contamination control measures were considered and incorporated into the methodology. Airborne microplastics, particularly fibres, are a known problem causing contamination in microplastic research (Woodall et al., 2015). To address this issue measures were taken to reduce airborne contamination. Tissue extract samples were covered with aluminium foil at every

possible stage in the procedure where they were at risk of exposure to airborne particles. The time with which the samples were exposed to air during dissection and tissue sample processing was kept to a minimum.

While carrying out tissue extractions and handling samples, a 100% cotton lab coat was worn at all times (Van Cauwenberghe and Janssen, 2014). Measures were also taken to ensure that all glassware and equipment used in the extraction, processing and handling of samples were washed sufficiently to remove contaminant particles (Claessens et al., 2013). All glassware used in the digestion and filtration procedures was acid washed overnight, rinsed thoroughly in MilliQ water and covered in aluminium foil and dried in an oven at 60□C. All equipment and glassware were then covered with aluminium foil for next use.

The use of procedural blanks was helpful in developing methods for contamination control. These were prepared identically to the tissue samples and analysed under the microscope to check for contamination (Vandersmeersch et al., 2015). They allowed for the identification and elimination of potential contaminant sources during the preliminary investigation. For instance, during the preliminary investigation, black particles were observed on both the tissue and blank filters. A visual inspection of the equipment used in the procedure was then carried out to identify the source of contamination. A black, rubber seal inside the lids of the glass bottle containers was identified as being a possible source of contamination. These were subsequently removed from the lids. Further sample runs and examinations of filters showed that the presence of black particles had been substantially reduced and suggested the rubber seal was the source.

3.0 Results

3.1 *Oyster size*

Generally, the size dimensions of the oysters from each location were relatively similar (Table 2). Shell lengths ranged between 51.01 and 68.19 mm, heights between 52.10 and 71.04 mm and widths between 16.54 and 31.71 mm. However, there were two outliers; oyster 2 from Weston was noticeably larger than the other oysters with length, height and width 83.54, 93.82 and 31.71 mm respectively. One Calshot oyster was also found to be uncharacteristically large. The length, height and width of oyster 5 was measured as 87.17, 112.04, 51.77 mm respectively.

Table 2. Shell dimensions (length, height and width) of the sampled oysters.

Sampling location	Oyster replicate	Shell length (mm)	Shell height (mm)	Shell width (mm)
Calshot	1	60.30	60.99	17.28
	2	55.95	64.87	20.57
	3	68.19	71.04	18.22
	4	51.01	52.10	16.54
	5	87.17	112.04	51.77
Weston	1	55.01	56.88	21.11
	2	83.54	93.82	31.71
	3	53.83	58.82	17.85
	4	54.93	65.33	18.16
	5	55.49	65.42	16.23
Langstone	1	63.45	67.43	18.55
	2	67.12	61.86	14.09
	3	53.77	65.76	19.59
	4	56.64	67.36	18.59
	5	65.23	71.50	20.50

3.2 *Abundance of microplastics in O.edulis*

Figure 4 indicates that Langstone had the greatest mean microplastic count of the three locations. The mean microplastic counts observed in gill and digestive tissue were 28 and 33.2 respectively. Weston followed with a mean of 15.6 in gill tissue and 23 microplastics in digestive tissue. Tissue extracts from Calshot oysters were observed to have the lowest mean microplastic counts overall. A mean count of 17.9 was observed in the gill tissue compared to a lower mean count of 11.6 in the digestive tissue. Conversely, in the other two sample sites, the mean count was observed to be greater in the digestive tissue. The mean counts observed in the procedural blanks for each sampling location were considerably lower than the counts found in tissue.

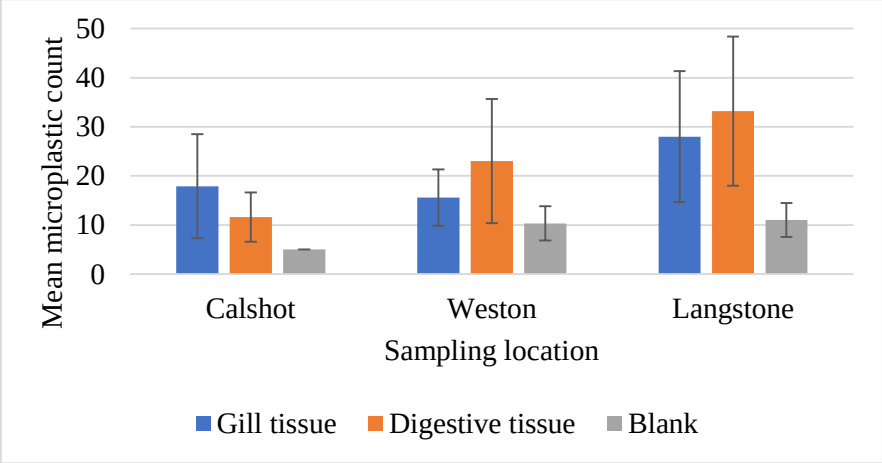

Figure 4. Comparison of mean microplastic count observed in gill tissue, digestive tissue and procedural blanks between each of the three locations (mean ± standard error). Five oysters were used from each location (n=5). From each of these, two replicates were taken for gill tissue and digestive tissue (n=2).

The results from the one-way ANOVA indicate that overall the differences observed in the microplastics counts between the sampling locations is highly significant ($P<0.001$). The Post Hoc test (Tukey HSD) showed that although there was no significant difference between the microplastic counts from Calshot and Weston ($P = 0.254$), a significant difference was found between the microplastic counts from Weston and Langstone ($P = 0.007$) and a highly significant difference was found between counts from Calshot and Langstone ($P<0.001$).

When calculated as percentages, the proportion of microplastics from Calshot oysters which were found in gills was 61.72% compared to 38.30% found in digestive tissue. Whereas the percentage of microplastics found in gill and digestive tissue in Weston and Langstone Harbour RSPB Reserve was more evenly distributed. In Weston oysters 40.41% of the total microplastics were found in gills and 59.59% were found in digestive tissue. In Langstone oysters, 45.75% and 54.25% of the microplastics were found in gill and digestive tissue extracts respectively. The one-way ANOVA testing the differences between the two tissue types suggests that there is no significant difference in microplastic count between gills and digestive tissue ($P = 0.617$).

Figure 5 suggests that there is no clear relationship between the length of the oyster shell and mean microplastic count. The majority of the shell lengths range between 52.1 and 71.5 mm, but within this size range the mean microplastic counts appears quite varied, ranging from 10.25 to as high as 47. Two oysters from Langstone between 60 and 70 mm in length have mean microplastic counts which are considerably higher than the other oysters (39, 47). Another two oysters from Calshot and Weston have much greater shell heights than the others; 93.82 and 112.04 mm respectively and their mean microplastic counts are at the lower end of the range. The oyster from Weston had a mean count of 14.5 and the oyster from Langstone, 7.25, notably the lowest mean microplastic count overall.

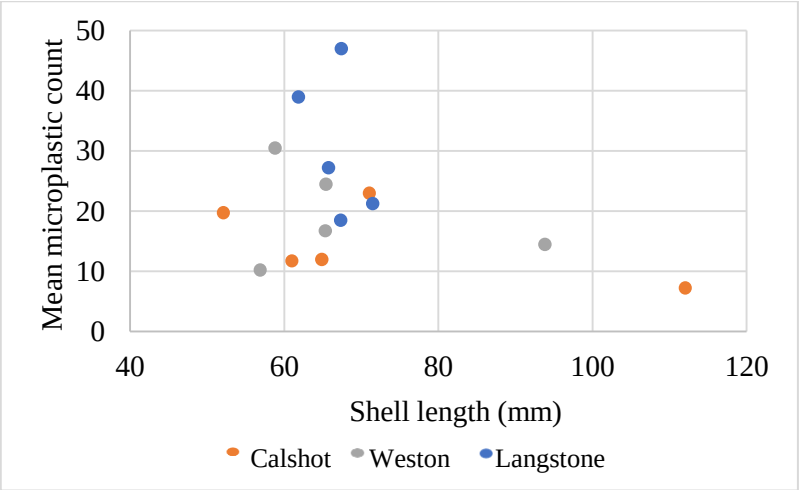

Figure 5. A comparison of shell length and mean microplastic count from the three sampling locations.

3.3 *Types of microplastics in O.edulis*

A range of different types of microplastics were observed on the filters of the *O.edulis* tissue extracts. These were classified into three main types; fibres, irregular and round microplastics, as illustrated in Fig.6.

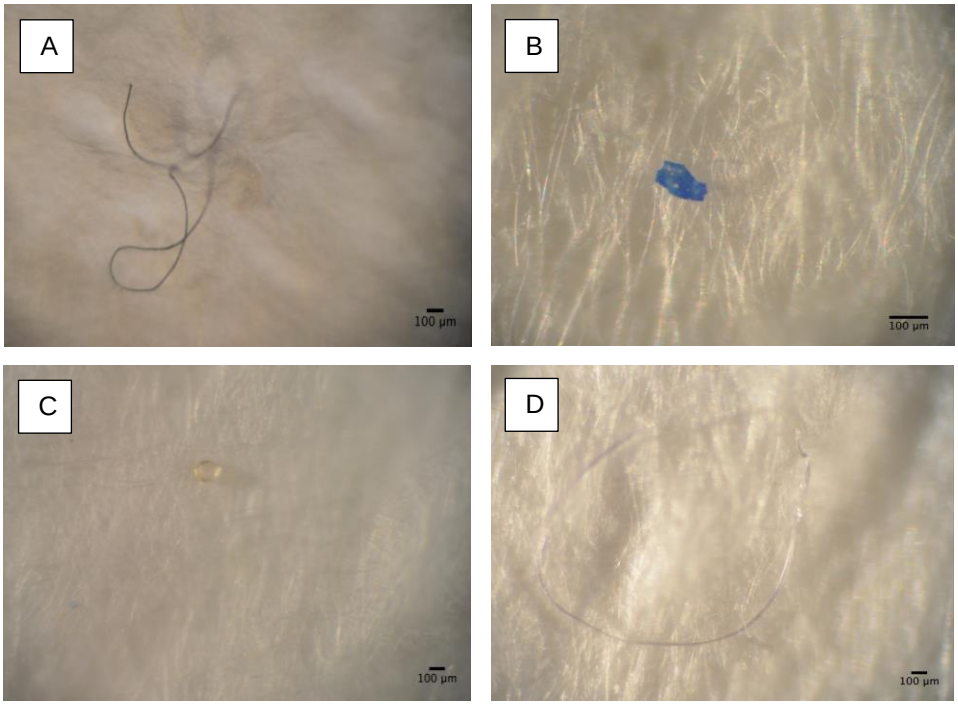

Figure 6. Photographs showing examples of the different types of microplastics found in *O.edulis* tissue extracts. Examples of fibres (A, D), an irregular microplastic (B) and a round microplastic (C) are shown. All photographs (A-D) were captured directly on the filter paper. A and D also illustrate two of the most common colours of fibres observed in the *O.edulis* samples. Scale bar = 100µm.

Fibres were found to have the greatest diversity in terms of colour. In total, 10 different colours were observed. Irregular microplastics were found to have the next greatest colour range (7), followed by round microplastics (4). The commonest colours among the observed microplastics were transparent-white and black; which accounted for 27.69% and 17.22% of the microplastics respectively. Photographs of typical examples of these types of microplastics are illustrated in Fig.6A and D. Yellow and amber were among the commonest colours observed for round microplastics, as exemplified in Fig.7.

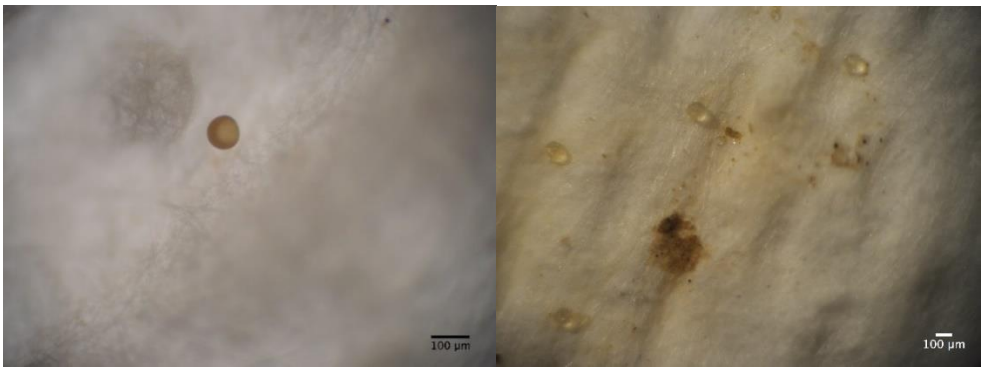

Figure 7. Photographs showing examples of the commonest colours of round microplastics found in *O.edulis* tissue extracts. The photographs were captured directly on the filter paper. Scale bar =100 µm.

The results suggest there is a general pattern regarding abundance of microplastic type. Fibres account for the greatest proportion of the mean microplastic count across each location (Fig.8A, B and C). For the majority of the oyster replicates, especially from Langstone, the mean count of fibres is over half than that of the other microplastic types. The abundance of irregular microplastics is generally much lower than fibres. Round microplastics have an even lower abundance and were not observed at all in two of the digestive tissue replicates (Fig.8A). The proportion of fibres in the procedural blanks across all three sampling sites is also considerably greater than irregular or round fibres.

Overall, the abundance of the different microplastic types observed in the digestive tissue appears to follow a similar pattern to the gill tissue samples. However, one difference was observed in the digestive tissue replicate 3 from Weston (Fig.8B), where the proportion of round particles was considerably larger than the other two microplastic types and compared to the gill tissue counts. There also appears to be a greater proportion of round particles in replicates 5 from Weston (Fig.8B) and 1 and 2 from Langstone (Fig.8C) than in other digestive tissue samples. Digestive tissue replicate 5 from Calshot is also different; it has a considerably lower mean microplastic count consisting entirely of fibres (Fig.8A). The proportion of fibres in the mean microplastic counts of the procedural blanks is also considerably greater than the proportion of irregular or round fibres.

The results of the Kruskal Wallis H test showed that no significant difference in mean microplastic count was found between the different tissue types and the microplastic types; fibre (P= 0.738), irregular (P=0.939) and round (P=0.419).

