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Agenda item 5: Main elements of the Integrated Monitoring and Assessment Programme related to Ecological Objectives 5, 9, 10

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Note by the Secretariat

This document has been prepared based on the discussion held at the Integrated CorrMon meeting held on 31 March-April First 2015 in Athens, Greece.

The meeting reviewed and approved the proposed elements on monitoring for EO5, 9 & 10 as presented in this document and recommended its submission to the MED POL Focal Point meeting for further review and agreement as appropriate.

In addition, the Corr Mon meeting highlighted a number of issues with regards to the assessment criteria for Ecological Objectives 5 & 9 to the attention of Med Pol Focal point meeting.

Chapter I
Eutrophication chapter and related fact sheet

I. MONITORING AND ASSESSMENT METHODOLOGICAL GUIDANCE ON EO5: EUTROPHICATION

1. Introduction

Eutrophication is a process driven by enrichment of water by nutrients, especially compounds of nitrogen and/or phosphorus, leading to: increased growth, primary production and biomass of algae; changes in the balance of nutrients causing changes to the balance of organisms; and water quality degradation. The consequences of eutrophication are undesirable if they appreciably degrade ecosystem health and/or the sustainable provision of goods and services. These changes may occur due to natural processes. Management concern begins when they are attributed to anthropogenic sources. Additionally, although these shifts may not be harmful in themselves, the main worry concerns 'undesirable disturbance': the potential effects of increased production, and changes of the balance of organisms on ecosystem structure and function and on ecosystem goods and services.

In the Mediterranean, the UNEP/MAP MED POL Monitoring programme included from its inception the study of eutrophication as part of its seven pilot projects approved by the Contracting Parties at the Barcelona meeting in 1975 (UNEP MAP, 1990a,b). The issue of a monitoring strategy and assessment of eutrophication was first raised at the UNEP/MAP MED POL National Coordinators Meeting in 2001 (Venice, Italy) which recommended to the Secretariat to elaborate a draft programme for monitoring of eutrophication in the Mediterranean coastal waters. In spite of a series of assessments reviewing the concept and state of eutrophication, there are important gaps in the capacity to assess the intensity of this phenomenon, even more to compare or grade the various sites. Efforts have been devoted to define the concepts to assess the intensity and to extend experience beyond the initial sites in the Adriatic Sea admittedly the most eutrophic area in the entire Mediterranean Sea.

GES with regard to eutrophication is achieved when the biological community remains well-balanced and retains all necessary functions in the absence of undesirable disturbance associated with eutrophication (e.g. excessive algal blooms, low dissolved oxygen, declines in sea-grasses, kills of benthic organisms and/or fish) and/or where there are no nutrient-related impacts on sustainable use of ecosystem goods and services. The conceptual model of eutrophication is presented in Figure 1 for information purposes.

2. The choice of indicators for monitoring and assessing eutrophication

Despite the great variability born by the water layers subject to active hydrodynamic processes, monitoring the characteristics of the seawater is still the most direct way of assessing eutrophication. A number of parameters have been identified as providing most information relative to eutrophication e.g. chlorophyll, dissolved oxygen, inorganic nutrients, organic matter, suspended solids, light penetration, aquatic macro-phytes, zoo benthos, etc. They all may be determined either at the surface or at various depths. However even though these variables are routinely determined by most marine laboratories they may pose some problems to some less specialized institutions. Remote sensing may also be employed and with great success when eutrophication extends over large areas such as in the case of the northern Adriatic Sea.

