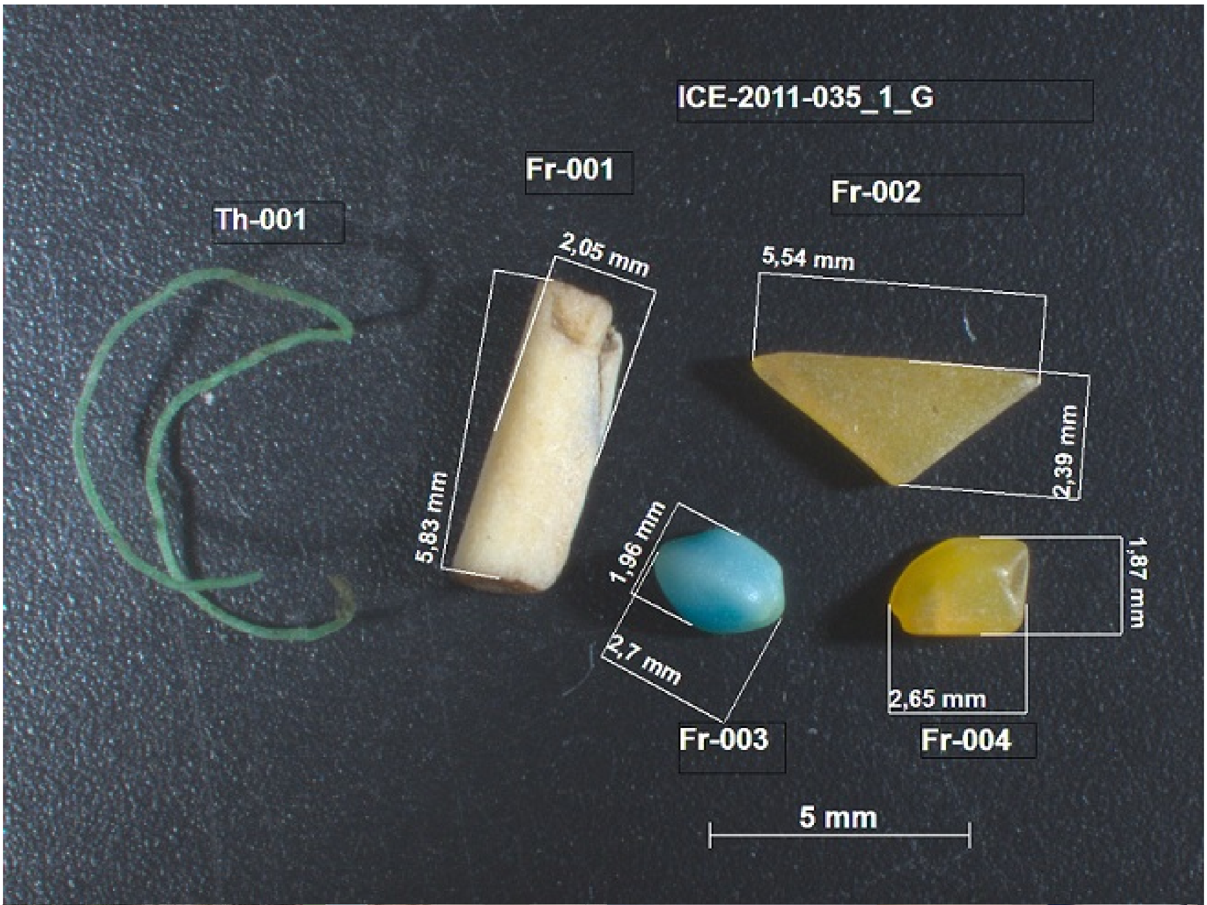
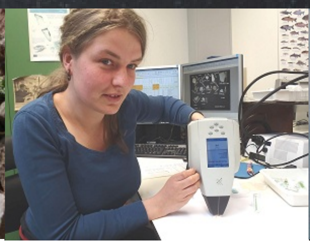
Composition and characteristics of plastic in the stomachs of northern fulmars (*Fulmarus glacialis*)

Bachelor Thesis
Coastal Zone Management

Susanne Kühn





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Composition and characteristics of plastic in the stomachs of northern fulmars (*Fulmarus glacialis*)

Bachelor Thesis

Project number: 594000

Bsc student: Susanne Kühn (870122004)

Supervisors: Jan Andries van Franeker (IMARES)
Arjen Strijkstra (Van Hall Larenstein)
Peter Hofman (Van Hall Larenstein)

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University of applied science "Van Hall Larenstein", Leeuwarden

IMARES - Institute for Marine Resources and Ecosystem Studies, Texel

Photos first page: Plastic pieces from stomachs of Icelandic northern fulmars (by S. Kühn); northern fulmar on Iceland (by S. Kühn); measurements with the Phazir NIR (by J.A. van Franeker); typical graph of polyethylene on the Phazir (by S. Kühn); typical result of the Phazir (by S. Kühn)

Preface and acknowledgements

In the framework of this bachelor thesis of Coastal Zone Management the characteristics of plastic, ingested by northern fulmars in Europe, has been studied. My previous research dealt with regional differences in quantities of plastics by northern fulmars, and added Iceland as a new location to the existing dataseries (Kühn & Van Franeker 2012). Finding evidence for possible harm of plastic to birds is a difficult process. This bachelor thesis research at IMARES/Texel supervised by Jan van Franeker is a contribution to better understanding by firstly identification of plastic types found in the birds, and secondly by studying the mechanical breakdown process of plastics in the stomachs.

I am especially thankful for the guidance of my supervisor Jan Andries van Franeker. Thanks to his knowledge, experience and critical mind I have learnt a lot. Our conversations were always inspiring and with his dedication to this work he will always be a great role model for me. Further I want to thank my supervisors from university, Arjen Strijkstra and Peter Hofman for their confidence and advise whenever necessary.

Arend Bolt of Van Gansewinkel Groep made it possible for us to work with their handheld infrared spectroscope “Phazir” to identify plastic types, without which this project would have been impossible .For further detail and validation of the Phazir data we received infrared spectroscope support from Denka Hristova and Pauline Schmit of the Department of Chemical Engineering of the Technical University Eindhoven. At the research institute IMARES Elisa Bravo Rebolledo did not only provided a lot of necessary data but also good company. André Meijboom always knew answers and solutions for all my laboratory questions and problems. Martin de Jong helped with taking time for my research. All colleagues from IMARES welcomed me warmly and were good company during my stay.

After long days working, there were not only a cup of tea and a warm meal waiting for me but also Job ten Horn, thank you for that!

Susanne Kühn
Texel, 20. August, 2012

Summary

This bachelor thesis reports on a study conducted at the research institute IMARES in the Netherlands. It focused on plastics ingested by northern fulmars (*Fulmarus glacialis*) around the North Sea region as a contribution to a better understanding of the fulmar in its role as an indicator for marine litter pollution in European marine policies.

The research was divided in two parts. The first part concerned the identification of plastic materials using infrared spectroscopy, and analyses the differences between regions and time periods. Identification of plastic types is not only relevant in the assessment of chemical hazards from ingestion, but it also contributes to the monitoring of fulmars as an indicator for Good Environmental Status (GES) study in the European Marine Strategy Framework Directive (MSFD). Preponderance of floating plastics such as polyethylene and polypropylene indicates that the fulmar is largely a surface pollution indicator, and that indirect secondary ingestion from deeper water layers through fish is less important.

The second part of the research looked into the ability of fulmars to grind plastic materials in their stomachs. In a blind test, colleagues were asked to categorize plastic pieces from unknown origin into four categories of ‘wear’. Results provide evidence for gradual grinding of plastics in the muscular stomach. Regional differences in wear suggest that part of plastics seen in stomachs of fulmars from higher latitudes may have been picked up by these birds in more polluted wintering areas.

Both study components are new and provide essential building stones for environmental monitoring and associated policy decisions concerning marine debris in European seas and beyond.

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1. Introduction

Plastic in stomachs of seabirds and especially northern fulmars (*Fulmarus glacialis*) is a well-recognised problem (van Franeker *et al.,* 2011). Plastic products are lost or dumped by sea-based activities and also via air and rivers the stream of plastic products reaches the oceans. (Wurpel *et al.,* 2011). Especially smaller plastic particles are an issue of growing concern. Light-weighted plastic floats on the surface or in pelagic waters although it gets bio-fouled and may sink on the ocean floor. Heavier plastic materials can sink directly to the ocean floor and remain in the sediment. Especially floating plastic has many environmental concerns. Entangled animals result in mortality and animals ingesting plastics run into danger of constipation and a false feeling of satiety (Gregory, 2009).

Most plastics are polymers from fossil raw materials, mainly petroleum. Polymers are formed by long-chained monomers. Substances are added to influence characteristics such as colour, softness, flame retardance, flexibility, etc.. Some of these “additives” are not integrated into the plastic structure and can leach out (Sakai *et al.,* 2000). At sea plastics are known to adsorb surrounding Persistent Organic Pollutants (POPs) such as toxic substances as pesticides (Mato *et al.,* 2001).

Plastic pollution of marine habitats is a problem of global scale. Plastic can reach every part of the sea. High densities of plastic close to urban sources distribute up to polar regions via currents and local winds (Barnes *et al*., 2009). As global plastic production increase 5% per year (Andrady & Neal, 2009), also the disposal and loss of plastic increases (Gregory, 2009). Insufficient lifecycle assessments and human behaviour intensify the problem (Wurpel *et al.,* 2011).

North Atlantic seabirds are known to consume plastic regularly (Moser & Lee, 1992). Procellariiformes as the northern fulmar are used to measure the quantity of floating plastic in different regions of their distribution area (van Franeker *et al.,* 2011). Since the 1980ies van Franeker measures the trends of pollution levels in fulmars, as they are known to forage exclusively at sea and do not regurgitate harder items regularly (van Franeker, 1985; van Franeker & Meijboom, 2002). 95% of fulmars in the North Sea contain plastic in their stomachs (van Franeker *et al.,* 2011).

This long-term research is used by the “Oslo and Paris Conventions for the protection of the marine environment of the North-East Atlantic” (OSPAR) to implement goals for the protection of the sea, called “Ecological Quality Objectives” (EcoQO). One of these goals aims the reduction of plastic pollution in the OSPAR area. The fulmar monitoring has been included as one of the indicators for a good environmental status.(OSPAR, 2008). In the European “Marine Strategy Framework Directive” (MSFD) the avoidance of “harm” is a key issue. For environmental harm of plastic in the sea it is substantial to identify acceptable levels that do not harm the environment (Galgani *et al.,* 2010). In 2020 a “Good Environmental Status” (GES) has to be reached by EU member states.

As plastic is known to contain and adsorb chemicals from the surrounding sea water the question arises to what extend fulmars, grinding up plastic particles in their stomachs, are affected by leaching substances that can be adsorbed through their tissues. Consequences are potential carcinogenic, toxic or endocrine disruption effects (Thompson *et al.,* 2009). Earlier research has shown that there seems to be a positive correlation between plastic ingestion by seabirds and the exposure of PCB (Yamashita *et al.,* 2011; Ryan *et al.,* 1988).

