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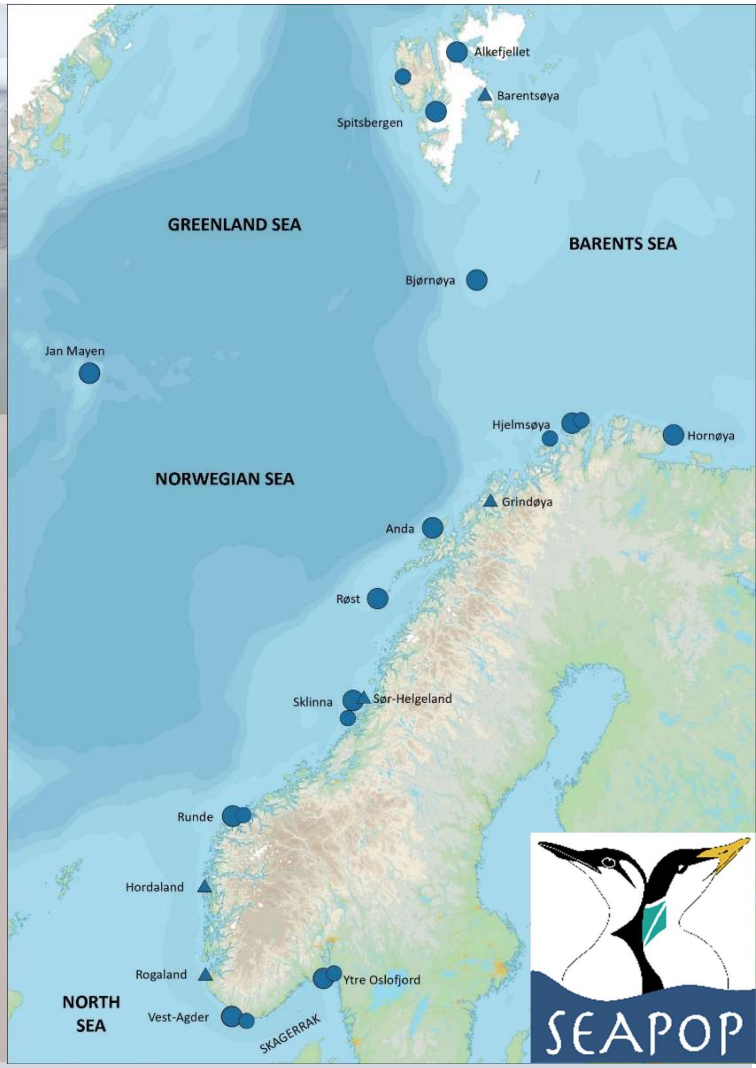
Seabirds as indicators of distribution, trends and population level effects of plastics in the Arctic marine environment

Workshop Report

Nina Dehnhard, Dorte Herzke, Geir Wing Gabrielsen, Tycho Anker-Nilssen, Amalie Ask, Signe Christensen-Dalsgaard, Sebastien Descamps, Ingeborg Hallanger, Sveinn Are Hanssen, Magdalene Langset, Laura Monclús, Nina O'Hanlon, Tone Kristin Reiertsen, Hallvard Strøm



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Framsenteret

Norwegian Institute for Nature Research

Dehnhard, N., Herzke, D., Gabrielsen, G.W., Anker-Nilssen, T., Ask, A., Christensen-Dalsgaard, S., Descamps, S., Hallanger, I., Hanssen, S.A., Langset, M., Monclús, L., O'Hanlon, N., Reiertsen, T.K., Strøm, H. 2019. Seabirds as indicators of distribution, trends and population level effects of plastics in the Arctic marine environment. Workshop Report. NINA Report 1719. Norwegian Institute for Nature Research.

Trondheim, November 2019

ISSN: 1504-3312
ISBN: 978-82-426-3470-2

AVAILABILITY
Open

PUBLICATION TYPE
Digital document (pdf)

QUALITY CONTROLLED BY
Børge Moe

SIGNATURE OF RESPONSIBLE PERSON
Research director Svein-Håkon Lorentsen (sign.)

COVER PICTURE
Herring gull with soccer ball (top left) © Nina O'Hanlon
Plastic found in a northern fulmar stomach caught as bycatch in Norway (bottom left) © Magdalene Langset
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KEY WORDS
Norway
Svalbard
Spitsbergen
Seabird Population Monitoring Programme (SEAPOP)
Seabirds
Monitoring
Plastic ingestion
Bioaccumulation

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Abstract

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Plastic pollution is a global and increasing threat to ecosystems. Plastics in the oceans are unevenly distributed, are transported by currents and can now be found in the most remote environments, including Arctic sea ice. The entanglement of wildlife by large plastic debris such as ropes is an obvious and well documented threat. However, the risks associated with the ingestion of smaller plastic particles, including microplastics (< 5mm) have been largely overlooked. Recent studies show that microplastic accumulates in the food web. Even in the Arctic and the deep sea, fish frequently contain microplastics in their guts. This, together with the fact that small microplastic particles can pass from the gut into blood and organs and also leach associated toxic additives raises health concerns for wildlife that ingest microplastic.

Within the North Atlantic, plastic ingestion in seabirds has been studied systematically only in the northern fulmar (*Fulmarus glacialis*), for which plastic particles > 1mm found in the stomachs of dead (beached or bycaught) birds are quantified. With the origin of these birds being unknown, it is, however, impossible to assess how plastics affect populations even of this one monitored species, let alone for other seabird species that differ in their foraging behaviour and risk to ingest plastics.

This report sums up the results of a workshop which aimed to identify possibilities for long-term monitoring of (micro-) plastic ingestion by seabirds in the framework of SEAPOP, the basal programme monitoring the performance of Norwegian seabird populations (www.seapop.no). The key conclusions were: 1) There is a need for baseline information on plastic ingestion across all seabird species to identify which species and populations are most suitable for monitoring. To obtain this information, the best approach is to investigate the stomach contents of dead birds (i.e. comparable methodology across all species). For long-term monitoring, not only species with high plastic ingestion are of interest, but also those with low plastic prevalence. 2) In the absence of information from (1), eight species that are complementary in their foraging behaviour and have a wide distribution range were selected as preliminary species of interest to monitor plastic ingestion. 3) For minimally invasive monitoring, regurgitates, fresh prey items and faeces are most suitable; 4) More information on prevalence of plastic ingestion is needed to identify optimal sample sizes for long-term monitoring. We therefore highlight the need for several pilot studies before establishing a plastic monitoring protocol within SEAPOP.

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Contents

Foreword

This NINA Report is the outcome of a workshop held in Tromsø on the 1st and 2nd of October 2019 and funded by The Fram Centre in the framework of the 2019 Plastic in the Arctic Research Programme. The workshop aimed to assess sampling and analytical methods for minimally/non-invasive monitoring of plastic ingestion by seabirds in the framework of the basal monitoring programme for Norwegian seabird populations (SEAPOP). All workshop participants read and approved the final version of the report.

8th of November 2019, Nina Dehnhard

1 Introduction

Plastic pollution is an increasing global problem which is receiving increasing public awareness in recent years (Borrelle et al. 2017, Eriksen et al. 2014, Haward 2018). Annually, between 1.2 and 12.7 million tons of plastic are estimated to enter the oceans on a global scale (Jambeck et al. 2015, Lebreton et al. 2017). Plastic pieces have reached the most remote areas of the planet such as the Arctic (Lusher et al. 2015, Peeken et al. 2018), Antarctic (Waller et al. 2017) and uninhabited islands in the South Pacific (Lavers & Bond 2017). Within the ocean, plastics are spread with wind and ocean currents and thus distribute unevenly in space and time, accumulating especially in the areas of ocean gyres (Law 2017, Lusher 2015) as well as in marine sediments (Thompson et al. 2004).