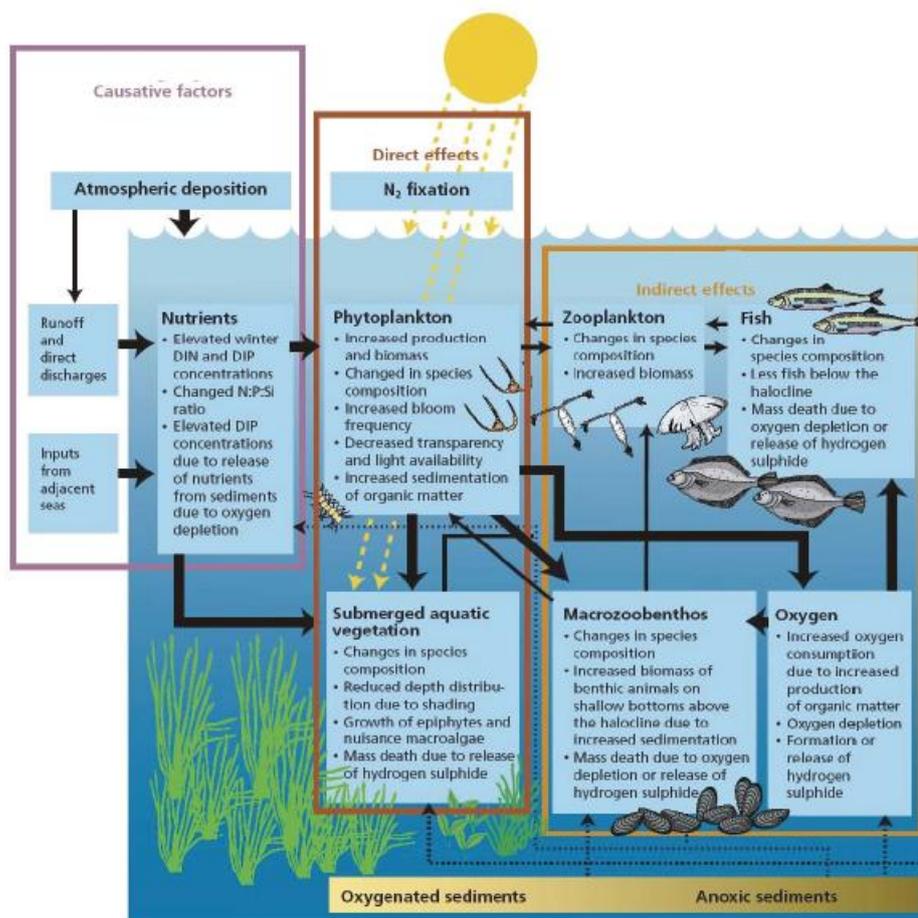


Figure 1. Conceptual model of eutrophication. The arrows indicate the interactions between different ecological compartments. A balanced marine ecosystem is characterised by: (1) a pelagic food chain (phytoplankton ► zooplankton/zoobenthos ► fish), which effectively couples production to consumption and minimises the potential for excess decomposition (2) natural species composition of plankton and benthic organisms, and (3) if appropriate, a natural distribution of submerged aquatic vegetation. Nutrient enrichment results in changes in the structure and function of marine ecosystems, as indicated with bold lines. Dashed lines indicate the release of hydrogen sulphide (H₂S) and phosphorus, under anoxic conditions at the sediment-water interface, which is positively related to oxygen depletion. In addition, nitrogen is eliminated by denitrification in anoxic sediment.

If only limited means are available, determination of those parameters that synthesize the most information should be retained. Chlorophyll determinations for example, although not very precise representations of the system, are data which provide a great deal of information. Reliable data on nutrients are extremely useful indicators of potential eutrophication. Turbidity and seawater colour (Forell scale, Wernard and van der Woerd, 2010) may also be a good measure of eutrophication, except near the mouths of rivers where inert suspended solids may be extremely abundant. Dissolved oxygen is one parameter that integrates much information on the processes involved in eutrophication, provided it is measured near the bottom or, at least, below the euphotic zone where an oxycline usually appears.

- 2.1. The choice of eutrophication indicators to be monitored under the LBS Protocol and the draft Integrated Monitoring and Assessment Programme (Common indicators 7 and 8, Concentration of key nutrients in the water column and Chlorophyll-a concentration in the water column)

Decision 21/3 of COP 18 of the Contracting parties to the Barcelona Convention (Istanbul, December 2013) provides for assessing eutrophication for the Ecosystem Approach by combining the information on nutrient levels, direct effects (specifically chlorophyll – a concentration and water transparency for the 2016 ECAP monitoring activities) and indirect effects (oxygen concentration for the 2016 ECAP monitoring activities). These elements to be monitored reflect the short term eutrophication monitoring strategy of UNEP/MAP MED POL Phase III and IV (UNEP (DEC) WG.231/14) according to which pilot monitoring programmes were implemented in different Mediterranean locations to build capacity in setting up and implementing integrated eutrophication monitoring programmes, (in which phytoplankton total abundance, abundance of major groups and bloom dominance would also be monitored on a discretionary basis). It is considered that the aim would now be focused within the ecosystem approach framework towards developing complete coherent datasets at the entire regional sea level.

In addition it is fundamental to link up to budgets of nutrient sources and loads (e.g. terrestrial, airborne) so the load can be associated with impairment and successful management measures can be developed from that relationship. Such an inventory of pollution sources and loads from land based activities (**NBB**) is prepared periodically by UNEP/MAP MED POL in the framework of the implementation of the LBS Protocol and the Strategic Action Programme (**SAP-MED**) to Address Pollution from Land Based Activities (adopted in 1997 and launched in 2000). The third cycle of the NBB reporting is currently ongoing and expected to be finalized in early 2015.

No single analytical tool is adequate to measure the degree of eutrophication of a given body of water. Instead, most experts believe the best approach is to measure many different parameters and to synthesize the results into a general model providing an overall, somewhat integrated degree of eutrophication for the water. Unless proper selection of the parameters to be measured is made, the amount of work required to assess the extent and intensity of eutrophication may be rather costly.

Measurement strategy and sampling design are therefore keys to the success in monitoring eutrophic areas. It will certainly have to adapt to the morphological characteristics of the area to be monitored, its hydrodynamics and the sources of nutrients. It should be realized that simple measuring and sampling schemes will not provide much insight into an extremely complex phenomenon. Depending on the importance of the impact of eutrophication (plankton blooms, HABS, anoxic events) the amount of effort needed to be put into a monitoring plan can be assessed.

3. Monitoring strategy

3.1. Considerations regarding eutrophication monitoring methods

Traditional methods for eutrophication monitoring in coastal waters involve in situ sampling/measurements of commonly measured parameters such as nutrients concentration, chlorophyll 'a' concentration, phytoplankton abundance and composition, transparency and dissolved oxygen concentration. Concerning available methods for in situ measurements, ships provide flexible platforms for eutrophication monitoring, while remote sensing provides opportunities for a synoptic view over regions or sub-regions. Besides traditional ship measurements, ferry-boxes and other autonomous measuring devices have been developed that allow high frequency and continuous measurements.