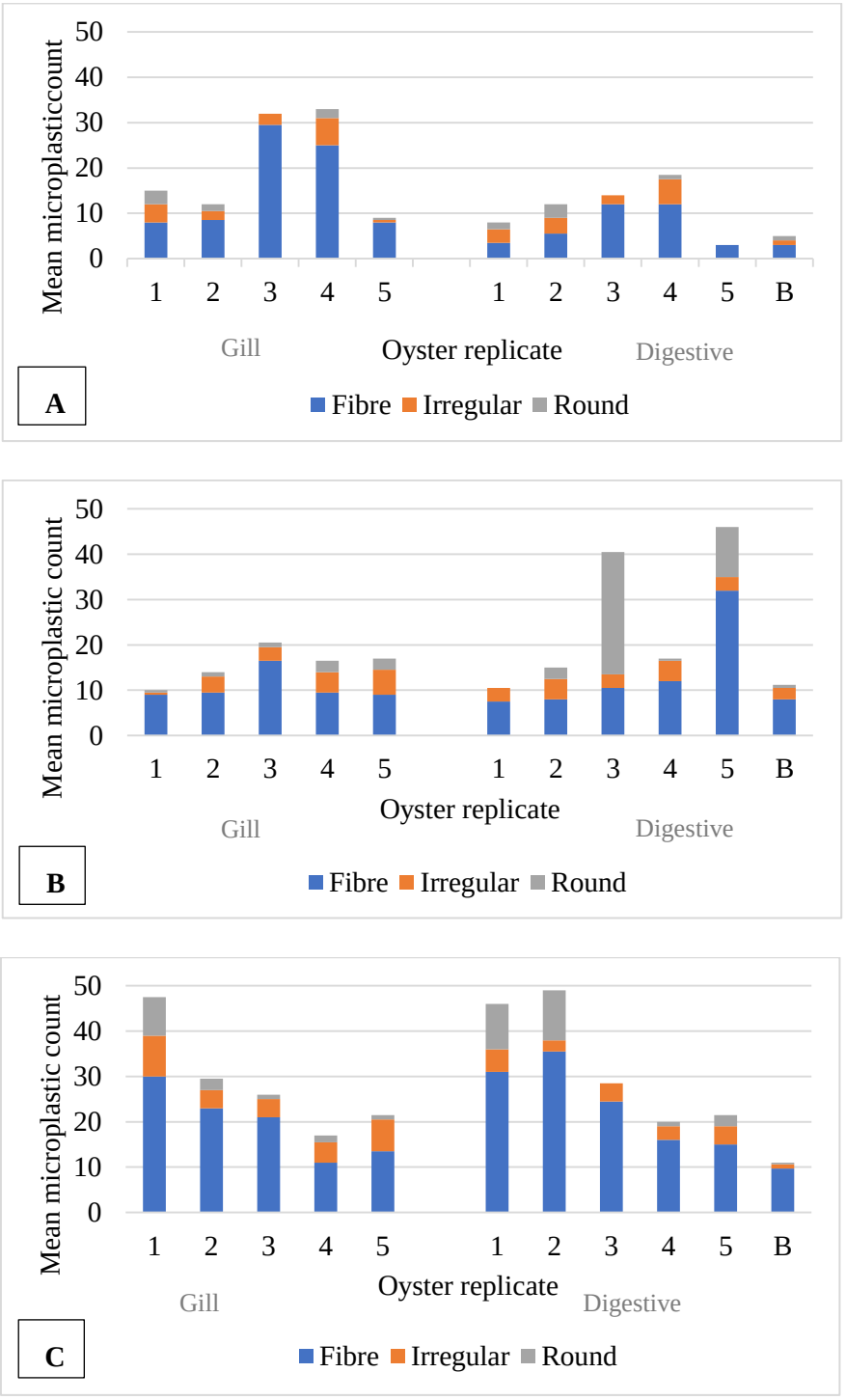

Figure 8. A, B and C show a comparison of the mean counts of each type of microplastic (fibre, irregular, round) in gill and digestive tissue samples (n=2) from Calshot, Weston and Langstone RSPB Reserve oysters respectively (n= 5). The numbers on the x-axis correspond to the oyster replicate from which the tissue extract was dissected. B represents the mean count from the procedural blanks.

3.4 *Size of microplastics in O.edulis*

Microplastic sizes ranged from 10.31 (0.01031 mm) to 4856.04 μm (4.85 mm). Generally, the minimum and maximum sizes were quite varied across oyster replicates. However, Weston oysters appear to have the most consistent size ranges per oyster in comparison to the other two locations. In particular, the highest and lowest minimum sizes, which range between 24.98 μm to 73.36 μm (Fig.9B). Also of note is the difference between the largest and smallest maximum sizes from Weston, which range between 2839.33 and 3471.64 μm. This difference (632.31) is considerably smaller than the differences observed in Langstone (2745.87) and especially Calshot (3963.62). In contrast to the Weston oysters, microplastic sizes found in the Calshot oysters appear to have a much greater range; between 12.12 (oyster 2) and 320.54 μm (oyster 1) of the digestive tissue extracts. However, Langstone's sizes ranged between 10.32 and 47.68 μm and so were the smallest of all the three sites.

Some of the replicates from Langstone appear to have the greatest size ranges (Fig.9C). Oyster replicates 2 and 3 from the gill tissue extracts and 5 from the digestive tissue extracts have particularly large ranges. The minimum and maximum sizes of replicates 2 (13.82, 4525.16 μm) and 3 (14.97, 4083.55 μm) give ranges of 4511.34 and 4068.58 respectively. Replicate 5 has a minimum size of 10.32 μm and maximum size of 3605.69 μm, giving a range of 3595.37. Furthermore, the smallest microplastics overall were also found in Langstone oysters, specifically replicate 5 of the digestive tissue extracts (10.32 μm) and replicates 2 (13.82 μm) and 3 (14.97 μm) of the gill tissue extracts. Between each location, the blank results appear quite varied. However, the Calshot and Weston blanks show that generally, the microplastics were larger than those observed in the tissue extracts.

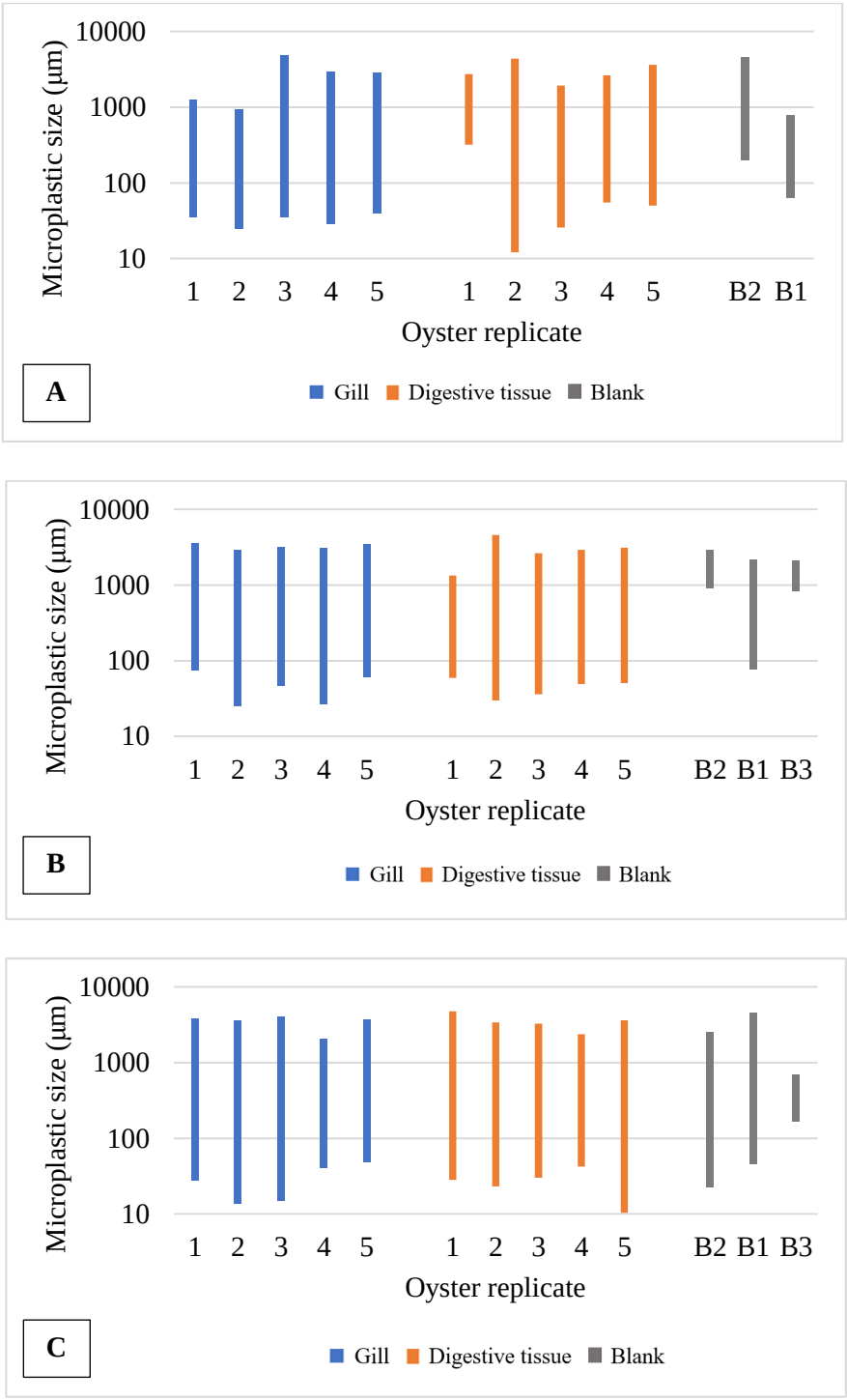

Figure 9. A, B and C show a comparison of the minimum and maximum microplastic sizes found in oysters from Calshot, Weston and Langstone oysters (n= 5). Numbers on the x-axis correspond to the oyster from which the tissue extract was dissected. B represents the procedural blanks.

3.5 *Round microplastics*

The diameters of a subsample of round microplastics, with similar appearances to the one shown in Fig.7, were determined and are presented in Table 3. On the whole, the diameters of the subsampled particles range between 41.13 and 121.10 µm. The Calshot particles were notably

smaller than those from other locations (41.13 - 92.39 μm). Whereas the diameters of particles sampled from Weston (86.70 - 121.10 μm) and Langstone were higher (62.52 -118.98 μm).

Table 3. The diameters of the round microplastics subsampled from Calshot, Weston and Langstone.

Sampling location	Microplastic	Diameter (μm)	Diameter (mm)
Calshot	1	78.37	0.07
	2	92.39	0.09
	3	42.78	0.04
	4	69.74	0.06
	5	41.13	0.04
Weston	1	115.53	0.11
	2	120.33	0.12
	3	86.70	0.08
	4	101.11	0.10
	5	121.10	0.12
Langstone	1	117.02	0.11
	2	118.98	0.11
	3	62.52	0.06
	4	105.88	0.10
	5	101.08	0.10

4.0 Discussion

The generated results suggest that the development and modifications to the methodology were successful in producing an effective protocol for the extraction and enumeration of microplastics in *O.edulis.*

4.1 *Abundance of microplastics*

The results indicate that microplastics are present in wild populations of *O.edulis* in the Solent region. Microplastics were found in both gill and digestive tissue of all oysters sampled from Calshot, Weston and Langstone. These findings are supported by previous research which have reported the presence of microplastics in the digestive system and gills of bivalves (Van Cauwenberghe and Janssen, 2014; von Moos et al., 2012). However, the quantities of microplastics detected in this study were greater than those found in marine bivalves in the literature (Li et al., 2015). While this could be partially attributable to contamination in the laboratory, it is more likely a result of the varying methods used to digest, filter and identify microplastics in the literature (Lusher et al., 2017). For example, most studies have used filters with larger pore sizes, leading to loss of microplastics in the smaller size fractions (Van Cauwenberghe and Janssen, 2014; Li et al., 2015).

The Langstone oyster tissue extracts had the greatest abundance of microplastics (Fig.4). The mean microplastic counts were significantly greater than those of Calshot and Weston. This finding is unsurprising for a range of reasons. Microplastics are found in greater quantities near densely-populated areas (Browne et al., 2011; Eriksen et al., 2014; Yonkos et al., 2014). As the recipient of litter and road runoff from the most densely-populated city outside of London (Office for National Statistics, 2012) Langstone Harbour is likely to be subject to high microplastic contamination. Furthermore, a total of 49 public and private wastewater pipes have been identified to discharge directly into the harbour (Friends of Langstone Harbour, n.d.). Sewage discharges into Langstone Harbour are a common occurrence; a total of 1118 storm-water discharge notifications have been issued every year since January 2010, with 9 notifications given the month preceding sampling (Langstone Harbour Board, 2017). Microplastics are among the suspected contaminants released in these discharges (Solent Forum, 2013). It is therefore likely that there are elevated concentrations of microplastics suspended in the water column and accumulating in the sediments within Langstone Harbour; potentially serving as an explanation for the greater microplastic abundances in the Langstone oysters. Furthermore, as a semi-enclosed system (Soulsby et al., 1978), Langstone Harbour is less able to flush these contaminants (Owen and Sandhu, 2000). As a result, they have a tendency to persist in close proximity to their sources (Dassenakis et al., 2003). In this study, the Langstone oysters were collected from the northern end of the harbour, near to several major pollutant sources including the Southern Water storm-water outfall at Budd's Farm (Langstone Harbour Board, 2017) (Appendix C). A study by Claessens et al. (2014) and Vianello et al. (2013) found that the microplastic concentrations of sediments were overall greater in the innermost parts of this type of system, potentially providing a further explanation as to why the Langstone oysters were found to have the greatest quantities of microplastics.

