European Marine Strategy Framework Directive
Working Group on Good Environmental Status
(WG-GES)

Monitoring Guidance for Marine Litter in European Seas

Draft Report

CHAPTER 6

LITTER IN BIOTA

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Draft Guidance Report:

TSG-ML was tasked to deliver guidance so that European Member States could initiate programmes for marine litter monitoring. As monitoring must be operational by 2014, first guidance was required by mid-2013. The draft Guidance report provides the basis for the marine litter programme however since new information continues to be compiled TSG -ML can review and update this guidance later in 2013.

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6. Litter in Biota

This Chapter focuses on indicator 10.2.1 of descriptor 10 MSFD "*Trends in the amount and composition of litter ingested by marine animals*." For this indicator the Commission Decision (2010/477/EU) expresses the need for further development based on the experience in some sub-regions (e.g. North Sea), to be adapted in other regions and on emerging knowledge about other impacts beside the ingestion of litter by marine organisms.

Therefore, the primary task for the implementation of appropriate monitoring for this indicator is to develop tools for investigating trends in ingested litter that cover all the MSFD marine regions. As no single species can provide full coverage over all Europe's marine sectors, a range of species is needed to monitor ingested litter. Some spatial overlap between regionally restricted monitoring species is desirable to link pollution measurements in the different areas.

In addition the issue of entanglement of marine organisms in litter is the second main impact to be considered when dealing with criteria 10.2. *Impacts of litter on marine life*.

Furthermore the COM Dec states that the improvement of knowledge concerning impacts on marine life (affected species, species used as indicators, the standardisation of methods and the determination of thresholds) is also needed. Hence, a next issue to be dealt with is the development of strategies for assessing harm/impacts, which will be done in the further run of the work of the TSG ML.

6.1. Scope & key questions to be addressed

- In the North Sea, an indicator is available, which expresses the impact of marine litter (OSPAR EcoQO). It measures ingested litter in Northern Fulmar and it is used to assess temporal trends, regional differences and compliance with a set target for acceptable ecological quality in the North Sea area (Van Franeker *et al*., 2011). A combined protocol is here proposed which can be used for seabirds in general and applied in most North-East-Atlantic countries, e.g. to be applied in regular monitoring for fulmars in areas that are currently not covered or for shearwaters in the Southern part of the NE Atlantic and in parts of the Mediterranean.
- Alternative tools for indicator 10.2.1 are needed for the Baltic Sea, the Mediterranean Sea, the Black Sea, and southern parts of the North-East-Atlantic.
- On the basis of available information and expertise, this report proposes a monitoring protocol for sea turtles with focus on relevant parameters for application in the Mediterranean and some parts of the Southern Atlantic. Another protocol is proposed for a MSFD marine litter monitoring of ingested litter in fish.
- Microlitter occurrence in Biota (birds, fish, and invertebrates) can be incorporated in the provided protocols as a complementary analysis (see Chapter 7).
- The approach taken for the development of the protocols for ingestion consists of the application of the same categorization of marine litter for all ingestion studies of vertebrates. The applied standard categories follow the existing fulmar methodology, in which a number of plastic categories is counted, and weighted as a unit.
- Additionally further knowledge is being compiled on the occurrence of entanglement events in marine organisms. Based upon these findings a harmonised protocol for the assessment of the use of plastic litter as nesting material and associated entanglement mortality in birds breeding colonies is proposed for immediate application.
- Additional paragraphs reflect on entanglement in beached animals, entanglement in live animals (others than in relation to seabird nests), ingestion of litter by marine mammals, ingestion of litter by marine invertebrates and research on food chain transfer. Only ingestion of and entanglement in marine litter by marine mammals are considered for further development whereas the other aspects are crucial issues for research but not suitable to be recommended for wide monitoring application at this stage. Ingestion protocols for invertebrates such as crustaceans, shellfish, worm or zooplankton are not included in this report but should be guided by methodological details as outlined in chapter 7 on microlitter monitoring.

Further development of existing tool sheets are presented in the following protocols.

6.2. Seabirds

Tool name

MSFD Protocol for the monitoring of litter ingested by seabirds (Procellariiformes, like fulmars or shearwaters). Based on tool 10.2.1_T1 – Fulmar and Tool 10.2.1_T2 – Shearwater.

Tool description

The methodology of this tool follows the OSPAR Ecological Quality Objective (EcoQO) methods for monitoring litter particles in stomachs of northern fulmars (*Fulmarus glacialis*). The stomach contents of birds beached or otherwise found dead are used to measure trends and regional differences in marine litter. Background information and the technical requirements are described in detail in documents related to the fulmar EcoQO methodology. A pilot study evaluating methods and potential sources of bias was conducted by Van Franeker & Meijboom (2002). Bird dissection procedures including characters for age, sex, cause of death etc. have been specified in Van Franeker (2004). Further OSPAR EcoQO details were given in OSPAR (2008, 2010a, b) and in Van Franeker *et al.,* (2011a, 2011b).

Related marine compartments

Seabirds like fulmars or shearwaters are feeding on the surface of the sea. Therefore the water column and especially the water surface is the marine compartment addressed when quantifying litter in the stomachs of fulmars.

6.2.1. Technical requirements

Bird corpses are stored frozen until analysis. Standardized dissection methods for Fulmar corpses have been published in a dedicated manual (Van Franeker, 2004) and are internationally calibrated during annual workshops. Stomach content analyses and methods for data processing and presentation of results were described in full detail in Van Franeker & Meijboom (2002) and updated in later reports. The methodology has been published in peer reviewed scientific literature (van Franeker *et al*., *2011*). For convenience, some of the methodological information is repeated here in a condensed form.

At dissections, a full series of data is recorded to determine sex, age, breeding status, likely cause of death, origin, and other issues. Age, the only variable found to influence litter quantities in stomach contents, is largely determined on the basis of development of sexual organs (size and shape) and presence of *Bursa of Fabricius* (a gland-like organ positioned near the end of the gut which is involved in immunity systems of young birds; it is well developed in chicks, but disappears within the first year of life or shortly after). Further details are provided in Van Franeker 2004.

After dissection, stomachs of birds are opened for analysis. Stomachs of Fulmars have two 'units': initially food is stored and starts to digest in a large glandular stomach (the *proventriculus*) after which it passes into a small muscular stomach (the *gizzard*) where harder prey remains can be processed through mechanical grinding. For the purpose of most cost-effective monitoring, the contents of proventriculus and gizzard are combined, but optional separate recordings should be considered where possible.

Stomach, contents are carefully rinsed in a sieve with a 1mm mesh and then transferred to a petri dish for sorting under a binocular microscope. The 1 mm mesh is used because smaller meshes become easily clogged with mucus from the stomach wall and with food-remains. Analyses using smaller meshes were found to be extremely time consuming and particles smaller than 1 mm seemed rare in the stomachs, contributing little to plastic mass.

If oil or chemical types of pollutants are present, these may be sub-sampled and weighed before rinsing the remainder of stomach content. If sticky substances hamper further processing of the litter objects, hot water and detergents are used to rinse the material clean as needed for further sorting and counting under a binocular microscope.

Litter Categories – source related information

In the Fulmar EcoCO, stomach contents are sorted into the following categories (Table 7), and this categorisation is followed for marine biota monitoring ingestion in seabirds, marine turtles and fish.

BIOTA categories for contents of digestive tract *(oesophagus, stomach(s), intestine)*			
PLA	**PLASTIC**	**acronym**	all plastic or synthetic items: note number of particles and dry mass for each category
IND	**pellets**	**ind**	industrial plastic granules (usually cylindrical but also oval spherical or cubical shapes exist
	probab ind?	**pind**	suspected industrial, used for the tiny spheres (glassy, milky, ...) occasionally encountered
USE	**sheet**	**she**	remains of sheet, eg from bag, cling-foil, agricultural sheets, rubbish bags etc
	thread	**thr**	threadlike materials, eg pieces of nylon wire, net-fragments, woven clothing; includes 'balls' of compacted such material
	foam	**foam**	all foamed plastics so polystyrene foam, foamed soft rubber (as in matras filling), PUR used in construction etc
	fragments	**frag**	fragments, broken pieces of thicker type plastics, can be bit flexible, but not like sheetlike materials
	other	**Poth**	any other, incl elastics, dense rubber, sigarette-filters, balloon-pieces, softairgun bullets; objects etc. DESCRIBE!!
RUB	**OTHER RUBBISH**	**acronym**	any other non synthetic consumer wastess: note number of particles and (in principle) dry mass for each category
RUB	**paper**	**pap**	newspaper, packaging, cardboard, includes multilayerd material (eg Tetrapack pieces) and aluminium foil
	kitchenfood	**kit**	human food remains (galley wastes) like oinion, beans, chickenbones, bacon, seeds of tomatoes,grapes, peppers, melon etc
	other user	**rva**	other consumber waste, like processed wood, pieces of metal, metal air-gun bulletes; leadshot, painchips. DESCRIBE
	FISHHOOK	**hoo**	fishing hook remains (NOT FOR HOOKS ON WHICH LONGLINE VICTIMS WERE CAUGHT - THOSE UNDER NOTES)
POL	**POLLUTANTS (INDUS/CHEM WASTE)**	**acronym**	other non synthetic indusrial or shipping wastes (number of items and mass per category (wet for paraffin)
POL	**slag/coal**	**sla**	industrial oven slags ('looks like non-natural pumice) or coal remains
	oil/tar	**tar**	lumps of oil or tar (also not n=1 and g=0.0001g if other particles smeared with tar but cannot be sampled separately)
	paraf/chem	**che**	lumps or mash of unclear paraffin, wax like substances (NOT stomach oil!) if needed subsample and estimate mass
	featherlump	**rva**	lump of feathers from excessive preening of fouled feathers (n=1 with drymass) (NOT for few normal own feathers)
FOO	**NATURAL FOOD**	**foo**	various categories, depends on the species studied, and aims of study
NFO	**NATURAL NON FOOD**	**nfo**	anything natural, but which can not be considered as normal nutritious FOOD for the individual

Table 7: Categories for classification of items for Biota

The fulmar categorisation of stomach contents is based on the general 'morphs' of plastics (sheet-like, filament, foamed, fragment, other) or other general rubbish or litter characteristics. This is because in most cases, particles cannot be unambiguously linked to particular objects. But where such is possible, under notes in datasheets, the items should be described and assigned a litter category number using the "Master List" developed by the TSG ML group (Chapter 8 – Annex 8.1).