Aim of this research is to increase the understanding of important processes occurring when fulmars ingests plastic, regarding processes of accumulation, degradation, gut passage and the potential of leaching toxic chemicals. Therefore it is important to know what kind of plastic do fulmars ingest (chapter 5.1. and 6.1.) and how it is fragmented inside birds (chapter 5.2. and 6.2.). Such issues are basic elements to a better understanding of harm levels to marine wildlife. Information collected will contribute to publications about the problem of accumulating plastic in the marine environment. The results can be used in future European projects that provide a scientific and technical basis for monitoring the European seas within the context of the MSFD.

2. Research questions

Different kind of plastic vary in the ability to adsorb chemicals during manufacturing and after release to the ocean (Teuten *et al.,* 2009), thus it is essential to know what kind of plastic do northern fulmars ingest. This forms the first part of this bachelor thesis and can be the basis of future ecotoxicological research.

1. Which plastic types (PE, PP, PET, etc.) occur in the stomachs of northern fulmars?

    1a. Can heavy plastic be identified that fulmars ingest via other prey?
    1b. Is there a correlation between the region and the characteristics of plastic pieces?

If the plastic is identified the specific weight can be associated. At the moment research on fulmars assumes that the plastic is taken up on the water surface. However, heavier plastic could express the ability to take up plastic via fish that consumed plastic. This information can be important to assess the fulmar as an indicator for plastic pollution, as until now, the fulmar is only used for surface assessments.

It is important to understand how plastic behaves inside a bird. From Antarctic research it is hypothesised that birds are grinding plastic in their stomachs until they can excrete microscopically small particles (van Franeker & Bell, 1988). Van Franeker suggests that harder plastic items may lose up to 75% of their mass in one month. Ryan and Jackson (1987) came to the result that polyethylene pellets have a half-live time of minimum one year. The research questions are listed below, the methods are explained in chapter 5.

2. How do northern fulmars digest plastic particles?

    2a. How do plastic particles fragment in stomachs?
    2b. Are there differences in plastic categories?

3. Plastic characteristics

BOX 1
Plastic types and their characteristics

To answer the research questions it is important to know the plastic characteristics. The information of this box origins from Abts (2010), if not cited different.

Plastic has a successful history. In less than one century plastic developed from almost unknown towards a product we cannot imagine living without. In 1950 1.5 million tons of plastic were produced globally, in 2009, 230 million tons entered our world (Plastic Europe, 2010).

Plastic use convinces through its positive characteristics of flexible design, slow degradation, water resistance, air impermeability, low weight and low production costs (Andrady & Neal, 2009).

Lithner *et al.* (2011) classified plastic polymers following Annex VI of the EU classification, labelling and packaging regulation (CLP). The hazard level are categorized into 5 hazard classes with *I* the less harmful and *V* the most harmful level (details shown in Annex I). This categorization can be useful when thinking in terms of ecotoxicity because it is based on leaching behaviour of plastic. For this study, it just gives a first indication for this study because it only copes with hazard classification standards and not with exposure.

Polyethylene (PE)
PE is the plastic which is most produced during the last years with a share of 30%. Its hazard category is II (Lithner *et al.,* 2011). PE is divided into high density polyethylene (HDPE) (density: 0.96 g/cm³) and low density polyethylene (LDPE) (0.914 g/cm³). PE is generally used for packaging material and plastic bags (jerrycans, pipes, isolation material, etc.). It is highly resistible against many acids, bases, oils and fats, however to be inflammable and weatherproof, additives as flame retardants and UV stabilizers are necessary.

Polypropylene (PP)
PP is with 20% share the second highest plastic produced, but at the moment it is the plastic material with the highest production growth. It is comparable to Polyethylene but much more harder and tighter than PE. PP is more sensitive for acids and oxidants as PE and also flammable and sensitive for UV radiation, wherefore additives are necessary. The density of PP is 0.90-0.907 g/cm³. PP is generally used for ropes, bottle caps and netting materials (Andrady, 2011).

Research on PP industrial pellets has shown that they absorb toxics like PCB and DDT from surrounding seawater (Mato *et al.*, 2001). However, in the hazard classification data it ranks at one of the less toxic plastics, in category I (Lithner *et al.,* 2011).

Polyvinylchloride (PVC)
PVC has the third biggest share of global production with 15%. It has a high share of chlorine which leads to a high density. Unplasticised PVC (PVC-U) has a density of 1.38-1.4 g/cm³ and the plasticised PVC (PVC-P) density depends on the amount of plasticisers added. Because of the softness of PVC necessary stabilizers as heavy metals (lead, cadmium) can influence the density and can also affect organisms' health. Plasticisers (e.g. Phthalates) are also discussed for having effects on health because they leach out of the plastic easily. Following the EU categories of Lithner *et al.* (2011), PVC belongs to the most hazardous plastic types of category V. PVC is resistible against acids, bases, oils, fats and alcohols. It is used as construction material as pipes, floor covers and foams.

Polystyrene (PS)
PS has a global production share of 10%. It belongs to the less hazardous plastics (II) (Lithner *et al.*, 2011). PS is resistible against low concentrations of acids and bases but not against oxidants. PS is used for packaging and, if foamed as insulation or packaging material. The density of PS is 1.03-1.05 g/cm³.

Acrylonitrile Butadiene Styrene (ABS)
ABS is used for car interiors and applied for electronica. The density is between 1.03-1.07 g/cm³. The toxicity is, according to Lithner *et al.* (2011), high (category V).

Polyamide (PA)
PA is usually produced as fibres. It is a tough and strong material, also known as nylon. The density varies between 1.02 g/cm³ and 1.14 cm/g³. PA belongs to the hazard categories III (Lithner *et al.*, 2011). It is sensitive to acids, concentrated formic acids are already breaking up the material. For the utilization outside, UV stabilizers need to be added. Its common use is car interiors, engine applications and especially fishery gear.

Polyethylene Terephthalate (PET)
Fibres of PET are used for packaging, but they are also produced from recycled PET beverage bottles. Most of the bottles are recycled to polyester fibres for fleece jackets. 7% of the world plastic production is PET (Andrady, 2011). According to Lithner *et al*. (2011) PET belongs to the less hazardous plastics in category II. The density is 1.37 g/cm³.

3.1. Negative effects of plastic

As well as there are many positive characteristics of plastic (see BOX 1), the same characteristics become problematic for disposal issues. Due to single-use applications and slow degradation, plastic fragments accumulate in the environment (Barnes *et al.,* 2009). Plastic can degrade up to some point through photo degradation, oxidation and abrasion (Andrady, 2003) but especially in marine environments cooling effects and salt water slow this process down (Gregory, 1999).

Almost half of the plastic materials are buoyant (US EPA, 2006) and float around the ocean surfaces, until they get too heavy through bio-fouling (Lobelle & Cunliffe, 2011). Other types of plastic (e.g. PET, nylon) have a higher density than water and can sink to the ground, where degradation takes even more time due to a lack of UV radiation (Barnes *et al.,* 2009).

3.1.1. Socio-Economic effects

Disposing plastic causes socio-economic disadvantages, regarding that plastic is made of fossil fuel and often dumped after single use, a valuable resource is lost. For the production of plastics 4% of the whole petroleum feedstock is used and almost the same quantity is needed for production processes (Andrady & Neal, 2009). Disposed plastic is not only aesthetically unattractive so that people avoid e.g. polluted beaches, it also costs municipalities a lot of money and effort to clean it up. At sea plastic debris also causes economic problems. Fishermen are confronted with plastic by-catch and ships risk manoeuvrability if plastic entangles propellers or obstructed cooling water intakes (van Franeker & Meijboom, 2002).

3.1.2. Ecological effects

During the last decades also ecological concerns increased. Already in 1997 Laist made a list of 250 marine species that were either entangled or had ingested plastic, including almost all top predators of the oceans (seabirds, whales, seals, otters, fish and also crustacean). Entanglement and ghost net fishing causes, beside death also sub-lethal effects as skin lesions, ulcerating wounds, interruption of feeding activities and failed predator avoidance (Gregory, 1991).

Heavier plastic can reach sea floors at all depths (Galgani *et al.,* 2000). Plastic also becomes bio-fouled and sinks to the sea floor, it is not known how much longer degradation needs in such an environment.

The community of marine benthic organisms may be disturbed (Katsanevakis *et al.,* 2007) and plastic sheets can lead to anoxia on the sea floor due to interrupted gas exchanges (Goldberg, 1997).

The smaller the plastic particle, the higher the ratio surface and within the possibility to adsorb chemicals. Ingested by organisms it also can be accumulated by top predators up to humans on the top of the food chain (Koch & Calafat, 2009).

As mentioned earlier, the lifespan of plastic in organisms is difficult to predict. Early research of Ryan & Jackson (1987) has shown that plastic pellets of polyethylene ingested by petrels do have a half-life-time of one year. Studies from Antarctica cape petrels show a loss of hard plastic particles of 75% in one month (van Franeker & Bell, 1988). The particles are most likely gradually wearing off and braking into “dust” or pieces, small enough to pass the guts of the birds. Softer particles like foam or sheets, seem to be easier digested (van Franeker *et al.*, 2011). Fulmars do generally not regurgitate hard prey items, as other sea birds do, they only spit in case of danger or when feeding their chicks. However most of the hard items are saved in the smaller muscular stomach (gizzard) from where regurgitating seems to be impossible (Ryan & Jackson, 1987, van Franeker *et al.,* 2011).

4. European Policy

In 1972 first steps in international policy measures, regarding plastic pollution of the sea, were taken. The London Dumping convention came in 1972, later MARPOL was introduced. The MARPOL agreement provides regulation in Annex V for future NO-DISCHARGE regulations for ships. Annex V totally prohibits the dump of plastic into the sea (MARPOL, Annex V, 1973). On European level, in 2000 the EU directive on port reception facilities for ship-generated waste and cargo residues came in force (EU directive 2000/59). This directive regulates the (illegal) dump of waste into the marine environment through providing port facilities. All of these conventions are aimed to avoid sea-based pollution.

The Oslo-Paris Convention for the protection of the marine environment (OSPAR) came in force in 1998 amongst other declarations for all regions of European seas. It covers the whole North Sea region and large parts of the North-East Atlantic and has been ratified by bordering countries and other EU states. The goal is the protection of the marine environment of the North-East Atlantic. Increasing human activities lead to a high pressure on the marine environment. Especially the North Sea is heavily affected through fishery, shipping, pollution, oil and gas extraction (Johnson, 2011). “Ecological Quality Objectives” (EcoQO) are assessments and monitoring programs to meet the chosen goals. The effect of humans in the marine environment are measured. Clear indicators are required to measure the effects and to manage the OSPAR region. For each indicator desired levels of quality has been designed.

In 2008 the OSPAR Commission defined the objective for plastic litter in the North Sea as follows:

“There should be less than 10% of northern fulmars (Fulmarus glacialis) having more than 0,1 g plastic particles in the stomach in samples of 50 to 100 beach-washed fulmars from each 4 or 5 areas of the North Sea over a period of at least five years.”

This measurement is seen as the representation of an ecological indicator to measure the amount of plastic in the North Sea (OSPAR, 2008).