Although Weston is also in close proximity to a densely-populated city; Southampton has a population density of 4,810 km^2 (Office for National Statistics, 2015), a WWTW (Yates, 2015) and plastics industry (Gallagher et al., 2016), lower quantities of microplastics were observed in the oysters. A possible explanation for this is that the site is located in an open system in the Solent, an ebb dominant estuary where a greater proportion of water and sediment is transported out of the estuary than in (Gallagher et al., 2016). Unlike Langstone, microplastics are more likely to be transported out of the estuary and away from intertidal sites within the Solent such as Weston, due to its rapid flushing time (Lockwood et al., 1985). The quantities of microplastics in Calshot oysters were even lower still, which may be explained by the comparatively low population density in the surrounding area (Office for National Statistics, 2015), low levels of industrial activity and absence of any major wastewater discharges in the local vicinity. The location of the site near the mouth of the estuary (Williams and Muxagata, 2006) makes it subject to higher levels of flushing and higher energy and exposure to open sea than Weston. These

findings support previous studies which also found greater microplastic quantities in more populated areas (Gordon, 2000; Andrady, 2011).

4.2 *Oyster size*

Comparisons of oyster shell length and mean microplastic count suggests there is no clear relationship between these two factors. Whereas, a study by Walne (1972) suggests that a positive correlation exists between filtration rate and shell length of *O.edulis.* Thus, it would be expected that oysters with larger shells, filtering larger volumes of water are more likely to uptake greater quantities of microplastics.

Interestingly, two atypically large specimens were found to have among the lowest mean microplastic counts. The largest oyster had, by far, the lowest microplastic count overall. As shell length is an indicator of age (Ridgway et al., 2010), this may suggest that *O.edulis* does not retain all microplastics it intakes throughout its life and may release them into the surrounding environment. One of the functions of the gills in bivalves is for the sorting and rejection of unwanted filtered material as pseudofaeces (Gosling, 2004; Xu et al., 2016). A study by Xu et al. (2016) suggests that this process limits the retention of microplastics within the clam, *Atactodea striata*, and may explain why quantities of microplastics is not necessarily proportional to oyster size. However, as the sample size for this study was small, it is not possible to draw definitive conclusions. Further studies using larger sample sizes are required to investigate to determine whether microplastic loadings are related to *O.edulis'* size and whether they change during the species' life cycle.

4.3 *Type of microplastic*

The majority of the microplastic particles observed in the oysters from each sampling location were fibres (Fig.8). In particular, at Langstone, which is somewhat unsurprising due to the aforementioned numerous storm-water discharges to the poorly flushed area (Langstone Harbour Board, 2017). The finding that fibres are the dominant type is supported by several microplastic studies (Thompson et al., 2004, Browne et al., 2010; Browne et al., 2011; Lusher et al., 2013; Gallagher et al. 2016; Waite, 2017). There are a range of potential sources of fibres in the Solent region. As a densely-populated area, there are numerous WWTWs discharging large volumes into the Solent. This is considered a major source (Murphy et al., 2016), as thousands of fibres pass through filtering systems of washing machines (Gallagher et al., 2016) and are released into wastewater as a result of washing synthetic clothing (Napper and Thompson, 2016). Moreover, the region is popular for recreational sailing and commercial shipping (Hampshire County Council, 2010) hosting a diverse range of fisheries (Southern IFCA, 2014). Materials and gear used in these activities, such as nets and ropes, are considered significant sources of fibres (Andrady, 2011) and may serve as a further explanation for their prevalence. However, Gallagher (2016) proposes that these activities are perhaps not the main source of the fibres in the Solent region, suggesting sewage and WWTWs containing fibres from clothing, cosmetic and cleaning

products as more probable sources. An alternative explanation for the prevalence of fibres, as highlighted by Napper and Thompson, (2016), is that plastic films may be misidentified as fibres as they break down into threads and filaments. The polythene bag and sheet wrapping organisation (Gallagher et al., 2016) near the Weston site could be a potential source of these plastic films.

Generally, irregular microplastics were found to be the next most common, followed by round particles. This differs from the findings of other similar studies, which show that round microplastics are the most commonly encountered after fibres (Thompson et al., 2009; Browne et al., 2011; Gallagher et al., 2016). It is likely that the prevalence of the irregular microplastics are as a result of road runoff from the surrounding densely-populated areas, as tyre fragments have been found to be a major source of microplastic pollution (Boucher and Fridot, 2017). However, there were some exceptions. In Weston and Langstone, the proportion of round particles in some replicates were noticeably larger than in Calshot, such as digestive tissue replicate 3 from Weston (Fig.8B). This may be attributable to the proximity of wastewater treatment discharges to these sites; which are likely to contain cosmetic microbeads (Eriksen et al., 2013; Tanaka and Takada, 2016). The reported ongoing discharges into Langstone Harbour from Southern Water's combined sewer overflows (Solent s, 2015), and the proximity of the Weston site to Woolston WWTP.

No significant difference was found between tissue type and type of microplastic. As the shape of microplastics may influence toxicity to the organisms ingesting them (Wright et al., 2013), this may be an important factor to investigate in future studies, as it could aid the identification of the most harmful microplastics and help prioritise which microplastic sources should be managed and minimised.

4.4 *Size of microplastics*

Across the three locations, there was a variety of different microplastic sizes found in the oysters, ranging from 10.31 to 4856.04 μm (Section 3.4). Some of the Langstone oysters were found to have the largest microplastic size ranges, which may be attributable to the diversity of potential sources such as wastewater discharges and pollutants from the surrounding settlements. Inhabiting a poorly-flushed, continually polluted system, the Langstone oysters may have been exposed to microplastics released into the system years ago which have degraded and fragmented over time (Ng and Obbard, 2006) as well as larger, more recently discharged microplastics. The Calshot microplastics also appear to have a large range of sizes, despite having comparatively fewer local pollutant sources than the other sites. The area's proximity to the mouth of the estuary (Williams and Muxagata, 2006) may increase its exposure to a wider range of pollutants sourced from the Solent tributary rivers, the waters around the Isle of Wight and potentially from the Atlantic. The Weston oysters, despite being close to a number of potential microplastic sources were found to

have the smallest range of microplastic sizes overall, which could be explained by the dominance of one particular source such as WWTPs over other potential sources.

On closer inspection of the round particle sizes from each sampling location revealed that the diameters are not consistent with those reported in the literature. Due to their uniform, spherical shape, sizes and colouring, the particles were suspected to be microbeads. However, they do not appear to fit the size profile of the cosmetic microbeads, stated to be >0.1 mm in size (Environment and Climate Change Canada, 2015). While this applies to some of the observed round particles found in this study, several are evidently smaller. This suggests that perhaps the round particles are in fact something else or potentially nanoplastics; particles <1 μm (Bergmann et al., 2015). The possibility that the round particles detected on the filters were caused by laboratory contamination is considered unlikely as the majority of the round particles were translucent and yellow; reported to be a common colour change for clear microplastic pellets which have undergone degradation in the marine environment (Acosta-Coley and Olivero-Verbel, 2015). The colouring is suggested to be indicative of photo-oxidative damage, meaning the particle has persisted in the marine environment for a long period of time (Acosta-Coley and Olivero-Verbel, 2015). Raman spectroscopy could be used determine the composition of the suspected microplastics (Ribeiro-Claro et al., 2017) and ultimately help identify the source of the contaminant.

Microplastic size is an important factor to consider as small particles have a greater surface area and propensity to absorb toxic pollutants (Bouwmeester et al., 2015). Other studies have shown that larger particles may be retained in the guts of bivalves for shorter periods of time than smaller particles (Brillant and MacDonald, 2000; Bouwmeester et al., 2015) and that smaller microplastics are more susceptible to translocation into the circulatory system (Browne et al., 2008). Further studies could investigate whether this is the case for *O.edulis.* Definitive conclusions cannot be made regarding the sizes of microplastics due to the limited sample size. However, further research could help identify the most dominant sizes found in the oysters and potentially give an indication as to the source of the microplastics. Further research is also needed to determine whether the microplastics found in *O.edulis* are trapped within the gills or lumen of the digestive tract, or whether they have migrated into the tissues or cells; both cases have been found in the digestive tract of *M.edulis* in a study by von Moos et al. (2012). Whereas in filter feeding *Carcinus* maenas, microplastics were identified on the gill surface but not within the tissue (Duis and Coors, 2016). This could potentially be determined by histological analysis (von Moos et al., 2012) and would provide an insight into the impacts of microplastics on *O.edulis*.

4.5 *Wider implications*

The discovery of microplastics in wild O.edulis could be an indication that microplastics are accumulating in filter feeders. This is a cause for concern for a number of reasons. Not only have the microplastics themselves been found to threaten the functioning of filter feeders (Murray and

Cowie, 2011), their associated POPs may also pose health risks to these species (Gallagher et al., 2016), and potentially the predators and humans consuming them (Nerland et al., 2014). There is therefore an urgent need for action to reduce further inputs of microplastics into the marine environment (GESAMP, 2016). The use of *O.edulis* as a biomonitoring species for marine microplastic pollution could help determine the extent, distribution and sources of microplastics affecting filter feeders, potentially informing management measures to reduce their discharge to the marine environment. This information, combined with further research into the ecological effects of microplastics could possibly drive policy on the production of plastics (Rochman, 2016) to address a major environmental problem (Dehaut et al., 2016).

4.6 *Methodological developments*

This study reinforces the recommendations made in the literature for the development of a standardised procedure for extracting, identifying and quantifying microplastics (Vandermeersch et al., 2015; Hidalgo-Ruz et al., 2016, Karlsson, 2014, Lusher et al., 2017; Catarino et al., 2017). The methodological findings of this study could potentially aid the progress towards such a procedure. It is recommended that the following areas are addressed for the purposes of improving and standardising future microplastic analysis in biological tissue samples.

4.7 *Contamination control*

The abundance of microplastics in procedural blanks was considerably lower than the abundance observed in the tissue extracts (Fig.4), suggesting that the majority of microplastics were from the oysters and not the laboratory environment. An earlier study claims to have found no microplastics in the blanks and very low numbers in tissue extracts (Van Cauwenberghe and Janssen, 2014), which is surprising considering the widely-acknowledged issues associated with contamination in microplastic research (Claessens et al., 2013, Cole et al., 2013; Woodall et al., 2015). However, as that study used a destructive acid digestion protocol, it is possible that microplastic counts were underestimated as a result. This study shows that despite best efforts to incorporate mitigation strategies in the methodology (Section 2.6), contamination remains an issue. Approaches, such as using a laminar flow hood and working in areas with low foot traffic have been used in previous studies (Claessens et al., 2013) but were not feasible for this study. Future research should strive to adopt a standardised laboratory protocol, using all viable measures to eliminate contamination.

4.8 Microplastic identification

There is a possibility that a proportion of the particles found on the filters were misidentified as microplastics, a problem recognised in the literature (Eriksen et al., 2013; Kyong Song et al., 2015; Gallagher et al., 2016). For the more accurate identification of microplastics Raman spectroscopy is recommended to support visual identification, as it is considered relatively accurate in determining polymer type (Remy et al., 2015).

4.9 Enzymatic digestion protocol

As previously mentioned, the use of enzymes in digestion procedures has been found to yield the best microplastic recoveries (Catarino et al., 2017). However, this study and previous studies have found there are still digestion efficacy issues (Dehaut et al., 2016) and so further research is required to improve this protocol for the complete digestion of tissue.

5.0 Conclusion

To the best of our knowledge, this is the first study to investigate the presence of microplastics in wild populations of *O.edulis*. In response to the claims in the literature that comparability of data from microplastic research is constrained, this study has attempted to develop what could potentially be a standardised method for the extraction and quantification of microplastics from oyster tissue. In particular, the limitations of other studies concerning microplastic recovery were addressed by the development of an enzymatic digestion protocol. The consistency in the findings suggest that the methodology has been somewhat successful in yielding effective results. The study also attempts to address the widely-acknowledged issues concerning incidental contamination of samples (Claessens et al., 2013). However, despite the rigorous measures taken to mitigate this contamination, some microplastics were still found in the procedural blanks. The adoption of further contamination mitigation measures such as the use of a laminar flow hood are therefore recommended for future studies. To aid the visual identification of microplastics, Raman spectroscopy is suggested as an effective technique and could help identify polymer type potentially useful in identifying the most dominant microplastic sources.

The study findings indicate that, like many previous studies on bivalves, microplastics are present within *O.edulis* populations within the Solent region. Microplastics were detected in gill and digestive tissue of oysters from all three study locations; Calshot, Weston and Langstone Harbour. The results demonstrate that the quantities of microplastics found in the oysters differ depending on sampling location. These differences are likely to be attributable to the varying number and sources of pollution within the Solent region, such as WWTWs, plastics industry, CSOs, commercial fishing activities as well as being influenced by differences in hydrodynamic conditions. The comparison between the sampling sites revealed that oysters from Langstone contained significantly larger quantities of microplastics, followed by the Weston oysters. Conversely, the Calshot oysters contained the smallest quantities. This was an unsurprising discovery as Langstone harbour is poorly flushed and affected by a plethora of pollutant sources discharging into the area (Langstone Harbour Board, 2017), whereas Calshot is surrounded exposed to greater tidal mixing and surrounded by a less polluted area.

In agreement with the literature, fibres were found to be the commonest microplastic type in this study (Gallagher et al., 2016). This is generally considered to be attributable to the numerous WWTWs and storm-water discharges associated with the densely-populated settlements surrounding the Solent Region. Furthermore, materials and gear such as nets and ropes used in

recreational sailing, commercial shipping and fisheries in the region are also deemed to be significant sources of these fibres. However, contrary to other studies, irregular fibres were found to be the next commonest type, which may be as a result of litter and tyre fragments entering the Solent from the surrounding cities via terrestrial and road runoff.

The presence of microplastics in the marine environment is only predicted to rise in the future, due to increased production and fragmentation of existing plastics in the oceans (Bergmann et al., 2015). This is a concerning prospect, considering microplastics' adverse impacts on marine species and potential to enter the food chain; with possible implications for human health. Thus, there is an urgent need to monitor their distribution and effects. As filter feeders are considered to be particularly at risk, species such as *O.edulis* could be used as bioindicators to monitor and provide useful information concerning the extent, distribution and sources of microplastic pollution in filter feeding species. This information could be used to guide management decisions to reduce the release of microplastics into the marine environment, addressing what has become a major environmental problem.

6.0 References

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7.0 Appendix

Appendix A – Southern IFCA Dispensation forms.