For each litter category/subcategory an assessment is made of:

1) **incidence** (percentage of investigated stomachs containing litter);
2) **abundance by number** (average number of items per individual), and
3) **abundance by mass** (weight in grams, accurate to 4th decimal)

Because of potential variations in annual data, it is recommended to describe '**current levels'** as the average for all data from the most recent 5-year period, in which the average is the 'population average' which includes individuals that were found to have zero litter in the stomach.

As indicated, EcoQO data presentation for Northern Fulmars is for the combined contents of glandular (proventriculus) and muscular (gizzard) stomachs. Results of age groups are combined except for chicks or fledglings which should be dealt with separately. Potential bias from age structure in samples should be checked regularly.

Size range

In the fulmar monitoring scheme, stomach contents are rinsed over a sieve with mesh 1 mm prior to further categorisation, counting and weighing. The size range of plastics monitored is thus ≥ 1 mm. Unpublished data on particle size details in stomachs of fulmars show that a smaller mesh size would not be of use because smaller items have passed into the gut.

In the OSPAR Fulmar EcoQO approach, the focus is on mass of categories of litter, rather than on the size of individual particles. However, the litter descriptor of the MSFD makes a distinction between macro- and micro-particles of litter, defined as objects with largest measurement over or below a limit of 5 mm. Both size groups are common in seabird stomachs. For comparative purposes it is then useful to know proportions of micro- and macro litter found in seabird stomachs. Whether such assessment of particle size is incorporated into standard monitoring methods, or is evaluated on a more incidental basis, will depend on practical and financial considerations. In the current Fulmar project, particle size assessment is not standard procedure (particle number and combined mass per litter category only give 'average' size information), but a dedicated study is currently assessing exact sizes of all particles in a large number of samples from different locations and time periods. Such dedicated detailed work can be repeated at appropriate moments.

In the seabird studies it is standard to filter stomach contents over a 1 mm sieve, and these thus largely ignore potential presence of micro-plastics below the 1 mm size. In the stomachs such sizes seem extremely rare, but potentially they could be present in gut material in the intestines resulting from break up of larger items in the stomach of from secondary ingestion with zooplankton or fish. For study of particles in such size range in bird intestines, methods as described in Chapter 7 on microplastics in biota should be followed.

Spatial coverage

Dead birds are collected from beaches or from accidental mortalities such as long-line victims, fledgling road kills etc. (for methodology see Van Franeker, 2004).

Survey frequency

Continuous sampling is required. A sample size of 40 birds or more is recommended for a reliable annual average for a particular area. However, also years of low sample size can be used in the analysis of trends as these are based on individual birds and not on annual averages. For reliable conclusions on change or stability in ingested litter quantities, data over periods of 4 to 8 years (depending on the category of litter) is needed (Van Franeker & Meijboom, 2002).

Maturity of the tool

The method is mature and in use.

Regional applicability of the tool

The tool is applicable to the MSFD marine regions where fulmars occur; the Greater North Sea, the English Channel, and the Celtic Seas For similar seabird species such as any of the family of the tubenoses, the methodology can follow this protocol. This could for example be applied to shearwater species occurring further south in the Atlantic or in the Mediterranean Sea.

6.2.2. Cost estimate

A cost estimate for the fulmar biota monitoring can be based on current level of funding available for the monitoring project in the Netherlands. This currently amounts to approximately 50 k€ annually, almost completely for scientist staff costs (covering roughly 300 man hour or 7.5 workweek – Euro cost based on contract rates by Wageningen UR). This concerns the time invested in coordinating the collection program by volunteer and other groups (c. 10 k€), lab dissections, stomach analyses and data-analyses of approximately 40-50 birds annually (20 k€), formal report writing and production (15 KE) and associated post reporting activity (5 k€). Material costs for transports and lab disposables are minor in the Netherlands, c. 1 K€/year, but occasionally more if providing volunteer groups with materials like freezers. The actual field work in this approach is conducted without cost by volunteer beach bird surveyors or other persons/organisations regularly surveying beaches. Their 'reward' is provided by the coordinator, spending considerable part of his effort on a good back-reporting to the participants about the programs outcomes (reports, webpage, individual contacts).

In the Dutch program, some limited account is taken of assisting other countries and integrating report writing for OSPAR (to allow this international component, data analyses and reporting were reduced from annual effort to once in two years). Costs for separate national programs may be reduced significantly if such integration of analyses and reporting by a single lead partner is more structurally arranged and financially supported.

6.2.3. Quality Assessment /Quality Control

The methodology referred to in this tool is based on an agreed OSPAR methodology which has been developed over a number of years with ICES and OSPAR and which has received full quality assurance by publication in peer reviewed scientific literature (Van Franeker *et al.,* 2011a). The EcoQO methodology has been fully tested an implemented on Northern Fulmars *Fulmarus glacialis*, including those from Canadian Arctic (Provencher *et al.,* 2009) and northern Pacific areas (Avery-Gomm *et al.,* 2012). All methodological details can be applied to other tubenosed seabirds (Procellariiformes) with no or very minor modifications. Trial studies are being conducted using shearwaters from the more sourthern parts of the north Atlantic and Mediterranean. In other seabird families, methods may have to be adapted as stomach morphology, foraging ecology, and regurgitation of indigestible stomach contents differ and can affect methodological approaches.

Trend assessment

In the Fulmar EcoQO, statistical significance of trends in ingested litter, i.e. plastics, is based on linear regression of ln-transformed data for the mass of litter (of a chosen category) in individual stomachs against their year of collection. 'Recent' trends are defined as derived from all data over the most recent 10-year period. The Fulmar EcoQO focuses on trend analyses for industrial plastics, user plastics, and their combined total.

Target definitions

In OSPAR the target for the Ecological Quality Objective is defined by the proportion of birds which exceeds a particular limit of plastic mass in the stomach. For the North Sea, the current, undated target is defined as

> *"There should be less than 10% of Northern fulmars having 0.1 gram or more plastic in the stomach in samples of 50-100 beached fulmars from each of 5 different regions of the North Sea over a period of at least 5 years".*

Other ways of target definitions are of course possible, e.g. in terms of average mass of plastic to be achieved by a specific date, or significance levels of rates of change that can be assessed on the basis of the data collected.

6.3. Sea turtles

Tool name

MSFD Protocol for the monitoring of litter ingested by sea turtles *(Caretta caretta*) and MSFD Protocol for sampling litter excreted by live sea-turtles (faecal pellet analysis) (optional) are based on tool 10.2.1_T3 – Sea Turtle.

Tool description

The stomach contents of stranded Loggerhead sea turtles *Caretta caretta* (Linnaeus, 1758) are used to measure trends and regional differences in marine litter. A pilot study evaluating methods and potential sources of bias was conducted during 2012 by ISPRA, CNR-IAMC Oristano, Stazione Zoologica Napoli; University of Siena, University of Padova, ArpaToscana. Dissection procedure, measurement, and litter analysis are shown below.

Related marine compartments

Caretta caretta feeds in the water column and at the seafloor. Therefore these two marine compartments are addressed when quantifying litter in the stomachs of stranded Loggerhead sea turtles.

6.3.1. Technical requirements

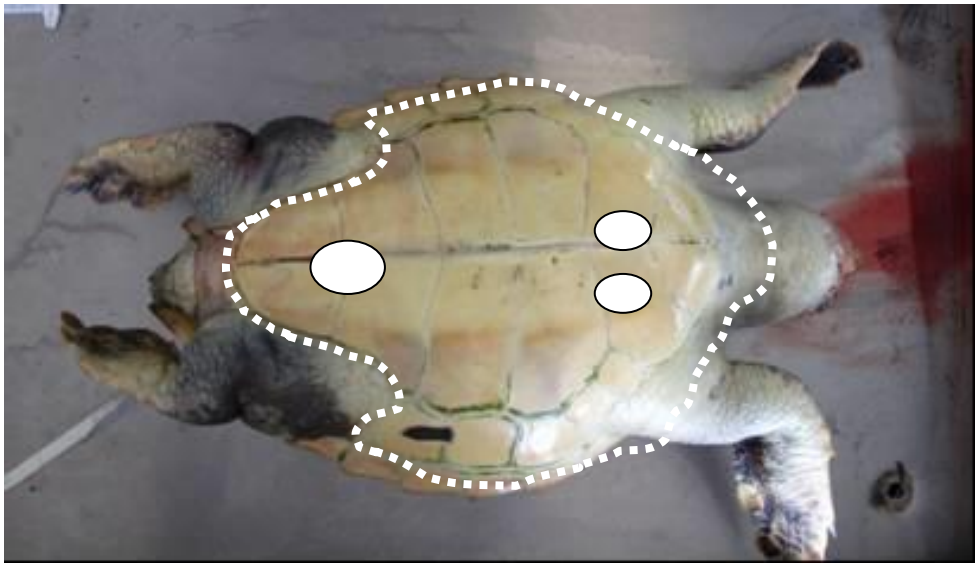
The Loggerhead sea turtle *Caretta caretta* is a protected species (CITES Appendix I), therefore only authorized people can handle them.