The EU Marine Strategy Framework Directive (MSFD) expects in 2012 a first assessment of marine litter in EU waters. Until 2020 every country has to designate a “good environmental status” (GES) about objectives they want to reach to improve the health of the marine environment (Galgani *et al.,* 2010). The GES of descriptor 10 “pollution at sea”, is defined as follows (EC, 2010):

“The characteristics and the amount of waste at sea may not have any negative effects on the coastal and marine environment.”

Therefore an indicator is necessary. For descriptor 10 the indicator 10.2.1. is determined as:

“Trends in the amount and composition of litter ingested by marine animals (e.g. stomach analysis”

The stomach analysis of fulmars will be used in areas where the birds occur. A Good Environmental Status could be formulated as:

“...e.g. x % annual reduction in the abundance of ingested litter”

For European policy the northern fulmar is a suitable indicator. This research can be a valuable component for future use of the fulmar as policy tool.

5. Methods & Material

5.1. Research question 1: Plastic characteristics

5.1.1. Material

The material to answer the question comes from different countries around the North Sea. It is known that number and mass of plastic found in stomachs of fulmars decreases with higher latitude (van Franeker *et al.,* 2011; Kühn & van Franeker, 2012).
That is why the number of birds used for this research differs as well as the number of pieces. From Iceland 50 stomach samples with plastic were available but the number of pieces is relatively low (340 pieces), however the Netherlands only have 22 samples from 2010 and a high number of pieces (695 pieces) (see table 1).
The sample from the Netherlands 1980's includes samples from 1982 to 1989.
A random sample is collected from the stormline (driftline) at Breezanddijk, Netherlands (53.0208˚N and 5.2060˚E) at February 19, 2012. The plastic was divided manually from the organic matter. A plankton splitter generated a random sample of 135 plastic pieces, of which 19 are industrial pellets and 116 are fragments. This sample can be used for comparison of plastic materials that beached among the coast line and plastic which was ingested by birds.

Table 1: Material used for identification with infrared spectroscopy

Country	n stomachs with plastic	n plastic pieces	Percentage
Iceland 2011	50	340	13.5
Faroe 2011	46	536	21.3
Netherlands 2010	22	695	27.6
Netherlands 1980's	58	812	32.2
Stormline 2012	n.a.	135	5.4
Total	**176**	**2518**	**100.0**

5.1.2. Method

Near Infrared spectroscopy "Phazir"

To identify the material of the plastic, Infrared spectrometers usually are used (Yamashita *et al.,* 2011; Browne *et al.,* 2011, Harrison *et al.,* 2012). For this research the Phazir Handheld Near Infrared Material analyser is used (DTS-PHAZIR-1624 for 1600-2400 nm) provided by the Dutch waste service provider "van Gansewinkel Groep b.v.". The reflection of the light source (in this case a standard light bulb) leaves a unique "fingerprint" of adsorbed light on a detector, which is linked to an integrated reference library (Blanco & Villarroya, 2002). The results are given as a spectrum of the plastic piece and three most comparable types of plastic that match best from the library ranked by the percent of conformity (see figure 1). It is a non-destructive technique that can scan one sample per second.

Figure 1: A typical Phazir result. A. suggestion of the most likely plastic. B. Graph of the reflection of infrared. C. Top categories, most likely types of plastic with additional percentage of certainty.

However, its ability is restricted. It cannot measure fully black and truly transparent objects and difficulties occur depending on size and shape, e.g. of very small items and threads. The table below (table 2) shows the materials the Phazir can identify and its abbreviations, also used in this report.

Table 2: Materials that can be identified by the Phazir

Abbreviations	Means
ABS	Acrylonitrile butadiene styrene
CA	Cellulose acetate
EVA	Ethylene-vinyl acetate
PA	Nylon (polyamide)
PB	Polybutylene
PBT	Polybutylene terephthalate
PC	Polycarbonate
PE	Polyethylene
PET	Polyethylene terephthalate
PETG	Polyethylene terephthalate glycol
PI	Polyimide
PMMA	Polymethyl metacrylate
PMP	Polymethyl pentane
POM	Acetal (Polyoxymethylene)
PP	Polypropylene
PPO	Polyphenylene oxide
PPS	Polyphenolyne sulfide
PS	Polystyrene
PSO	Polysulfone
PTT	Polytrimethylene terephthalate
PUR	Polyurethane
PVC	Polyvinyl chloride
SAN	Styrene acrylonitrile
TPV	Thermoplastic elastomer
Elastomer	Elastomer
Ionmer	Ionmer
Nylon/ABSblend	Nylon/ABSblend
Styrenic terpolymer	Styrenic terpolymer

The arbitrary decision was to consider plastic pieces for being “identified” from 80% accordance onwards. This decision was made to get a high sample number to work with. Unidentifiable pieces (<80% reliability) are labelled as “NoID”. In the presentation of results plastic categories that abundance is less than 1% each are grouped into “other”. This category includes cellulose acetate (CA), ethylene-vinyl acetate (EVA), Nylon-ABS blend, polybutylene (PB), Polycarbonate (PC) and polymethyl methacrylate (PMMA).

Again samples from Iceland, Faroe Islands and the Netherlands (different time and periods) are used for temporal and spatial comparison. Through getting insight in the characteristics of plastic, heavier pieces (that cannot float on seawater surface) must be ingested as secondary prey.

Using the information about plastic densities (see BOX 1) a comparison with water density is possible. Assuming that North Sea water has in average 35,000 mg salt per litre, with the average summer water temperature of 17˚C the density of the water is 1.02554 g/cm^3. In winter, with an average water temperature of 6 ˚C the density rises slightly to 1.02758 g/cm^3. Table 3 summarizes the densities of different plastic materials and compares it to sea water at different temperatures.

Table 3: Densities of plastic materials in comparison with salt water

	Saltwater 6˚C	Saltwater 17˚C	PE	PP	PVC	PS	ABS	PA	PET
Density (g/cm^3)	1.027	1.025	0.91-0.96	0.90-0.91	1.16-1.55	1.05-1.05	1.04-1.06	1.02-1.14	1.37-1.37

5.1.3. Method validation

This is the first time, that plastic from stomachs is identified by Phazir near infrared spectroscopy. Therefore a careful validation of the method and the data is necessary.

18 plastic pieces from different birds, beached at the Dutch coast, were measured with a Fourier transform infrared spectroscope (FTIR Varian 610IR) provided by the Department of Chemical Engineering of the Technical University Eindhoven. Absorption and transmission of infrared light are measured, after passing a sample (see figure 2). The results were compared with an integrated library and the 3 most comparable possibilities are presented. The results of the FTIR were compared to the results of the Phazir NIR to verify these results.

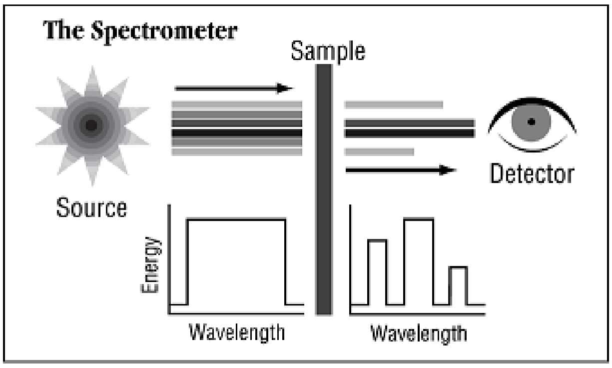


Figure 2: Function of an infrared spectroscope (ThermoNiclet, 2001)

For further validation of Phazir performance known plastic types were measured. Many plastic object are marked with a polymer recycling code (see figure 3). 16 pieces were measured with the Phazir NIR and compared to their “real” plastic type.

Figure 3: Measuring daily plastic items with the Phazir NIR and comparing it to the labelled plastic type (Photo: S. Kühn)

5.2.1. Background

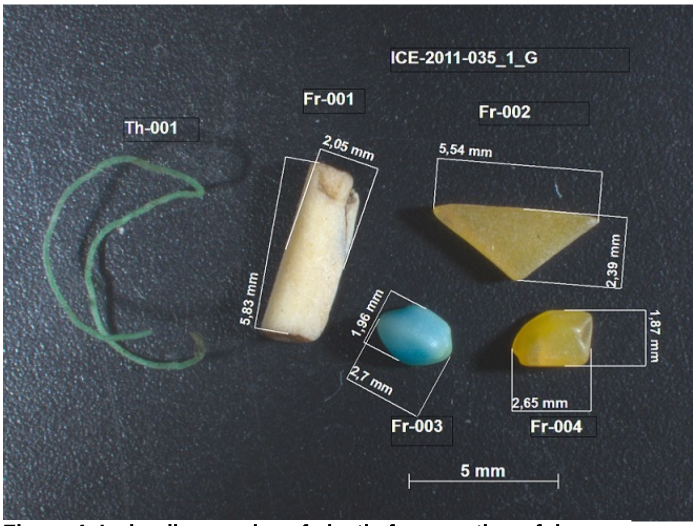


Figure 4: Icelandic samples of plastic from northern fulmars (S. Kühn)

To answer the second part of this study it was necessary to measure plastic particles in the different stomachs (Bravo Rebolledo, 2011). Samples from Faroe Islands, the Netherlands and Iceland from 2010/2011 were divided into the two stomachs (Proventriculus and Gizzard) and sieved separately on 1 mm mesh size. Research from Bravo Rebolledo (2011) has shown that there are only negligible quantities of plastic in stomachs and guts, sieved on 0.3 mm mesh size. The sample first was handled following the standard protocol of van Franeker and Meijboom (2002). Afterwards photos of every single plastic particle were taken with a Zeiss Stereo Microscope “Discovery V8” with an integrated camera. With the connected computer program Axio Vision 4.7. it was possible to measure length and width of each particle manually (see figure 4). With a digital sliding calliper the height was taken. To calculate the volume of the particle, a volume factor was used, assuming that a perfect cube has the factor 1. The plastic pieces have all different kinds of forms so the loss of the perfect cube volume between 0.1 up to 1 was estimated, keeping in mind that, for example a perfect spherule has the volume factor of 0.5236 and for cylinders 0.7853. Also the colour of each plastic piece was determined, using the range of colours standardized with the RAL system and the transparency of the plastic piece. Finally all pieces were weighed individually on a 0.0001 gram scale. Until now more than 8000 pieces have been measured individually. Using these data it was possible to measure the rate of movement from Proventriculus to the Gizzard and to compare the grinding rate between Proventriculus and Gizzard (Bravo Rebolledo, 2011).

5.2.2. Material grinding process

The sample used to answer this research question is a subsample of the first question. Van Franeker and Bell (1988) described that plastic can be digested in a stomach trough seasons. To avoid seasonal bias our current study samples were taken from birds that died in April/May. For the Faroe Islands and Iceland it means that birds are coming back from their wintering areas at sea to their breeding areas. From the Netherlands 12 birds were available. To keep up a comparable number of plastic pieces the first 28 birds from Iceland were included following the random numbering during earlier process (see table 4).