The risks of entanglement and ingestion for wildlife with acute risks of death due to strangulation or by blocking of the stomach or gut passage are well documented for macroplastics (> 200 mm) and mesoplastics (5-200 mm) (Gregory 2009, Worm et al. 2017). Especially the highly visible incorporation of plastic into seabird nests and incidents of strangulations at nest sites are raising awareness among the general public of the threats that plastic pollution poses for wildlife.
The documentation of such interactions between wildlife and macro- and mesoplastics is also helped by the current expansion of citizen-science projects, enabling a future assessment of frequency of occurrence and identification of vulnerable species (e.g. www.birdsanddebris.com/).

In contrast, the environmental impact of microplastics (< 5 mm) has largely been overlooked (GESAMP 2015). Microplastics form a heterogeneous mix of polymers which differ in shape (e.g. pellets/beads, fibres, fragments and films), density (Andrady 2011) and additives such as plasticizers (e.g. phthalates and chlorinated paraffins), polybrominated diphenyl ethers (PBDEs; used as flame retardants) and heavy metals (Kwan & Takada 2019, Massos & Turner 2017). Additional hydrophobic organic chemicals (HOCs) can accumulate on the surface of microplastics (Ziccardi et al. 2016).

Due to their size, microplastics are commonly ingested by many invertebrates (including zooplankton) and fish as they are mistaken for food (reviewed in Lusher et al. 2017). Via secondary ingestion, they accumulate in higher trophic level organisms, a process that has been shown along several food web linkages in laboratory experiments (Cedervall et al. 2012, Farrell & Nelson 2013, Setälä et al. 2014). Outside the laboratory, microplastics have been found in the guts of many pelagic and demersal fish species, including species of commercial interest such as mackerel (*Scomber japonicus* and *Trachurus trachurus*), whiting (*Merlangius merlangus*) and blue whiting (*Micromesistius poutassou* (Lusher et al. 2013, Neves et al. 2015) and in juvenile polar cod (*Boreogadus saida*) living under the Arctic sea ice (Kühn et al. 2018). A recent study also showed that 73% of deep-water fish from the Northwest Atlantic had ingested microplastics (Wieczorek et al. 2018). Due to the accumulation of microplastics in sediments, benthic feeders might be at higher risk to accumulate microplastic than pelagic or surface-feeding fish, but support for higher microplastic accumulation through the benthic food web is mixed (Lusher et al. 2013, McGoran et al. 2017, Neves et al. 2015).

One of the risks involved with ingesting microplastics is the exposure to plasticizers, HOCs (including PBDEs) and heavy metals if these leach out of the plastics into the organisms (Rochman et al. 2013, Tanaka et al. 2013, Tanaka et al. 2015, 2018, Teuten et al. 2009). Exposure to HOCs and heavy metals in general can severely affect health and reproductive success of organisms and ultimately population trends (Burger 2008, Erikstad et al. 2013, Letcher et al. 2010). Laboratory studies that investigated the effects of HOCs leaching from microplastics (both virgin particles and particles that had accumulated additional HOCs from the marine environment) found signs of liver toxicity in fish (Rochman et al. 2013). Finally, plasticizers such as the frequently used phthalates and Bisphenol A commonly act as endocrine disrupters, causing hormonal imbalance and affecting reproduction and development in organisms (Kang et al. 2002, Meerts et al. 2001, Morrissey et al. 1987). Another risk is the uptake

of plastic particles into tissues (Rochman 2013). Small microplastics and nanoplastics (particles < 1 µm; Gigault et al. 2018) can trigger inflammation processes in tissues (Brown et al. 2001, Espinosa et al. 2017, Pedà et al. 2016) and reduce the survival of zooplankton (Mattsson et al. 2017). In fish, nanoplastics can pass from the gut into the blood stream and lymphnodes (Hodges et al. 1995) and have recently even been found to pass into the brain, causing significant behavioural disruptions (Mattsson et al. 2017).

There is therefore cause for concern that ingested micro- and nanoplastics, even when not blocking the guts, may seriously affect higher trophic level organisms (Andrady 2011) with implications for wildlife and humans (Smith et al. 2018). However, up to today, there is no method in place to systematically quantify the effects of plastic on wildlife populations, e.g. through lowered fecundity, offspring survival and/or adult survival. Seabirds are ideal sentinels of marine pollution since they are long-lived top predators and thus prone to bio-accumulation (Elliott & Elliott 2013). They can easily be accessed on land in their breeding colonies, where it is often feasible to study the same individuals year after year (Burger & Gochfeld 2004, Durant et al. 2009). From a conservation point of view, (micro-) plastics pose an additional risk for seabirds which already are under pressure from various other threats inflicted upon them by humans, with – on a global scale – the top dangers assessed to be invasive alien species, bycatch, hunting and trapping, and the effects of global climate change (Dias et al. 2019).

Most studies on plastic ingestion in seabirds have focussed on surface-feeding species that mistake plastic pieces for food (especially Procellariiformes such as the Northern fulmar (*Fulmarus glacialis*) or Cory's shearwater (*Calonectris diomedea*); O'Hanlon et al. 2017, Rodríguez et al. 2012, van Franeker et al. 2011, van Franeker & Law 2015). In the North Sea and North East Atlantic, the Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR) uses stomach contents of beached northern fulmars as a monitoring tool to assess ingestion of floating plastic. This is currently the only systematic monitoring of plastic ingestion by wildlife in the whole of Europe. However, the current protocol ignores plastic particles that are smaller than 1 mm (van Franeker et al. 2011) and thus the majority of microplastics. OSPAR has defined an Ecological Quality Objective (EcoQO) of 0.1 g plastic per stomach, which is currently exceeded at all monitoring sites from Svalbard to the North Sea (https://www.ospar.org/work-areas/eiha/marine-litter/plastic-particles-in-fulmars). The relationship between stomach plastic load and health effects in fulmars is, however, not known since the EcoQO was primarily developed to monitor changes in ingestion rates with 0.1 g chosen as an arbitrary threshold. Also, since the OSPAR monitoring is based on beached (and thus dead) northern fulmars, we lack information not only on their origins (i.e. what breeding population they belong to), but also on sub-lethal and long-term effects of plastic ingestion on seabirds and wildlife in general.

In the meantime, plastic ingestion is not limited to surface-feeding species such as northern fulmars, but has been documented in many species across the North East Atlantic (O'Hanlon et al. 2017) as in the rest of the world (Wilcox et al. 2015). Among species, the probability to ingest plastics appears to differ between species that are feeding at the surface versus on pelagic or benthic prey (O'Hanlon et al. 2017), and such differences may even prevail within species (Álvarez et al. 2018, Battisti et al. 2019, Hammer et al. 2016). Nevertheless, O'Hanlon et al. (2017) concluded that still very little information is available about the current prevalence of plastic ingestion for many species and highlighted the need for standardised monitoring, at least across the North Atlantic.

With the current monitoring of northern fulmars and the scarce data on plastic ingestion available for other species, it is impossible to assess the potential of plastic pollution to affect health and behaviour and, ultimately, key life-history parameters (breeding success, survival and thus population demography and trajectories) of seabirds. Closing these knowledge gaps has proven difficult so far since measuring the exposure to plastics is complex, especially when live organisms are to be sampled.

2 The workshop

Our workshop aimed at identifying a suite of state-of-the art sampling methods for plastic ingestion that are feasible to use, minimally/non-invasive and ensures the best analytical outcomes, given the existing logistics and population monitoring systems already in place in mainland and Arctic Norway. SEAPOP (www.seapop.no), the basal monitoring programme for Norwegian seabird populations, forms an ideal framework to collect seabird samples to measure plastic exposure of seabirds of known origin and link this with key data on diets and demographic parameters of their populations. SEAPOP covers a wide range of seabird species and populations from Svalbard in the high Arctic to the North Sea and Skagerrak (**Figure 1a**), a latitudinal gradient of 2500 km over which plastic intake in fulmars is known to increase (Trevail et al. 2015, van Franeker & Law 2015). Through the SEATRACK project (http://www.seapop.no/en/seatrack/), we know the spatial distribution of the monitored seabird populations, which can cover large areas of the North Atlantic and Arctic Ocean (**Figure 1b**). Even seabirds that breed in central or southern Norway may visit Arctic waters in the non-breeding season, i.e. in autumn, winter or early spring. This knowledge about foraging areas and habitat use throughout the year is imperative for explaining spatio-temporal variations in plastic ingestion.