i) Protocol for application in case of finding of a dead sea turtle

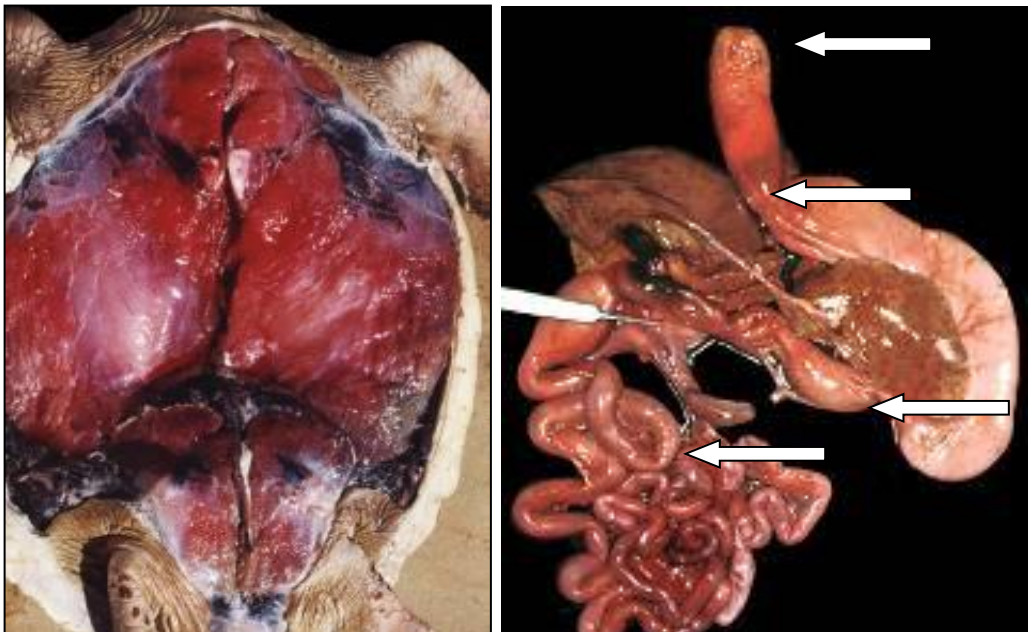
Upon finding the animal, its discovery should be reported to the main authorities and the operation of coordinated with the local authorities (depending on national law). Based on initial observations and if possible still at the place of discovery, some data should be recorded (See "Identification Data" Sheet in Annex 6.1). The animal should be transported to an authorized service centre for necropsy. In case the body is too decomposed, the integrity of the digestive tract should be assessed before disposal at the licensed contractor. If the necropsy cannot be carried out immediately after recovery, the carcass should be frozen at -16 ° C, in the rehabilitation facility.

Before the necropsy operation, morphometric measurements should be collected (see Annex 6.1). External examination of the animal should be conducted, including inspecting the oral cavity for possible presence of foreign material. To remove and separate the plastron from the carapace, an incision should be made on the outside edge, as shown as a dashed line in Picture 2. Once the inside of the plastron is accessed, the ligament attachment of the pectoral and pelvic girdle should be cut, as indicated in white circles in Picture 2. Qualitative evaluation of the trophic status of the animal should be made, including the atrophy of pectoral muscles (none, moderate, severe), fat thickness in the articular cavities and on the coelomic membrane (abundant, normal, low, none).

Removal of pectoral muscles and the heart should expose the gastrointestinal system (GI) (Picture 3, Left). The different portions of the GI should be isolated by means of plastic clamps, fixed on esophagus proximal to the mouth, on the esophageal valve, on the peg and on the cloaca, as close as possible to the orifice anal, as indicated by arrows in Picture 3 (Right). The entire GI should be removed and placed on the examination surface. This is easier if done by at least 2 operators: one person keeps the animal lying on its side, while the other separates the ligaments of the different organs and the membranes of the carapace by extracting the GI from the animal. The sex of the animal should be recorded. The 3 parts of the GI (esophagus, stomach, intestines) should be separated, affixing a second clamp at the cut edge to prevent spillage of the contents.



Picture 2: Dead sea turtle - cutting line and location of main plastron ligament (Wyneken, 2001)



Picture 3(Left): The ventral pectoral and pelvic musculature covers most of internal organs, which must be removed to expose the peritoneal cavity; **(Right):** Sea turtle gastrointestinal different portion

The following sampling procedure of GI contents can be applied to any section of the GI: the section of the GI should be placed in a graduated beaker of of adequate size, pre-weighed on electronic balance (accuracy of ± 1g). The section of GI should be open and the contents emptied into the beaker with the help of a spatula, followed by the record of the net weight and volume of the content. The section of the GI should be observed and any ulcers or any lesions caused by hard plastic items should be recorded.

The contents should be inspected for the presence of any tar, oil, or particularly fragile material that must be removed and treated separately. The liquid portion, mucus and the digested unidentifiable matter should be removed, by washing the contents with freshwater through a filter mesh 1 mm, followed by a rinse of all the material collected by the filter 1mm in 70% alcohol and finally again in freshwater. The retained content should be enclosed in plastic bags or pots, labelled and frozen, not forgetting the sample code and corresponding section of the GI. Finally, the contents can then be sent for analysis.

NOTE: If the contents are stored in liquid fixative, remember to take note of the compound and the percentage of dilution and communicate them to the staff in charge for the further analysis.

For the analysis of the contents of the GI, the organic component should be separated from any other items or material (marine litter). The fraction of marine litter should be analysed and categorised with the help of a stereo-microscope, following the approach used in the protocol for ingestion in birds (see section 6.2 above) (Van Franeker et al., 2005; 2011b; Matiddi et al., 2011) and using a data-sheet as the one provided in Annex 6.2.

The fraction of marine litter should be dried at room temperature and the organic fraction at 30°C. Both fractions should be weighted, including the different categories of items identified within the marine litter fraction. The volume of the litter found should also me measured, through the variation of water level in a graduated beaker, when the items are immersed without air. If possible, different categories of "food" should also be identified. Otherwise, the dry contents should be kept in labelled bags and sent to an expert taxonomist.

ii) Optional protocol for application for sampling litter excreted by live sea-turtles (faecal pellet analysis) in case of finding a specimen alive:

Upon finding the animal, its discovery should be reported to the main authorities and the operation of coordinated with the local authorities (depending on national law). Based on initial observations and if possible still at the place of discovery, some data should be recorded (See "Identification Data" Sheet in Annex 6.1). The animal should be transported to an authorized rehabilitation facility

At the rehabilitation facility, the remaining morphologic parameters should be recorded (annex 6.1) and the animal placed in the rehabilitation tanks. As soon as the animal begins to feed, a coloured plastic ball should be added to the food in order to assess the rate of gastrointestinal transit (size of plastic ball must be related to animal size). In most cases, the observed standard time for gastrointestinal transit is approximately 1.5 months after the first evacuation. The faeces should be sampled from the tank for the entire period between the arrival of the animal and the expulsion of the first coloured ball. The digested part should be removed by washing the sample with freshwater through a filter mesh 1mm and drying the retained fraction at room temperature. To analyse the content and identify the different categories of possible litter, the same approach as for the bird stomach content should be followed, as indicated above (Van Franeker et al., 2005; 2011b; Matiddi et al., 2011) and using a similar template as in Annex 6.2.

Extraction of data:

Following the protocol for seabirds, abundance by mass (weight in grams, accurate to 3th decimal) is the main information useful for monitoring program.

Other information as colour of items, volume of litter, different type of litter, different incidence of litter in oesophagus, intestine and stomach, incidence and abundance by number per litter category, are useful for research and impact analysis.

Data entry as described in Annex 6.2.

Litter Categories - source related information

For turtle analyses, stomach contents are sorted into the categories as given above for birds (Table 7). Following the protocol for seabirds, abundance by mass (weight in grams, accurate to 3th decimal) is the main information useful for monitoring program. Other information as colour of items, volume of litter, different type of litter, different incidence of litter in oesophagus, intestine and stomach, incidence and abundance by number per litter category, are useful for research and impact analysis.

The proposed form for data recording is given in Annex 6.2.

Size range

≥1 mm (stomach contents are rinsed over 1 mm mesh sieve)

Spatial coverage

Dead sea turtles are collected from beaches or at sea from accidental mortalities such as victims of long-line fishing (bycatch) or of boat collisions.

Survey frequency

Continuous sampling is required. Minimum sample population size for year and period of sampling must be established for reliable conclusions on change or stability in ingested litter quantities.

Maturity of the tool

Not mature at this stage. Specific monitoring programs are required.

Regional applicability of the tool

The tool is applicable to the MSFD marine regions where sea turtles *Caretta caretta* occur; in particular Mediterranean Sea country and a part of Atlantic East coast, not in Black sea.

6.3.2. Cost estimates

A cost estimate for the sea turtle litter monitoring is difficult to estimate due to the lack of dedicating monitoring programs at national level. Cost to be intended per single sea turtles rescue centre in an assessment area and monitoring programs can be integrated with stranding monitoring programs or collaboration with other research programs on the chemical pollution and diseases in this species.