In situ measurements are more suitable:

- In (sub) regions/areas/sites with an increasing eutrophication problem,
- When a sub-region/area/site is close to or under GES for eutrophication
- When the status with respect to eutrophication is still unclear
- In sub-regions/areas/sites where for other reasons accurate and reliable data are needed (generally these are coastal sub-regions, in particular close to rivers).

Modelling and remote sensing should also be considered as alternatives or in addition to in situ measurements, depending on the requirements with respect to data. In general, in situ measurements always remain necessary to validate and calibrate the models and data calculated from satellite measurements.

Model generated data are more suitable:

- In (sub) sub-regions with a stable, predictable eutrophication status
- In sub-regions in GES or where the eutrophication problem is decreasing
- In offshore areas where taking in situ measurements is costly and where nutrient levels are correlated with levels in the coastal zone (extrapolation)
- In case satellite data are inaccurate or not available
- Where there is a need for an average picture of the local eutrophication status; models are very good at calculating this average picture combining hydraulic models and in situ measurements of standard sampling sites (interpolation)

As with models, remote sensing generally allows the production of data with a higher spatial and temporal resolution than in situ measurements. Thanks to the use of satellites it is possible to have synoptic measurements over large areas. This makes the satellite data particularly useful for large-scale studies and observations and/or for studies of temporal trends.

Satellite data are more suitable:

- In (sub) sub-regions/areas/sites with a stable, predictable eutrophication status
- In sub-regions/areas/sites in GES or where the eutrophication problem is decreasing
- In offshore sub-regions/areas/sites where taking in situ measurements is costly and where nutrient levels are correlated with levels in the coastal zone
- In case models are inaccurate or not available
- For comparisons of the eutrophication status over large sub-regions
- For validation and calibration of the information on spatial distribution
- In sub-regions/areas where funds are limiting
- In sub-regions/areas where for other reasons the accuracy can be lower than provided by in situ measurements (generally these are offshore areas)
- In addition to in situ measurements

However, satellite data need to be supported by ground truth data.

A good strategy appears to be a combination of remote sensing and scanning of the area known or suspected to be affected with automatic measuring instruments such as thermo-salinometer, dissolved oxygen sensors and in vivo fluorometer and/or nephelometer. Sampling for the determination of “in vitro” fluorescence and nutrient analysis may be carried out with relatively little effort if a proper pump and hose are mounted on the ship. The measurements may be done at the surface or just below it with a water intake on the hull of the vessel or at fixed or varying depths with a towed “fish” and pumping system.

Processing and evaluating the data should be carried out having a predefined model of the system under study. Models of the aquatic ecosystem may be good tools for monitoring eutrophication efficiently. Since none of the eutrophication indicators alone can provide an absolute account of the extent and /or intensity of eutrophication, numerical models in which quantitative relationships among the various characteristics are given, allow an overall assessment of the phenomenon to be made with a small number of field and/or laboratory measurements.

3.2. The frequency of eutrophication monitoring and location of sampling sites

The extent of eutrophication shows spatial variation, for instance coastal regions versus the open sea. The frequency and spatial resolution of the monitoring programme should reflect this spatial variation in eutrophication status and pressures following a risk based approach and the precautionary principle.

The first factor promoting eutrophication is nutrient enrichment. This explains why the main eutrophic areas are to be found primarily not far from the coast, mainly in areas receiving heavy nutrient loads.

However, some natural symptoms of eutrophication can also be found in upwelling areas. Additionally, the risk of eutrophication is linked to the capacity of the marine environment to confine growing algae in the well-lighted surface layer. The geographical extent of potentially eutrophic waters may vary widely, depending on:

- (i) the extent of shallow areas, i.e. with depth ≤ 20 m;
- (ii) the extent of stratified river plumes, which can create a shallow surface layer separated by a halocline from the bottom layer, whatever its depth
- (iii) extended water residence times in enclosed seas leading to blooms triggered to a large degree by internal and external nutrient pools; and
- (iv) upwelling phenomena leading to autochthonous nutrient supply and high nutrient concentrations from deep water nutrient pools, which can be of natural or human origin.

Sub-regions/areas that are in sub-GES status in terms of eutrophication, or that could be considered at risk of not achieving GES generally require more intense monitoring than regions shown to be achieving GES.

Flexibility should be incorporated into the design of the monitoring programme to take account of differences in each marine sub-region/area. Furthermore in cooler regions winter is an optimal period for measuring nutrients since the data are not disturbed by (variable) uptake by algae/macrophytes. In those regions, spring/summer is an optimal period of the algal growing season and therefore for measuring effects of high nutrient availability. In warmer regions productivity continues during (a large part of) the winter period. In these regions, year round measurements of nutrients may be more appropriate.