Table 4: Material used for grinding processes

Country	n birds	pieces	Percentage	Cumulative Percentage
Iceland 2011	28	128	23.4	23.4
Faroe Islands 2011	13	129	23.6	47
Netherlands 2010	12	155	28.3	75.3
Stormline 2012	n.a.	135	24.7	100
Total	**53**	**547**	**100**	

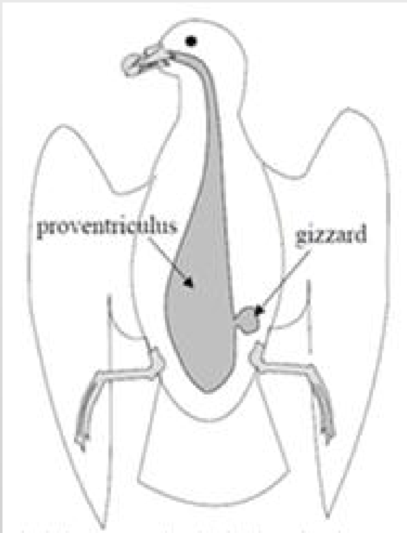
5.2.3. Method

A subsample of the material used in the first research question of this report was used. Samples from the Dutch coastline are taken to verify that the grindings come from a bird stomach and not from floating on the water surface or being grinded at beaches. As mentioned in chapter 5.1.1. also samples from the Dutch coastline were collected to compare grinding rates in birds and on coasts. To determine the grindings-grade 4 categories were introduced (0 not grinded – 3 very much grinded). The calibration of these categories were made individually by scientists from IMARES, already working with fulmar plastic (Jan Andries van Franeker, André Meijboom, Martin de Jong, Elisa Bravo Rebolledo). The categorization of pieces was made on photos made by Bravo Rebolledo and Kühn during the measurement process mentioned above (see chapter 5.2.1.).
For this question only fragments and pellets were used for determination because e.g. threads and foam are difficult to categorize into grinding rates that are used for this study.
Conducting "blind tests" the scientists did not know where the samples came from or what the purpose of the research was. An average per grinding grade and country were calculated. The results were tested on significance using Excel chi square tests.

The digestive tract of a fulmar has two stomachs (see BOX 2). Regarding the different tasks of Proventriculus and Gizzard, the plastic should be more worn in the gizzard because of its grinding activities. Therefore grinding rates between the 2 stomachs were compared.

BOX 2

Stomach morphology (van Franeker & Meijboom, 2002):



"Stomachs of Fulmars are basically structured as a two-unit system. Below the oesophagus ('throat') lies a large, soft-walled stomach. This so-called proventriculus is a bag-like structure extending throughout the abdominal cavity to nearly the position of the cloaca. During the breeding season, birds can store large quantities of food here, of over 25% of their body-mass (van Franeker, 2001), probably somewhat less during the nonbreeding seasons. Digestive processes start in the proventriculus. The fulmar and its allies are remarkable in the fact that they tend to accumulate considerable quantities of fatty fluids in the proventriculus, extracted from the food. The stomach oil is not only a valuable energy reserve under adverse conditions, it is also a powerful defence system against predators or competitors. They can spit out this oil with remarkable force and accuracy. The second stomach (gizzard) is much smaller, and has a hard muscular wall lined with a rough inner surface. Its function is to grind harder bits and pieces in the food mass to sizes that can pass on into the intestines. The hardest and indigestible prey items, like fish eye lenses and squid-jaws are not easily grinded and tend to accumulate in the gizzard over longer periods of time. The passage from proventriculus to gizzard is narrow in most petrels, and it seems that items once in the gizzard cannot go back. Because of this, if stomach contents are regurgitated, as in birds feeding chicks or birds spitting oil, it is only contents of the proventriculus that are ejected: the gizzard is not emptied. Like squid-jaws, hard plastic items accumulate in the gizzard and from there can only leave the body via the intestines. Although usually it seems that only amorf pre-digested substance can pass on into the intestines, the odd plastic particle can be found further down the intestine. Apparently, not always hard items need to be worn down completely in the gizzard before they are excreted. When ingestion rates of hard prey remains and plastic items are in excess of the rate of processing in the gizzard, the latter becomes totally filled and hard items also start accumulating in the proventriculus. This 'overflow' of accumulating plastics into the proventriculus is frequently the case in Fulmars from the North Sea region"

6. Results

6.1. Results Plastic identification

Table 5 shows the distribution of the 2518 plastic pieces that were used, divided into regions and years.
In each country around 19% of the pieces were impossible to identify due to the Phazir analyser restrictions, using the 80% identification level to split identified from not identified plastic.

Table 5: Distribution of birds and identified plastic pieces. First all plastic from bird stomachs was totalled up, then plastic from the Dutch coastline was added.

	number of birds	number of pieces	pieces identified	Percentage identified
Iceland	50	340	261	76.8
Faroe Islands	46	536	447	83.4
Netherlands 2010	22	694	569	82.0
Netherlands 1980's	58	813	672	82.7
Total plastic from stomachs	**176**	**2383**	**1949**	**81.8**
Stormline	n.a.	135	127	94.1
Total all pieces		**2518**	**2076**	**82.5.**

6.1.1.a Plastic types in fulmar stomachs

To calculate the composition of plastic pieces in northern fulmars, data from the stormline 2012 were excluded. All pieces from stomachs were categorized following standard protocol of van Franeker *et al.,* (2011).

Table 6: Distribution of measured plastics in categories in fulmar stomachs (without stormline)

	industrial pellet	probably industrial	sheet	thread	foam	fragment	other plastic	Total
Iceland 2011	14	9	39	25	55	197	1	**340 (14.3%)**
Faroe Islands 2011	36	1	49	35	50	361	4	**536 (22.5%)**
Netherlands 2010	80	0	77	42	188	299	9	**695 (29.2%)**
Netherlands 1980's	368	0	87	44	88	220	4	**811 (34%)**
Total	**494 (20.7%)**	**10 (0.4%)**	**253 (10.6%)**	**145 (6.1%)**	**384 (16.1%)**	**1079 (45.3%)**	**18 (0.8%)**	**2383 100%**

The composition of plastic materials from fulmar stomachs of all countries and all years is summarized in table 7. PE has the biggest share of the group with more than the half of the pieces (52%).

Table 7: Results of Phazir identification from fulmar stomachs using the 80% identification rule.

Material	Frequency (n)	Percentage (%)
PE	1245	52.2
PP	380	15.9
PVC	161	6.8
PET	66	2.8
PA	17	.7
PS	26	1.1
ABS	31	1.3
other	23	1.0
NoID	434	18.2
Total	**2383**	**100.0**

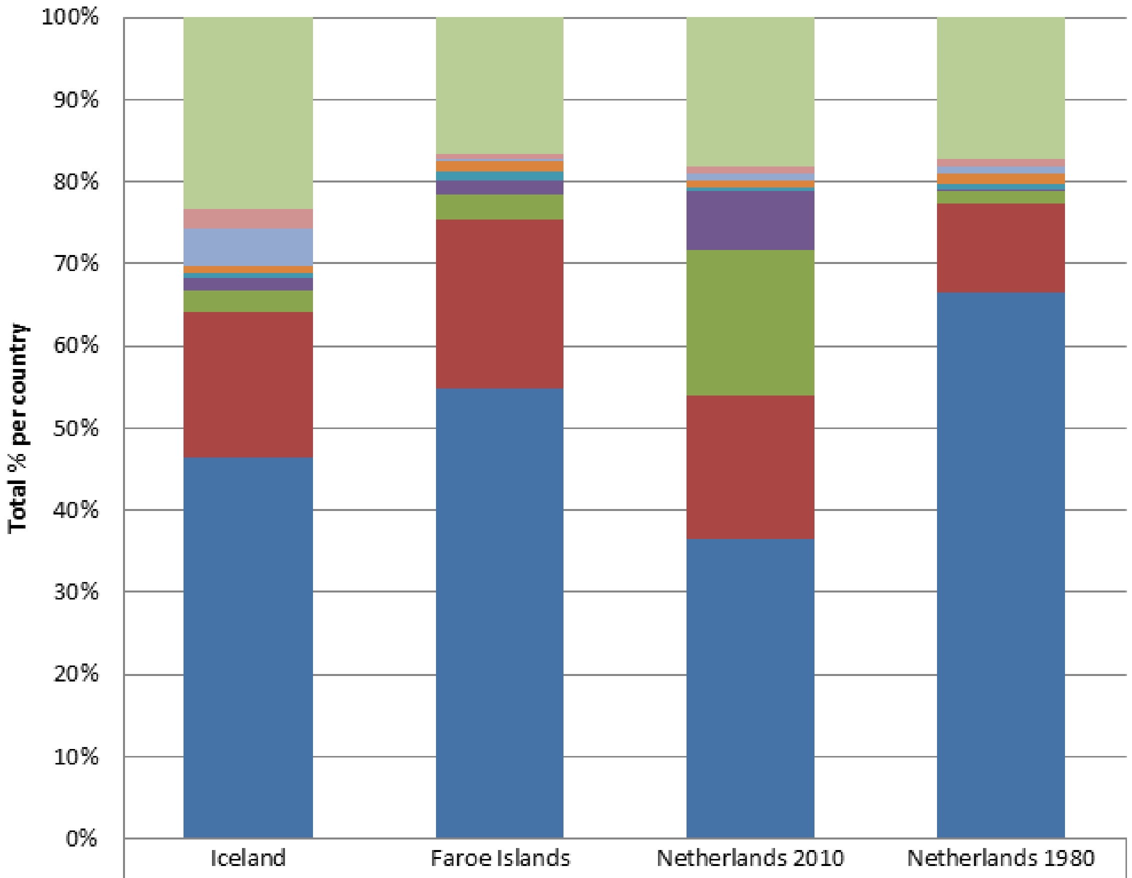
6.1.1.b Plastic types on the Dutch coastline

The random sample of plastic from the Dutch coastline was measured as well. The results of the measurements are shown in table 8. As mentioned before only fragments and industrial pellets were considered.