The costs presented below are calculated on the base of the activity at the Stazione Zoologica of Naples, where main equipment and facilities are already present.

Cruise cost	**€2 k**	**Gasoline and truck for the collection of the carcasses**
Staff costs	**€4.5 k**	**Coordinator (1 researcher x 1 month/year)**
	€9 k	**Dissection (1 researcher x 2 months /year)**
	€7 k	**Dissection and field collection (1 technician x 2 months/year)**
Capital Equipment cost	**€1 k**	**Consumable**
	€2 k	**Deep Freezer**
	€1 k	**Dissection table**
	€3 k	**Stereomicroscope**
Cost Processing/analysing samples	**€12 k**	**300 €/Turtle (including carcass disposal costs). Estimated 40 turtles/year**

Table 8: Estimation of costs for analysis of litter ingestion in marine turtles

6.3.3. Quality assurance/quality control

There is a lack of quality assurance/quality control (QA/QC) due to lack of long monitoring programs. Data available are poor and based on few years (Matiddi *et al.,* 2011; Bentivegna *et al.*, 2013; Camedda *et al.,* 2013; Travaglini *et al.,* 2013). More publications in peer reviewed scientific literature are required.

Trend assessment

Specific long monitoring programs are required.

Target definitions

Specific long monitoring programs are required.

6.4. Protocol for litter ingestion by fish

Tool name

MSFD Protocol for the monitoring of macrolitter ingested by fish.

Tool description

The methodology of this tool follows methods described in the literature for monitoring macrolitter items > 5mm in stomachs of fish. but can be complemented by analysis of microliter fraction (see Chapter 7). The stomach contents can be employed to measure trends and regional differences in marine litter.

Related marine compartments

The tool is proposed for application for pelagic and benthic feeding fish species. Therefore the water column as well as the seafloor of the marine compartment is addressed when quantifying litter in the stomachs of different fish species.

6.4.1. Technical requirements

As a number of regular fish monitoring programmes is in existence fish samples can be easily obtained from these. For the North Sea a list of surveys is available at http://www.cefas.defra.gov.uk/publications-and-data/fishdac.aspx. Similarly data may be found at www.ices.dk including Baltic surveys. The Mediterranean is covered by http://www.sibm.it/SITO%20MEDITS/.

A list of suggested species will not be provided here. However, the most common ones both from an ecosystem perspective as well as from commercial importance should be investigated. These may include e.g. herring (*Harengus harengus*), cod (*Gadus morhua*), tuna species or anchovy (*Engraulis encrasiccous*).

The following parameters should be recorded immediately after sampling:

- location
- trawl/fishery type
- species
- length and standard length
- age
- sex
- visible deformations and skin condition (e.g. ulcers)

Note that no common procedure for litter ingested by fish has so far been developed. For large fish e.g. adult cod, procedures similar to those followed for seabirds and turtles might be adequate, but for smaller fish or juvenile life stages, methods may need to be more in line with details for microlitter studies as described in the Chapter 7. Procedures for size ranges of herring and smaller, as given below, might be subject to amendments as knowledge advances.

A sample size of at least 50 specimens per species and age group is recommended although data on variability are still missing. As more data become available this number may be reduced or increased depending on the relative loads found, i.e. a statistically relevant number of samples is required. Furthermore, when procedures become routine, pooling of samples to reduce workload may also be considered.

When examination directly after sampling is not possible fish are stored deep frozen.

Remove stomach and rinse exterior with deionised water to avoid secondary contamination of the contents. Small stomachs are treated with 10 % KOH or 30 % H_2O_2[27] at ambient temperature to degrade natural organic matter. Depending on the amount this treatment has to be repeated several times as necessary, i.e. until the reaction has visibly stopped.

[27] Note that the effectivity of the oxidative treatment still has to be fully investigated.

Chemical treatment of stomach contents has to be carried out carefully as the action of hydrogen peroxide on organic matter may lead to strong reactions such as intense foaming. Hence gloves and goggles have to be used.

Note that this treatment does not degrade chitin completely but weakens it only structurally. So far no appropriate solvent has been found that will degrade marine chitin under mild conditions. The potential occurrence of chitin remains from e.g. zooplankton or crab remnants interferes with the quantification of fragments.

Larger stomachs are opened and contents removed. Again a peroxide treatment may be necessary to remove natural organic matter such as food-derived fat adhering to plastic items.

After oxidation the remaining material may be washed through a series of sieves to obtain defined size fractions. In order to differentiate between macro- and microlitter at least a 5 mm sieve separation is to be carried out. The retained material is visually inspected and counted under a dissecting microscope where necessary.

In cases where the identification of plastic by visual inspection is ambiguous, i.e. for smaller items, confirmation might be sought by spectroscopy, e.g. FT-IR or Raman, or the "hot needle" technique may be employed.

The fraction passing a 5 mm sieve may then be used for an analysis of microlitter (see Chapter 7 for details).

For carnivorous species fish bones may be removed by extended treatment with c-HCl. Most polymer types are not degraded by up to 5 % hydrochloric acid while polyamide, polycarbonate and some of the less regularly occurring ones such as polyoxymethylene are affected at higher concentrations (see e.g. http://www.kuhnke.de/fileadmin/templates/content/Automation/Branchen/Medizintechnik/764343chemische_bestaendigkeit.pdf).

As an additional method to separate smaller plastic litter from natural inorganic matter in stomach samples, density separation may be applied (see Chapter 7). Nevertheless, this method will require removal of natural organic matter as described above.

With density separation, also surface-tension phenomena should be taken into account. For example, considerable numbers of sand grains may remain at the liquid-surface of a jar in which stomach contents are shaken for separation. Only when surface tension is broken by e.g. lightly stirring the surface with a tweezer, such sand grains drop, and true density separation is reached.

The categorisation of stomach contents is based on the general morphology of plastic items found, i.e. sheetlike, filament, foamed, fragment or other (see list given under a- birds). In most cases, smaller fragments will not be unambiguously related to a particular defined item. Where this is, however, possible items should be described and assigned a litter category number using the masterlist developed by the TSML group (Chapter 8).

For each litter category/subcategory an assessment is made of:

- **incidence** (percentage of investigated stomachs containing litter);
- **abundance by number** (average number of items per individual), and
- **abundance by mass** (weight in grams, accurate to 4th decimal)

Because of potential variations in annual data, it is recommended to describe '**current levels'** as the average for all data from the most recent 5-year period, in which the average is the 'population average' which also includes individuals that were found to have zero litter in the stomach.

Litter categories - source related information

For fish analyses, stomach contents are sorted into the categories as given above for seabirds (Table 7).

Size range

Both juveniles and adults and, wherever possible, also intermediate stages have to be considered. However, depending of the type of litter to be determined, i.e. macro- *vs*. microlitter, different size ranges may be preferred. In general it depends on fish size and choice of litter particle size considered. For micro-sized plastics below mm range, methods using KOH etc., density separation, acids etc. are given in the microlitter report detailed explanation and precautionary recommendations.

Spatial coverage

As mentioned above sampling for analysis of litter in fish should be part of already established surveys.

Survey frequency

Continuous sampling is required.

Maturity of the tool

Not mature at this stage. Specific monitoring programs are required. Methods for the analysis of fish stomach contents, although restricted to natural food items, have been reviewed by Hynes (1950), Pillay (1952), Natarajan and Jhingran (1961), Hyslop (1980) and Cortes (1997) while statistical techniques, i.e. cluster analysis, have been addressed by Rice (1988) and Tirasin and Jørgensen (1999).

Regional applicability of the tool

The tool is applicable anywhere. Species/size selection should be optimized for regional comparison and, wherever possible, overlapping species must be chosen in adjacent areas.

6.4.2. Cost estimates

The most significant costs arise from sampling, i.e. when dedicated cruises become necessary. This can be overcome by obtaining samples from established monitoring programmes.

Overall temporal requirements for the analysis of one stomach is estimated at about one to two man-hours.

Quality assurance / quality control

The methodology needs to be further developed. There is presently a lack of quality assurance/quality control (QA/QC) due to non-existence of long-term monitoring programmes. Only few data are available which usually are based on single surveys (e.g. Anonymous, 1975; Davison and Asch, 2011; Foekema *et al.,* 2011, 2013; Possatto *et al.,* 2011; Anastasopoulou *et al.,* 2013;).

Trend assessment

Due to the lack of maturity of the tool specific long-term programmes have to be developed.

Target definitions

Specific targets have to be developed, e.g. based on the OSPAR recommendation for seabirds (see above).

6.5. Plastic as nest material & entanglement in Bird colonies

Name of protocol

MSFD Protocol for the monitoring of plastic litter as nesting material in seabird breeding colonies and associated entanglement mortality.

Tool description

Seabirds are apex predators in marine systems and are particularly vulnerable to entanglement with plastics and other marine litter (Votier *et al.,* 2011). Seabirds such as northern gannets (*Morus bassanus*), shags (*Phalacrocorax aristotelis*) or kittiwakes (*Rissa tridactyla*) tend to incorporate marine litter, much of it originating in fisheries, into their nests, at times resulting in entanglement. Depending on the regional occurrence and distribution of breeding colonies the nesting material of different species can be assessed for marine litter. In addition, the associated entanglement mortality can be studied as well. Ideally both components should be assessed in combination. The share of plastic items in nests of certain species of birds can be used as an indicator of the amount of litter in the natural environment in the vicinity of their breeding site and to assess entanglement risk of animals. The associated entanglement mortality can serve as an indicator for the direct harm caused by the incorporation of marine litter in nests of breeding colonies.