In brief the geographical scale of monitoring for the assessment of GES for eutrophication will depend on the hydrological and morphological conditions of an area, particularly the freshwater inputs from rivers, the salinity, the general circulation, upwelling and stratification. The spatial distribution of the monitoring stations should, prior to the establishment of the eutrophication status of the marine sub-region/area, be risk-based and proportionate to the anticipated extent of eutrophication in the sub-region under consideration as well as its hydrographic characteristics aiming for the determination of spatially homogeneous areas. Consequently, each Contracting Party would be required to determine the optimum frequency per year and optimum locations for their monitoring stations. Each Contracting Party is responsible for the choice of the most representative sampling stations in order to detect a change over a selected period.

Salinity gradients can be a proxy for river discharge and salinity and nutrient concentrations are often strongly correlated. Salinity can thus be used to determine an optimal spatial distribution of sampling sites, in particular if a model is available to couple salinity and hydrodynamics to nutrient levels. Salinity and temperature are also important parameters supporting the interpretation of eutrophication indicators. Therefore, annual and seasonal temperature regime and, where relevant, spatial and temporal distribution of salinity should be measured in both GES and non-GES regions.

The current national eutrophication monitoring programme implemented so far by the Contracting Parties in the framework of the UNEP/MAP MED POL programme should be used as a sound basis for monitoring under the EcAp complemented with the additional elements based on the above mentioned considerations and each country/sub region/area specificity.

3.3 Characterization of Ecological Quality Status of coastal marine waters with regard to eutrophication

The TRIX index (Vollenweider et al., 1998) may be used for a preliminary assessment of the trophic status of coastal waters in relation to eutrophication providing that its advantages and shortcomings are taken into account (Primpas and Karydis, 2011). The adopted UNEP/MAP MED POL short term eutrophication monitoring strategy monitored parameters to support the TRIX index. This Index is widely used to synthesize key eutrophication variables into a simple numeric expression to make information comparable over a wide range of trophic situations:

$$\text{TRIX Index} = (\text{Log}_{10} [\text{ChA} \cdot \text{aD}\% \text{O} \cdot \text{DIN} \cdot \text{TP}] + k) \cdot m$$

where:

ChA = Chlorophyll a concentration as $\mu\text{g/L}$;

aD%O = Oxygen as absolute % deviation from saturation;

DIN = Dissolved Inorganic Nitrogen, N-(NO₃+NO₂+NH₄) as $\mu\text{g/L}$;

TP = Total Phosphorus as $\mu\text{g/L}$.

$$k=1.5$$

$$m = 10/12 = 0.833$$

The parameters k and m are scale coefficients necessary to fix the lower limit value of the Index and the extension of the related Trophic Scale, i.e. from 0 to 10 TRIX units. Referring to the ChA and a DO% components, these factors are direct indicators of productivity, in terms of both the amount of phytoplankton biomass produced and the dynamic of that production, respectively. In other words, the TRIX Index summarises what the coastal system does (by including the contribution of the direct indicators of productivity, as “actual productivity”) and what the coastal system could do (contribution of the nutritional factors components, as “potential productivity”). As a result of the Log transformation of the four original variables, the annual distributions of TRIX over homogeneous coastal zones are usually of normal kind, and show a fairly stable variance, with STD around 0.9. As for the interpretation of TRIX values, those exceeding 6 TRIX units are generally associated to highly productive coastal waters, where the effects of eutrophication are represented by frequent episodes of anoxia in bottom waters. Values lower than 4 TRIX units are typical of scarcely productive waters, while values lower than 2 are generally associated to the open sea.

The TRIX index used for the assessment of trophic status of coastal waters has been applied in many European seas (Adriatic, Tyrrhenian, Baltic, Black Sea, and North Sea). However, all these waters are characterized by high nutrient levels and phytoplankton biomass; an index calibration based on systems that are principally eutrophic may introduce bias to the index scaling. In the work of Primpas and Karydis, 2011, the TRIX trophic index is evaluated using three standard sets of data characterizing oligotrophy, mesotrophy, and eutrophication in the Aegean (Eastern Mediterranean) marine environment. A natural eutrophication scale based on the TRIX index that is suitable to characterize trophic conditions in oligotrophic Mediterranean water bodies is proposed. This scale was developed into a five-grade water quality classification scheme describing different levels of eutrophication.

It is recommended also that the contracting parties rely on the classification scheme on chl-a concentration ($\mu\text{g/l}$) developed by MEDGIG as an assessment method easily applicable by all Mediterranean countries based on the indicative thresholds and reference values adopted therein (see Table 2).

4. Development of assessment thresholds and identifying reference conditions for eutrophication in order to be able to monitor the achievement of GES

Three approaches may be used for GES determination:

- a. In order to assess quantitatively the achievement of GES in relation to eutrophication, a measurable assessment threshold may be set, including the definition of reference conditions. GES assessment thresholds and reference conditions (background concentrations) may not be identical for all areas, especially where the marine environment is already disturbed by human presence for many years. In these cases a decision has to be made whether to set the threshold value for GES achievement independently to the setting of the reference conditions. The approach is based on the recognition that area-specific environmental conditions must define

threshold values. A threshold value could include provisions to allow for statistical fluctuations (example: No nutrients and chl-a values exceeding the 90th percentile are present in a frequency more than statistically expected for the entire time series). GES could be defined on a sub-regional level, or on a sub-division of the sub-region (such as the Northern Adriatic), due to local specificities in relation to the trophic level and the morphology of the area.