The workshop took place on 1st and 2nd of October 2019 at The Fram Centre in Tromsø and was attended by scientists from the two SEAPOP programme partners (Norwegian Institute for Nature Research (NINA) and Norwegian Polar Institute (NPI)) as well as the Norwegian Institute for Air Research (NILU), the Norwegian University for Science and Technology (NTNU) and the University of the Highlands and Islands in Scotland (see Appendix 1 for a list of workshop participants). In order to design an optimal study design, we reviewed the existing literature and discussed possibilities for minimal invasive sampling (see overview in Section 3.1) and a selection of species of interest (Section 4.1). We further reviewed existing guidelines for sampling in the field to avoid contamination of samples during collection and subsequent analyses (Section 3.2) and summed these up to a field sampling protocol (Appendix 2). Finally, possible further analyses, ranging from pure quantification to identification of different polymers (e.g. polypropylene, polyethylene etc.) were discussed (Section 3.3). The workshop only dealt with ingestion of plastic, not the entanglement of seabirds in plastic debris. The latter is certainly an ethical problem, but its effects on seabird populations would be very difficult to study in quantitative terms.

In the course of the discussions, it became apparent that before starting with systematic sampling to assess plastic ingestion for even just a limited number of species along the SEAPOP monitoring sites, we would first need more information on the prevalence of plastic particles in these species to determine sample sizes needed for the different sampled materials of interest (e.g. regurgitates versus faeces). Therefore, preliminary studies are needed to optimize the study design for long-term monitoring of plastic exposure across the SEAPOP network of sites (detailed in Sections 4.2 and 4.3). Once this is in place, sampling could also be extended to other regions, possibly other bird species and provide relevant information to define EcoQOs by OSPAR and others.

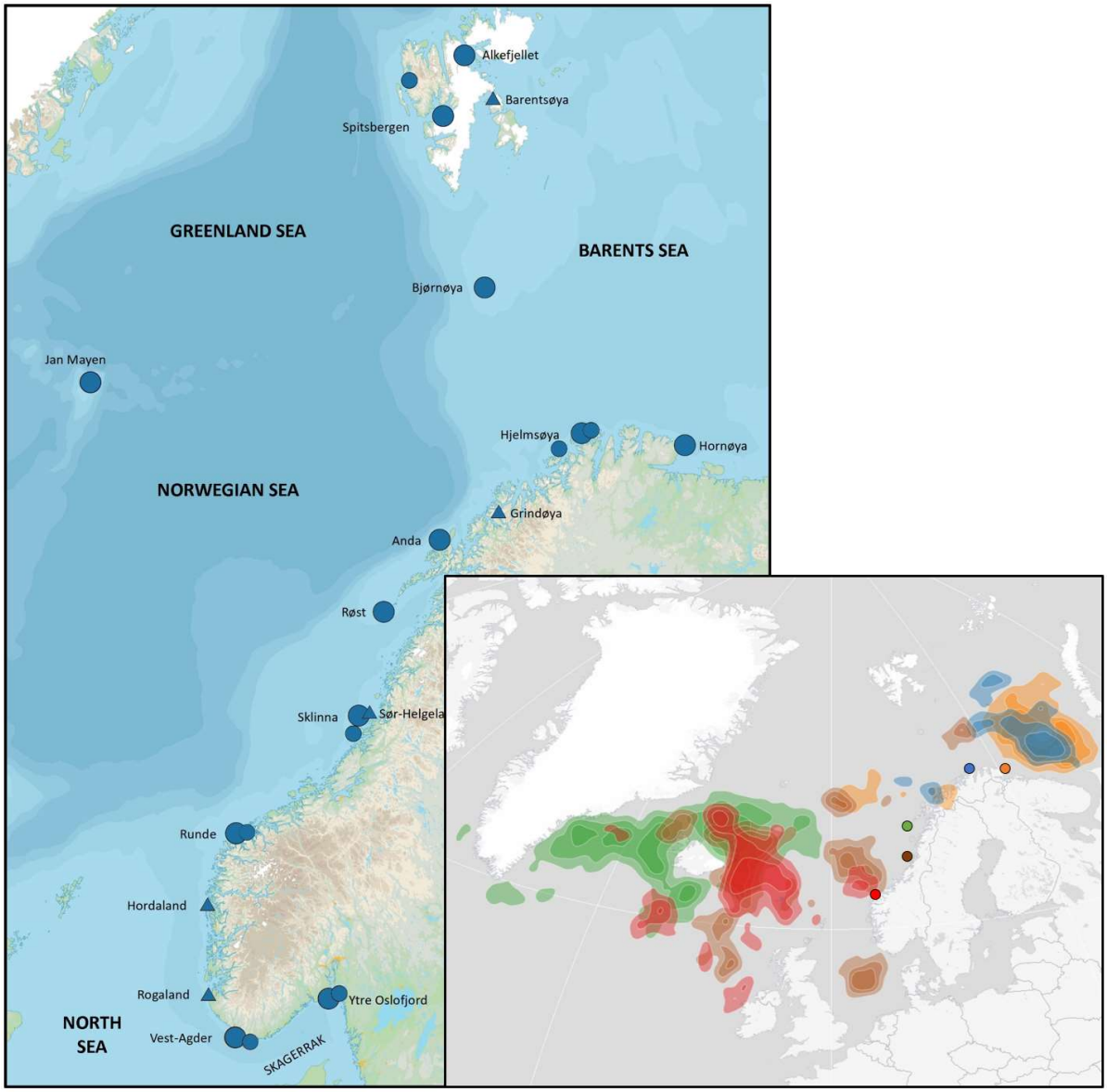

Figure 1a. *Main map: SEAPOP key monitoring sites ranging from the Arctic to the North Sea. Circular symbols indicate multi-species monitoring sites, triangles single-species monitoring sites. © SEAPOP.* ***Figure 1b.*** *Inserted map: Winter distribution of Atlantic puffins (Fratercula arctica), from five of the monitoring key sites on the Norwegian mainland. Different colours refer to origin from different breeding colonies, marked with dots of the same colour. © SEATRACK*

3 Review of existing methodology

3.1 Sampling materials to monitor plastic exposure

3.1.1 Regurgitates - pellets

A number of seabird species, e.g. great cormorants (*Phalacrocorax carbo*), European shags (*P. aristotelis*) and great skuas (*Catharacta skua*) regurgitate pellets to excrete indigestible prey items such as fish bones and otoliths. Pellets can be easily collected and do not necessitate the handling of seabirds. Although pellets in general were deemed useless to assess microplastic ingestion in a recent review (Provencher et al. 2019), microplastic particles in pellets have been documented in two independent studies (Acampora et al. 2017a, Álvarez et al. 2018). Besides the occurrence of plastic particles in the pellets, it is also possible to use the same pellets to identify the diet of the seabirds (Acampora et al. 2017a, Álvarez et al. 2018, Barrett et al. 2007). This may help to interpret if the diet affects the probability to ingest plastics (Álvarez et al. 2018).

Disadvantages of using pellets compared to other methods are that comparisons among species are limited to the few species that produce pellets. Furthermore, it is unclear how much plastic is regurgitated with the pellets, and how much plastic remains in the birds and is excreted in other ways (Provencher et al. 2019). Plastic that is regurgitated with the pellets will likely have low or no impact on the birds but may contaminate the breeding/roosting sites with plastic.