Southern
Inshore Fisheries and Conservation Authority

Dispensation for: University of Southampton
Byelaw(s):
Oyster Closed Season,
Temporary Closure of Shellfish Beds
Oysters
Oysters, Clams, Mussels – Prohibition of Night Fishing

AUTHORISATION TO FISH FOR SCIENTIFIC PURPOSES

Southern Inshore Fisheries and Conservation Authority's, Oyster Closed Season, Temporary Closure of Shellfish Beds, Oysters and Oysters, Clams, Mussels – Prohibition of Night Fishing Byelaws shall, unless otherwise specified, apply to the whole of the District, provided that nothing in this byelaw(s) shall apply to any person fishing while the Temporary Closure of Shellfish beds is in place, Oysters and Oysters, Clams, Mussels –

Prohibition of Night Fishing for scientific relaying purposes, under written authority of the Southern Inshore Fisheries and Conservation Authority and in accordance with the conditions contained in that Authority.

The Authority herby authorises:

Organisation(s):	**University of Southampton**
Person(s):	**Dr Malcolm Hudson**
	Miss Ilna Zapata Restrepo
	Miss Katie Bawden

To conduct scientific oyster survey, within the confines of the Solent and Southampton water within the Southern IFCA District.

Vessel(s):	**N/A**
Requirements:	**Byelaw(s)**
Purpose:	**Scientific**
Period of Authorisation:	**1st July to October 31st 2016**

Details of the authorisation:

1. The dispensation applies to the byelaw(s) for the purpose of oyster sampling only. No more than 5 to be taken from areas that are low in density and a maximum 10 when densities are of sufficient numbers.
    - Oyster closed season
    - Temporary Closure of Shellfish beds
    - Oysters
    - Oysters, Clams, Mussels – Prohibition of Night Fishing

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Southern
Inshore Fisheries and Conservation Authority

2. Prior to the sampling (to which this dispensation applies) verbal notification to the office of the Southern IFCA is required (contact number 01202 721373), if no officer is available, a message should be left on the answer machine. At all times during the sampling, an individual named on this authorisation must be present. An Officer or Officers of the Authority may be required to be required to be aboard the vessel during operation to which this dispensation applies.

3. This permission is granted in respect to the removal of oyster for scientific/sampling purposes only; where by hand gathering will take place in the following areas only where possible mud shoes should be worn to reduce impacts on the intertidal soft mud and areas:

Weston Shore to Hamble point: **50.886546, -1.375198 and 50.848875, -1.310616**

Cracknore Hard: **50.897487, -1.429386 and 50.895701, -1.425824**

Hythe Marina frontage: **50.848875, -1.310616 and 50.874558, -1.397450**

One cleaner reference site on the shore between Calshot and Lepe, avoiding sea grass beds, **50.820284, -1.307572 and 50.820284, -1.307572**

4. This dispensation only applies to the byelaw(s) (as detailed above) of the Southern IFCA, it should be noted that other restrictions may apply to other species.

5. Southern IFCA has a Prohibition of gathering (Sea Fisheries Resources) in Seagrass beds byelaw within EMS and MPAs throughout the District. **This dispensation does not give you authorisation to collect sea fisheries resources**

using rake, spade or fork or any similar tool in any of the above byelaw prohibited areas.

6. This dispensation must be carried on board by the named vessels during any activity where the dispensation applies. This dispensation should be made available to any Officer of the Southern IFCA upon demand.

7. The Authority has the right to rescind this authorisation without notice, if the above conditions are not met as specified.

Granted and signed by the Deputy Chief Executive Officer on behalf of the Southern Inshore Fisheries and Conservation Authority.

Neil Richardson 1st July 2016

Deputy Chief Officer

64 Ashley Road Parkstone Poole Dorset BH14 9BN

T/F: 01202 721373 E: enquiries@southern-ifca.gov.uk W: www.southern-ifca.gov.uk

Southern
Inshore Fisheries and Conservation Authority

Dispensation for: University of Southampton
Byelaw(s):
Oyster Closed Season,

Temporary Closure of Shellfish Beds

Oysters

Oysters, Clams, Mussels – Prohibition of Night Fishing

AUTHORISATION TO FISH FOR SCIENTIFIC PURPOSES

Southern Inshore Fisheries and Conservation Authority's, Oyster Closed Season, Temporary Closure of Shellfish Beds, Oysters and Oysters, Clams, Mussels – Prohibition of Night Fishing Byelaws shall, unless otherwise specified, apply to the whole of the District, provided that nothing in this byelaw(s) shall apply to any person fishing while the Temporary Closure of Shellfish beds is in place, Oysters and Oysters, Clams, Mussels –

Prohibition of Night Fishing for scientific relaying purposes, under written authority of the Southern Inshore Fisheries and Conservation Authority and in accordance with the conditions contained in that Authority.

The Authority herby authorises:

Organisation(s):	**University of Southampton**
Person(s):	**Dr Malcolm Hudson**
	Mr Wesley Smith

To conduct a scientific oyster survey within the confines of the RSPB Intertidal Shingle Bar in Langstone Harbour, of the Southern IFCA District.

Vessel(s):	**The Good Tern**
Skipper:	**Wesley Smith**
Requirements:	**Byelaw(s)**
Purpose:	**Scientific**
Period of Authorisation:	**10th July to October 31st 2016**

Details of the authorisation:

1. The dispensation applies to the byelaw(s) for the purpose of oyster sampling only. No more than 5 to be taken from areas that are low in density and a maximum 10 when densities are of sufficient numbers.

    - Oyster closed season
    - Temporary Closure of Shellfish beds
    - Oysters

T/F: 01202 721373 E: enquiries@southern-ifca.gov.uk W: www.southern-ifca.gov.uk

2. Prior to the sampling (to which this dispensation applies) verbal notification to the office of the Southern IFCA is required (contact number 01202 721373), if no officer is available, a message should be left on the answer machine. At all times during the sampling, an individual named on this authorisation must be present. An Officer or Officers of the Authority may be required to be required to be aboard the vessel during operation to which this dispensation applies.

3. This permission is granted in respect to the removal of oyster for scientific/sampling purposes only; where by hand gathering will take place in the following area only where possible mud shoes should be worn to reduce impacts on the intertidal soft mud and area:

RSPB Intertidal Shingle Bar	**50.817642, -1.0083550**

4. This dispensation only applies to the byelaw(s) (as detailed above) of the Southern IFCA, it should be noted that other restrictions may apply to other species.

5. Southern IFCA has a Prohibition of gathering (Sea Fisheries Resources) in Seagrass beds byelaw within EMS and MPAs throughout the District. **This dispensation does not give you authorisation to collect sea fisheries resources using rake, spade or fork or any similar tool in any of the above byelaw prohibited areas**.

6. This dispensation must be carried on board by the named vessels during any activity where the dispensation applies. This dispensation should be made available to any Officer of the Southern IFCA upon demand.

7. The Authority has the right to rescind this authorisation without notice, if the above conditions are not met as specified.

Granted and signed by the Deputy Chief Executive Officer on behalf of the Southern Inshore Fisheries and Conservation Authority.

Katherine Bawden

Neil Richardson 4th July 2016

Deputy Chief Officer

64 Ashley Road Parkstone Poole Dorset BH14 9BN

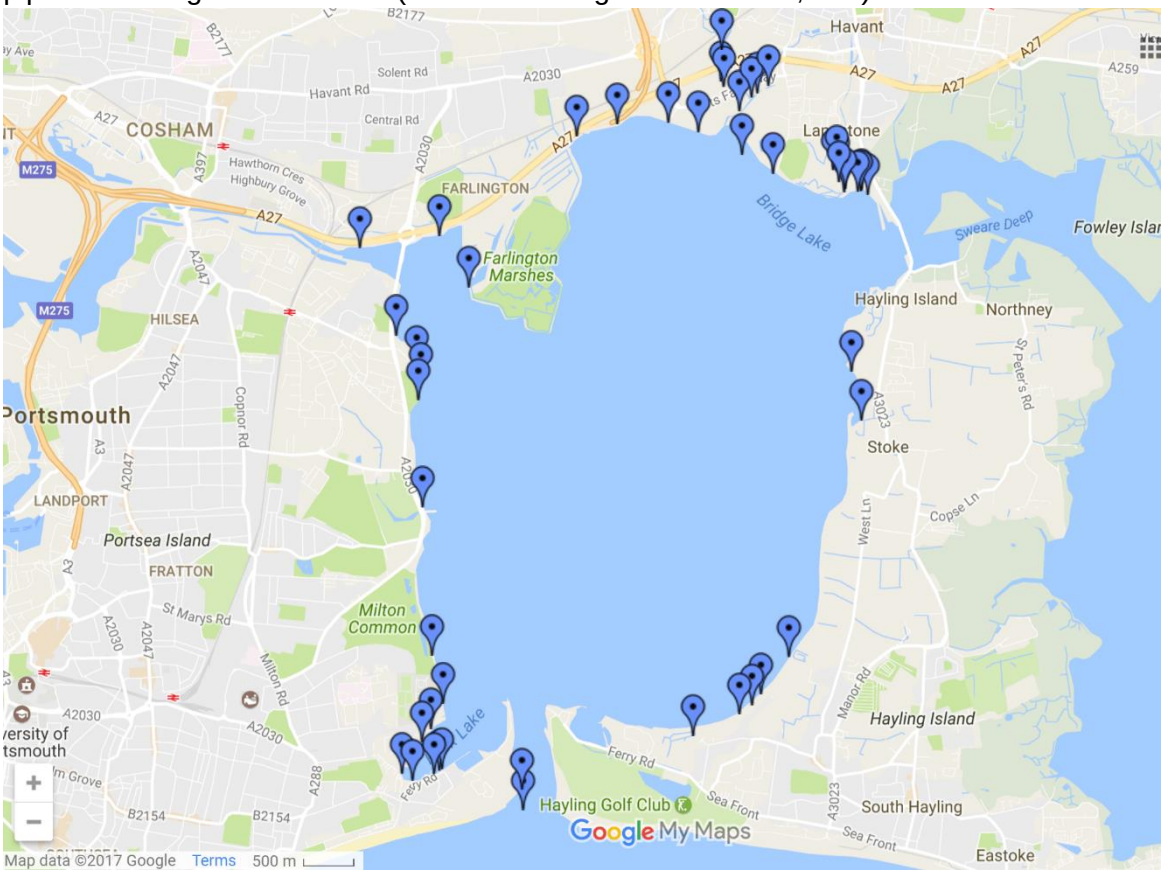
T/F: 01202 721373 E: enquiries@southern-ifca.gov.uk W: www.southern-ifca.gov.uk

Appendix B – Colour classification table.

	Colour classification	
Type of microplastic	Colours	Included colour variations (if any)
Fibre	Blue	Medium, light and dark blue
	Black	
	Orange	Dark, amber
	White	Clear-white, opaque-white
	Pink	
	Green	Light green, dark green, green-brown
	Yellow	Clear-yellow
	Red	Clear-red

	Purple	Dark purple, lavender
	Brown	Dark, medium
	Grey	Dark, medium, light
Irregular	Black	
	Green	Dark, medium
	Orange	Amber
	White	Transparent-white, opaque-white
	Blue	Dark, medium, light
	Red	
	Grey	Dark, light
Round	Blue	Dark, medium, light
	Grey	
	Black	
	Yellow	Transparent-yellow, brown-yellow
	White	Transparent-white, opaque-white

Appendix C – Map of Langstone Harbour Discharges. Public and private wastewater pipes into Langstone Harbour (Friends of Langstone Harbour, n.d.).



Appendix D - ERGO Animal application form – Ethics form

All mandatory fields are marked (M*). Applications without mandatory fields completed are likely to be rejected by reviewers. Other fields are marked "if applicable". Help text is provided, where appropriate, in italics after each question.

1. APPLICANT DETAILS

1.1 (M*) Applicant name and email:	**Katherine Bawden** kb9g14@soton.ac.uk
1.2 (M*) Supervisor name and email:	**Dr Malcolm Hudson** mdh@soton.ac.uk
1.3 Other researchers/collaborators and external personnel involved in study (if applicable): ***Name, address, email, telephone***	**Ian Williams – idw@soton.ac.uk** Chris Hauton – ch10@noc.soton.ac.uk Anthony Jensen – acj@noc.soton.ac.uk **Lina Maria Zapata Restrepo- lmzr1g15@soton.ac.uk**

2. STUDY DETAILS

2.1 (M*) Title of study:	Quantification of microplastics in the tissues of the European flat oyster, Ostrea edulis and the Pacific oyster, Crassostrea gigas within wild and cultured populations.
2.2 (M*) Type of study (*e.g. Undergraduate, Doctorate, Masters, Staff*):	Undergraduate
2.3 i) (M*) Proposed start date (allow at least 1 month):	05/10/16
2.3 ii) (M*) Proposed end date:	30/09/17

2.4 (M*) What are the aims of this study?
The study aims to assess the quantity of microplastics in the tissues of the European flat oyster and Pacific oyster in within wild and cultured populations.

2.5 (M*) What are the objectives of this study?

The main objectives of the study are:

1. To assess the quantity of microplastics in the tissues of the European oyster and Pacific oyster; specifically, in the gills, gonads and digestive tract.
2. To determine the most prevalent polymers in the gills, gonads and digestive tissue of the oysters.
3. To determine whether there are differences in the quantities of plastic found between wild oysters and non-native species and those grown in aquaculture.
4. To determine whether there is any spatial variation in microplastic uptake across different sample locations.

2.6 (M*) Background to study (*a detailed and thorough rationale for conducting the study, listing all relevant publications*)**:**

Large amounts of plastic waste are generated every year, and improper disposal and increased global production has led to accumulation in the oceans [1]. The long degradation times of plastics mean they can persist for decades, and has resulted in their ubiquitous distribution in the marine environment [2]. Of particular concern are microplastics; particles<1mm [3] which may be ingested by marine fauna; affecting their ability to survive and reproduce [4].
As filter feeders are particularly vulnerable to microplastic ingestion, the survival of species such as the European flat oyster (*Ostrea edulis)*, which is already classified as a Threatened Species [5] may be at greater risk [6]. The Solent, once the largest native oyster fisheries in Europe [7] has suffered a significant decline in oyster stocks. The 2015 Inshore Fisheries and Conservation Authority Stock Report showed that out of a total of 13 different sites sampled in the Solent, oysters were absent from hauls taken at five of the sites [8]. The study aims to quantify the microplastic content of wild oyster samples from different locations within the Solent and compare these to cultured oyster samples from a hatchery in Scotland.
The enzyme, Proteinase- K, will be used to quantify microplastics in the oyster tissue as, unlike acid, it does not degrade microplastics, and thus will improve the accuracy of estimation quantities and identification of plastic types [9].