In terms of European findings to develop a protocol for the use of plastic litter as nesting material and associated entanglement in birds, surveys of breeding colonies might be a powerful indicator regarding

inflicted mortality for seabirds due to marine litter. Negative effects can be documented rather easily and clearly compared with the often more indirect and sublethal effects of e.g. plastic ingestion.

An advantage is that many seabird colonies are already regularly surveyed in many European countries to document the number of breeding pairs and/or breeding success. Thus, a protocol on entanglement in marine litter might potentially be filled out alongside with other existing investigations without too much extra effort.

Related marine compartments

The litter is collected by seabirds for nest construction in the surroundings of the colonies on beaches and at the sea surface.

6.5.1. Technical requirements

Select a (part of) a colony which is easily viewed from fixed viewpoint(s) and for which the borders of the study section(s) can be easily described. If only a part is monitored this should be representative of the whole colony and at least comprise 5 to 10% of all nests (at least several tens of nests). Subsampling of a representative plot can allow for calculating pollution/entanglement for an entire colony, but this is also a function of frequency. If frequency of occurrence of marine litter is low, a large number of nests need to be monitored to be able to accurately monitor trends.

Using GPS and ground-marks, fix the point(s) from which observations will be made, and ensure that such spot(s) can be easily found again in later years for continued monitoring.

Using photography, document exactly which are the borders of the study plot. In principle select an area fully defined by 'natural' borders, so that it is easily reproduced.

Decide on standard dates at which surveys should be conducted: as a minimum a first count should be made prior to the nesting season, to establish potential remainders of entangled corpses still present from the previous year. The second count should be conducted during the peak of the breeding season to receive the maximum number of 'apparently occupied nets' (AON) and respective total number of breeding birds for all species in the colony/monitoring plot. The third survey should be planned shortly after fledging of the chicks, to establish litter rates in the nests, and presence of (new) corpses of birds that died from entanglement. Intermediate or later counts may refine the picture, and may be combined with surveys of breeding effort and success.

For the surveys, use a prescribed observation tool, e.g. binoculars or a telescope of fixed type and magnification ('standardizing the likelihood of observing details in nest structures). When the location and accessibility to the colonies allow, *in situ* observations can be made.

Make a detailed count of the number of nests in the study plot and document number of nests with (digital) photographs whenever possible. This helps to ensure consistent monitoring of plots regarding the number of breeding birds, categorization of litter types and entanglement rates.

Make a detailed count of the structures in above count that contain visible marine synthetic litter, document pollution with digital photographs whenever possible. The `nest litter rate` is assessed as the number of nests containing visible litter divided by the overall number of nests in the study plot

Depending on situation, try to specify details of relative abundance of different types of litter, e.g. roughly as threadlike, sheets, foams, fragments or other, or in more detail using standard MSFD categorization of litter items, try to identify source of litter as e.g. fishing, shipping, recreational. Make a count of birds visibly entangled, recording separately species (other species than the breeders may become entangled), and age (adults, immature or chick) and if alive or dead. Document entanglement with (digital) photographs whenever possible. Ideally this count is done at a standard date, which needs to be defined, shortly AFTER fledging of main number of chicks from the colony.

Impact level from litter in nests is then assessed as the number of dead or dying animals (specified for species and age classes) divided by the overall number of breeding birds in the study plot *('entanglement mortality rate').* The number of live birds that are cut loose and released should be specifically recorded as such but included in the totals for individuals mortally entangled, because without human intervention they would have died; in situations where colonies are intensively surveyed for population monitoring, entanglement rates can be compared also to number of breeders, numbers of chicks etc.).

If possible conduct this type of survey in a number of different plots to provide a measure for local variability (known to be high e.g. in neighboring shag colonies in France (Cadiou *et al.,* 2011).

Above observation survey types can be conducted easily without entering study plots and without or with little interfering with the breeding of birds. As a general rule for repeated monitoring, it is NOT recommended to collect nest structures after the breeding season to quantify proportions of litter included. In many cases, nests are multi-year structures, and removal may negatively affect breeding of site-owners and their neighbors in the next season, either by extra efforts to construct a new nest, disputes with neighbors over remaining nests and materials, or quality of the nest affecting nesting success. This type of work is recommended only as incidental effort by specialized researchers in dedicated research projects. Selected details from some earlier studies are specified in Annex 6.3.

Litter categories – source related information

There are issues to be aware of in interpreting results from this type of monitoring.

Different seabird species have different ranges from colonies when looking for nesting material and may use different types of litter into their nests depending on species and location.

The litter in nests of Northern Gannets (e.g. Montevecchi 1991, Votier *et al.,* 2011, Bond *et al.,* 2012) originates exclusively from the sea, whereas Kittiwakes also pick up litter as nesting material from land (e.g. Clemens & Hartwig 1993, Hartwig *et al.,* 2007). The latter may also apply to cormorants and possibly also shags.

Votier *et al*., (2011), described that gannets seemed to prefer certain type of plastics such as synthetic rope for building nests compared with its proportion found on adjacent beaches. This apparent selectivity needs to be considered if seabirds are used as indicators for measuring trends in certain types of litter. More background info on above mentioned species can be found in Annex 6.3.

Size range

Detection of all visible litter particles from macro- to microlitter is possible.

Spatial coverage

This protocol is designed for application in breeding colonies of seabirds.

Survey frequency

In general, well-built nest are found during incubation and during the rearing period the nest is frequently more or less destroyed by the young; to investigate entanglement rate the best period is after fledging but to investigate the occurrence rate of marine litter the best period is during incubation.

Maturity of the tool

Not mature at this stage. So far no standard protocols to document entanglement in seabird colonies could be identified to be in use although several studies seem to have used a consistent methodology and a number of studies have been conducted on Northern Gannets, European Shags and Black-legged Kittiwakes.

Regional applicability of the tool

This tool can be applied in all regions wherever breeding colonies exist. A partial overview of breeding colonies for especially suitable species can be found in Annex 6.3. It could also be used in waters such as the Baltic or Black Sea where species as Cormorants and Shags breed that build litter into their nests but where other suitable biomonitors such as Northern Fulmars or Sea Turtles are absent.

6.5.2. Costs estimates

In general no special cruise costs are required in case this protocol can be applied within other monitoring or studies in existing study colonies (on breeding pairs/success, or any study involving capture/banding of adults and/or chicks). In case dedicated monitoring is carried out just for this reason one cruise day to the colony with one day of fieldwork (driver of the boat is required). In addition staff-costs for two observers incurred to survey around 100 nests in 20-30 minutes each (and then take the mean) in

addition to the costs for the boat is needed. At regularly-worked colonies, multiple surveys each year are possible.

The equipment costs are low consisting of binoculars/scopes which in most cases will be part of already existing field equipment. Data entry requires additional 1-2 hours of work. The costs for reporting depend on the venue and come down to around 10 hours for untrained technical to summarize data and prepare the report.

In the special case of the monitoring in the *Iroise Marine Natural Park* on shags, about 5 days of fieldwork for the different colonies (1 boat + 1 pilot + 2-4 observers according the colonies) and 2 days for data processing, analyses and annual short report are required.

6.5.3. Quality assurance / quality control

Having 2 observers (or even >2) count independently can produce error estimates. The methodology needs to be further developed.

Trend assessment

Data analysis and trend assessments can be carried out by time series analyses (found in most statistic packages).

A problem is the longevity of plastic litter in nests as in many locations these materials may persist for many years if they are not blown or washed away by storms, rain and flooding or taken away by humans.

Thus, nests may contain the plastic litter of several breeding seasons, and trends in the indicator values may show delays and may thus have functionality for assessing long term rather than short term trends. Finally, as indicated variability scales in the indicator need to be assessed (e.g. Cadiou *et al.,* 2011)

Target definitions

At this stage it seems premature to identify targets reflecting good environmental status or to specify requirements for trend calculations to assess speed of change towards achievement of GES.

6.6. Considerations on further options for monitoring impacts of marine litter on biota

6.6.1. Entanglement rates among beached animals

Direct harm or death is more easily observed and thus more frequently reported for entanglement than for ingestion of litter (CBD 2012). This applies to all sorts of organisms, marine mammals, birds, turtles, fishes, crustaceans etc.

It is, however, difficult from simply looking at the outside appearance of an animal to identify whether a particular individual has died because of entanglement in litter rather than from other causes, mainly entanglement in active fishery gear (bycatch). Nevertheless it is possible to differentiate between animals that have died quickly due entanglement and sudden death in active fishing gear and those suffering a long drawn out death after entanglement in pieces of nets, string or other litter items, because entangled birds, which have been entangled for a time before death are emaciated.

Proportions of sea birds found dead with actual remains of litter attached as evidence for the cause of mortality are extremely low. For beached birds, entanglement rates in the Netherlands are far below 1%, and only for Gannets may reach up to a few percent (Camphuysen, 2008). The possible use of entangled beached birds as an indication of mortality due to litter will be further investigated.

In marine mammals, numbers of beached animals and especially cetaceans are often high (e.g. of harbour porpoises at shores of the North Sea (and even at the Baltic Sea compared to predicted population numbers) or of common dolphins at beaches of the Eastern North Atlantic) and many have body marks suggesting entanglement, although remains of ropes or nets on the corpses are mostly rare. Given that in a lot of places well working stranding networks are already in place, dead marine mammals should, whenever possible, become subject to pathologic investigations which need to include an assessment for the cause of disease and death and the relevance of marine litter in this connection.