3.1.2 Regurgitates – partly digested food

When being handled for other tasks, many seabird species regurgitate partly digested food as a defence mechanism. Similarly as for pellets, these regurgitates can be used to investigate both diet and contained plastic (Acampora et al. 2017b). One disadvantage of using fresh regurgitates as an indicator of plastic ingestion is that it is unknown how much of the stomach content is regurgitated and whether the amount of plastic regurgitated is representative of the entire stomach content. It is likely that only contents of the proventriculus, but not of the gizzard are regurgitated, while the gizzard is known to hold the majority of plastics at least in northern fulmars (Van Franeker & Meijboom 2002). Furthermore – similarly as for pellets – the method is limited to those species that regurgitate spontaneously (black-legged kittiwakes, fulmars, chicks of large gulls and cormorants). When sampling regurgitates from chicks, it should be considered that diet (and therefore plastic ingestion) may differ between adults and chicks.

3.1.3 Fresh prey items

Auk species feed their chicks by bringing entire fish back to the colony. These fish can be collected when working in the colony, e.g. when catching adult guillemots with a noose pole, or when catching puffins with mist nets. In these fresh prey items, plastic particles can be quantified on the individual level of the prey, e.g. plastic particles per sandeel (Ammodytidae), in a similar manner as in studies focusing on plastic ingestion by fish (Wieczorek et al. 2018). While the sampling of fresh prey items is limited to members of the auk family, the results may be extrapolated to other species, if their diet and foraging areas overlap.

3.1.4 Faeces

Plastic particles have been reported in faeces of ducks (Reynolds & Ryan 2018) and in faeces precursors of northern fulmars (Provencher et al. 2018). Faeces samples can be collected from all species, but practically this may be difficult if faeces are very liquid. In the ideal case, this is a non-invasive method and when combined with DNA studies (McInnes et al. 2016), it could be possible to investigate both plastic exposure and diet from the same sample. In contrast to

regurgitates and fresh prey items, faeces reflect the excretion of plastics, and the quantification of how much plastic is taken up with one meal or a given quantity of fish is difficult. Furthermore, retention times of plastic in the digestive tract are unknown.

3.1.5 Blood

Blood samples may reveal information about organisms being in contact with plastic earlier in life, and therefore showing increased values of certain additives that are typically present in plastic, or their metabolites. The advantage of blood sampling is that it would allow a quantification of the additives in the same manner across different species and age classes (e.g. chicks versus adults). However, due to the impossibility to distinguish between additives that are taken up with the prey (i.e. accumulation of pollutants through the food web) versus those taken by the bird directly from ingested plastic, alternative chemical tracers stemming directly from the plastic polymer need to be identified. The identification of additives and their metabolism pathways is still ongoing, hampered by the confidential composition of plastic products as well as the large variety of potentially used additives. Furthermore, the more additives are to be quantified, the more blood is needed (minimum amount: 2 ml).

3.1.6 Feathers

Feathers allow the possibility to determine external contamination from water and air with (micro-)-plastic particles by brushing off externally attached plastic particles (Reynolds & Ryan 2018). However, this appears little meaningful for comparisons among seabird species that spend different amounts of time swimming, diving and flying, and there is a high risk for contamination of samples while handling birds and storing the feathers.

Feathers can further be used to identify additives – similar as for blood – that are bound in the feather matrix (Eulaers et al. 2014) or applied on the feathers during preening with preen gland oil. Advantages are that collecting feathers (even from live birds) is a low effort and low impact for the birds, and feathers can also be used to determine other potential markers of interest, e.g. diet via stable isotopes (Bond & Jones 2009), heavy metal exposure (Fenstad et al. 2017) or stress levels based on feather corticosterone (Harms et al. 2015).

The feather matrix remains metabolically inert after their formation, and therefore additives stored in the feather matrix, stable isotopes and corticosterone levels reflect the moult period – which is often spent elsewhere than the breeding period. Disadvantages of using feathers are similar as those for blood: It is difficult to differentiate if additives accumulated via the food chain or reflect direct plastic exposure of the bird. Furthermore, feathers are easily contaminated with dust particles and therefore need to be stored in a dust-free environment and suitable containers (glass, aluminium foil).

3.1.7 Preen gland oil

Preen gland oil can be used to quantify additives contained in plastics or metabolites thereof, e.g. phthalates (Hardesty et al. 2015) or other contaminants (Eulaers et al. 2014). As for blood and feathers, with this approach it remains unclear if the bird itself was in contact with plastic particles, or if the concentrations of additives are due to accumulation via the food web. Sampling of preen gland oil from live birds can be difficult when birds are fasting or have naturally low amounts of body fat (i.e. during incubation and chick-rearing). Furthermore, the fasting and mobilisation of internal fat stores may affect concentrations of additives found in preen gland oil. Also, in addition to a little understood mechanism and kinetics of additive transfer from stomach to preen gland oil, there might be a time lag between plastic exposure and production/sampling of preen gland oil, which we know very little about, challenging the estimation of the time frame

and extend of plastic exposure. Finally, non-lipophilic additives and metabolites will not be detectable in preen gland oil.

3.1.8 Subcutaneous fat

Sampling of subcutaneous fat through biopsy from live birds has been practiced without detected adverse health effects and could offer direct comparability of samples obtained from live and dead birds (Rocha et al. 2016). Nevertheless, biopsies appear to be a more invasive and time-consuming compared to other options named above. Furthermore, it can be expected that additives found in subcutaneous fat would be similar as in preen gland oil, since both are fat-based. Similar as for preen gland oil, sampling will be most difficult when birds are fasting and have naturally low amounts of body fat (i.e. during incubation and chick-rearing). No direct link between concentrations in fat and plastic findings in stomachs is possible, since fat is accumulated over a longer period of time.

3.1.9 Eggs

Eggs could be another matrix to analyse additives that are related to plastic ingestion. Eggs can be collected with a relatively low sampling effort and are already collected in limited amounts for long-term storage at the Norwegian Environmental Specimen Bank (www.miljoprovebanken.no). Transfer mechanisms for additives from females to their eggs are little understood hampering the evaluation of measured concentrations. For example, the concentrations of additives may differ among species that are income or capital breeders (Bustnes et al. 2010). Furthermore, within a clutch, the egg laying order can affect pollutant concentrations (Dehnhard et al. 2017), and egg laying order can be difficult to determine in the field. Egg predation may also make it difficult to document if the first egg found is the first egg laid. Finally, since egg laying dates vary between years, it can be difficult to collect fresh eggs, which again can affect analytical outcomes.

3.1.10 Dead birds

Dead birds can be opportunistically sampled either in the breeding colonies, or from bycatch or wrecks of seabirds (e.g. because of oiling or starvation), in which cases their breeding locations are usually unknown. Dead birds allow a holistic approach, e.g. the quantification of plastic in different parts of the digestive tract, subsequent identification of plastic polymers, and finally a determination of additives found in different body tissues.

The disadvantages are that sample sizes are unpredictable and, especially for those birds collected in breeding colonies, usually too low to allow comparison of plastic ingestion among sites. Furthermore, freezer space can be limited, and carcasses found in colonies may not be sufficiently fresh. Finally, one needs to consider the cause of death. Diseased birds or those in poor body condition may have behaved differently than the rest of the population and therefore cause a bias in results.

3.2 Sampling strategies & precautions needed

When aiming for particles below 1 mm, there is a high risk for contamination of samples, both in the field and in the laboratory. It is therefore crucial to minimise the risk for contamination using suitable sampling equipment (as much glass and metal as possible, as little plastic as possible) and in addition use blanks to control for contamination along the entire way from sampling in the field to the final step in the laboratory. The contact of samples with plastic may not always be avoidable (e.g. when sampling blood with plastic syringes or collecting regurgitates or faeces

from birds while handling them), however it is possible to account for this contamination in the lab when the materials are known. Therefore, the same type of syringes, gloves or funnels should be used at all field sites.

We here largely suggest to follow the guidelines recommended by Uhart et al. (2017) for sampling of ACAP species in the southern ocean as well as the recommendations of Provencher et al. (2019).