Table 8: Results of Phazir identification from the Dutch coastline, using the 80% identification rule

	Industrial pellet	Fragment	Total
NoID	2	6	**8**
PE	6	39	**45**
PP	11	71	**82**
Total	**19**	**116**	**135**

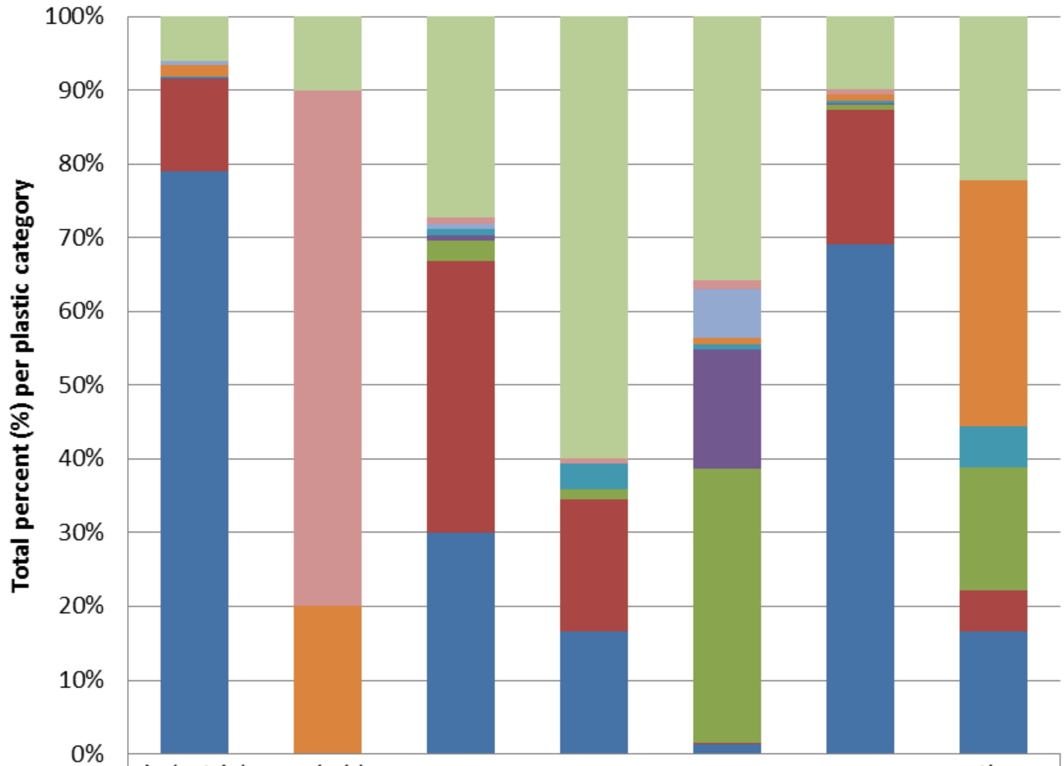
In figure 5 the distribution of plastic materials in different countries and the temporal comparison between Netherlands 2010 and Netherlands 1980's are shown. In the Netherlands 2010 remarkable differences are visible. Many pieces of plastic there are made of PVC (17.7%) and PET (7.1%), meanwhile the average of Iceland (ICE), Faroe Islands(FAE) and Netherlands 1980's is 2.4% for PVC and 1.2% for PET. In terms of number all countries differ significantly from each other (ICE-FAE p <0.001, ICE-NET2010 p <0.001, FAE-NET2010 p <0.001). Also on temporal scale all countries differ from the results of the Netherlands 1980 (p <0.001).



	Iceland	Faroe Islands	Netherlands 2010	Netherlands 1980
NoID	79	89	125	141
other	8	3	6	6
ABS	16	2	6	7
PS	3	6	7	10
PA	2	6	3	6
PET	5	10	49	2
PVC	9	16	123	13
PP	60	110	122	88
PE	158	294	253	540

Figure 5: Distribution of plastic material per area and time period

Looking into more detail to materials in figure 6 the plastic is divided into the standard categories (van Franeker *et al.,* 2011). Virgin industrial pellets and fragments are dominantly made of polyethylene. Sheets and threads are often made of polypropylene. Threads also have a relatively large share of polyamide, also known as nylon. In threads also the share of unidentified items is the largest. Foam differs remarkably, most of the pieces are made of PVC (37.1%) and PET (16.2%). Because of the small expected numbers no chi square test was conducted.

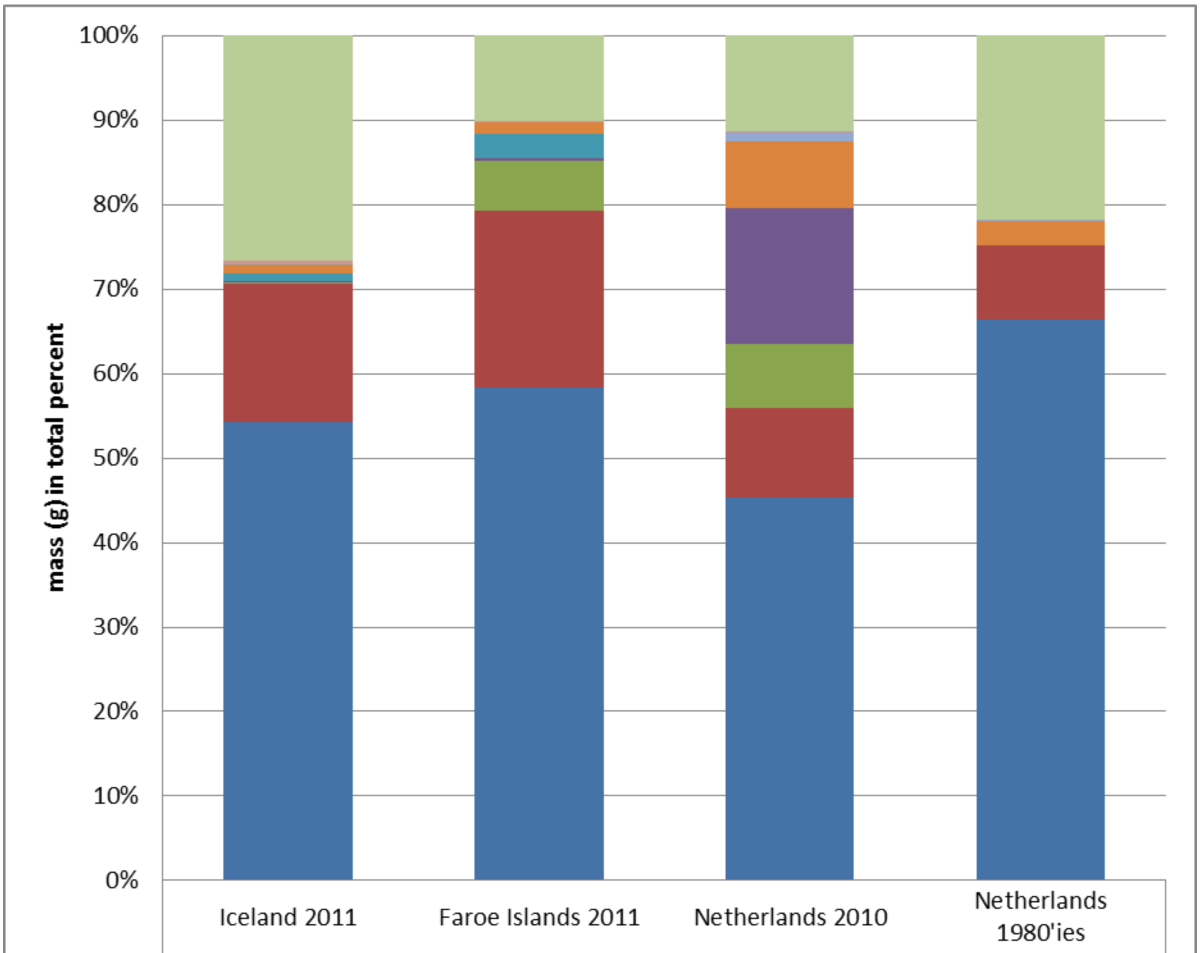


	industrial pellet	probably industrial	sheet	thread	foam	fragment	other plastic
NoID	30	1	69	87	137	106	4
other	0	7	2	1	5	8	0
ABS	3	0	2	0	25	1	0
PS	7	2	0	0	3	8	6
PA	1	0	2	5	3	5	1
PET	1	0	2	0	62	1	0
PVC	0	0	7	2	142	7	3
PP	62	0	93	26	1	197	1
PE	391	0	76	24	5	746	3

Figure 6: Distribution of plastic materials per category

6.1.2. Mass

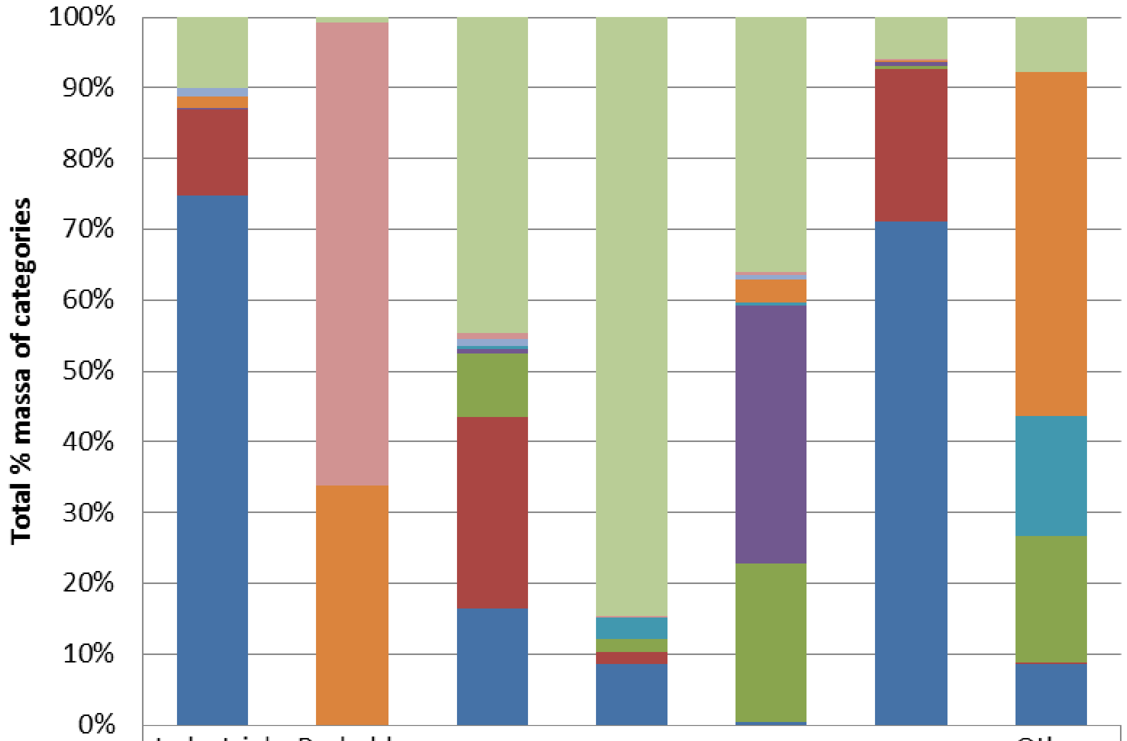
To measure the effects of plastic on birds rather than looking at the data by number of items it is more important to know the distribution of plastic in terms of mass. The numbers are calculated as an average per country and plastic type. The results are shown in figure 7. The picture given is almost similar to the results in numbers. PE again takes the biggest share for all countries, also in terms of mass. Only in the Netherlands 2010 PET is heavier in relation to PE which leads to a shift in the graph.



	Iceland 2011	Faroe Islands 2011	Netherlands 2010	Netherlands 1980'ies
NoID	0.0316	0.0175	0.034	0.0744
other	0.0003	0.0003	0.0008	0
ABS	0.0002	0.0001	0.0031	0.0004
PS	0.0013	0.0023	0.0235	0.01
PA	0.001	0.0049	0.0003	0
PET	0.0001	0.0006	0.0484	0
PVC	0.0003	0.0102	0.0227	0
PP	0.0193	0.0362	0.03186	0.03
PE	0.0642	0.1011	0.1365	0.2259

Figure 7: Mass of plastic pieces in different countries

The distribution of mass on different categories is shown in figure 8. Heavy threads seem difficult to determine, reflecting the general difficulty to identify threads (figure 6). Heavy foams (PVC & PET) form the largest share of mass in their category.