This issue will be further investigated and the development of a dedicated monitoring protocol for the entanglement of marine mammals in marine litter will be considered in the next report of the TSG ML.

6.6.2. Entanglement rates among live animals (other than in relation to seabird nests)

Sightings records and a photo identification catalogue from a haul out site in southwest England were used to establish entanglement records for grey seals. Between 2004 and 2008 the annual mean entanglement rates varied from 3.6 % to 5%. Of the 58 entanglement cases, 64% had injuries, which were deemed serious. Of the 15 cases where the entangling litter was visible, 14 were entangled in fisheries materials (Allen *et al.,* 2012). This sort of study is extremely valuable to estimate impacts from marine litter, but requires high levels of specialist research effort. Rare opportunities for this type of study and high costs prevent a recommendation as standard monitoring tool, but dedicated research efforts are highly recommended where possible.

6.6.3. Ingestion of litter by marine mammals

Samples of 107 stomachs, 100 intestines and 125 scats of harbor seals from the Netherlands were analyzed for the presence of plastics. Incidence of plastic was 11% for stomachs, 1% for intestines, and 0% for scats. Younger animals, up to 3 years of age, were most affected (Rebolledo *et al.,* 2011). In this paper, ingestion rates, although of serious concern, were considered too low, and in combination with low sample availability and high cost led to the conclusion that they would not provide a useful MSFD monitoring tool. However, further studies are recommended, as in each of 19 analyzed samples of faces from harbor and grey seals in the German Lower Saxony Wadden Sea, microplastics mainly from granular origin and fibers were found ranging from some milligram to a few grams per sample (personal comment by G. Liebezeit), but that needs to be confirmed by peer-reviewed literature. Determination for microplastics should be implemented in the systematic analyses before final conclusions can be taken.

A recent study described a case of mortality of a sperm whale related to the ingestion of large amounts of marine litter in the Mediterranean Sea. The results show how these animals feed in waters near an area completely flooded by the greenhouse industry, making them vulnerable to its waste products if adequate treatment if this industries waste is not in place (Stephanis *et al*., 2013).

Ingestion of litter by a wide range of whales and dolphins is known. Although known rates of incidences of ingested litter are generally low to justify a standard MSFD monitoring recommendation at this point, it can also be argued that the number of pathologically studied animals is low as well. Dead marine mammals should, whenever possible, become subject to pathologic investigations which need to include an assessment for the cause of disease and death and the relevance of ingested marine macro- and microlitter in this connection.

Therefore the development of a monitoring protocol for the ingestion of marine litter in the different size categories by marine mammals will be considered in the next report of the TSG ML.

6.6.4. Ingestion of litter by marine invertebrates

As concluded in the chapter on microplastics, it would be premature to recommend monitoring programs for specific organisms such as zooplankton species, shellfish like mussels and others as there is insufficient view on frequency of occurrence of ingested litter and species specific requirements in fairly complicated research methods. General methods for dedicated microplastics research in invertebrate biota have been described in chapter 7. Further research into litter ingestion and impacts is highly recommended.

6.6.5. Research on food chain transfer

More and more studies are available, which indicate the affiliation of toxic substances by marine organisms when ingesting plastic litter. E.g. in three of 12 analyzes in abdominal adipose of oceanic seabird (short-tailed shearwaters) higher-brominated congeners (polybrominated diphenyl ethers 10 (PBDEs)) were detected, which are not present in the natural prey (pelagic fish). The same compounds were present in plastic–derived chemicals from ingested plastics to the tissue of marinebased organisms (Tanaka *et al*., 2013).

In a study by Fossi *et al.,* 56 % of surface neustonic/planktonic samples in the Mediterranean contained microplastic particles. The highest abundance (9.63 items/m^3) was found in the Portofino MPA (Ligurian Sea). High concentrations of phthalates (DEHP and MEHP) were detected in the neustonic/planktonic samples. The concentrations of MEHP found in the blubber of stranded fin whales suggested that phthalates could serve as a tracer of the intake of micro-particles.

Although highly relevant, impacts of trophic transfer of microplastics through marine food chains with relevance also on human consumption, are beyond the scope of MSFD monitoring, but are highly important in future research.

6.7. References

Anastasopoulou, A., Mytilineou, C., Smith, C.J., Papadopoulou, K.N., 2013. Plastic debris ingested by deep-water fish of the Ionian Sea (Eastern Mediterranean). Deep-Sea Res. II 74, 11-13.

Anonymous, 1975. Plastic cups found in fish. Mar. Poll. Bull.. 6, 148.

Avery-Gomm, S., O'Hara, P.D., Kleine, L., Bowes, V., Wilson, L.K. and Barry, K.L. 2012. *Northern fulmars as biological monitors of trends of plastic pollution in the eastern North Pacific*. Marine Pollution Bulletin 64: 1776-1781.

Bentivegna F., Travaglini A., Matiddi M., Baini M., Camedda A., De Lucia A., Fossi M.C., Giannetti M., Mancusi C., Marchiori E., Poppi L., Serena F. and Alcaro L., 2013. *First data on ingestion of marine litter by loggerhead sea turtles, Caretta caretta, in Italian waters (Mediterranean Sea).* Proceedings of the Biology and ecotoxicology of large marine vertebrates: potential sentinels of Good Environmental Status of marine environment, implication on European Marine Strategy Framework Directive. 5-6 June, Siena.

Boerger, C.M., Lattin, G.L., Moore, S.L. and Moore, C.J. 2010. *Plastic ingestion by planktivorous fishes in the North Pacific Central Gyre.* Mar Poll Bull 660: 2275-2278.

Bond, A.L., Montevecchi, W.A., Guse, N., Regular, P.M., Garthe, S., Rail, J.F. 2012. *Prevalence and composition of fishing gear debris in the nests of northern gannets (Morus bassanus) are related to fishing effort.* Marine Pollution Bulletin 64(5): 907-911.

Cadiou, B., Pouline, P., Dugue, L. 2011. *Occurrence of marine debris in European shag´s nests as indicator of marine pollition.* Poster at the Seabird Group 11th International Conference, 2-4 September 2011, Plymouth, UK.

Camedda A., Matiddi M., Massaro G., Coppa S., Perilli A., Ruiu A., Briguglio P. and de Lucia A., 2013. *Five years data on interaction between loggerhead sea turtles and marine litter in Sardinia.* Proceedings of the Biology and ecotoxicology of large marine vertebrates: potential sentinels of Good Environmental Status of marine environment, implication on European Marine Strategy Framework Directive. 5-6 June, Siena.

Camphuysen, C.J. 2008. *Verstrikkingen van zeevogels in zwerfvuil en vistuig, 1970-2007 (Entanglements of seabirds in marine litter and fishing gear, 1970-2007).* SULA 21(2): 88-92.

Cortes, E. 1997. *A critical review of methods of studying fish feeding based on analysis of stomach contents: application to elasmobranch fishes.* Can J Fish Aquat Sci 54: 726-738.

Dantas, D., Barletta, M. and da Costa, M. 2011. *The seasonal and spatial patterns of ingestion of polyfilament nylon fragments by estuarine drums (Sciaenidae).* Environ Sci Poll Res: 1-7.

Davison, P. and Asch, R.G. 2011. *Plastic ingestion by mesopelagic fishes in the North Pacific Subtropical Gyre*. Mar Ecol Prog Ser 432: 173-180.

Dierschke, J., Dierschke, V., Hüppop, K., Hüppop, O. and Jachmann, K.F. (2011) *Die Vogelwelt der Insel Helgoland*. OAG Helgoland.

Foekema, E.M., Gruijter, C.D., Mergia, M.T., Kwadijk, C., Kotterman, M., Klok, C., Franeker, J.A.V., Murk, A.J. and Koelmans, A.A. 2011. *Inventory of the presence of plastics in the digestive tract of North Sea fishes.* Last accessed 22 Novemeber 2012 online at: http://documentsplantwurnl/imares/afval/vissen/plastics-fish-2011pdf

Foekema, E.M., Gruijter, C.d., Mergia, M.T., Kwadijk, C., Kotterman, M., Klok, C., Franeker, J.A.v., Murk, A.J., Koelmans, A.A., 2011. *Inventory of the presence of plastics in the digestive tract of North Sea fishes*. http://documents.plant.wur.nl/imares/afval/vissen/plastics-fish-2011.pdf, visited Nov 22, 2012.

Hartwig, E., Clemens, T., Heckroth, M. 2007. *Plastic debris as nesting material in a Kittiwake-(Rissa tridactyla) colony at the Jammerbugt, Northwest Denmark*. Marine Pollution Bulletin 54 (2007) 595-597.

Hynes, H.B.N. 1950. *The food of the freshwater stickle-backs (Gasterosteus aculeatus and Pygosteus pungitius) with a review of methods used in the studies of the food of fishes*. Anim Ecol 19: 36-58.

Hyslop, E.J. 1980. *Stomach contents analysis-a review of methods and their application.* J Fish Biol 17: 411-429.

Jackson, G.D., Buxton, N.G. and George, M.J.A. 2000. *Diet of the southern opah Lampris immaculatus on the Patagonian Shelf; the significance of the squid Moroteuthis ingens and anthropogenic plastic*. Mar Ecol Prog Ser 206: 261–271.

JNCC 2009: http://jncc.defra.gov.uk/page-2875-theme=textonly

Matiddi, M., van Franeker, J.A., Sammarini, V., Travaglini, A. and Alcaro, L. 2011. *Monitoring litter by sea turtles: an experimental protocol in the Mediterranean.* Proceedings of the 4th Mediterranean Conference on Sea Turtles. 7-10 November, Naples.