3.2.1 Preparation of sampling equipment

Prior to the field season, prepare the sampling equipment, consisting of glass vials, metal spatulas, metal forceps, metal scalpel blades and aluminium foil (commercial household aluminium foil). All of the re-usable equipment should be thoroughly cleaned first, and rinsed with water. Glass vials will be covered on the top with a double-layer of aluminium foil. To remove any remaining organic material, heat all sampling equipment (including the aluminium foil and the aluminium-foil covered glass vials) to 450°C overnight. Pack all equipment in pre-heated aluminium foil to avoid contamination during transport to the field site. Make sure glass vials are stored upright.

Prepare **transport blanks – one per fieldsite / species / sampling material** (e.g. one for faeces samples of common guillemots at Hornøya): Close these transport vials already in the lab prior to the field season with a plastic lid (keep the aluminium foil under the lid, as you will later do with the real samples). Keep transport-blank vials closed and in upright position. Keep them with the remaining sample vials (but do not open them at the field site). Upon return of samples to the lab, these transport blanks serve as controls to assess contamination during work in the lab.

Obtain nitrile gloves in a light-blue or other obvious colour to be used in the field, same brand at all field sites. Obtain funnels and PVC-aprons in unusual (e.g. yellow or pink) colours.

3.2.2 Sampling in the field

Sampling equipment like forceps, spatulas, scalpel blades for dissections of dead birds are single use in the field. Exceptions have to be made for funnels and PVC-aprons, that have to get cleaned and rinsed with filtered water/ethanol before being used again.

Follow the field protocol (Appendix 2) for sampling the different materials. Where possible, only use metal spatulas / forceps, but if this is not possible (e.g. when picking up pellets) use nitrile gloves. For collecting regurgitates or faeces while handling birds, the use of funnels or PVC aprons is unavoidable.

Take at least **three environmental blanks per fieldsite / material / species** sampled. Use a standard glass sampling vial, open it at the field site by removing the aluminium foil. Keep it open for the same amount of time as for a real sample. Cover the glass vial again with the same aluminium foil as used before, place the plastic lid on top. Store in an upright position and bring it back with the actual samples. Upon return of samples to the lab, these field blanks serve as controls to assess contamination during handling in the field and the lab combined.

3.3 Laboratory analyses

3.3.1 Necropsies of dead birds and materials of interest

Dissections of birds and general health assessment should follow the current standard procedure for OSPAR monitoring of northern fulmars (van Franeker 2004). Deviating from this protocol, it would be desirable to remove not only the stomach but the entire intestinal system, to screen for macro-, meso- and microplastics, similarly as in Provencher et al. (2018). Stomach and intestine contents could be treated similar to regurgitates, the organic material could be enzymatically digested and any potential plastic particles further analysed (see 3.3.10).

The liver, kidneys and spleen, samples of breast muscle, abdominal and subcutaneous fat and the skull with the brain should be collected, wrapped in double aluminium foil, and stored frozen for further analyses (see 3.3.11).

3.3.2 Treatment of regurgitates, fresh prey items and faeces

For analyses of the contained (micro-)plastic, the organic material within regurgitate / fresh diet and faeces samples has to be digested. This can be done using an enzyme mix as in von Friesen et al. (2019), or alternatively potassium hydroxide (Kühn et al. 2017). A saturated and filtered solution of Biotex ® or other enzymatic washing powders, which have been used previously for similar purposes in diet studies (Hillersøy & Lorentsen 2012), could be an additional alternative option for digestion of organic material.

The remains of the samples can then be filtered through sieves of consecutive order (e.g. mesh sizes 1000 µm, 300 µm and 50 µm), and plastic particles retrieved.

During these procedures in the lab, especially when targeting plastic particles < 1 mm, it is crucial to minimise potential pollution. This implies working in a clean lab environment, possibly with filtered air and under a fume hood/under-pressure keeping samples covered as much as possible, or the use of a pyramid glove box for certain work steps (Provencher et al. 2019, Torre et al. 2016). Lab staff should wear cotton lab coats possibly in an uncommon colour (e.g. pink or orange) for easy detection of fibres originating from lab clothes (Provencher et al. 2019). Finally, blanks should be used to quantify the risk of airborne microplastic pollution.

Even if there is no standard method available for detecting, quantifying and identifying plastic particles yet, a number of methods in use are applicable for a certain targeted resolution and depth of information. Below a selection of available and most suitable methods is listed:

Visual inspection

Microplastic particles of a size range larger than 1 mm can be identified by visual inspection using the naked eye or a microscope by a trained person. Despite being a fast process, the possibilities of false positives are high with a high personal bias. Also dark particles might be under-reported due to their similarity with natural particles. Particles smaller than 1 mm found under the microscope that might be made of plastic should be further characterised by either Nile Red staining, FTIR or Raman spectroscopy (see below).

Nile Red staining

Originally developed for the staining of tissues for clinical investigations, fluorescent tagging by Nile Red staining offers a simple, low-cost method of determining the presence of plastic particles. However, it relies on the complete removal of all other organic materials to avoid false positives. Briefly, a Nile Red solution is carefully added to a processed MP sample (on a filter), rinsed with acetone to remove excess dye, and then washed with copious amounts of ultrapure water. The filter is inspected under a light microscope (e.g. Leica DMI 4000), with fluorescent

excitation (360 nm, 450–490 nm, or 515–560 nm) and fluorescent particles are counted (Cole 2016, Erni-Cassola et al. 2017, Maes et al. 2017).

FTIR spectroscopy
Fourier-transform infrared spectroscopy (FTIR) relies on the absorption of infrared light by distinct building blocks of plastic polymers and the following spectra containing individual signals characteristic for specific polymers (fingerprints). The method is fast, requires trained personal operating a range of available instrumentation, targeting a varying size range (from 5 mm to 10 µm). However, for measurements of size ranges below 500 µm advanced FTIR instruments are required. Weathering and biofouling can impact the FTIR spectrum, causing misinterpretations of the identity of the polymer type.

Raman spectroscopy
Raman spectroscopy is as FTIR a non-destructive spectroscopic technique that provides a structural fingerprint, as for the FTIR technique (da Costa et al. 2019).

3.3.3 Treatment of sampled materials to identify additives

The determination of additives leached out from plastic particles is based on the removal of all matrix from the chemical along with a number of concentration steps to enable the measurement of the additive with suitable analytical instumentation. The whole process is highly dependent on the targeted additive and the material type. Only trained personnel and a suitable laboratory is able to carry out this work. However, much lower concentrations of additives can then be determined (in the pg and ng/g range) than what is today possible for the determination of plastic particles (µg/g). The challenge today is the identification of a suitable additive that is not present in seabird tissues due to other exposure sources besides plastic ingestion.

4 Conclusions and recommendations of the workshop

4.1 Selection of study species

The monitoring on SEAPOP keysites is focussed on populations of 19 different seabird species (**Table 1**; Anker-Nilssen et al. 2019). For the majority of these species, very little information on plastic ingestion has been collected in colonies in mainland Norway or Svalbard (see supplement of O'Hanlon et al. 2017). Furthermore, except for the well-studied northern fulmars (Herzke et al. 2016, Trevail et al. 2015, van Franeker et al. 2011) and one master thesis on great skuas (Knutsen 2010), all existing data on plastic ingestion by Norwegian seabirds are from the 1980s (see supplement of O'Hanlon et al. 2017). A few more recent studies from other areas in the Northeast Atlantic, especially in the North Sea, have revealed plastic to be ingested also by European shags, great cormorants, great black-backed gulls, lesser black-backed gulls, herring gulls, black-legged kittiwakes, common guillemots, Brünnich's guillemots, Atlantic puffins and little auks (see supplement of O'Hanlon et al. 2017). While northern fulmars represent the species with the best knowledge and highest prevalence of plastic ingestion, the workshop participants agreed that in order to assess potential effects of plastic on seabird populations, it is necessary to also monitor species with a likely lower plastic ingestion rate.