	Industrial pellet	Probably industrial	Sheet	Thread	Foam	Fragment	Other plastic
NoID	0.4877	0.0001	0.2525	1.4476	1.00068	0.8148	0.0964
other	0	0.0085	0.0046	0.0026	0.0105	0.0193	0
ABS	0.0602	0	0.0061	0	0.0202	0.0001	0
PS	0.0795	0.0044	0	0	0.0911	0.0428	0.5968
PA	0	0	0.002	0.0533	0.0073	0.0085	0.2093
PET	0.0076	0	0.0037	0	1.0152	0.072	0
PVC	0	0	0.0512	0.0294	0.6176	0.0692	0.2182
PP	0.58066	0	0.1522	0.029	0	2.95325	0.0025
PE	3.624	0	0.0931	0.1472	0.013	9.81605	0.1067

Figure 8: Mass of plastic pieces per plastic category

6.1.3. Plastic densities

Assuming that 12.6% of the number of plastic is of material which has a higher density than water (PA, PVC, PET, ABS, PS) it is necessary to look how these heavy plastics are produced. Most of them are foamed and float because of its integrated air spaces. Excluding foam, equal to 16% of the total n of pieces measured, only 3.3% “heavy”, in this case: heavier than saltwater, plastic remains (see table 9). Table 10 shows the distribution in terms of numbers of materials of all countries without foam. Taking away other and not identifiable plastics the percentage increases slightly to 3.7%.

Table 9: Distribution of plastic materials without foam by number of items

Density	Material	Frequency	Percentage	Cumulative percentage
light plastics	PE	1240	62.0	81.0%
	PP	379	19.0	
heavy plastics	PS	22	1.1	3.3%
	ABS	6	0.3	
	PVC	19	1.0	
	PET	4	0.2	
	PA	14	0.7	
	other	18	0.9	15.8%
	NoID	297	14.9	
Total		**1999**	**100**	**100%**

Table 10: Distribution of plastics without foam, others and not identified plastics by number of items

Density	Material	Frequency	Percentage	Cumulative percentage
light plastics	PE	1243	73.7	96.3%
	PP	381	22.6	
heavy plastics	PS	23	1.4	3.7%
	ABS	3	0.2	
	PVC	19	1.1	
	PET	4	0.2	
	PA	14	0.8	
Total		**1687**	**100.0**	**100%**

6.1.4. Toxicity

Following the indication of toxicity of Lithner *et al.* (2011) the plastics are grouped per level of toxicity (see table 11). Toxicity grade I is the less toxic category, V the most toxic one. None of the plastic found in the stomachs belonged to category IV.

Table 11: Levels of toxicity following Lithner *et al*. (2011)

Toxicity	I	II	III	IV	V
Plastic	PP	PE, PS, PET	PA	n.a.	PVC

The graph below (figure 9) compares the composition of toxic plastics per country based in their weight. Birds of Iceland and the Netherlands 1980's ingested mostly less toxic plastic. The Netherlands 2010 and the Faroe Islands show the biggest share of the most toxic category (V). Using chi square test, including the category "NoID" to test the differences Iceland differs significantly from the Faroe Islands ($p < 0.05$) and from the Netherlands 2010 ($p < 0.05$) but not from the Netherlands 1980's ($p = 0.25$). The Faroe Islands do not differ from the Netherlands 2010 ($p = 0.15$) but from the Netherlands 1980's ($p < 0.001$). The Netherlands 2010 and 1980's do not differ significantly ($p = 0.27$).

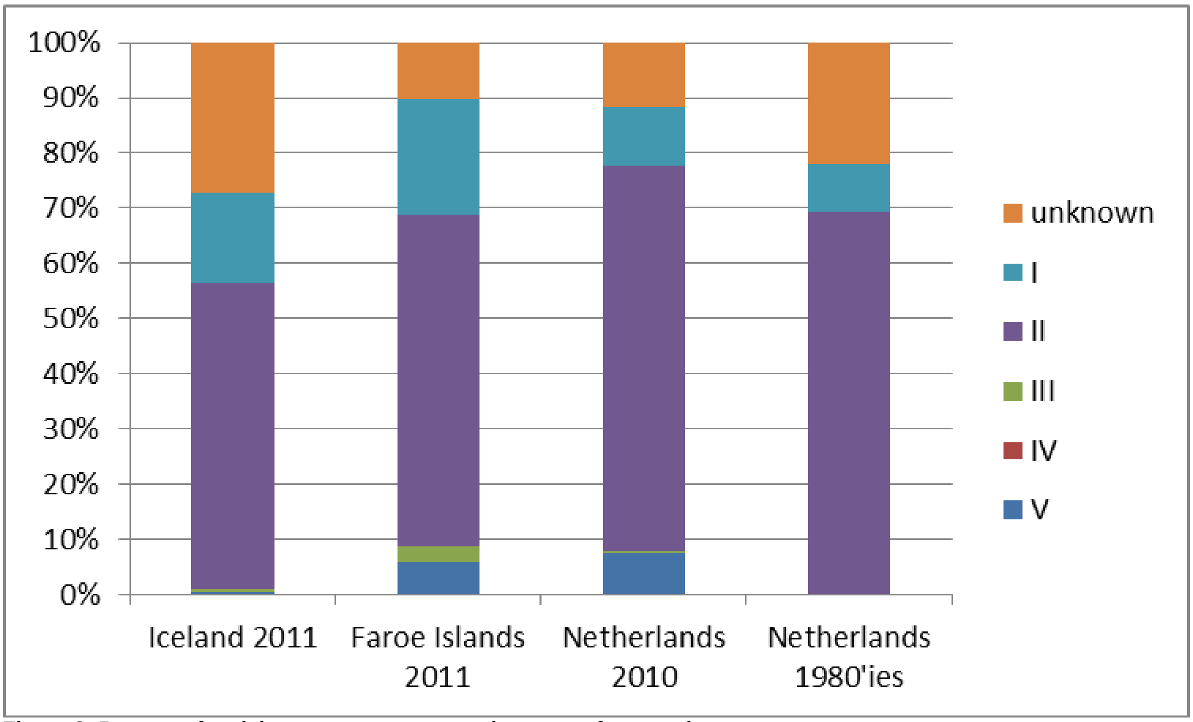


Figure 9: Percent of toxicity groups per country by mass of categories

6.1.5. Trend

Figure 10 compares the plastic materials from fulmar stomachs of the Netherlands in the 1980's and 2010. Polyethylene decreases significantly ($p < 0.001$) in comparison of other materials that became more common in later years, such as PVC and PET and PP.

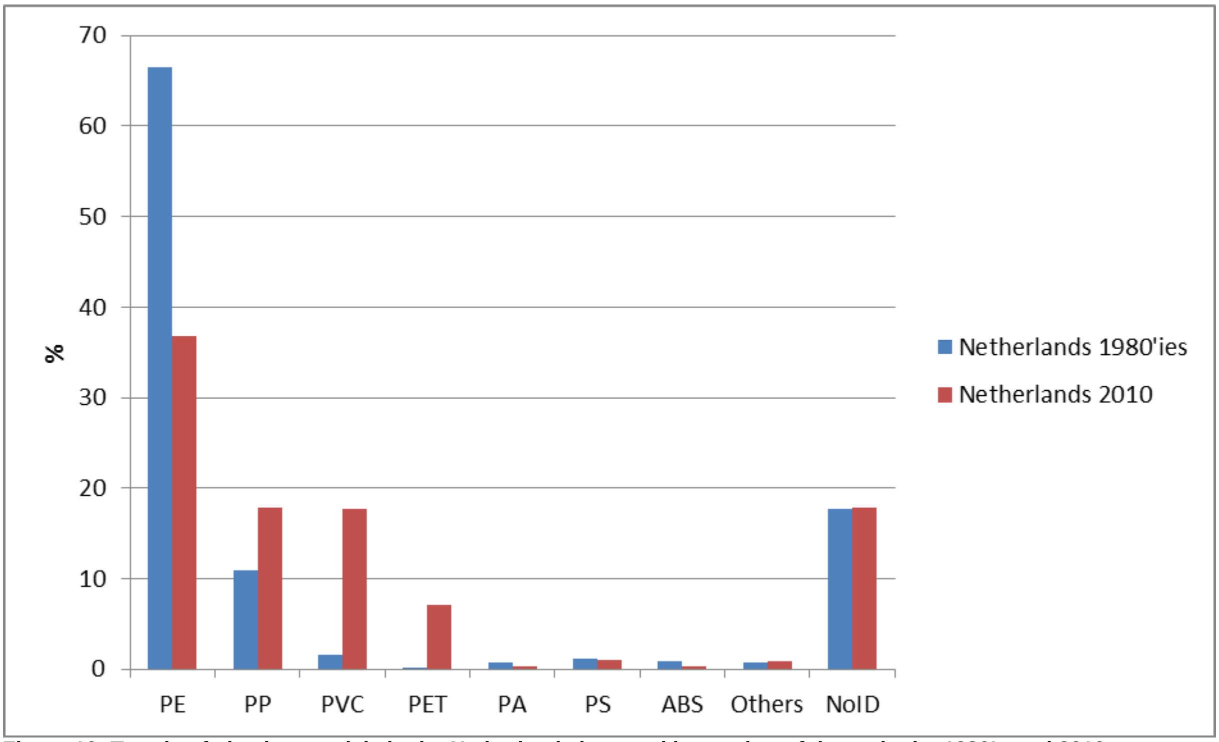


Figure 10: Trends of plastic materials in the Netherlands ingested by northern fulmars in the 1980's and 2010

6.1.6. Results method validation

As this method has not been used before, different ways of method validation were applied. Except of one piece all pieces that were identified by the Phazir NIR (>80% accuracy) were also approved by the FTIR. One sheet that the Phazir identified as PVC with an accuracy of 83%, the FTIR gave as result PE. Five pieces were not identifiable by the Phazir and also the FTIR struggled in accuracy. However, in 2 of this cases both techniques suggest PE as the most likely material. A complete table of this comparison can be found in Annex II.
Testing the Phazir through measuring known materials turned into 12 of 15 pieces, where the Phazir and the given material match. In one case two categories shared the same probability. In another case the right material got the second place, in one case the third. Only one piece was wrongly identified. Also this table can be found in Annex III.

6.2. Results Fragmentation process

To answer the question how birds grind plastic pieces in their stomachs individual plastic pieces were categorized by four colleagues, that made their own arbitrary classification. The results are presented below.
Calculating the average number (n) and standard deviation (±sd) for every grinding category the following table appear. The standard variation looks high and has to do with the decision of one scientist not to apply the 0 (not grinded) category (see table 12).