There is some knowledge concerning the ecological and economic implications of this decline, but it is unclear as to the extent to which microplastics are accumulating in the oyster tissues and its possible effects. This study will assess the extent to which microplastics are accumulating in the oyster tissues and thus help determine whether this may affect the oysters' ability to survive and reproduce.

[1] Browne, M. Dissanayake, A., Galloway, T., Lowes, D. and Thompson, R. (2008). Ingested Microscopic Plastic Translocates to the Circulatory System of the Mussel, Mytilus edulis (L.). *Environmental Science and Technology*. 42 (13), P5026-5031.
[2] von Moos, N., Burkhardt-Holm, P. and Köhler, A. (2012). Uptake and Effects of

Microplastics on Cells and Tissue of the Blue Mussel Mytilus edulis L. after an Experimental Exposure. *Environmental Science and Technology*. 46 (20), P11327-11335.
[3] Claessens, M., Van Cauwenberghe, L, Vandegehuchte, M. and Janssen, C. (2013). New techniques for the detection of microplastics in sediments and field collected organisms. *Marine Pollution Bulletin*. 70 (1-2), P227-233.
[4] Wright, S., Thompson, R. and Galloway, T, (2013). The physical impacts of microplastics on marine organisms: A review. *Environmental Pollution*. 178, P483-492.
[5] OSPAR Commission, (2009). *Background document for Ostrea edulis and Ostrea edulis beds*. [pdf] OSPAR Commission. Available at: (http://www.ospar.org/documents?v=7183). [Accessed: 10/02/16].
[6] Sussarellu, R., Suqueta, M., Thomas,Y., Lambert, C., Fabioux, C., Pernet, M., Goica, N., Quillien, V., Mingant C., Epelboin, Y., Corporeau, C., Guyomarch, Robbend, J., Paul-Pont, I., Soudant, P. and H. (2015). Oyster reproduction is affected by exposure to polystyrene microplastics. *Proceedings of the National Academy of Sciences*. 113 (9), P2430-2435.
[7] BLUE Marine Foundation. (2015). *BLUE PLANS TO RESTORE OYSTERS TO THE SOLENT.* Available at: (http://www.bluemarinefoundation.com/2015/04/20/blue-plans-to-restore-oysters-to-the-solent/). [Accessed: 10/02/16].
[8] Southern Inshore Fisheries and Conservation Authority, (2008). *Solent Oyster Fishery 2015 Stock Report- Baird Dredge*. [pdf]. Southern IFCA. Available at: (http://www.solentforum.org/). [Accessed 18/02/16].
[9] Vandermeersch, G., Van Cauwenberghe, L., Janssen, C., Marques, A., Granby, K., Fait, G., Kotterman, M., Diogene, J., Bekaert, K., Robbens, J. and Devriese, L. (2015). A critical view on microplastic quanitification in aquatic organisms. *Environmental Research*. 143, P46-55.

2.7 (M*) Has this work been done before? If so, what are you adding to previously published work?

This study differs from previously published work in that it uses different methods to assess the quantity of microplastics within oyster tissues and has a specific focus on the European oyster.

2.8 (M*) What are the benefits of this study?

The Solent's population of *O.edulis* was once the largest self-sustaining population in Europe, but since the 1970s has been in decline and is now almost fully diminished [8]. This research project could help identify other factors threatening the ability of *O.edulis* to survive and reproduce; potentially providing information from which native oyster restoration schemes can be improved.

- Provided with information on the quantity of microplastics, it may be possible to determine whether microplastics have a significant impact on the ability of populations of *O.edulis* to survive and

reproduce. Therefore, the success of restoration schemes attempting to reestablish the populations; such as those in Ireland and France [9] may be limited. Strategies may be able to be developed in order to reduce the impact of the microplastics on the ability of *O.edulis* to reproduce and survive.
- It may give an indication as to whether there is a need to develop mitigation strategies to reduce the ingestion of microplastics among bivalves in the Solent.
- It may also help determine whether there is a risk to human health in consuming oysters and indicate whether there is a need to limit human consumption.

[9] Lallias, D., Boudry, P., Lapègue, S., King, J. and Beaumont, A. (2010). Strategies for the retention of high genetic variability in European flat oyster (Ostrea edulis) restoration programmes. *Conservation Genetics*. 11 (5), P1899-1910.

2.9 (M*) Study design and detailed protocol (*Give a clear detailed protocol*)

Outline what approach is being used, why certain methods have been chosen and include statistical design.

Samples of *Ostrea edulis* and *Crassostrea gigas* will be obtained from areas sampled by IFCA in the 2016 stock survey and from other shoreline sites. Samples of *O.edulis* will also be obtained from Loch Ryan, a hatchery in Scotland, and used as a control.
Dissection of the oysters will be undertaken to remove the focus tissues; gills, digestive tract and gonads.
To determine the microplastic quantities present in the gills, digestive tract and gonads of *O.edulis* and *C.gigas*, the enzyme Proteinase- K will be used. This chemical was chosen for the study as unlike the acid digestion methods, it does not degrade plastics; giving greater accuracy in the estimation of microplastics. Each sample of 0.2g of tissue will be digested using 8mg of Proteinase -K. The digested tissue will undergo heating at 60 °C for 1 hour, followed by 1 hour boiling at 100°C, a method which has been proven to yield the highest recovery efficiencies [3]. The digested tissue will then undergo filtration using filter paper of pore size 1 µm to maximize the quantity of microplastics collected. Microscopic analysis using Infrared spectroscopy or Raman microscopy will be undertaken. The filtrate will be examined for the identification of microplastics.

3. SAMPLE AND SETTING

3.1 (M*) State numbers (or predicted numbers) to be used for study:
The intention is to have a good spatial coverage of Solent locations and, if possible, a suitable number of *O. edulis* and *C.gigas* from each site (approximately 5 of each). Samples may be limited due to low numbers of wild native oysters and so advice will be taken from Southern IFCA on sustainable numbers to use for this project. If insufficient samples are found of *O.edulis*, samples of non-natives (*C.gigas*), will be used as a proxy organism living in the same environment with similar characteristics.

3.2 (M*) What species is the proposed sample and where is it located (e.g private land, university land, overseas, specific location)?
Ostrea edulis and *Crassostrea gigas.* Samples of *O.edulis* will be obtained from a hatchery in Loch Ryan Scotland as well as from the IFCA 2016 oyster stock survey. Samples of *C.gigas* will be obtained from shoreline locations along the Solent (Hamble point, Weston, Calshot, Langstone Harbour, Marchwood and two offshore Southern IFCA samples).

3.3 (M*) Are endangered or protected species involved (intentionally or possibly inadvertently)
C.gigas are neither endangered nor protected species. *Ostrea edulis* is not included in the list of threatened species by IUCN, but it is a UK Species of Principal Importance/Priority Species.

3.4 (M*) If so has relevant permission and licence been obtained?
Permission for sampling in shoreline locations within nature reserves and for samples from the IFCA 2016 oyster stock has been obtained.

3.4 (M*) Please list and upload licences required and name of person holding it.
No licenses are required for this study, but permission from Southern IFCA and the RSPB to gain access to shoreline sites will be obtained. ..\Dissertation\Southampton University Dispensation July to 31st Oct 2016.pdf

3.5 (M*) Which laws apply?
N/A

3.6 (M*) What is the relationship between researchers and external funding

organisation if any?
The study will receive funding from the Solent Forum Professor Mike Clark Award.

4. RESEARCH PROCEDURES, INTERVENTIONS AND MEASUREMENTS

4.1 (M*) Give a brief account of the procedure as experienced by the participant *(Make clear who does what, how many times and in what order. Make clear the role of all assistants and collaborators.*
Oyster samples will be collected from the shoreline locations by Lina Maria Zapata Restrepo, Malcolm Hudson and Katherine Bawden and will be stored in freezers the National Oceanography Centre (NOC) and. The samples will be dissected and tissue samples digested and filtered in the NOC laboratory. The procedure will be carried out by Katherine Bawden on all oyster samples collected and will be overseen by Chris Hauton and assisted by Lina Maria Zapata Restrepo.

5. ANIMAL WELFARE

5.1 (M*) Will the animal be exposed to psychological or physical discomfort and/or distress?
It is unlikely that the organisms will be exposed to a high level of psychological or physical discomfort during the study.

5.2 (M*) Explain how you intend to alleviate any psychological or physical discomfort and/or distress that may arise? (if applicable)
The oysters will be transported from the field to the laboratory as quickly as possible and will be frozen quickly to avoid suffering.

5.3 Explain how you will care for any living organisms in the study (if applicable)?
The oysters will be collected and immediately stored in a cool bag in the field. Once transported to the laboratory they will be frozen as soon as possible.

5.4 What is the fate of the organisms at the end of the study?
Death.

5.5 Have you undertaken any animal handling training (if applicable)?
No, but I will receive appropriate training from Chris Hauton and Lina Maria Zapata Restrepo.

5.6 (M*) How will data from this study be used? *Researchers should be aware of, and compliant with, the Data Protection policy of the University. You must be able to demonstrate this in respect of handling, storage and retention of data.*
Data from this study will be used to complete my BSc Environmental Sciences dissertation, a copy of which will shared with the Solent Forum.

Katherine Bawden

Appendix E -

Deproteinizing biological tissue samples – prior to assay for microplastic – including the preparation of a solution of 5M sodium perchlorate. HAUTON 033

Work in 346/32, or 456/07 and 454/01

Wear lab coat, surgical gloves and safety glasses when performing this assay.

Wear acid-resistant and elbow-length nitrile gloves/gauntlets when handling solutions of sodium perchlorate

WHEN PREPARING SOLUTIONS OF 5M SODIUM PERCHLORATE WORK IN AN APPROPRIATE CLEANED FUME CUPBOARD AND AWAY FROM ORGANIC MATERIAL

When using 5M sodium perchlorate, work in a plastic tray and away from organic material.

Preparation of reagents:

1) 50 ml of 5M sodium perchlorate.

This solution should only be made up in 50 ml volumes and should be stored in a glass-stoppered bottle, in an appropriate drip tray.

Stock solutions of 5M sodium perchlorate should only be produced using the fume cupboard in lab 346-32. Before preparing the solution the fume cupboard should be emptied of any stroed equipment and the internal surfaces of the fume cupboard should be washed and rinsed with laboratory de-ionised water, to remove any organic traces. Once rinsed the fume cupboard should be allowed to air dry.

Place the laboratory balance into the fume cupboard inside an appropriate drip tray. The balance should also be cleaned and allowed to air dry. Ensure the balance is levelled before each use.

Weigh out 30.61g of sodium perchlorate (Sigma Aldrich #410241) and place into a clean 50 ml Falcon tube. Screw the tube shut and place to one side. Re-clean the laboratory balance and remove from the fume cupboard back to the bench, re-levelling the balance as necessary.

Working in a drip tray, dissolve the 30.61 g of powder in 50 ml of de-ionised water. Once dissolved – transfer the 5 M solution to a clean and dry 100 ml glass bottle and store in an appropriate secondary container.

Any small spills contained within the drip tray should be diluted and flushed to waste using very copious amounts of water. **Do not soak up any concentration of sodium perchlorate solution with organic material, such as laboratory towel.**

Once the solution has been prepared, wash down all surfaces before the fume cupboard is returned to general use.

2) Homogenization solution (500ml).

On a ventilated laboratory bench, weigh out the following components:

63.04g Trizma-HCl (to 400mM; from Sigma Aldrich #T5941-500G)

22.33g EDTA (to 60mM; from Fisher as disodium salt #D/0700/50)

8.766g NaCl (150 mM; from Fisher #S/3120/60)

0.5g SDS (1%; from Sigma Aldrich #436143-25G)

Dissolve to 450ml in de-ionised water, adjust to pH8.0 and then make up to 500ml. Store in a stoppered glass bottle at 4 ^{o}C until required.

3) Assay protocol:

Work on a clean bench in 346-32. Remove all organic sources of combustion before working. Wash surfaces with first ethanol and then de-ionised water before starting. Allow to air dry.

When handling solutions of sodium perchlorate use the fume cupboard and work in a clean drip tray.

a) Remove oysters from the freezer (**need to find out how long they need to defrost so they are soft enough to be dissected and weighed**).
b) Dissect the oysters, separating the gonads, digestive tract and gills.
c) Weigh 0.2g of each tissue for each oyster, placing in separate 100ml glass containers.
d) Add 15ml homogenisation solution. Samples should be homogenised using a pipette and incubated at 50 °C for 15 minutes, using the oven in 454/07.
e) Add 8mg of Proteinase-K to the solution, then incubate for 2 hours at 50 °C using the oven in 454/07.
f) Working in a white plastic tray in a fume cupboard, add 375 µl 5M sodium perchlorate.
g) Seal the glass containers.
h) Place containers on shaking table at room temperature for 20 minutes.
i) Incubate at 60 °C for 20 minutes
j) Filter digested material through a Whatman GF/C filter (nominally 1.2 µm pore diameter) and rinse the filter with > 100 ml of ultrapure water (milli-Q).
k) Store filter papers wrapped in aluminium foil in an appropriate container for analysis.

Katherine Bawden

Appendix F -

CHEMICAL RISK ASSESSMENT FORM *Version Sept 2013*

You will need the most recent MSDS (available from supplier) and the Guidance Notes (available on NOC H&S Website) to fill out this form. Contact the NOC Safety Adviser for further guidance. This assessment ***only addresses the risk of harm to health*** *form the substances listed. Additional risk assessment may be required to control the risk from other hazards associated with this work/the procedure used.*

Department:	OES/FNES	**Location of use:**	346/32, 454/01 and 454/07	**Persons involved:**	Katie BAWDEN, Lina ZAPATA RESTREPO, Chris HAUTON
Lab procedure ref:	Hauton027			**MSDS supplier and revision date:**	Sigma Aldrich according to Regulation (EC) No. 1907/2006. Version 5.0, Revision Date 19.07.2016
Describe the task:	Making a solution of 5M sodium perchlorate and using 375ul volumes to digest biological tissue for microplastic analysis (Hauton 033).				