***Table 1**. List of typical seabird species breeding in significant numbers at SEAPOP keysites, their distribution and foraging characteristics. Species identified as interesting for plastic ingestion (see text for selection criteria) are highlighted in bold.*

Species name	Scientific name	Distribution	Foraging behaviour
Northern fulmar	*Fulmarus glacialis*	Arctic & temperate	Surface/opportunistic, offshore
Northern gannet	*Morus bassanus*	Arctic & temperate	Surface-diving, coastal-offshore
Great cormorant	*Phalacrocorax carbo*	Arctic & temperate	Benthic, coastal
European shag	*Phalacrocorax aristotelis*	Arctic & temperate	Benthic, coastal
Common eider	*Somateria mollissima*	Arctic & temperate	Benthic, coastal
Great skua	*Catharacta skua*	Arctic & temperate	Surface/opportunistic, coastal-offshore
Arctic skua	*Stercorarius parasiticus*	Arctic & temperate	Surface/opportunistic, coastal-offshore
Great black-backed gull	*Larus marinus*	Arctic & temperate	Surface/opportunistic, coastal-offshore
Herring gull	*Larus argentatus*	temperate	Surface/opportunistic, coastal
Lesser black-backed gull	*Larus fuscus*	temperate	Surface/opportunistic, coastal
Glaucous gull	*Larus hyperboreus*	Arctic & temperate	Surface/Opportunistic, coastal
Black-legged kittiwake	*Rissa tridactyla*	Arctic & temperate	Surface, coastal-offshore
Ivory gull	*Pagophila eburnea*	Arctic	Surface/opportunistic, Pelagic
Common guillemot	*Uria aalge*	Arctic & temperate	Diving, coastal-offshore
Brünnich's guillemot	*Uria lomvia*	Arctic	Diving, coastal-offshore
Razorbill	*Alca torda*	Arctic & temperate	Diving, coastal-offshore

Black guillemot	*Cepphus grylle*	Arctic & temperate	Benthic, coastal
Atlantic puffin	*Fratercula arctica*	Arctic & temperate	Diving, coastal-offshore
Little auk	*Alle alle*	Arctic	Surface, coastal-offshore

The first conclusion was that it would be desirable to establish baseline information on plastic ingestion for every seabird species, possibly best (since this allows for the most standardised comparison among species) by screening dead birds found in colonies or caught as bycatch for plastic contained in their entire digestive tract. Realistically, it will likely take several years for this baseline information to be collected. Until this information is present, we therefore agreed to identify species of special interest for plastic monitoring based on the current information present, namely that plastic is distributed unevenly in the oceans (Law 2017, Law et al. 2010, van Franeker & Law 2015), that plastic ingestion differs between species that are feeding at the surface versus on pelagic or benthic prey (O'Hanlon et al. 2017) and that only a selection of the species are included in ongoing monitoring of their performance in terms of diets, breeding success, survival rates and/or population trends. We therefore made a selection of species that are complementary in their foraging behaviour/habitat (i.e. surface/diving/benthic and coastal versus offshore), are distributed over a wide spatial range from the Arctic to temperate zones, and are part of the monitoring schemes at several SEAPOP keysites (**Table 1**, **Figures 2 and 3**).

We included northern fulmars as the species with the currently best knowledge base on plastic ingestion in the North Atlantic. Northern fulmars are classical opportunistic surface feeders with offshore foraging distribution and a high prevalence of plastic ingestion due to their habit of mistaking floating plastic debris for food. Unfortunately, only very few northern fulmars are breeding along the coast of mainland Norway, and colonies in Spitsbergen are difficult to access. Most work on northern fulmars within SEAPOP therefore takes place at Bjørnøya and Jan Mayen. Current work on Bjørnøya already involves stomach flushing and blood sampling, and this could be extended further to sampling of faeces.

European shags were included as the most-widespread and – within the SEAPOP network – easiest to work-with species with benthic and coastal feeding habitats all year around (Christensen-Dalsgaard et al. 2017, Lilliendahl & Solmundsson 2006).

Great skuas and great black-backed gulls were included due to their mainly predatory and opportunistic foraging behaviour, which may increase their risk for accumulation of plastic (Hammer et al. 2016, O'Hanlon et al. 2017). While working with adult birds can be challenging, chicks of both species regurgitate partly digested prey when being handled (e.g. for ringing), and can therefore be easily sampled.

Black-legged kittiwakes as surface feeders are particularly prone to plastic ingestion, yet unlike fulmars they are less opportunistic and likely ingests plastic mostly with their prey. Black-legged kittiwakes feed in both coastal and offshore areas during the summer (Christensen-Dalsgaard et al. 2018) and are fully pelagic during the non-breeding season (Frederiksen et al. 2012).

Atlantic puffins forage by pursuit-diving in both coastal and offshore areas during the summer (Shoji et al. 2015) and are fully pelagic during the non-breeding season (Fayet et al. 2017). Common and Brünnich's guillemots have similar foraging habits, being pursuit divers in coastal to offshore areas during summer and offshore areas only during winter (Mehlum et al. 1998, Mehlum et al. 2001, Thaxter et al. 2012, Thaxter et al. 2010), but in some areas the diet of common guillemots indicates that they feed in more shallow and near-shore waters than puffins. The guillemots also feed on larger fish than Atlantic puffins, while common and Brünnich's guillemots are somehow complementary in their distribution (common guillemots more temperate, Brünnich's guillemots strictly Arctic; **Figure 3**), but apart from that overlap in their foraging habits.