Table 12: Average number (n) and standard deviation (±sd) of grinding categories in different countries

	Iceland 2011		Faroe Islands 2011		Netherlands 2010		Stormline 2012		Total
	n	**± sd**	**n**	**± sd**	**n**	**± sd**	**n**	**± sd**	
not grinded	3.5	*± 5.7*	3.25	*± 5.9*	8	*± 8.8*	25.75	*± 25.4*	**40.5**
little grinded	23.25	*± 5.4*	16.75	*± 5.7*	61	*± 9.1*	67.25	*± 8.1*	**168.3**
much grinded	49	*±14.2*	37	*± 11.9*	51.25	*± 6.2*	36.75	*± 22.6*	**174**
very much grinded	52.25	*±21.4*	72	*± 22.1*	31.75	*± 11.2*	5.25	*± 3.9*	**161.5**
Total	**128**		**129**		**152**		**135**		**544**

Figure 11 shows the results of the different countries. Particles of the category "not grinded" are mostly found on the Dutch coastline (20%). Together with the category "little grinded" (50%) pieces of the stormline have the highest rate of unworn pieces and differ significantly from all plastic that came from bird stomachs.
In stomachs of birds the "not grinded"-category is the lowest with 3% for Iceland, 3% for the Faroe Islands and 5% for the Netherlands. Most pieces occur in categories "very much grinded" (maximum 56% in the Faroe Islands) and "much grinded". Birds from the Faroe Islands therefore contain the most grinded pieces followed closely by Iceland (however, in terms of statistics the difference is not significant (p=0.1178)). Compared to the other countries birds from the Netherlands contain many "not" or "little grinded" pieces (45%) and differs significantly from Iceland (p <0.001), the Faroe Islands (p <0.001) and also the Dutch stormline (p <0.001).

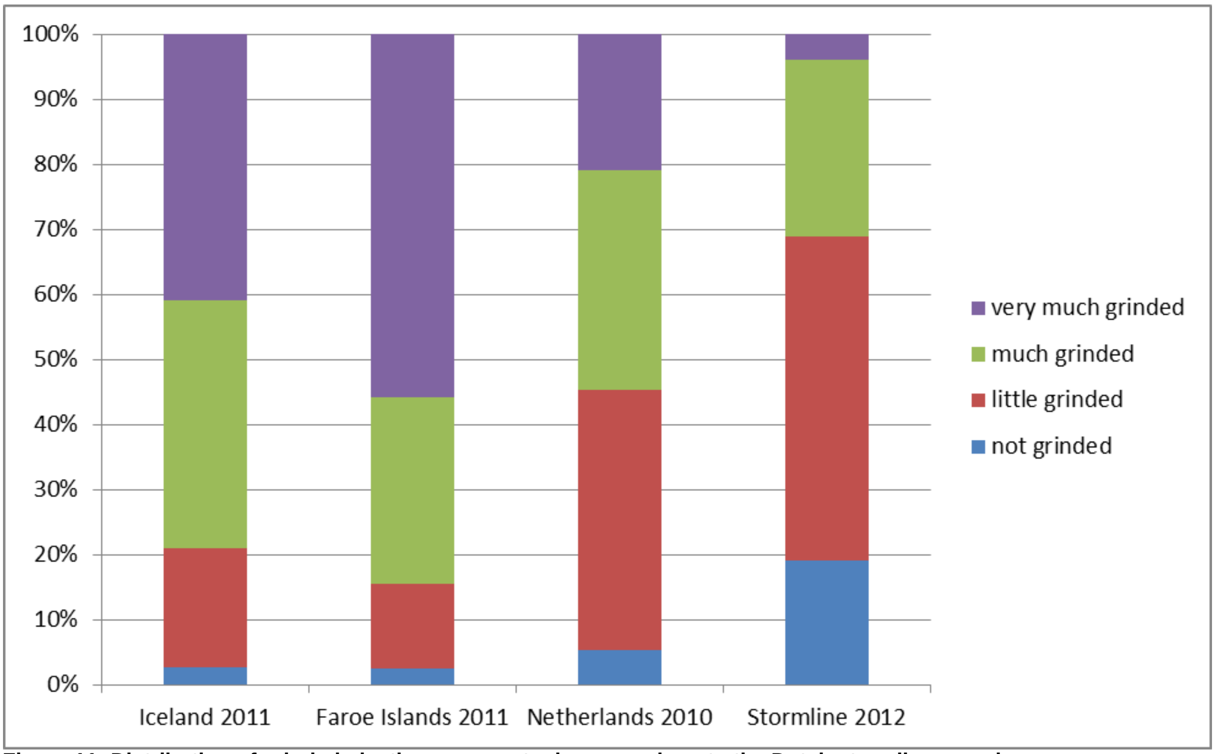
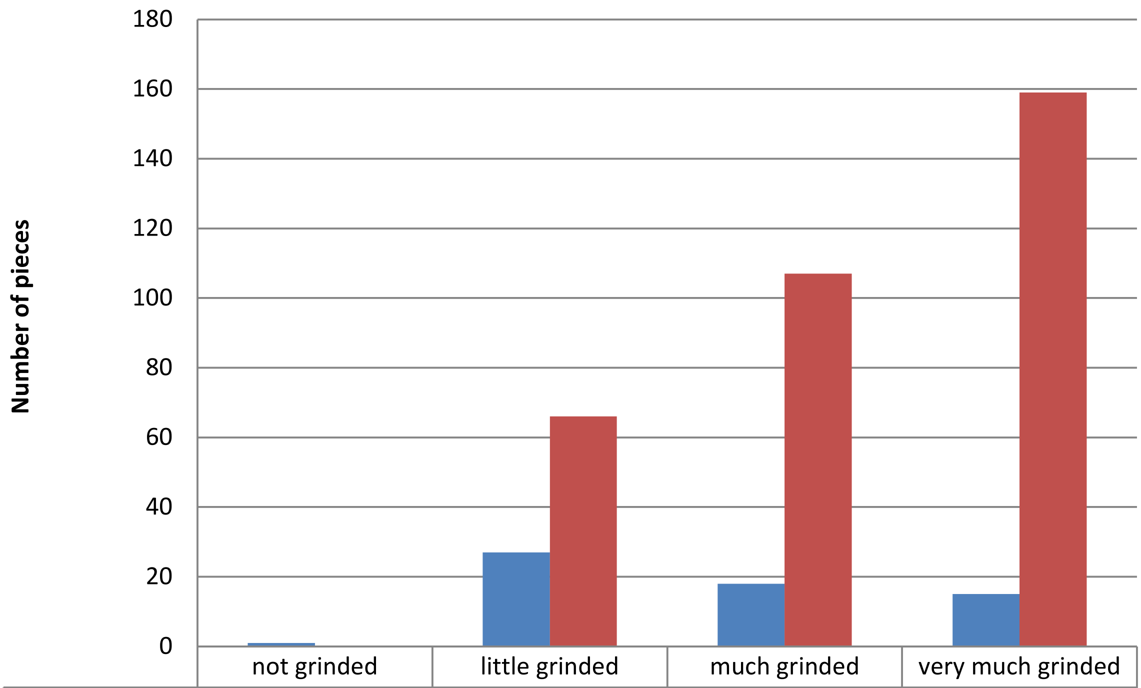


Figure 11: Distribution of grinded plastics per country in comparison to the Dutch stormline sample

To find out more about grinding processes in the stomachs of fulmars, it is important to distinguish between the two stomachs. Therefore plastic from the stormline was excluded (135 pieces) as well as two birds from the Netherlands 2010, where the stomachs had not been divided during sorting process (16 pieces). The proventriculus is used as a storage place for food, grinding takes place in the much smaller muscular gizzard. The remaining 393 plastic pieces were divided into two stomachs and compared. In the gizzard the pieces are much more grinded than in the proventriculus (see figure 12).



	not grinded	little grinded	much grinded	very much grinded
Proventriculus	1	27	18	15
Gizzard	0	66	107	159

Figure 12: Comparison of grinding rates between proventriculus and gizzard

7. Discussion

To measure the pollution of the North Sea, OSPAR and the EU use the northern fulmar as indicator species, since it forages regularly on plastics. Probably also plastic pieces from the seafloor may be taken up via fish but it was totally unknown to what level such secondary ingestion occurred. One part of this research focused on plastic characteristics to assess this possibility. Material was identified with infrared spectroscopy. Samples of birds, beached in different countries were used for spatial comparison, as well as stomach contents from the 1980's for temporal comparison.
For validation a FTIR was used to verify the method. The results suggest the assumption that in most cases the Phazir NIR identified the pieces correctly. When measurement by the Phazir was restricted also the results, offered by the FTIR became less probable.
Identifying known materials show a high reliability, in a few cases the right material was offered in the 3 most likely plastic types.
The distribution of plastic materials did not differ much, except for the data from the Netherlands in 2010. There were a high amount of foam, made of PVC and PET. Most of the foam, made from PVC and PET was present in one bird (in terms of number 74% of PVC, 96% of PET). Therefore it seems to be a bias due to low sample numbers in birds. The high proportion in means of weight is caused by the high specific mass of PET and PVC.
A low sample number also influenced the results of two other categories; "probably industrial" material (10 pieces) and "other plastic" (18 pieces). The high number of identified PS pieces in the last category originated from soft airgun bullets that can be found in stomachs. The method to find out if birds are able to consume heavy plastic types via fish is based on theoretical assumption of densities.
The toxicity of plastic is very difficult to determine. As mentioned above, plastic is variable regarding added toxics during manufacturing processes that can leach into the environment, as well as the ability of plastics to adsorb chemicals from the marine environment. It is impossible to estimate the composition of these factors for each individual piece without expensive chemical analysis. Using Lithner (2011) as an approach in this research, only a rough estimation based on one factor known (leaching toxicity during manufacturing processes) is made and offered only a first estimation of probable harm. To get to know more about the possible harming effects of toxics from plastics inside birds, further research will be necessary.
Foam has specific characteristics that causes possible effects on birds. Due to its soft substance, it is likely to be digested fast and therefore plastic toxics may not have too much time to release. On the other hand, the same characteristics could be responsible for an opposite effect: through the softness of foam the toxics can be squeezed out of the plastic and become available to the birds. However there is no research that has focused on these details yet.
Comparing results from the Dutch data of the 1980´s and 2010 there is a remarkable change, from one major plastic type (PE) to more variable types of plastic (PVC,PET). Unfortunately it was not possible to find something about exact production rates of different materials in the 1980's to compare them to actual numbers and to evaluate if the development changed.

To understand the effects of plastic on birds it is important to get insight in the digestive processes. By measuring the level of grinding of plastic pieces, details become visible. However, the methodology is based on subjective opinions of four researchers, already working in fulmar projects. To get a more exact result it might be necessary to ask more specialists. Another possibility is to use high definition techniques, as an electronic microscope (Eriksson & Burton, 2003; Göpferich, 1996), however there is still a lack of applied categorizing methods.
For this research only harder items as fragments and industrial pellets were used, where grinding is more visible. However in the future also threads and foams could be categorized on grinding rates.