HAZARD IDENTIFICATION AND CONTROL				
Chemical(s) or Product Name (As listed in the chemical catalogue or in the MSDS. If mixing chemicals creates a dangerous mixture please note and complete a separate line for this mixture)	**Risk Phrases/Hazard Statements** (Numbers and wording - full list available on H&S website.). If more than one R-phrases [H-statements] choose one that gives rise to most severe classification.	**Hazard Group** (A,B,C,D,E) Select from Appendix 1	**Exposure Potential** (High / Med / Low) Assess using Appendix 2	**Exposure Control Approach (ECA)** Select from Appendix 3
Sodium Perchlorate (Sigma Aldrich 410241)	H271 May cause fire or explosion; strong oxidizer. H319 Causes serious eye irritation. H373 May cause damage to organs through prolonged or repeated exposure. H302 Harmful if swallowed.	A	Medium	ECA1

TAB TO THE END OF TABLE TO INSERT NEW ROWS

For multiply chemicals what is the highest ECA required for this task?	

Will you be using a lower level ECA (only allowed for those denoted by*)? *If yes, list the ECA and justify why?*	No

SPECIAL CONSIDERATIONS	
Could a less hazardous substance be used instead? *If yes, then detail why this cannot be used.*	No
Does the substance present additional risks to certain groups or individuals? *(e.g. young people, expectant mothers)*	No
Do your chemicals have risk phrases or hazard statements that require a DSEAR assessment? *See appendix 1. If yes, complete and attach a DSEAR Checklist (available on the H&S website)*	Yes

PERSONAL PROTECTIVE EQUIPMENT (PPE)

State any PPE required for this task/method. Include which type and when they are to be worn. Note: PPE is to be used as the "last resort".

Eye protection:	Yes for handling all chemicals	**Hand protection:**	Yes, acid resistant ntrile gauntlets when handling solutions of 5M sodium perchlorate. Examination gloves when handling any dilute solutions.
Face protection:	Yes, for diluting sodium perchlorate	**Special clothing:**	Gauntlets when handling very small volumes of 5M sodium perchlorate in a fume cupboard.
Respiratory protection: *(Requires specialist training & monitoring)*	No, as chemicals are handled in a fume cupboard	**Any others:**	Lab coat, closed shoes and long trousers in all cases

EMERGENCY PROCEDURES	
Eye contact:	**Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.**.
Inhalation:	**If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.**
Skin contact:	**Wash off with soap and plenty of water. Consult a physician.**
Ingestion:	**Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.**
Spill procedure:	**Dilute small spills with copious volumes of water and, when dilute, run to waste. DO NOT dry up spills using absorbent material and do not place contaminated material in waste bins.**

HEALTH MONITORING

Is health surveillance required for the protection of the health of employees?

This is required when: (a) there is a disease associated with the substance in use (eg Asthma, Dermatitis, Cancers); (b) it is possible to detect the disease or adverse change and reduce the risk of further harm; (c) the conditions in the workplace make it likely that the disease will appear. Please refer to Guidance for COSHH Health Surveillance on the H&S Website.

No

SPECIAL TRAINING REQUIREMENTS

Decide whether any special training is required to carry out the task safely. In most cases, on the job training will be sufficient.

INSTRUCTIONS FOR SAFE STORAGE

How should the substance be stored? (e.g. locked cupboard which is correctly labelled, away from other substances, etc.)

Is there any other substance that this substance must not come into contact with?

Sodium perchlorate: Keep container tightly closed in a dry, cool ad well-ventilated place. Do not store near combustible materials.

DISPOSAL PROCEDURES *Detail fully how the chemical waste is to be disposed of (down sink, by specialist contractor, etc)*

Are chemicals with Risk Phrases R50-R59 (environmental hazards) involved?

No

ASSESSMENT OF RISK USING CONTROLS DETAILED ABOVE
Are the hazards/risks suitably controlled, using the control measures detailed above? If not, state the further actions required, e.g. Requirement for a standard operating procedure (SOP), restricting access, prohibiting lone working, specifying supervision, etc in the box below.
Yes

ACCREDITATION, VERIFICATION AND REVIEW

I am satisfied that the control measures outlined above are adequate to control the risks to health from the hazardous substances used in the work activity described to the lowest level reasonably practicable.

Assessor:	Katie Bawden	**Signature:**		**Date:**	31-8-16
Approved by:	Chris Hauton	**Signature:**		**Date:**	30-8-16

Verification by users *(Continue on a separate sheet if necessary)*

I have read and understood the information contained in this Assessment and agree to abide with all safety controls.

Name	Signature	Date

Appendix 1: Hazard Group A – E

Appendix 2: Exposure Potential

EXPOSURE POTENTIAL						
	LOW		**MEDIUM**		**HIGH**	
Quantity used	<1g or ml		1 to 100g or ml	X	>100g or ml	
Duration	≤ 1 min per day		> 1- 15 min per day	X	> 15 min per day	
No. of persons involved	1 to 2	X	3 to 4		5 or more	
Volatility (liquids)	BP ≥ 150ºC or VP ≤ 500 Pa / 3.75 mmHg	X	BP 50 – 150ºC or VP 500 – 25000 Pa/ 3.75 – 187.5 mmHg		BP ≤ 50ºC or VP ≥ 25,000 Pa/ 187.5 mmHg	
Dustiness (particulates)	Pellets and non-dusty solids		Granular or crystalline (coarse dusts)	X	Fine solids and light powder	
Nature of operation	Low energy eg careful handling	X	Medium energy e.g. pouring from low heights or stirring, use of hand tools		High energy, e.g. spraying, grinding, high speed stirring, sonication etc	
Overall Exposure Potential: The more boxes for individual factors that are ticked on the right hand side of the form, the higher the overall exposure potential should be. However, the assessment cannot be based on a simple count of high or low factors, but must rely on the judgement and experience of the assessor.					**Low**	
					Medium	
					High	

Append
Exposur
Approa

EXPOSURE CONTROL APPROACH ***Note: NERC Guidance on 20 Standard Controls must be observed at all time (available on the H&S website)***				
ECA1: Work in a well constructed laboratory with good general ventilation (an air change rate in excess of 5x per hour) using good working practices to minimise spread / generation of high airborne concentrations of hazardous contaminants.				
ECA2: Work undertaken as above but with the application of engineering controls using LEV devices such as extract grilles, captor hoods or nozzles, partial enclosures with extraction and re-circulating single HEPA filtered enclosures.				
ECA3: As ECA 1 plus use of high efficiency partial containment devices such as NERC Class 1 fume cupboards which are ducted to external atmosphere or, for solids or aerosols, double HEPA filtered powder handling enclosures / safety cabinets.				
ECA4: Specially devised precautions applied after seeking specialist advice and writing a detailed risk assessment. The precautions applied will involve the highest levels of engineered controls and, although fume cupboards may be appropriate, consideration should be given to using total enclosure devices such as a dedicated laboratory or containment suite may also be appropriate.				
Hazard Group of Substance	***E***	ECA3*	ECA4*	ECA4
	D	ECA3*	ECA4*	ECA4*
	C	ECA2*	ECA3*	ECA3
	B	ECA1	ECA2*	ECA2
	A	ECA1	ECA1	ECA2*
** These approaches may be varied or relaxed (e.g. the next lower ECA used) as justified by risk assessment*		***Low***	***Medium***	***High***
		Overall Exposure Potential		

Appendix G

FNES / Ocean & Earth Science: General Risk Assessment Form

This form must be used in conjunction with the Risk Assessment Guidance Notes and Hazard Checklist *available on NOCSNET H&S section Version 1.3 (Jan 2013)

Faculty / Service / Academic Unit / Team / Department: *(see Note 1)*	OES/FNES	**Location / Room Number / Map Reference:**	346/32, 454/01, 454/07
RISK ASSESSMENT TITLE *MAIN ACTIVITY*	Preparation of 5M sodium perchlorate and use in enzymatic digestion of biological tissues for microplastic analysis.		

Katherine Bawden

Briefly describe the 'tasks' being assessed:	Preparing a 50ml solution of 5M sodium perchlorate. Using 375ul of that solution in an enzymatic digest of biological tissue. (Hauton 033)
Other assessments, documents or considerations which might also be required:	Accompanying protocol, COSHH, DSEAR and MSDS for sodium perchlorate.

THESE ARE SUMMARY GUIDELINES ONLY.

THIS FORM MUST BE USED IN CONJUNCTION WITH THE FULL GUIDANCE NOTES AND THE HAZARD CHECKLIST AVAILABLE FROM YOUR SUPERVISOR.

List (in column b below) the individual tasks associated with the activity being assessed. Use the hazard checklist to help identify the hazards that may be encountered when undertaking each task (list each one in column c). Next, identify who might be affected (e.g. yourself, other students, staff, others, or even members of the public) and indicate what level of harm might arise from that particular hazard. You can use the 'SEVERITY' rating to help with this and don't forget that additional consideration may be required for special groups. If you're completing a new assessment and there are no control measures in place, say so in column f. If there are already control measures to reduce or remove the harm, such as wearing safety glasses in a lab, list them in column f.

Finally, use the Risk Estimation Matrix (5x5 matrix) to assess the INHERENT RISK (if there are NO controls in place) or RESIDUAL RISK (if controls are already in place) and assign a HIGH, MEDIUM (MED) or LOW rating against that harm arising. You can list the 'severity' (1-5) and 'likelihood' (1-5) terms that you've used in the appropriate columns below. If the rating is LOW, then you can proceed with your activities once the assessment has been approved. If either the INHERENT or RESIDUAL risks are STILL rated as MEDIUM or HIGH, then further control measures (or post assessment actions) will be required.

Discuss these measures with your supervisor or manager and re-assess if necessary.

IF THE RISKS FOR ANY OF THE HAZARDS IN THE TABLE IS DEEMED TO BE HIGH, WORK MUST NOT PROCEED UNTIL FURTHER CONTROLS ARE PUT IN PLACE.

IDENTIFICATION OF REASONABLY FORESEEABLE HAZARDS *(see Notes 2i & 2ii)*				**INDICATE CONTROL MEASURES IN PLACE & EVALUATE THE INHERENT OR RESIDUAL RISKS** *(see Notes 3i & 3ii)*							
Reference (a)	***Task / Aspect of Work.*** *(b)*	***What are the hazards?*** *Refer to checklist* *(c)*	***Who might be harmed and how could that harm arise?*** *(i.e. Who, how and nature of harm)* *Any special considerations? (d)*	**SEVERITY 1-5**	**LIKELIHOOD 1-5**	**INHERENT RISK** *(e)*	***What are you already doing?*** ***List existing measures to control risk.*** *(f)*	**SEVERITY 1-5**	**LIKELIHOOD1-5**	**RESIDUAL RISK** *(g)*	**Further Controls Required? (YES/NO)**
0	LABORATORY WORK	TRIPS AND SPILLS	ANYONE WORKING IN THE LAB,	**1**	**3**	**LOW**	KEEP AILES CLEAR, USE STANDARD LAB PROCEDURES: CLOSED SHOES, LONG TROUSERS, LABCOAT, GLOVES AND GOGGLES WHERE APPROPRIATE.	**1**	**1**	**LOW**	**NO**

IF THE RISKS FOR ANY OF THE HAZARDS IN THE TABLE IS DEEMED TO BE HIGH, WORK MUST NOT PROCEED UNTIL FURTHER CONTROLS ARE PUT IN PLACE.

IDENTIFICATION OF REASONABLY FORESEEABLE HAZARDS *(see Notes 2i & 2ii)*				**INDICATE CONTROL MEASURES IN PLACE & EVALUATE THE INHERENT OR RESIDUAL RISKS** *(see Notes 3i & 3ii)*							
Reference (a)	***Task / Aspect of Work.*** *(b)*	***What are the hazards?*** *Refer to checklist* *(c)*	***Who might be harmed and how could that harm arise?*** *(i.e. Who, how and nature of harm)* *Any special considerations? (d)*	**SEVERITY 1-5**	**LIKELIHOOD 1-5**	**INHERENT RISK** *(e)*	***What are you already doing?*** ***List existing measures to control risk.*** *(f)*	**SEVERITY 1-5**	**LIKELIHOOD1-5**	**RESIDUAL RISK** *(g)*	**Further Controls Required? (YES/NO)**
1A	PRODUCING A SOLUTION OF 5M SODIUM PERCHLORATE	HEALTH, REACTIVE AND FIRE/EXPLOSION HAZARD	PERSONNEL HANDLING CONCENTRATED SODIUM PERCHLORATE, ANYONE ELSE WORKING IN THE SAME LAB	**3**	**4**	**HIGH**	SMALL VOLUMES OF 5 M SOLUTIONS PREPARED IN A FUME CUPBOARD INSIDE A SECONDARY DRIP TRAY. FUME CUPBOARD CLEANED AND RINSED BEFORE AND AFTER USE TO RMOVE ORGANIC TRACES/CONTAMINANTS ANY SPILLS WILL BE WASHED AWAY DOWN THE FUME CUPBOARD SINK USING PLENTY OF WATER. NO OTHER EQUIPMENT TO BE STORED IN CUPBOARD WHILST BEING USED FOR SODIUM PERCHLORATE. FUME CUPBOARD MUST BE WASHED DOWN AFTER WORK HAS FINISHED GOGGLES AND ACID RESISTANT NITRILE GLOVES TO BE WORN WHEN HANDLING SODIUM PERCHLORATE SOLUTIONS	**1**	**3**	**LOW**	**YES**

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IF THE RISKS FOR ANY OF THE HAZARDS IN THE TABLE IS DEEMED TO BE HIGH, WORK MUST NOT PROCEED UNTIL FURTHER CONTROLS ARE PUT IN PLACE.											
IDENTIFICATION OF REASONABLY FORESEEABLE HAZARDS *(see Notes 2i & 2ii)*				**INDICATE CONTROL MEASURES IN PLACE & EVALUATE THE INHERENT OR RESIDUAL RISKS** *(see Notes 3i & 3ii)*							
Reference (a)	***Task / Aspect of Work.*** *(b)*	***What are the hazards?*** *Refer to checklist* *(c)*	***Who might be harmed and how could that harm arise?*** *(i.e. Who, how and nature of harm)* *Any special considerations? (d)*	**SEVERITY 1-5**	**LIKELIHOOD 1-5**	**INHERENT RISK** *(e)*	***What are you already doing?*** ***List existing measures to control risk.*** *(f)*	**SEVERITY 1-5**	**LIKELIHOOD1-5**	**RESIDUAL RISK** *(g)*	**Further Controls Required? (YES/NO)**
1	ADDING DILUTED SODIUM PERCHLORATE TO ORGANIC MATERIAL	EXPLOSION, FIRE AND SEVERE BURNS	ANYONE HANDLING THE SUBSTANCE. HEATING MAY CAUSE EXPLOSION; CONTACT WITH ORGANIC/ COMBUSTIBLE MATERIAL MAY CAUSE FIRE. THIS CAN LEAD TO SEVERE BURNS. IN CASE OF SPILLS OR SPLASHES ONTO ORGANIC MATTER E.G. LABCOAT, THE DRIED OUT SUBSTANCE CONCENTRATES SODIUM PERCHLORATE, WHICH CAN LEAD TO A REACTION CAUSING FIRE	**3**	**4**	**HIGH**	CONCENTRATED SODIUM PERCHLORATE IS IN FLAKE FORM, MINIMISING RISK OF DUST AND POTENTIAL CONTACT WITH COMBUSTIBLE MATERIAL. DILUTED SODIUM PERCHLORATE SHOULD BE ADDED UNDER A STANDARD FUME CUPBOARD WITH SASH AT MINIMUM HEIGHT AND WEARING STANDARD PPE THE DILUTION SHOULD BE CARRIED ON A WHITE PLASTIC TRAY, AND THE ADDING OF SODIUM PERCHLORATE TO BIOLOGICAL SAMPLES SHOULD ONLY BE CARRIED OUT ON THAT TRAY ALSO.	**2**	**3**	**LOW**	**NO**

IF THE RISKS FOR ANY OF THE HAZARDS IN THE TABLE IS DEEMED TO BE HIGH, WORK MUST NOT PROCEED UNTIL FURTHER CONTROLS ARE PUT IN PLACE.