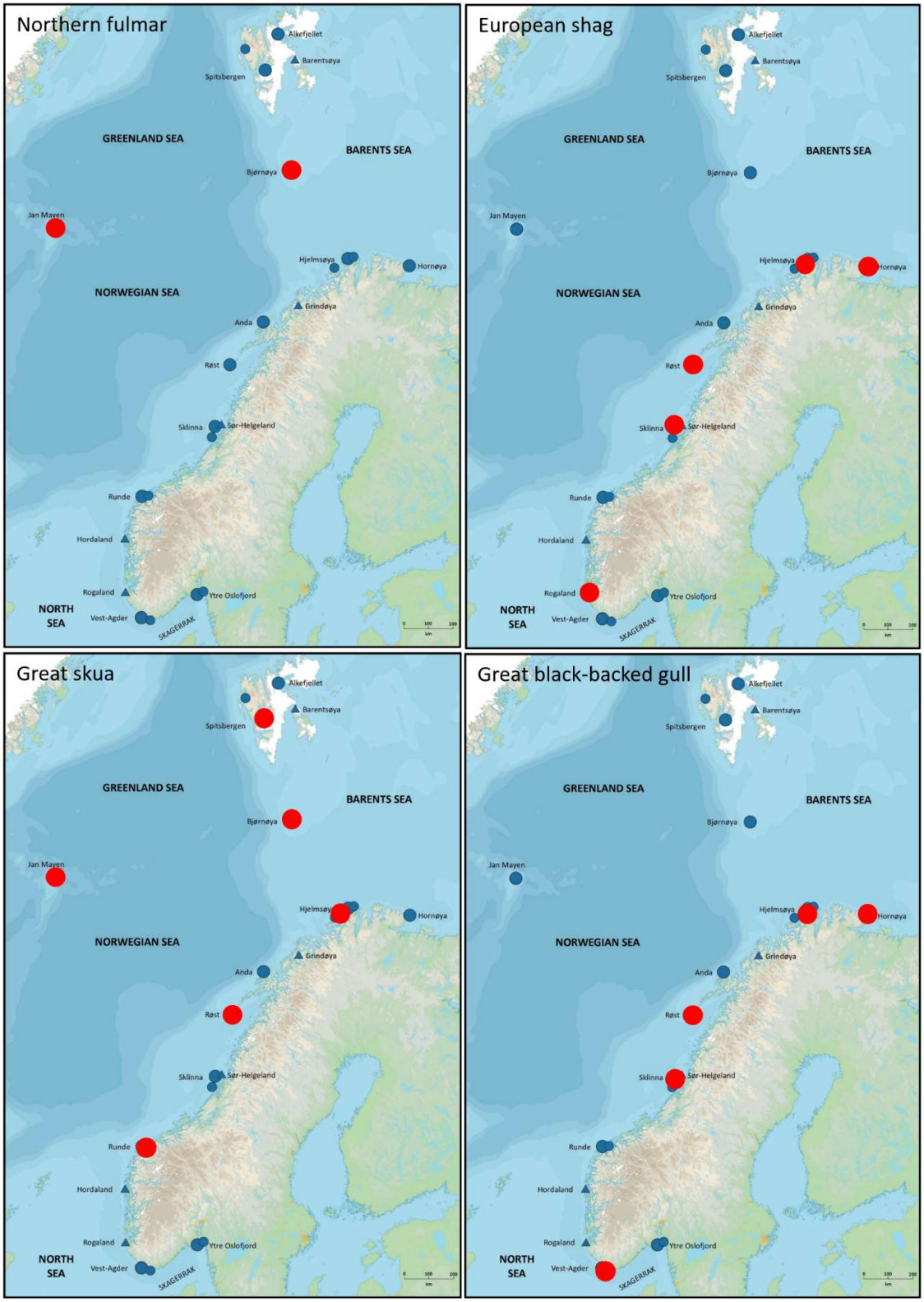

***Figure 2**. Possible study sites (in red) marked among SEAPOP keysites (in blue) for species selected for plastic monitoring (continued in **Figure 3**).*

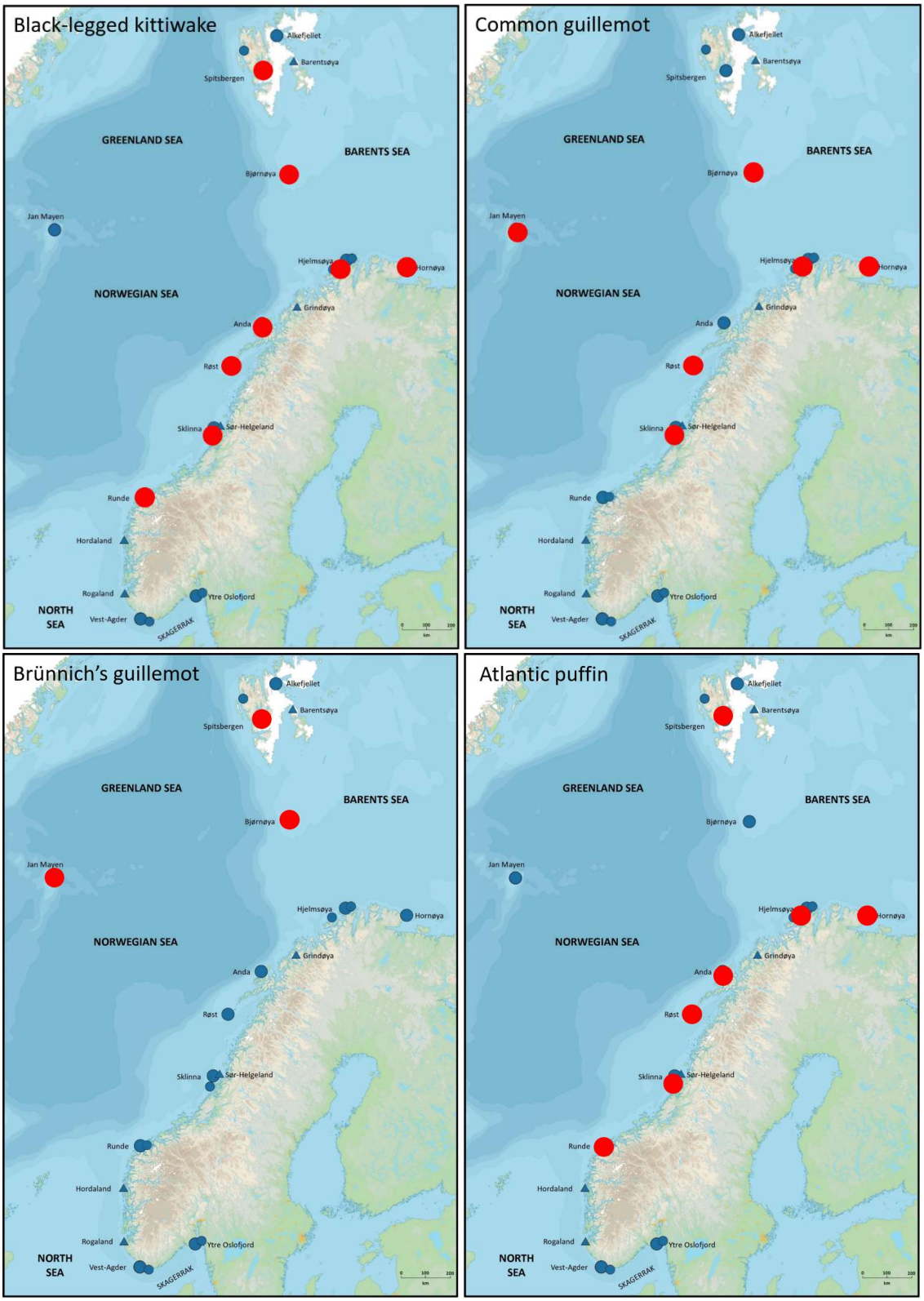

Figure 3. Continuation of **Figure 2**. Possible study sites (in red) marked among SEAPOP keysites (in blue) for species selected for plastic monitoring.

4.2 Selection of sampling materials, sample sizes and analytical procedures

Analytical procedures for determination of additives are complex and developing methods that can distinguish between ingestion of plastic by the birds versus accumulation of the same additive through the food web are currently still under development. For these kinds of studies, we furthermore need to get better information about which plastic polymers are ingested by the birds. Collecting materials that do not directly reflect the plastic ingestion (i.e. blood, feathers, preen gland oil, subcutaneous fat and eggs) is therefore not the right way forward.

When focussing on live birds, the study design is limited to non-destructive collection of faeces and regurgitates as well as fresh prey items carried by some species. Dead birds should therefore be collected in addition where / whenever possible and used as comprehensively as possible, prioritizing analyses of plastics contained in their stomachs and remaining intestinal system.

From an economical and scientifically relevance point of view, the quantification of plastic particles, including the fraction of ≥ 250 µm ≤ 1 mm (both in number and mass, if appropriate) is the main priority, followed by the determination (second priority) of the polymer types via FTIR or Raman spectroscopy. The determination of additives in tissue samples (e.g. from dead birds) only forms the final (third) step of the analytical procedure.

For the northern fulmar in the North Sea, a sample size of at least 40 birds was required annually over a period of 4-8 years, to detect a 25% change in the mass of ingested plastic (O'Hanlon et al. 2017, van Franeker et al. 2011). However, the annual sample sizes required to detect a change depend inevitably on species, location, the prevalence of ingested plastic and the level of change over time (Provencher et al. 2015). For our set of selected species, we neither know their current prevalence of ingested plastic, nor variation among sample sites. Determining optimal sample sizes with regards to both scientific outcome and feasibility from a practical and financial point of view is therefore impossible at the current state in time. Before starting a long-term monitoring effort for plastic ingestion under SEAPOP, we therefore see the strong need for some pilot studies that help us identify this basic information and optimize the study design for the envisaged long-term monitoring of plastic ingestion by seabirds within SEAPOP.

4.3 Recommended preliminary studies

4.3.1 Determine frequency of occurrence of plastic in regurgitates/fresh prey items and faeces of different seabird species at different breeding sites

Focussing on the species of key interest (section 4.1), the aim is to obtain samples (realistically 20-30 per species / material / colony) from all available field sites (highlighted in red in **Figures 1 and 2**) to determine baseline information about the frequency of occurrence of plastic in both regurgitates and faeces. With this information, it will be possible to assess comparability in results between regurgitates and faeces, spatial variation in frequency of occurrence in plastic in each of the species and determine the minimum required sample sizes for long-term monitoring.