- b. A second approach to determine GES for eutrophication is to use trends for nutrients contents, and direct and indirect effects of eutrophication. When using the trend approach, a reference value representing the actual situation is needed, for comparison. In the case of nutrients and chl-a, such reference values exist due to data availability in most areas. Therefore, GES could be defined as no increasing trends in nutrient and/or chlorophyll-a concentrations over a defined period of time in the past (ex. 6 years), which are not explained by hydrological variability. For indirect effects, GES could ask for no decreasing trend in oxygen saturation beyond what would be statistically expected.
- c. GES thresholds and trends are recommended to be used in a combined way, according to data availability and agreement on GES threshold levels. In the framework of UNEP/MAP MED POL there is experience with regard to using quantitative thresholds. It is proposed that for the Mediterranean region, quantitative thresholds between “good” (GES) and “moderate” (non GES) conditions for coastal waters could be based as appropriate on the work that is being carried out in the framework of the MED GIG intercalibration process of the EU Water Framework Directive (WFD), a project closely followed by the UNEP/MAP MED POL programme.

In this context regarding the definition of subregional thresholds for chlorophyll a water typology is very important for further development of classification schemes of a certain area. Within the MEDGIG exercise the recommended water types for applying eutrophication assessment is based on hydrological parameters characterizing a certain area dynamics and circulation. The typological approach is based on the introduction of a static stability parameter (derived from temperature and salinity values in the water column): such a parameter, on a robust numerical basis, can describe the dynamic behaviour of a coastal system.

On the basis of surface density and salinity values three major water types have been defined:

Table 1

	Type I	Type II	Type III
σ_t (density)	<25	25<d<27	>27
salinity	<34.5	34.5<S<37.5	>37.5

The three different water types, in an ecological perspective, can be described as follows:

- Type 1 coastal sites highly influenced by freshwater inputs
- Type 2 coastal sites not directly affected by freshwater inputs
- Type 3 coastal sites not affected by freshwater inputs

As suggested by the on line expert group on eutrophication established by the Contracting parties it is recommended that with regard to nutrient concentrations, until commonly agreed thresholds have been determined, negotiated and agreed upon at a sub regional or regional level, GES may be determined on a trend monitoring basis.

With regards to chlorophyll a, the on line Mediterranean eutrophication group recommend the reference and threshold values of the MEDGIG approach to be used for assessing ewutrophication status as presented in in Table XX.

Table 2:

Coastal waters Typology	Reference conditions of Chla ($\mu\text{g L}^{-1}$)		Boundaries of Chla ($\mu\text{g L}^{-1}$) for G/M status	
	G_mean	90% percentile	G_mean	90% percentile
Type I	1.4	3.93	6.3	17.7
Type II-FR-SP		1.28		3.50
Type II-A Adriatic	0.33	0.8	1.5	4.0
Type II-B Tyrrhenian	0.32	0.77	1.2	2.9
Type III-W Adriatic			0.64	1.7
Type III-W Tyrrhenian			0.48	1.17
Type III_W FR- SP		0.79		1.89
Type IIIE		0.1		0.4

Note: The MEDGIG exercise phase III is in progress, therefore an update of the above table may occur, which will be considered, accordingly.

In conclusion it is recommended to rely on the classification scheme on chl-a concentration ($\mu\text{g/l}$) as a parameter easily applicable by all Mediterranean countries based on the indicative thresholds and reference values presented in table 2.

However following the evaluation of information provided by a number of countries and other available information it has to be noted that the Mediterranean countries are using different eutrophication assessment methods such as TRIX, Eutrophication scale, EI, HEAT, etc. These tools are very important to continue to be used as appropriate at sub-regional or national levels because there is a long term experience within countries which can reveal / be used for assessing eutrophication trends.

Indicators Monitoring Fact Sheets on Ecological Objective 5 : Eutrophication
ECOLOGICAL OBJECTIVE 05: Human- induced eutrophication is prevented, especially adverse effects thereof, such as losses in biodiversity, ecosystem degradation, harmful algal blooms and oxygen deficiency in bottom waters

Common Indicator Description	Description of Parameters and/or Elements, matrix	Assessment Method	Guidelines Reference Methods QA/QC	Recommendations /Additional Data needed
<p>Common Indicator 7, COP18 Indicator 5.1.1 : Concentra-tion of key nutrients in the water column With Ecological Objective 5.1 : Human introduction of nutrients in the marine environment is not conducive to eutrophication Pressure Indicator</p>	<p>Total Nitrogen (N $\mu\text{mol/L}$), Nitrate ($\text{NO}_3\text{-N}$ $\mu\text{mol/L}$)*, Ammonium ($\text{NH}_4\text{-N}$ $\mu\text{mol/L}$)*, Nitrite ($\text{NO}_2\text{-N}$ $\mu\text{mol/L}$)*, Orthophosphate (P-PO_4 $\mu\text{mol/L}$), Total Phosphorus*, Silicate (SiO_2 $\mu\text{mol/L}$)</p>	<p>UNEP/MAP MED POL State and Temporal Trend Monitoring Programme For coastal stations minimum sampling 4/year, 6-12 /year re-commended For open waters sampling frequency to be determined on a sub-regional level following a risk based approach</p>	<p>Guideline : Eutrophication Monitoring Strategy of UNEP/MAP MED POL UNEP(DEC) MED WG.231/14 Reference Methods : Sampling and Analysis Techniques for the Eutrophication Monitoring Strategy of UNEP/MAP MED POL (MAP Technical Reports Series No. 163) QA/QC : UNEP/MAP MED POL Inter-calibration exercises in agreement with QUASIMEME</p>	<p>*Units supporting the TRIX index, with Mediterranean sub-regional specifics</p>
<p>Common Indicator 7, COP18 Indicator 5.1.1 : Concentra-tion of key nutrients in the water column Proposed Sub-indicator (COP18 Indicator 5.1.2)</p>	<p>Si:N, N:P, Si:P</p>	<p>Nutrient monitoring under UNEP/MAP MED POL State and Temporal Trend Monitoring Programme For coastal stations minimum sampling 4/year, 6-12 /year</p>	<p>Guideline : Eutrophication Monitoring Strategy of UNEP/MAP MED POL UNEP(DEC)MED WG.231/14 Reference Methods : Sampling and Analysis Techniques</p>	