IDENTIFICATION OF REASONABLY FORESEEABLE HAZARDS *(see Notes 2i & 2ii)*				**INDICATE CONTROL MEASURES IN PLACE & EVALUATE THE INHERENT OR RESIDUAL RISKS** *(see Notes 3i & 3ii)*							
Reference (a)	***Task / Aspect of Work.*** *(b)*	***What are the hazards?*** *Refer to checklist* *(c)*	***Who might be harmed and how could that harm arise?*** *(i.e. Who, how and nature of harm)* *Any special considerations? (d)*	**SEVERITY 1-5**	**LIKELIHOOD 1-5**	**INHERENT RISK** *(e)*	***What are you already doing?*** ***List existing measures to control risk.*** *(f)*	**SEVERITY 1-5**	**LIKELIHOOD1-5**	**RESIDUAL RISK** *(g)*	**Further Controls Required? (YES/NO)**
2	ADDING 375 MICROLITRE VOLUMES OF 5M SODIUM PERCHLORATE TO A 15 ML DIGEST	SPILLS	PERSONNEL HANDLING THE SOLUTIONS SPILLS MAY OCCUR. IF SPILLS SOAK INTO ORGANIC MATERIAL, CAN PRESENT A FIRE RISK	**3**	**3**	**HIGH**	ONLY SMALL VOLUMES WILL BE TRANSFERRED USING A PIPETTOR. 5M SOLUTIONS WILL BE OPENED IN A FUME CUPBOARD AND ALL TRANSFERS WILL BE CONDUCTED INSIDE A DRIP TRAY.	**1**	**3**	**LOW**	**NO**

TAB TO THE END OF TABLE TO INSERT NEW ROWS

Ref	**Further Controls or Post Assessment Actions required** *(see Note 4)*	**Action by whom?**	**Action by when?**

ASSESSOR & SUPERVISOR / MANAGER TO COMPLETE *(see Note 5)*					
ASSESSOR: SIGNED	Katie Bawden	PRINT NAME	Katie Bawden	DATE	05-09-16
DECLARATION BY RESPONSIBLE SUPERVISOR / MANAGER: I confirm that this is a suitable and sufficient risk assessment for the above work task / activity.					
SIGNED	Chris Hauton.	PRINT NAME	Chris Hauton	DATE	30-08-16
ANNUAL REVIEW (OR WHEN ACTIVITIES CHANGED OR MODIFIED) **Assessment reviewed on** *(Date and reviewer initials – see Note 6)*					

All personnel working on the task: *I have read and understood the information contained in this Risk Assessment and agree to implement (or abide by) the safety controls indicated and will report to the responsible manager any incidents that occur or any shortcomings that I find in this assessment.*

Name	Signature	Date	Name	Signature	Date

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Health & safety risk assessment: A basic guide

(1) Identify all hazards and reasonably foreseeable 'worst case' consequences. A 'hazard' is anything with the potential to cause an adverse consequence, such as an injury or illness.

Reasonably foreseeable 'worst case' consequence: 'Worst case' means it is not necessarily the most likely consequence that should be considered, but 'reasonably foreseeable worst case' means that far-fetched, improbable hazards and consequences need not be considered.

(2) Estimate inherent risk for each hazard. 'Inherent' risk is that without any controls applied.

Risk: Is likelihood of hazard event and reasonably foreseeable 'worst case' consequence combined.

In estimating risk, consider factors that could exacerbate risk, such as reasonably foreseeable emergencies, lone work, inexperience, new & expectant mothers, waste disposal, potential effects on others such as contractors or visitors, etc. A separate 'row' for a particular hazard / consequence may be needed to account for these.

Estimate risk using the matrix on the next page, and indicate High, Medium (Med) or Low on the form.

'High' risks must be reduced before activity / task can commence or continue.
'Medium' risks must be reduced as much and as soon as is reasonably practicable.

(3) Devise controls for each hazard. A 'control' is a measure taken to reduce risk.

Controls: As a general principle, the 'hierarchy' of control that is to be applied (from most to least preferable) is: avoid the risk; substitute something less hazardous that gives same or similar outcomes; 'engineering' controls (ie, equipment and articles that mitigate or contain a hazard); safe system of work (i.e., a prescribed work method); and personal protective equipment ('PPE', e.g., gloves, helmet, boots, etc.). So, PPE is a last resort.

Other controls that should be considered: training and supervision, planning for possible emergencies, health surveillance, validation and maintenance of any engineering controls, and correct specification of any PPE.

'Low' risks, by definition, do not require controls.

(4) Estimate residual risk for each hazard. 'Residual' risk is that with controls applied.

Residual risk is estimated as above, and the objective is for all risks to be low so far as is reasonably practicable.

(5) The responsible manager, principal investigator, project leader, etc., must sign the Declaration on the assessment.

- Health & safety risk assessments must be 'suitable and sufficient', i.e., cover all relevant issues and include enough detail.
- It is activities / tasks should be risk assessed, and not, as such, substances (but rather use of substances), or equipment (but rather use of equipment), or locations (but rather activities therein), or people (but rather what they do).
- This template is for 'general' health & safety risk assessment, suitable for most hazards, but certain hazards require additional regulatory and technical detail (e.g., ionising radiations, biological agents, genetic modification, noise, hazardous chemicals, etc.).
- Health & safety risk assessments can be generic, provided they remain 'suitable and sufficient'.
- Health & safety risk assessments need to be reviewed periodically (at least every two years or sooner if inherent risk is high), and also after incidents, after significant changes to the activity / task, if staff raise any concerns, if there is a relevant change to the law or to other relevant standards, or if there is anything to suggest the assessment is not suitable or sufficient.
- It is not necessary to print this page and the page showing the matrix for the final assessment.

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Health & safety risk estimation matrix

High risk – requires controls to reduce risk before activity / task can commence (or continue).

Medium risk – requires controls to reduce risk as much and as soon as is reasonably practicable.

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Low risk – all risk should be reduced to this tolerable level, so far as is reasonably practicable.

Severity: Reasonably foreseeable worst case consequence **Likelihood: [3] of hazard event**	**Minor** superficial injury; or slight and temporary health effect (1)	**Moderate** significant injury or illness [1]; or temporary minor disability (2)	**Major** serious injury or illness [2]; or significant or permanent disability (3)	**Critical** fatal injury or illness; or substantial and permanent disability (4)	**Catastrophic** fatal injury or illness for multiple persons (5)
Likely high probability, 1 in 10 chance or higher, once in two weeks or longer for activities on a daily basis (5)	medium risk	high risk	high risk	high risk	high risk
Possible significant probability, 1 in 100 chance or higher, once in six months or longer for activities on a daily basis (4)	low risk	medium risk	high risk	high risk	high risk
Unlikely low probability, 1 in 1,000 chance or higher, once in four years or longer for activities on a daily basis (3)	low risk	low risk	medium risk	high risk	high risk

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Rare very low probability, 1 in 10,000 chance or higher, once in a decade or longer for activities on a daily basis (2)	low risk	low risk	low risk	medium risk	high risk
Almost never extremely low probability, less than 1 in 100,000 chance, once in a century or longer for activities on a daily basis (1)	low risk	low risk	low risk	low risk	medium risk

[1] 'Significant injury' could include, for example, laceration, burn, concussion, serious sprain, minor fracture, etc.
'Significant illness' could include, for example, dermatitis, minor work-related musculoskeletal conditions, partial hearing loss, etc.

[2] 'Serious injury' could include fracture or dislocation (other than digits), amputation, loss of sight, penetration or burn to eye, electric shock, asphyxia, or any injury leading to unconsciousness or requiring resuscitation or admittance to hospital for more than twenty-four hours. 'Serious illness' could include, for example, requiring medical treatment after chemical, biological or radiological exposure, severe debilitating musculoskeletal conditions, severe dermatitis, asthma, etc.

[3] For likelihoods in between the listed values, use the higher likelihood to estimate risk. These probability definitions are only a guide.

Katherine Bawden

Appendix H

Dangerous Substances and Explosive Atmospheres Regulations 2002 (DSEAR) Risk Assessment Checklist

If the substance(s) that you are working with, handling or storing is flammable, extremely flammable, highly flammable, oxidising, explosive, or capable of producing an explosive atmosphere, this checklist must be completed and should be attached with the standard Chemical Risk Assessment Form.

Note here names of product, or substance being handled, stored, or produced:

Sodium perchlorate 5M	

Control measures

Process/activity (Where appropriate to the nature of the activity or operation)	**Yes**	**No**	**N/A**
Has the quantity of the dangerous substance held/used been reduced to a minimum?	✓		
Have steps been taken to avoid, or minimise releases (intentional or unintentional)?	✓		
Have steps been taken to control releases at source?	✓		
Have steps been taken to prevent the formation of an explosive atmosphere?			✓
Have steps been taken to collect, contain and remove any releases to a safe place (e.g. by ventilation)?	✓		
Have steps been taken to avoid adverse conditions (e.g. Exceeding the limits of temperature or other control settings)?	✓		
Are incompatible substances kept apart in storage and, so far as is practicable, in use (e.g. oxidisers and combustibles)?	✓		
Have the number of employees exposed to the dangerous substances or explosive atmosphere been reduced to the minimum?	✓		
Has plant been supplied that is explosion resistant?			✓
Is explosion suppression or relief provided on equipment?			✓
Have adequate measures been taken to control or minimise the spread of fire, or explosion?	✓		
Has suitable personal Protective Equipment (PPE) been provided, and have operatives been trained how to wear it correctly?	✓		
Comments: These measures have been taken in lab 346-32			

Workplace/process and management systems (Where appropriate to the nature of the activity or operation)	**Yes**	**No**	**N/A**
Is the workplace designed, constructed and maintained so as to provide adequate fire-resistance and/or explosion relief?	✓		
Is any assembly, construction, installation, rig, plant, equipment, protection system, etc., designed in such a manner as to minimise risk of fire and/or explosion?	✓		
Is any such assembly, construction, installation, rig, plant, equipment, protection systems, etc., used in such a manner as to minimise risk of fire and/or explosion?	✓		
Have appropriate safe systems of work, or other required procedural systems of organising work, been developed and communicated to the workforce, either by way of this form or another document?	✓		
Is a permit to work scheme required for working with the substance(s), or in the work area, and are these strictly enforced?			✓
In the case of explosive atmospheres; (*if not applicable tick here and proceed to storage*)			☑
Have all such areas been classified in to zones in accordance with Schedule 2 to the Regulations?			
Where necessary have such classified zones been marked at their entry points with the specified 'EX' hazard warning sign?			
Are all areas classified into such zones appropriately protected from sources of ignition, through the selection of equipment and protective systems compliant with the Equipment and Protective Systems Intended for Use in Potentially Explosive Atmospheres Regulations?			
Are employees working in zoned areas provided with clothing that does not create a risk of electrostatic discharge?			
Have areas where explosive atmospheres may be present, before their first operation, been verified as being safe by a person, or organisation competent in the field of explosion protection?			
Storage	**Yes**	**No**	**N/A**
Are all flammable substances kept in suitable fire resistant storage and are all quantities in excess of 50ltrs kept in dedicated and appropriately protected flammable stores?			✓
Are all petroleum spirits, or derivatives thereof, in excess of 50ltrs kept in dedicated and appropriately protected petroleum spirit stores?			✓
Are incompatible substances stored apart (e.g. flammables, oxidisers, combustibles, flammable gases, LPG)?	✓		
Where appropriate have storage areas been designed to provide explosion relief/resistance?			✓
Comments:			

Where any question relevant to a dangerous substance being used, produced, handled or stored has returned a No response, the subject area should be revisited to ensure that all required and reasonably practicable risk reducing measures have been implemented.