Montevecchi, W.A. 1991. *Incidence and types of plastic in gannets nests in the Northwest Atlantic.* Can. J. Zool. – Revue Canadienne De Zoologie 69, 295–297.

Moore, E., Lyday, S., Roletto, J., Litle, K., Parrish, J.K., Nevins, H., Harvey, J.T. and de Marignac, J. 2005. *Bird Entanglement observed during beach monitoring surveys*. Last accessed 13 June 2013 online at: http://www.farallones.org/

Natarajan, A.V. and Jhingran, A.C. 1961. *Index of preponderance'-a method of grading the food elements in the stomach analysis of fishes*. Indian J Fish 8: 54-59.

OSPAR 2008. *Background Document for the EcoQO on plastic particles in stomachs of seabirds.* OSPAR Commission, Biodiversity Series. Publication Number: 355/2008. OSPAR, London, pp. 13.

OSPAR 2010a. *Quality Status Report 2010.* OSPAR Commission, London. pp. 175.

OSPAR 2010b. *The OSPAR system of Ecological Quality Objectives for the North Sea: a contribution to OSPAR's Quality Status Report 2010*. OSPAR Publication 404/2009 (Update 2010). OSPAR Commission London, en Rijkswaterstaat VenW, Rijswijk. pp. 16.

Pillay, T.V.R. 1952. *A critique of the methods of study of food of fishes*. J Zool Soc India 4: 185-200.

Possatto, F.E., Barletta, M., Costa, M.F., Sul, J.A.I.d. and Dantas, D.V. 2011. *Plastic debris ingestion by marine catfish: An unexpected fisheries impact*. Mar Poll Bull 62: 1098-1102.

Provencher, J.F., Gaston, A.J. and Mallory, M.L. 2009. *Evidence for increased ingestion of plastics by northern fulmars (|Fulmarus glacialis|) in the Canadian Arctic*. Marine Pollution Bulletin 58: 1092-1095.

Rice, J.C. and Science Brmch. 1988. *Repeated cluster analysis of stomach contents data: method and appkation to diet of cod in NAFO division 3L*. Environ Biol Fishes 21: 263-277.

Simmonds, M.P. 2012. *Cetaceans and marine debris: the great unknown*. Journal of Marine Biology 2012, Article ID 684279, pp. 8. doi:10.1155/2012/684279.

Skóra, M.E., Sapota, M.R., Skóra, K.E. and Pawelec, A. 2012. *Diet of the twaite shad Alosa fallax (Lacépède, 1803) (Clupeidae) in the Gulf of Gdansk, the Baltic Sea*. Oceanol Hydrobiol Stud: 24-32.

Tirasin, E.M. and Jørgensen, T. 1999*. An evaluation of the precision of diet description.* Mar Ecol Prog Ser 182: 243-252.

Travaglini A., Matiddi M., Ciampa M., Alcaro L. and, Bentivegna F., 2013. *Marine litter in loggerhead sea turtles (Caretta caretta) from Central and Southern Italian waters: analysis from dead and alive turtles.* Proceedings of the Biology and ecotoxicology of large marine vertebrates: potential sentinels of Good Environmental Status of marine environment, implication on European Marine Strategy Framework Directive. 5-6 June, Siena.

Van Franeker, J.A. and Meijboom, A. 2002. *Litter NSV - Marine litter monitoring by Northern Fulmars: a pilot study.* ALTERRA-Rapport 401. Alterra, Wageningen. pp. 72.

Van Franeker, J.A., Heubeck, M., Fairclough, K., Turner, D.M., Grantham, M., Stienen, E.W.M., Guse, N., Pedersen, J., Olsen, K.O., Andersson, P.J. and Olsen, B. 2005. *'Save the North Sea' Fulmar Study 2002-2004: a regional pilot project for the Fulmar-Litter EcoQO in the OSPAR area*. Wageningen, Alterra, Alterra-rapport 1162. pp. 70.

Van Franeker, J.A., Blaize, C., Danielsen, J., Fairclough, K., Gollan, J., Guse, N., Hansen, P.L., Heubeck, M., Jensen, J.-K., Le Guillou, G., Olsen, B., Olsen, K.O., Pedersen, J., Stienen, E.W.M. and Turner, D.M. 2011a. *Monitoring plastic ingestion by the northern fulmar Fulmarus glacialis in the North Sea.* Environmental Pollution 159: 2609-2615. doi:10.1016/j.envpol.2011.06.008

Van Franeker, J.A. and the SNS Fulmar Study Group. 2011b. *Fulmar Litter EcoQO monitoring along Dutch and North Sea coasts in relation to EU Directive 2000/59/EC on Port Reception Facilities: results to 2009.* IMARES Report Nr C037/11. IMARES, Texel, pp. 52 + pp. 2.

Votier, S.C., Archibald, K., Morgan, G., Morgan, L. 2011. *The use of plastic debris as nesting material by a colonial seabird and associated entanglement mortality*. Marine Pollution Bulletin 62 (2011) 168-172.

Williams, D.D. and Williams, N.E. 1974. *Counterstaining technique for use in sorting benthic samples.* Limnol Oceanogr 19: 152–154.

Wyneken, J. 2001.*The Anatomy of Sea Turtles*. U.S. Department of Commerce NOAA Technical Memorandum NMFS-SEFSC-470, 1-172.

Annex 6.1 - Sea Turtle Necropsy Data Sheet

Identification Data	
Species, Tag/chip number	
Date of finding	
Circumstances (stranded, interaction with human activity (precise, and precise gear when interaction with fishing activity, death at rescue center)	
Date of necropsy (after or before freezing, if freezed indicate at which temperature)	
Trophic status atrophy of the pectoral muscles (None, Moderate, Severe) fat thickness in the articular cavities and on the coelomic membrane (Abundant, Normal, Low, None)	
Fresh/Decomposition status (categories to be explained)	
Date of turtle death	
Cause of death, if determined	
Location	
Coordinates	
Identification number (code) (International CITES code)	
Finder personal details (name, telephone, mel)	

Measurements	**Unit (cm)**
Carapace length (CCL)	
Overcurve width (CCW)	
Plastron length (CPL)	
Plastron width (CPW)	

External observation	Comments	Photo (if relevant)
Head		
Flipper		
Carapace		
Plastron		
Tail		
Sex-maturity		
Skeletal-damage		
Foreign bodies		
Cause of death		
Other		

Gastrointestinal tract	Observation/Comments	Photo (if relevant)
Oesophagus		
Stomach		
Intestine		

Annex 6.2 – Data sheet for recording of ingested items in sea-turtles

To do for each part of the gastrointestinal tract (oesophagus, stomach, intestine)

Oesophagus, Stomach or Intestine	

Type of Litter	**Presence yes/no**	**Abundance (items number)**	**Volume (ml H_2O)**	**Color (number)**	**Dry Weight (g)**	**Microlitter abundance (number items <5mm)**
IND ind						
IND Pind						
USE she						
USE thr						
USE foa						
USE fra						
USE Poth						
RUB pap						
RUB kit						
RUB rva						
RUB hoo						
POL sla						
POL tar						
POL che						
FOO						
NFO						

For litter categories see Table 7 inserted in the birds protocol.

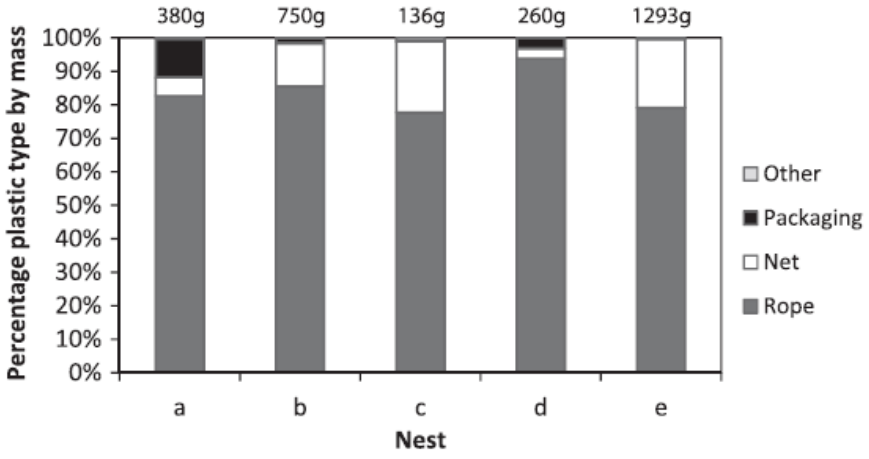
Annex 6.3 – Litter in nests of 3 species of European Sea-birds

Northern gannet (*Morus bassanus*)

The northern gannet is endemic to the North Atlantic and most breed in Canada, Britain and Ireland. There are 21 gannetries around the British Isles (JNCC 2009), with most being on remote offshore islands and stacks, and two on mainland cliffs. Between March and September Britain is in fact home to nearly 70% of the world's breeding gannet population, making their habitat internationally important.