4.3.2 Assess if frequency of occurrence of plastic in dietary samples varies with time over the course of the breeding season

Temporal variation in plastic intake within the breeding season is a realistic possibility, for example if parental birds are changing their foraging behaviour or diet from incubation to chick

rearing (present in great cormorants; Lehikoinen 2005). Being aware of such potential temporal variation is important before setting up a long-term monitoring programme. For practical and cost-related reasons, we here focus on two species at three locations: Atlantic puffins at Anda and Røst and European shags at Sklinna. Diet samples in the form of whole beak loads (1-40 individual whole fish per beak load, depending on species and age class) of Atlantic puffins have been collected during several years at both sites throughout the entire breeding season at 5-day intervals and are stored frozen and are readily available for analyses. Diet samples in the form of pellets can be collected from shags at Sklinna in a similar way (20-30 at 5-day periods over the entire breeding season).

4.3.3 Test and optimize techniques in the field and in the lab

Since we aim for a monitoring of particles in the range of ≥ 250 µm ≤ 5mm, contamination avoidance is very important both in the field and in the lab, but this can also increase costs dramatically and therefore threaten the feasibility of the planned monitoring. We therefore see the need to optimize the techniques in the field and in the lab. For example, using metal spatulas in the field only a single time means a large number of metal spatulas need to be purchased, which is a significant cost-factor. Possible alternatives could be to clean spatulas in the field and rinse them off using filtered water and/or ethanol or experimenting with single-use plastic free corn-starch spoons or wooden sticks (e.g. ice cream sticks or coffee stir sticks). Corn starch spoons could also be a more practical solution in the field when trying to manoeuvre a complete, yet sticky faeces sample from the ground into a sampling vial. Since corn starch can be enzymatically digested together with the remaining organic material of the sample, it could be possible to place the sampling stick or the part that touched the sample into the vial as well. In the lab, optimization is needed around the protocol to digest organic matter of different material and to screen samples for plastic particles.

4.3.4 Need for an exposure study

In addition to the above listed pilot studies, we identified the need for an experimental exposure study under controlled conditions, ideally on northern fulmars, to assess the actual health consequences of plastic ingestion for birds. This need arises since the 0.1 g threshold of plastic per bird defined in the OSPAR monitoring is an arbitrary threshold and we in fact – after more than 25 years of monitoring – still do not know the implications of ingesting this amount of plastic. However, such knowledge is desirable not only for OSPAR to assess the meaningfulness of the defined threshold and possibly adapting the threshold. Furthermore, this information from a controlled experiment would also help to assess the implications of plastic ingestion for wild seabird populations.

4.4 Final conclusions

Plastic pollution is ubiquitous, increasingly problematic and comes with potentially high risks for wildlife. Plastic pollution also receives growing attention by the general public, media and politicians. Yet, very little scientific evidence is currently available to determine the impact of plastic on wildlife health, survival and ultimately populations. While seabirds worldwide are among the most threatened groups of birds, and their populations are at risk from many different threats, the current state of knowledge and ongoing monitoring schemes do not allow a quantitative assessment of the impact of plastic pollution on seabird populations. Such information is much needed – not least for decision-making processes in national and international politics and conservation management.

Our workshop identified a road map to close some of the existing knowledge gaps and deliver much needed information for seabirds across a large geographic scale, from the high Arctic to southern Norway. With plastic pollution being a global phenomenon, we emphasize the importance for large-scale coordinated monitoring programmes, and therefore the relevance of this initiative also for OSPAR and possibly a trans-Atlantic coordination.

5 Acknowledgements

We would like to thank The Fram Centre for funding of this workshop as project # 112018 in the framework of the 2019 Plastic in the Arctic programme and providing excellent meeting facilities for the workshop at the centre. Geir Helge Systad provided helpful comments on an earlier draft of the manuscript.

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Appendix 1. List of workshop participants

Name	Organisation
Tycho Anker-Nilssen	Norwegian Institute for Nature Research (NINA)
Signe Christensen-Dalsgaard	Norwegian Institute for Nature Research (NINA)
Nina Dehnhard	Norwegian Institute for Nature Research (NINA)
Sveinn Are Hanssen	Norwegian Institute for Nature Research (NINA)
Magdalena Langset	Norwegian Institute for Nature Research (NINA)
Tone Kristin Reiertsen	Norwegian Institute for Nature Research (NINA)
Dorte Herzke	Norwegian Institute for Air Research (NILU)
Amalie Ask	Norwegian Polar Institute (NPI)
Sebastien Descamps	Norwegian Polar Institute (NPI)
Geir Wing Gabrielsen	Norwegian Polar Institute (NPI)
Ingeborg Hallanger	Norwegian Polar Institute (NPI)
Hallvard Strøm	Norwegian Polar Institute (NPI)
Laura Monclús	Norwegian University of Science and Technology (NTNU)
Nina O'Hanlon*	Environmental Research Institute (ERI), University of the Highlands and Islands

* attending via Skype

Appendix 2. Field sampling protocol

! Avoid fleece and synthetics while collecting samples, choose cotton or wool instead!

! Remember to take BLANKS – 3 per species / site / material!

! When opening glass sample vials, don't touch the inside of the aluminium foil which is covering the top. Keep the aluminium foil within reach

Sampling of pellets: Use a metal spatula and/or nitrile gloves (a new one for each pellet!) to lift pellets off the ground. Discard pellets that are not fresh, not complete, or where plastic contamination is visible on the ground surrounding the pellet. For blanks, wave the spatula or your hand with a nitrile glove over the opened vial for as long as it takes to place a pellet into it.

Sampling of regurgitates during handling of birds: Try to anticipate when the bird is regurgitating. Let it regurgitate into the funnel (with the sampling vial below) or onto a PVC-apron (and move the regurgitate with a spatula into the glass vial afterwards). For blanks, place the empty/clean funnel into the vial and remove it again, or scratch with the spatula over the clean apron and move the spatula into the vial, respectively.

Sampling of fresh prey items: Use forceps to place the entire prey items into glass vials. For blanks, wave the forceps above the opened glass vial for as long as it takes to place the fish into the vial.

Sampling of faeces from the ground: Apply the same technique as for pellets. Use a metal spatula or nitrile gloves (a new one for each sample!) to lift faeces samples off the ground. Discard faeces that are not fresh, have been disturbed (e.g. bird walking over it) or where plastic contamination is visible on the ground surrounding the faeces. If on loose soil, try to collect as little earth / sand as possible with the sample (take only the upper layer). For blanks, wave the spatula over the opened vial for as long as it takes to place the faeces sample into it.

Sampling of faeces from birds during handling: Similar as with the regurgitates during handling, if possible let the birds defaecate into a funnel with the glass vial underneath. Otherwise, collect the faeces from the apron. For blanks, place the empty/clean funnel into the vial and remove it again, or scratch with the spatula over the clean apron and move the spatula into the vial, respectively.

Clean funnels, aprons after every sample using filtered water /ethanol

AFTER placing the sample into the glass vial, put the original double-layer of aluminium foil back on the top of the vial. Then screw the plastic lid on top. Store upright and frozen (-20°C).

Initial suggestion of sample sizes per species and site for pilot studies:

- Pellets/fresh regurgitates: 20-30
- Fresh prey: 20-30 beak-loads. Depending on species, site and year one beak-load may vary between a single 20 cm long cod or 40 sandeel larvae. Collecting a diverse range of prey species and size/age classes is to be preferred over obtaining > 40 fish larvae of the same size/age class & species.
- Faeces: 20-30

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The Norwegian Institute for Nature Research, NINA, *is as an independent foundation focusing on environmental research, emphasizing the interaction between human society, natural resources and biodiversity.*

NINA was established in 1988. The headquarters are located in Trondheim, with branches in Tromsø, Lillehammer, Bergen and Oslo. In addition, NINA owns and runs the aquatic research station for wild fish at Ims in Rogaland and the arctic fox breeding center at Oppdal.

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NINA Report

ISSN: 1504-3312
ISBN: 978-82-426-3470-2

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