A hypothesis to explain higher grinding of plastic in the northern areas may be the migration pattern of fulmars. During winter, the number of fulmars in the North Sea, where pollution rates are higher, increases (Skov *et al*., 1995). Around April, the birds are returning to their breeding areas, taking accumulated plastic from southern areas, which degraded during this journey. Back on their breeding areas, less plastic is available, therefore less “not grinded” material can be found in birds from northern habitats (Kühn & van Franeker, 2012).
Looking at the results from the Dutch stormline, it seems, that the grinding process takes place inside the stomach, since pieces from the stormline were only little grinded even if they were traveling through saltwater, UV radiation and surge of waves among beaches. Possibly plastic floating around northern areas might be more grinded, coming from polluted southern areas, but also samples from the Dutch fulmars differ significantly from samples collected at the stormline.
The two stomachs of a fulmar fulfill different tasks. The plastic grinds in the smaller muscular gizzard. In the gizzard, the plastic degrades until it is small enough to enter the intestines. Through the grinding process inside the stomach, the surface of plastic pieces increases and therefore, also the possible toxicity through additives that can leach out of the plastic. These effects on birds are not described yet.

For the European program to monitor the amount of plastic in oceans, this research tends to confirm that the fulmar as an indicator species is largely reflecting surface plastic pollution. Most plastic with a higher density potentially available to birds via fish, seems less relevant for fulmars in the North Sea.

8. Conclusion

The research focussed on the characteristics of plastic ingested by northern fulmars. It assessed the actual EU monitoring program, where the fulmar is used to indicate the plastic pollution of the North Sea.
The report shows the variation of different plastic types occurring at sea and therefore in stomachs of northern fulmars. The results support the theory that plastic uptake takes place directly from the water surface. Only a small percentage of pieces are heavier than seawater and must be ingested on another way, probably through fish. The northern fulmar appears thus suitable for monitoring programs concentrating on surface plastic. To assess other habitats (e.g. sea floor) other methods are necessary.

To assess the possible consequences of ingested plastic it is necessary to understand the pathways of plastic through the digestive system of fulmars. Therefore grinding processes build one part of this big and complex issue. Fulmars digest plastic through grinding the plastic to small items until they can pass through the guts. This process takes place in the small muscular stomach, the gizzard. This research indicates that birds, coming from southern wintering areas, that are heavy polluted, back to their cleaner northern breeding areas, take the plastic with them and grind it in their stomachs.

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Annex I: Categories of toxicity (Lithner *et al.*, 2011)

Table 1
Sorting of CLP hazard classes and categories into 5 levels of hazards (I–V), with grades increasing by a factor 10 for each level (V = most hazardous).

Hazard class (category)	Abbreviation	Hazard level	Hazard grade
Carcinogenicity (cat. 1A; 1B)	Carc. 1A; Carc. 1B	V	10,000
Germ cell mutagenicity (cat. 1A; 1B)	Muta. 1A; Muta. 1B		
Reproductive toxicity (cat. 1A; 1B)	Repr. 1A; Repr. 1B		
Persistent, bioaccum., toxic/very persistent, very bioaccum.[a]	PBT/vPvB		
Hazardous to the ozone layer	Ozone		
Explosives (unstable)	Unst. Expl.		
Germ cell mutagenicity (cat. 2)	Muta. 2	IV	1000
Acute toxicity (cat. 1; 2 — oral; dermal; inhalation)	Acute Tox. 1; Acute Tox. 2		
Respiratory/skin sensitisation (cat. 1)	Resp. Sens. 1; Skin Sens. 1		
Specific target organ toxicity — single exposure (cat. 1)	STOT SE 1		
Specific target organ toxicity — repeated exposure (cat. 1)	STOT RE 1		
Hazardous to the aquatic environment (chronic cat. 1; 4)	Aq. Chronic 1; Aq. Chronic 4		
Carcinogenicity (cat. 2)	Carc. 2	III	100
Reproductive toxicity (cat. 2; lact.)	Repr. 2; Lact		
Acute toxicity (cat. 3 — oral; dermal; inhalation)	Acute Tox. 3		
Aspiration hazard (cat. 1)	Asp. Tox 1		
Skin corrosion/irritation (cat. 1A; 1B; 1 C)	Skin Corr. 1A; Skin Corr. 1B; Skin Corr. 1 C		
Serious eye damage/eye irritation (cat. 1)	Eye Dam. 1		
Specific target organ toxicity — single exposure (cat. 2)	STOT SE 2		
Specific target organ toxicity — repeated exposure (cat. 2)	STOT RE 2		
Hazardous to the aquatic environment (acute cat. 1; chronic cat. 2)	Aq. Acute 1; Aq. Chronic 2		
Explosives (Div 1.1)	Expl. 1.1		
Acute toxicity (cat. 4 — oral; dermal; inhalation)	Acute Tox. 4	II	10
Skin corrosion/irritation (cat. 2)	Skin Irrit. 2		
Serious eye damage/eye irritation (cat. 2)	Eye Irrit. 2		
Specific target organ toxicity — single exposure (cat. 3)	STOT SE 3		
Hazardous to the aquatic environment (chronic cat. 3)	Aq. Chronic 3		
Explosives (Div 1.2)	Exp. 1.2		
Explosives (Div 1.3; 1.5)	Expl. 1.3; Expl. 1.5	I	1
Flammable gas/aerosols/liquids (mainly cat. 1)	Flam. Gas 1; Flam. Aerosol 1; Flam. Liq.1 and 2		
Self-reactive substance or mixture (type A; B)	Self-react. A; Self-react. B		
Pyrophoric liquids/solids	Pyr. Liq. 1; Pyr. Sol. 1		
Oxidising liquids/solids	Ox. Liq. 1; Ox. Sol. 1		
Organic peroxide (type A; B)	Org. Perox. A; Org. Perox. B		

[a] The classification of PBT and vPvB substances are not yet included in the CLP-regulation. The Stockholm Convention on Persistent Organic Pollutants (POPs) has been searched.

Annex II: Comparison Phazir NIR and FTIR

bird code	which stomach? P=Proventriculus, G=Gizzard	item number within sample and category	Identified 80%	TOP 3 CATEGORIES	accuracy (%)	TOP 3 CATEGORIES	accuracy (%)	TOP 3 CATEGORIES	accuracy (%)	TOP 3 CATEGORIES	accuracy	TOP 3 CATEGORIES	accuracy	TOP 3 CATEGORIES	accuracy	remarks FTIR	colour	Conclusions
			Phazir (NIR)	Phazir (NIR)	Phazir (NIR)	Phazir (NIR)	Phazir (NIR)	Phazir (NIR)	Phazir (NIR)	FTIR	FTIR	FTIR	FTIR	FTIR	FTIR	FTIR		
JAFCODE3		catnr	ID80	NIR1		NIR2		NIR3		FTIR1		FTIR2		FTIR3				
NET-2010-001	G	In-001	PE	PE	96	ION	93	PP	86	PE	0.02962	PE	0.031113	PE	0.031452		beige	match
NET-2010-001	G	Th-002	PP	PP	91	EVA	89	PVC	84	PP	0.301412	PP	0.310468	PP	0.311302		salmon pink	match
NET-2010-002	P	Th-001	PE	PE	95	ION	87	PP	83	PE	0.060146	PE	0.060664	PE	0.072597		jet black	match
NET-2010-020	G	In-001	PE	PE	99	ION	97	PP	87	PE	0.2962	PE	0.031113	PE	0.0315		cement grey	match
NET-2010-020	G	In-002	PE	PE	99	ION	97	PP	85	PE	0.053749	PE	0.055872	PE	0.059004	UHMWPE	sand yellow	match
NET-2010-020	G	In-003	PE	PE	95	ION	90	PP	85	PE	0.045192	PE	0.53747	PE	0.05722	UHMWPE	golden yellow	match
NET-2010-020	G	In-006	PP	PP	99	EVA	91	PVC	88	PP	0.043222	PP	0.045785	PP	0.047066		light ivory	match
NET-2010-020	G	In-009	PE	PE	97	ION	94	PP	90	PE	0.019443	DEX	0.029976	PE	0.03646		pure white	match
NET-2010-020	G	In-010	PE	PE	99	ION	97	PP	86	PE	0.110167	PE	0.111811	PE	0.0120611		sand yellow	match
NET-2010-020	G	In-013	PE	PE	99	ION	97	PP	86	PE	0.035917	PE	0.036457	PE	0.038332		moss grey	match
NET-2010-020	G	Fo-047	NoID	PVC	70	PB	67	PPO	66	Kraton	0.267553	PS	0.390142	PS	0.439231	Kraton: mix PS, PE	ochre yellow	Phazir: NoID, FTIR: unsure
NET-2010-034	G	In-001	PE	PE	99	ION	96	PP	90	PE	0.027092	PE	0.034149	0	0.045458		ivory	match
NET-2010-034	G	In-005	NoID	PA	36	PMMA	22	PA/ABS	19	par	0.462285	ATR	0.465255	par	0.535061	PARVAN 3150	signal black	Phazir: NoID, FTIR: unsure
NET-2010-034	G	In-007	NoID	ABS	42	PPO	40	PA/ABS	39	par	0.0334347	ATR	0.340558	DEX	0.393807	PARVAN 3150	squirrel grey	Phazir: NoID, FTIR: parrafine
NET-2010-034	G	Sh-005	PVC	PVC	83	PP	81	PE	81	PE	0.093451	PE	0.097576	PE	0.143718		ivory	no match
NET-2010-034	G	Th-001	NoID	PP	57	PVC	52	PET	52	PP/PE	0.185697	PP/PE	0.214555	TPV	0.219132		sand yellow	Phazir: NoID, FTIR: unsure, both match PE
NET-2010-034	G	Th-002	NoID	PE	63	PP	57	ION	57	PE	0.043674	PE	0.044858	PE	0.053278		clay brown	Phazir: NoID, FTIR: sure, both match PE
NET-2010-034	G	Op-002	PS	PS	98	ABS	95	AS	88	PS	0.020972	PS	0.089809	PS	0.116132		golden yellow	match

Annex III: Comparison Phazir NIR and assigned plastic type

Plastic item	Plastic1	%	Plastic2	%	Plastic3	%	real type	remarks
climbing helmet	PPO	86	ABS	82	PS	82	ABS	**2.**
Pen Highlighter	PP	95	EVA	86	PVC	86	PP	**match**
Case drill	PS	90	ABS	90	PPO	82	ABS	**both 90%**
gull colour ring (Kees C.)	PPO	91	ABS	89	PS	87	PMMA	**no match**
champignon packaging	ABS	97	AS	96	PS	96	PS	**3.**
bursting bubbles packaging	PE	87	ION	83	PP	76	PE	**match**
creme cap	PS	95	ABS	94	AS	88	PS	**match**
plastic packaging "zip"	PE	96	ION	90	PP	88	PE	**match**
flower pot	PP	95	EVA	86	PVC	85	PP	**match**
screw box	PP	99	PVC	91	PET	83	PP	**match**
bread box	PP	100	PVC	92	PET	86	PP	**match**
sligro pass	PET	84	PBT	84	PC	66	PET	**match**
plastic box	PS	97	AS	96	ABS	93	PS	**match**
choco cream cap	PP	97	PVC	89	EVA	87	PP	**match**
pasta drip off	PP	93	PVC	86	EVA	82	PP	**match**