Common Indicator Description	Description of Parameters and/or Elements, matrix	Assessment Method	Guidelines Reference Methods QA/QC	Recommendations /Additional Data needed
Nutrient ratios (silica, nitrogen and phosphorus) where appropriate		recommended Simple mathematical derivation of ratios of nutrient concentrations	for the Eutrophication Monitoring Strategy of UNEP/MAP MED POL (MAP Technical Reports Series No. 163)	
Common Indicator 8, COP 18 Indicator 5.2.1 : Chlorophyll-a concentration in the water column With Ecological Objective 5.2 : Direct effects of nutrient over-enrichment are prevented State,Impact indicator	Chlorophyll –a concentration in seawater (µg/l)*	UNEP/MAP MED POL State and Temporal Trend Monitoring Programme For coastal stations minimum sampling 4/year, 6-12 /year recommended. Further discussions with Focal Points needed if sampling frequency could be increased on monthly basis, in order to sufficiently support the application of assessment methods (as TRIX) taking in account the rapid changes which may occur on chemical and biological variables or to achieve more frequent sampling in highly variable seasons and less frequently during more stable periods.	Guideline : Eutrophication Monitoring Strategy of UNEP/MAP MED POL UNEP(DEC)MED WG.231/14 Reference Methods : Sampling and Analysis Techniques for the Eutrophication Monitoring Strategy of UNEP/MAP MED POL (MAP Technical Reports Series No. 163) UNEP/MAP MED POL Inter-calibration exercises in agreement with QUASIMEME	*Unit supporting the TRIX index, with Mediterranean sub-regional specifics The indicative boundaries values for chlorophyll-a determined in the framework of MED GIG for the status classes required by the EU Water Framework Directive, namely between “good” and “moderate” status could be tested by non-EU Mediterranean countries to find out if they are relevant. Remote sensing techniques would be a useful tool for estimating chlorophyll concentrations. On a regional scale the remote sensing tool could be useful to identify emerging problem areas Pilot programmes are recommended to be carried out at the sub-regional scale to test the integration of remote sensing with in situ data

Common Indicator Description	Description of Parameters and/or Elements, matrix	Assessment Method	Guidelines Reference Methods QA/QC	Recommendations /Additional Data needed
<p>Common Indicator 8, COP 18 Indicator 5.2.1 : Chlorophyll-a concentration in the water column</p> <p>With Proposed Sub-Indicator of Water Transparency where relevant</p> <p>State,Impact Indicator</p>	<p>Water transparency measured as i.e.. Secchi depth or according to ISO 7027:1999 Water Quality-Determination of Turbidity</p>	<p>UNEP/MAP MED POL State and Temporal Trend Monitoring Programme</p>	<p>Guideline : Eutrophication Monitoring Strategy of UNEP/MAP MED POL UNEP(DEC)MED WG.231/14</p> <p>Reference Methods : Sampling and Analysis Techniques for the Eutrophication Monitoring Strategy of UNEP/MAP MED POL (MAP Technical Reports Series No. 163)</p> <p>ISO standard 7027:1999 Water quality -- Determination of turbidity</p>	
<p>Common Indicator 8, COP 18 Indicator 5.2.1 : Chlorophyll-a concentration in the water column</p> <p>With Proposed Sub-Indicator 5.3.1 : Dissolved oxygen near the bottom, i.e. changes due to increased organic matter decomposition and size of the area concerned</p> <p>Pressure,Impact indicator</p>	<p>Dissolved Oxygen concentration (mg/l) and Saturation (%)*</p>	<p>UNEP/MAP MED POL State and Temporal Trend Monitoring Programme</p>	<p>Guideline : Eutrophication Monitoring Strategy of UNEP/MAP MED POL UNEP(DEC)MED WG.231/14</p> <p>Reference Methods : Sampling and Analysis Techniques for the Eutrophication Monitoring Strategy of UNEP/MAP MED POL (MAP Technical Reports Series No. 163)</p>	<p>*Unit supporting the TRIX index as absolute % deviation form saturation, with Mediterranean specifics reflected on sub-regional level</p> <p>Daily variations of Dissolved Oxygen Profiles in the critical season along with T₀ and Salinity, performed through specific buoy applications</p>