A study by Votier *et al.,* (2011) investigated the use of plastics as nesting material by northern gannets for the years 1996-1997 and 2005-2010 in the third largest gannet colony in the world (Grassholm, Whales) where approximately 40.000 pairs of gannet breed. On average gannet nests contained 469.91 g (range 0–1293 g) of plastic, equating to an estimated colony total of 18.46 tons (range 4.47–42.34 tonnes). Litter in nests were categorized into four categories: rope made from synthetic fibers, fishing nets, packaging (plastic bags and strapping) and any other plastic which did not fit into the former three categories. The majority of nesting material was synthetic rope, which appears to be used preferentially. The relative contribution of the main types of macro-plastics were calculated and compared with shipping- and fisheries-derived plastics collected from nine nearby-beaches. Within these two categories the plastics were assigned to the same four categories as those used for gannets nests and presented in frequency of occurrence. Overall the plastic component was dominated by rope made from synthetic fibers (83%), followed by netting (15%), packaging (2%) and a very small proportion of other plastics (<1%) (Figure below).

The associated levels of mortality were assessed as well. Based on data from eight years of surveys to release entangled birds at the end of the breeding season, the number of entangled birds by year and age class was reported. On average 62.85 ± 26.84 (range minima 33–109) birds were entangled each year, totaling 525 individuals over eight years, the majority of which were nestlings. The number of entangled gannets showed no consistent linear trend over time. The percentage mortality also varied markedly among years and there was a tendency for higher mortality during later visits. The vast majority of entangled birds were fully-grown nestlings, ranging from 75% to <100% of the total numbers.



Percentage of four main plastic types found in five northern gannet nests. Values above bars indicate total dry weight (g) of plastic for each nest. We included a sixth nest in our analysis that contained no plastic. Values above bars are total plastic mass for each nest.

Already in the mid 1980ties 2.6 % of all (non-breeding) northern gannets observed at the island of Helgoland (south-eastern North Sea) were entangled in fishing gear (Schrey & Vauk, 1987). Today, virtually all nests of the breeding colony on Helgoland contain plastic litter (632 pairs in 2013, O. Hüppop, pers. comm.). Dierschke *et al.,* (2011) estimated that at least 20 to 30 gannets are annually killed in this colony by entanglement. The vast majority of nests here is not accessible by humans. Visual observations are possible, but not done yet at a routinely basis.

A study by Bond *et al*. (2012) assessed the prevalence and composition of fishing gear litter in the nests of northern gannets and found a relation to fishing effort. This long-term study was done in the Northwest

Atlantic Ocean, almost all gannet nests examined at two colonies situated in Newfoundland contained marine litter in the late 1980s, much of it being fishing gear litter. The proportion of nests with marine litter decreased following the fishery closure (investigated in 2007) and the proportion of nests with marine litter was related exponentially to the number of gillnets set around the breeding colonies.

Kittiwake (*Rissa tridactyla*)

The Kittiwake is a colonial breeding seabird and occurs discontinuously along the shores of north-west Europe, from the coasts of Portugal and Galicia (north-west Spain) in the south, through Brittany (France), Ireland and Britain, the German Island of Helgoland, Iceland and along Scandinavian coasts to the Kola Peninsula. In the UK, Kittiwakes occur on most coasts, although there are few colonies on the south and east coasts of England. A high percentage of the British Kittiwake population nests in northern Scotland and along the North Sea coast south to East Yorkshire.

The recording of the share of marine litter used as nest construction material by the Kittiwake colony at the Bulbjerg at the Jammerbugt in Northwest Denmark in 1992 has been taken up in 2005. Whereas in the year 1992 plastic litter items were included in 39.3% of 466 Kittiwake nests in the Bulbjerg colony, in 2005 57.2 % of 311 nests contained plastic litter (Hartwig *et al.,* 2007). Litter items detected in 1992 consisted of white, black, green, red, and blue synthetic strings, plastic foil and fishing net remnants, the ones identified on 2005 could be assigned to tight meshed netting and strings in various colours (red, blue and black).

The share of litter seems to correspond to the amount of litter of these categories on the beach and in the surroundings of the colony. This is supported by findings reported in Clemens and Hartwig 1993 for the Kittiwakes at the colony on Helgoland, where during the 1992 breeding season, of the 152 nets counted, spread over the entire colony, in 17 (=11,1%) nests visible litter particles such as net fragments, plastic strings, plastic foil and rubber band were found. Anyway, in both publications (Clemens & Hartwig, 1993, Hartwig *et al.,* 2007) there is no exact quantification of litter types given, neither in Kittiwake nests nor for litter in the surrounding environment of the colonies. Moreover, the size of this surrounding area which is assumed to act as source of the litter in nests is not defined either. Thus, the initial conclusion that the share of litter in Kittiwakes nest reflects the amount of litter of these categories on the beach and surroundings would need further specification and testing.

Shag (*Phalacrocorax aristotelis*)

The European Shag can be found along the entire Atlantic coast of Europe as far north as Finland and including Iceland, as far south as the coast of Morocco, and ranges in the entire Mediterranean nesting on parts of the coastline of most European (e.g. Italy, Turkey) and north African countries (e.g. Algeria, Libya), as well as parts of the Black Sea coast (e.g. Ukraine).

In Western Brittany marine litter in shag´s nests is used as indicator of marine pollution. This monitoring is carried in the Marine Natural Park (Cadiou *et al.,* 2011). A simple assessment method was developed to assess occurrence and abundance of marine litter in nests during annual census of breeding pairs, tested in 2010-2012.

Five abundance classes were distinguished, from MD 1-5 (1-5 items identified) to MD20+ (see Table below. Hereby an example how the data collection on one day but in different colonies is taken:

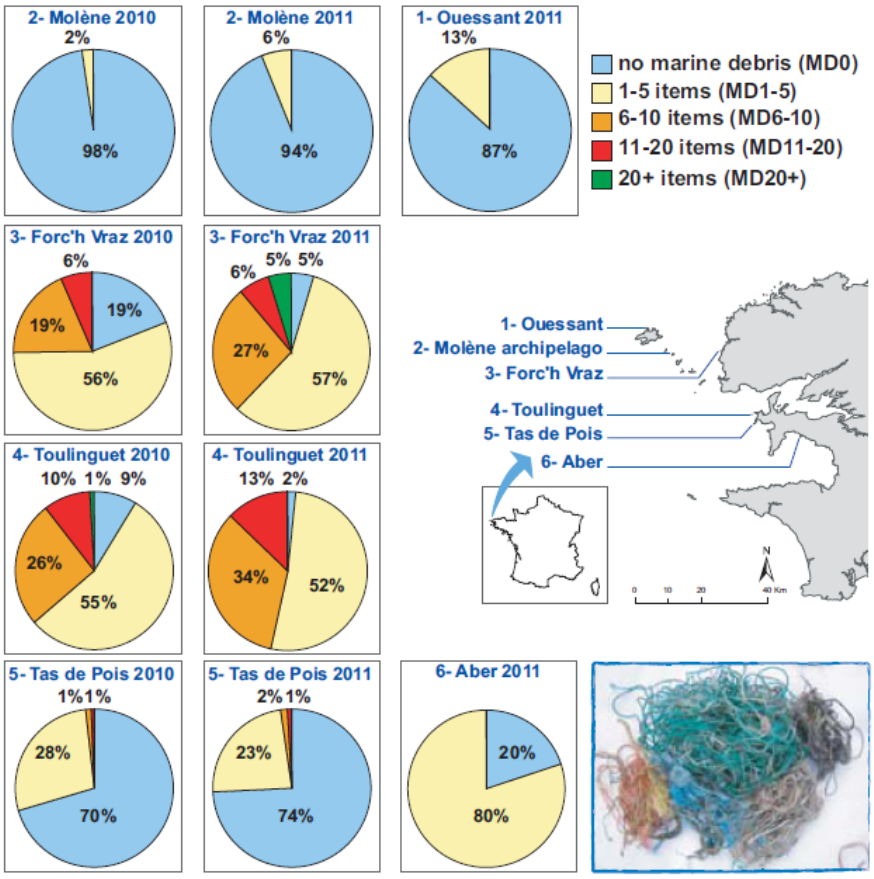
Date	Colony	Observers	Nest-content	marine-debris remarks
18.05.2012	Ar Gest	B. Cadiou	0	MD0
18.05.2012	Ar Gest	B. Cadiou	1E1D	MD0
18.05.2012	Ar Gest	B. Cadiou	2D	MD01-05
18.05.2012	Ar Gest	B. Cadiou	2B1W	MD01-05
18.05.2012	Ar Gest	B. Cadiou	0	MD01-05
18.05.2012	Ar Gest	B. Cadiou	3A	MD06-10
18.05.2012	Ar Gest	B. Cadiou	1D1W	MD06-10

18.05.2012	Ar Gest	B. Cadiou	0	MD11-20
18.05.2012	Ar Gest	B. Cadiou	2C	MD11-20
18.05.2012	Ar Gest	B. Cadiou	0	MD21+
				MD0 = no marine litter
				MD01-05 = 1-5 items of marine litter
				MD06-10 = 6-10 items
				MD11-20 = 11-20 items
				MD21+ = >20 items
			0 = empty nest	
			W = egg	
			A-G = age classes of chicks	
			e.g. 2B1W = 2 chicks (age class B) + 1 egg	
Bernard Cadiou - Bretagne Vivante-SEPNB, Brittany, France				

Example of table for data collection in different colonies

Samples of litter were randomly collected in different nets after fledging. Items were classified into different categories according to the OSPAR classification of marine litter, in order to identify their origin (fishery activities, domestic use etc.). Results pointed out high variability of occurrence and abundance of marine litter between colonies (table below). The abundance class MD50+ was not met so far. A few cases of entangled birds have been reported with breeding adults or young found dead in their nests. It is planned to further investigate in marine currents in the study area to investigate on possible explanations

about higher densities of floating litter in the vicinity of some breeding colonies.



Number of litter items in nests in different breeding colonies (Cadiou *et al.*, 2012)