Appendix I

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RECORD OF RISK ASSESSMENT

Title of the risk assessment	Field trip 28th July 2016
Date risk assessment carried out	26/07/2016
Describe the work being assessed	Field work (boat transport)
Describe the location at which the work is being carried out	Langstone Harbour, RSPB Reserve
Where appropriate list the individuals doing the work and the dates/times when the work will be carried out	Malcolm Hudson, Katherine Bawden, Wez Smith
List any other generic or specific risk assessments or other documents that relate to this assessment-use hyperlinks if possible	
Name and post of risk assessor	Katherine Bawden - Environmental Sciences Undergraduate

Reference number and version number of risk assessment	
List the names and posts of those assisting in compiling this risk assessment	
Name, post and where required, signature of the responsible manager/supervisor approving the risk assessment	Malcolm Hudson (Environmental Sciences)

Katherine Bawden

Assessment

Title of risk assessment	Field trip 28th July 2016

Katherine Bawden

Risk Acceptability	
1-3	Risk acceptable
4-6	Risk to be reduced if readily possible
7-14	Risk to be reduced if reasonably practicable
15-25	Risk unacceptable

Risk Matrix			Severity				
			very low	low	medium	high	very high
			1	2	3	4	5
Likelihood	certainty	5	5	10	15	20	25
	likely	4	4	8	12	16	20
	possible	3	3	6	9	12	15
	less likely	2	2	4	6	8	10
	improbable	1	1	2	3	4	5

ref	Task/Aspect of work	Hazard	Harm and how it could arise	Who could be affected?	Existing measures to control risk	Risk Factors: Overall Likelihood	Risk Factors: Overall Severity	Residual Risk score	Any changes or extra controls?
	Boat travel to site	Waves and currents	Risk of falling overboard and ultimately, drowning.	Any members of the party.	All members will wear buoyancy aids for the duration of the journey.	2	5	4	
	Boat travel to site	Docking	Risk of injury to hands and limbs during docking.	Any members of the party.	Keeping hands and limbs away from the edges of the boat when docking. Care will be taken with anchor chains to avoid tripping.	2	4	4	
	Getting on or off site	Traffic	Risk of accident crossing road- this is unlikely as the road is not a major route with little traffic	Any members of the party.	Care when crossing roads to access site.	1	4	4	
	Working and moving around site	Extreme weather	Exposure to extremes of hot or cold; heavy rain exacerbating other hazards.	Any members of the party.	All members to have warm and water proof clothes for conditions typical of October, or sun protection if needed. If conditions are too extreme the work will be postponed or cancelled	2	2	4	
	Working and moving around site	Microbes or toxic material in sediments	Hand to mouth contact	Any members of the party.	Eating, drinking and smoking will not be permitted until after hand washing	1	4	4	

	Working and moving around site	Tides	Risk of accident crossing road- this is unlikely as the road is not a major route with little traffic	Any members of the party.	Work will take place at or just after low tide having checked tide tables. Work will finish at least two hours before high tide after which the site will become partly flooded. Supervisor and students will have mobile phone for calling emergency services if required. No-one will be permitted to leave the groups working together.	1	5	5	
	Working and moving around site	Soft mud on unvegetated area, including creeks, gullies and mudflat	Getting stuck in soft mud; slips and trips	Any members of the party.	Unvegetated areas will be avoided unless the supervisors give permission for access having checked the surfaces are safe. Rope and a spade will be taken to site. All party will wear wellington boots or similar footwear. No-one will be permitted to leave the groups working together. Head counts to be made on arrival and departure.	2	2	4	
	Working and moving around site	Darkness	Getting stuck working at night	Any members of the party.	Work will finish on time keeping to daylight hours	1	5	5	
	Boat travel to site	Waves and currents	Risk of falling overboard and ultimately, drowning.	Any members of the party.	All members will wear buoyancy aids for the duration of the journey.	2	5	4	

Post Risk Assessment Actions

Title of risk assessment
Field trip 28th July 2016

Have any of the specialist control measures listed below been identified as required during this risk assessment? - indicate yes or no - if yes then include details on the post assessment action list below.	**yes/no**
is any exposure monitoring required?	no
Is any occupational health monitoring required?	no
Are there any hazards or other factors that could affect pregnant or nursing mothers?	no

Is any specific training required before people can carry out this work?	no

Are any additional procedures or risk assessments required as a result of this assessment?	no

Are any specialist disposal arrangements required?	no

Are any special emergency arrangements required?	no

	Post Assessment actions		
ref	**action**	**by whom**	**by when**

Appendix J

FNES / Ocean & Earth Science: General Risk Assessment Form

This form must be used in conjunction with the Risk Assessment Guidance Notes and Hazard Checklist *available on NOCSNET H&S section Version 1.3 (Jan 2013)

Faculty / Service / Academic Unit / Team / Department: *(see Note 1)*	OES/FNES	**Location / Room Number / Map Reference:**	456/07, 456/01, 346/32, 454/01, 454/07, 781/05
RISK ASSESSMENT TITLE *MAIN ACTIVITY*	**Deproteinizing biological tissue samples**		
Briefly describe the 'tasks' being assessed:	Deprotineization of biological tissue samples using Perchloric Acid (3.6%, 0.6M)		
Other assessments, documents or considerations which might also be required:	Protocol Hauton027, COSH, MSDS 60% Perchloric Acid & 3.6% Perchloric Acid		

THESE ARE SUMMARY GUIDELINES ONLY.

THIS FORM MUST BE USED IN CONJUNCTION WITH THE FULL GUIDANCE NOTES AND THE HAZARD CHECKLIST AVAILABLE FROM YOUR SUPERVISOR.

List (in column b below) the individual tasks associated with the activity being assessed. Use the hazard checklist to help identify the hazards that may be encountered when undertaking each task (list each one in column c). Next, identify who might be affected (e.g. yourself, other students, staff, others, or even members of the public) and indicate what level of harm might arise from that particular hazard. You can use the 'SEVERITY' rating to help with this and don't forget that additional consideration may be required for special groups. If you're completing a new assessment and there are no control measures in place, say so in column f. If there are already control measures to reduce or remove the harm, such as wearing safety glasses in a lab, list them in column f.

Finally, use the Risk Estimation Matrix (5x5 matrix) to assess the INHERENT RISK (if there are NO controls in place) or RESIDUAL RISK (if controls are already in place) and assign a HIGH, MEDIUM (MED) or LOW rating against that harm arising. You can list the 'severity' (1-5) and 'likelihood' (1-5) terms that you've used in the appropriate columns below. If the rating is LOW, then you can proceed with your activities once the assessment has been approved. If either the INHERENT or RESIDUAL risks are STILL rated as MEDIUM or HIGH, then further control measures (or post assessment actions) will be required.

Discuss these measures with your supervisor or manager and re-assess if necessary.

IF THE RISKS FOR ANY OF THE HAZARDS IN THE TABLE IS DEEMED TO BE HIGH, WORK MUST NOT PROCEED UNTIL FURTHER CONTROLS ARE PUT IN PLACE.

Katherine Bawden

IDENTIFICATION OF REASONABLY FORESEEABLE HAZARDS *(see Notes 2i & 2ii)*				**INDICATE CONTROL MEASURES IN PLACE & EVALUATE THE INHERENT OR RESIDUAL RISKS** *(see Notes 3i & 3ii)*							
Reference (a)	***Task / Aspect of Work.*** *(b)*	***What are the hazards?*** *Refer to checklist* *(c)*	***Who might be harmed and how could that harm arise?*** *(i.e. Who, how and nature of harm)* *Any special considerations? (d)*	**SEVERITY 1-5**	**LIKELIHOOD 1-5**	**INHERENT RISK** *(e)*	***What are you already doing?*** ***List existing measures to control risk.*** *(f)*	**SEVERITY 1-5**	**LIKELIHOOD1-5**	**RESIDUAL RISK** *(g)*	**Further Controls Required? (YES/NO)**
0	LABORATORY WORK	TRIPS AND SPILLS	ANYONE WORKING IN THE LAB, THIS CAN CAUSE SKIN OR EYE IRRITATION, AND BURNS IN SEVERER CASES	**1**	**3**	**LOW**	KEEP AILES CLEAR, USE STANDARD LAB PROCEDURES: CLOSED SHOES, LONG TROUSERS, LABCOAT, GLOVES AND GOGGLES WHERE APPROPRIATE.			**LOW**	
1A	DILUTING 60% (9.2M) PERCHLORIC ACID INTO 3.6% (0.6M)	HEALTH, REACTIVE AND FIRE/EXPLOSION HAZARD	PERSONNEL HANDLING CONCENTRATED PERCHLORIC ACID, ANYONE ELSE WORKING IN THE SAME LAB	**3**	**4**	**HIGH**	DILUTION ONLY CARRIED OUT IN SCRUBBED AND APPROVED FUME CUPBOARD FOR PERCHLORIC ACID, WITH THE SASH AT MINIMUM HEIGHT NO OTHER EQUIPMENT TO BE STORED IN CUPBOARD WHILST BEING USED FOR PERCHLORIC ACID. FUME CUPBOARD MUST BE WASHED DOWN AFTER WORK HAS FINISHED ADDITIONAL PPE TO BE USED: SAFETY GLASSES, FULL FACE VISOR, GOGGLES, GLOVES (2 PAIR OUTER BUTYL/THICK NITRILE), RUBBER APRON, ARM GUARDS	**1**	**3**	**LOW**	**YES**

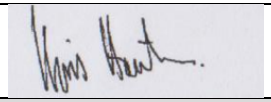
IF THE RISKS FOR ANY OF THE HAZARDS IN THE TABLE IS DEEMED TO BE HIGH, WORK MUST NOT PROCEED UNTIL FURTHER CONTROLS ARE PUT IN PLACE.											
IDENTIFICATION OF REASONABLY FORESEEABLE HAZARDS *(see Notes 2i & 2ii)*				**INDICATE CONTROL MEASURES IN PLACE & EVALUATE THE INHERENT OR RESIDUAL RISKS** *(see Notes 3i & 3ii)*							
Reference (a)	***Task / Aspect of Work.*** *(b)*	***What are the hazards?*** *Refer to checklist* *(c)*	***Who might be harmed and how could that harm arise?*** *(i.e. Who, how and nature of harm)* *Any special considerations? (d)*	**SEVERITY 1-5**	**LIKELIHOOD 1-5**	**INHERENT RISK** *(e)*	***What are you already doing?*** ***List existing measures to control risk.*** *(f)*	**SEVERITY 1-5**	**LIKELIHOOD1-5**	**RESIDUAL RISK** *(g)*	**Further Controls Required? (YES/NO)**
1B	ADDING DILUTED PERCHLORIC ACID (3.6%, 0.6M) TO ORGANIC MATERIAL	EXLOSION, FIRE AND SEVERE BURNS	ANYONE HANDLING THE SUBSTANCE. HEATING MAY CAUSE EXPLOSION; CONTACT WITH COMBUSTIBLE MATERIAL MAY CAUSE FIRE. THIS CAN LEAD TO SEVERE BURNS. IN CASE OF SPILLS OR SPLASHES ONTO ORGANIC MATTER E.G. LABCOAT, THE DRIED OUT SUBSTANCE CONCENTRATES PERCHLORIC ACID, WHICH CAN LEAD TO A REACTION CAUSING FIRE	**2**	**4**	**MEDIUM**	DILUTED PERCHLORIC ACID SHOULD BE ADDED UNDER A STANDARD FUME CUPBOARD WITH SASH AT MINIMUM HEIGHT AND WEARING STANDARD PPE THE DILUTION SHOULD BE CARRIED ON A WHITE PLASTIC TRAY, AND THE ADDING OF PERCHLORIC ACID TO BIOLOGICAL SAMPLES SHOULD ONLY BE CARRIED OUT ON THAT TRAY ALSO.	**1**	**2**	**LOW**	

IF THE RISKS FOR ANY OF THE HAZARDS IN THE TABLE IS DEEMED TO BE HIGH, WORK MUST NOT PROCEED UNTIL FURTHER CONTROLS ARE PUT IN PLACE.

IDENTIFICATION OF REASONABLY FORESEEABLE HAZARDS *(see Notes 2i & 2ii)*				**INDICATE CONTROL MEASURES IN PLACE & EVALUATE THE INHERENT OR RESIDUAL RISKS** *(see Notes 3i & 3ii)*							
Reference (a)	***Task / Aspect of Work.*** *(b)*	***What are the hazards?*** *Refer to checklist* *(c)*	***Who might be harmed and how could that harm arise?*** *(i.e. Who, how and nature of harm)* *Any special considerations? (d)*	**SEVERITY 1-5**	**LIKELIHOOD 1-5**	**INHERENT RISK** *(e)*	***What are you already doing?*** ***List existing measures to control risk.*** *(f)*	**SEVERITY 1-5**	**LIKELIHOOD1-5**	**RESIDUAL RISK** *(g)*	**Further Controls Required? (YES/NO)**
1C	PLACE ACID SAMPLE IN CENTRIFUGE TO MIX SAMPLE	SPILLS CAUSED BY FALCON TUBES OPENING IN WHILE CENTRIFUGE IS USED	PERSONNELL PLACING THE FALCON TUBES IN THE CENTRIFUGE AND REMOVING THEM SPILLS MAY OCCUR	**1**	**3**	**LOW**	ENSURE THAT THE FALCON TUBES ARE PROPERLY CLOSED AND SEALED BEFORE PLACING THEM INTO THE CENTRIFUGE	**1**	**2**	**LOW**	
2	POTASSIUM HYDROXIDE	CAUSES SEVERE BURNS, HARMFUL IF SWALLOWED	PERSONNEL DILUTING POTASSIUM HYDROXIDE AND ADDING IT TO THE ACID/BIOLOGICAL MATTER SOLUTION	**2**	**3**	**LOW**	USE ONLY UNDER FUME HOOD, WEARING PPE. DO NOT BREATH VAPOURS/DUST. DO NO INGEST	**1**	**2**	**LOW**	

TAB TO THE END OF TABLE TO INSERT NEW ROWS

Ref	**Further Controls or Post Assessment Actions required** *(see Note 4)*	**Action by whom?**	**Action by when?**
1A	**CONCENTRATED PERCHLORIC ACID SHOULD ONLY BE HANDLED BY PERSONNEL THAT RECEIVED TRAINING SPECIFIC PERCHLORIC ACID USE.**		Before use, 25.09.13

ASSESSOR & SUPERVISOR / MANAGER TO COMPLETE *(see Note 5)*					
ASSESSOR: SIGNED		PRINT NAME	KATIE BAWDEN	DATE	05-09-16
DECLARATION BY RESPONSIBLE SUPERVISOR / MANAGER: I confirm that this is a suitable and sufficient risk assessment for the above work task / activity.					
SIGNED		PRINT NAME	CHRIS HAUTON	DATE	30-08-16
ANNUAL REVIEW (OR WHEN ACTIVITIES CHANGED OR MODIFIED) **Assessment reviewed on** *(Date and reviewer initials – see Note 6)*					

All personnel working on the task: *I have read and understood the information contained in this Risk Assessment and agree to implement (or abide by) the safety controls indicated and will report to the responsible manager any incidents that occur or any shortcomings that I find in this assessment.*					
Name	**Signature**	**Date**	**Name**	**Signature**	**Date**