Chapter II
Contaminant chapter and related fact sheet

II. MONITORING AND ASSESSMENT METHODOLOGICAL GUIDANCE ON EO9: CONTAMINANTS

1. Introduction

In most Mediterranean countries, the monitoring of concentrations of a range of chemical contaminants in water, sediments and biota is undertaken in response to the UNEP/MAP Barcelona Convention, its Land-Based Protocol, UNEP/MAP MED POL monitoring programmes, international (e.g. WFD) or national drivers. The scope and scale of this monitoring varies, but should be considered as a base from which to introduce a greater degree of harmonisation between Contracting Parties and to ensure that contaminants and matrices of importance within assessment sub regions are covered by appropriate monitoring programmes. Biological effects monitoring is generally less widely established in both national or international programmes, and the number of countries undertaking such studies (and the intensity of the coverage) is much smaller. Therefore, it will be essential in coming years to expand and develop further the use of biological effects methods to cover properly the EO9.

GES under Ecological Objective 09 is achieved when contaminants cause no significant impact on coastal and marine ecosystems and human health. As the type and quantities of emissions have changed and environmental legislation has led to reductions in pollution for certain substances and areas, the monitoring of contaminants needs to be adapted and focused to address present and upcoming risks that might affect the achievement of GES (GES). However coverage from current national programmes is limited. Therefore, for pragmatic reasons, initial assessments of GES under Ecological Objective 9 will probably be based upon data of a relatively small number of contaminants and biological effects, reflecting the scope of current programmes and the availability of suitable agreed assessment criteria. Important development areas over the next few years will include harmonisation of monitoring targets (determinands and matrices) within assessment sub-regions, development of suites of assessment criteria, integrated chemical and biological assessment methods, and review of the scope of the monitoring programmes to ensure that those contaminants which are considered to be important within each assessment area are included in monitoring programmes. Through these, and other, actions, it will be possible to develop targeted and effective monitoring programmes tailored to meet the needs and conditions within each assessment sub-region.

A considerable amount of monitoring data from the past decades is available through the pollution monitoring and assessment component of UNEP/MAP MED POL Programme under UNEP/MAP-Barcelona Convention. These data have been used e.g. for the identification of significant marine contaminants and the development of monitoring strategies and guidance. With respect to implementing the requirements of the Ecosystem Approach Process, there are considerable benefits to be gained from taking advantage of monitoring data and information developed through the UNEP/MAP MED POL Monitoring programme. Such actions include (1) the use of existing experience in the design of monitoring programmes, (2) the use of existing guidance on analytical etc. methods to inform technical aspects of ecosystem approach monitoring, (3) the use of existing sampling station networks as a framework for ecosystem approach sampling networks, (4) the use of existing statistical assessment tools and work on assessment criteria as the basis for assessments of ecosystem approach data, (5) the use of existing data to describe the distributions of contaminants and effects in the sea, and (6) the use of existing time series as the basis of monitoring against a “no deterioration” objective. The availability of quality assured data with confirmed quality is of importance for the assessment of trends in pollutant concentrations.

Monitoring the pressure deriving from chemical contaminants over time and space is a basic requirement for a quantitative assessment of the environmental status of the seas. Baseline assessments are necessary in order to monitor trends and prevent deterioration. Monitoring plans need to be proactive, not reactive and combined with risk assessments. Monitoring instruments and assessment criteria need to be sensitive and comparable.

While all Land Based Sources and Activities (LBS) Protocol substances should ideally be considered, their monitoring in the marine environment might not be performed for all, due to the absence of sources or the physicochemical characteristics of the substances. The availability of source information is crucial to the selection of substances for monitoring.

In view of the adoption in COP19 of the UNEP/MAP Barcelona Convention Offshore Protocol¹ Action Plan, the development and adoption of Mediterranean monitoring procedures and programmes for offshore activities, is envisaged to take place in 2016 - 2017. building, inter alia, on the Integrated Monitoring and Assessment Programme of the EcAp.

Sampling a particular environmental compartment should be based on the anticipated pathway, fate and effect of each pollutant. Each compartment of the marine environment (water, sediments, biota) provides specific information about the pollution status, trends and sources of toxic substances.

The identification of pollution sources and how their associated inputs change over time is also fundamental to assess the effectiveness of the pollution mitigation strategies and to direct the further efforts needed to achieve GES. UNEP/MAP MED POL implements a periodic inventory of pollution sources and loads from land based activities, in the framework of the LBS Protocol and the Strategic Action Programme (SAP) to Address Pollution from Land-based Activities (adopted in 1997 and launched in 2000). The pollution sources database of UNEP/MAP MED POL holds 12,500 records of pollutants loads from industrial and municipal sources reported by the countries on a 5-year period (Data reported on 2003 and 2008). Each record indicates the emission of a substance for a given activity sector and sub-sector, in an administrative region and country. The database covers about 100 different substances or groups of substances and parameters according to national legislation and country development specificities. However a restricted number of substances are common to almost all national pollutant releases.

1. Monitoring Strategy for contaminants and effects (Applicable to all contaminants related indicators, ie Common Indicators 11-15)

1.1. The risk approach and precautionary principle

According to the risk approach monitoring needs to be carried out in coastal and marine areas where chemical contaminants have been found to represent significant risks to the marine ecosystems, and the data provided by the monitoring should serve the needs posed by the Ecosystem Approach process. Monitoring should allow the necessary statistical data treatments and long-term time-trend data analysis. Early warning of upcoming issues, such as emerging contaminants, should eventually become an integral part of the future monitoring systems.

The precautionary principle requires that, in doubt, protective measures should be implemented. In particular the marine environment is vulnerable due to possible accumulation of contaminants in the specific food chains and the irreversibility of impact on its ecosystems.

1.2. Selecting locations for environmental monitoring of contaminants and biological effects

The grid of monitoring stations will depend on the purpose of the specific campaigns. Most monitoring stations will be part of the UNEP/MAP MED POL monitoring schemes. It has been recognized that the open and deep sea is much less covered by monitoring efforts than coastal areas. There is a need to include within monitoring programmes also areas beyond the coastal areas in a representative and efficient way, where risks warrant coverage.

¹ The Protocol for the Protection of the Mediterranean Sea against Pollution from the Exploration and Exploitation of the Continental Shelf and the Seabed and its Subsoil (Offshore Protocol). The Protocol entered into force on 24 March, 2011 and according to the Offshore Action Plan Contracting Parties that have not already done so should endeavor to ratify the Protocol by 2017.

A joint strategy for monitoring should include master stations, distributed spatial spread and other approaches, such as transect sampling, if applicable.

The selection of sites for the monitoring of contaminants and biological effects in the marine environment is a direct function of the assessment of risks and the monitoring scope:

- Areas of concern identified on the basis of the review of the existing information and linked to UNEP/MAP MED POL and WFD assessments.
- Areas of known past and/or present release of chemical contaminants.
- Offshore areas where risk warrants coverage (aquaculture, offshore oil and gas activity, dredging, mining, dumping at sea...).
- Sites representative in monitoring of other sea-based (shipping) and atmospheric sources.
- Reference sites: For reference values and background concentrations.
- Representative sensitive pollution sites/areas at sub regional scale.
- Deep-sea sites/areas of potential particular concern

The selected sites should allow the collection of a realistic number of samples (e.g. be suitable for sediment sampling, allow sampling a sufficient number of biota for the selected species during the duration of the programme). Modelling tools can provide information for the best placement of monitoring stations with respect to ocean currents and input pathways.

Contracting Parties should provide their proposed sampling locations and the reasons for monitoring. It is essential that the monitoring strategies are being coordinated at regional and/or sub regional level. Coordination with monitoring for other Ecological Objectives is crucial for cost-effective approaches. The organization of cruises as a joint effort from different Contracting Parties might be an effective option.

1.3 Geographic scale of monitoring and assessment

The geographic scale of monitoring for the assessment of GES for contaminants and their effects depends on the specific conditions of an area that may influence the background concentration of contaminants, including local mineralogy, inputs from rivers, hydrodynamic conditions, sediment texture, etc. A risk based approach should be used in order to follow a screening procedure to decide the areas to be assessed and monitored more frequently.

The areas where greater pollution pressure occurs could be divided into smaller areas for assessment purposes and could be monitored more frequently than remote and non-affected marine waters.

Monitoring for the assessment of GES for totally anthropogenic contaminants such as organochlorine compounds, could be carried out on a regional scale, since the background concentration for these contaminants is zero. However, local specificities in the production and use of these compounds (pesticides and industrial compounds) have created a difference between the sub-regions that has to be considered.

Furthermore, although coastal levels of pollutants are mainly influenced by local processes (river runoff, coastal hot spots), open-sea biota and sediments are mainly influenced by regional or even super-regional pathways (atmospheric transport and deposition of pollutants emitted from remote areas). The latter is also true for PAHs.

Based on the above, it could be appropriate to consider monitoring for assessing a regional GES threshold for open sea and a different one for coastal zones.

For naturally occurring contaminants such as heavy metals in addition to the previous remarks, as local mineralogy plays an important role in the definition of the GES threshold, since metal deposits

are present in different Mediterranean locations, monitoring for the assessment of GES for heavy metals may need to be carried out on a subdivision of the sub-region according to local characteristics.

For contaminants biological effects and occurrence of oil spills, monitoring for the assessment of GES could be carried out on sub-regional or even regional level, provided appropriate information is available.

Also, for pathogenic microorganisms in bathing water, monitoring for the assessment of GES could be carried out on a sub-regional or even local level due to the nature of microbiological contamination (the impact is restricted to a relatively short distance from the pollution source due to the short survival time of microorganisms in seawater).

1.4 Monitoring frequency

Monitoring frequencies will be determined by the purpose of the sampling effort. They can range from shorter time scales for seasonally variable input, to large time scales for sediment core monitoring. For trend determination the timescales will depend on the ability to detect trends considering the variability in the whole analytical process and the number of replicates. It can be possible to decrease the monitoring frequency in cases where established time series show concentrations well below levels of concern, and without any upward trend over a number of years. For multiannual parameters, opportunities for joint organization between Contracting Parties and between or within Regional Seas Conventions should be considered.

3. Development of assessment criteria for the definition of threshold limit values for chemical environmental status monitoring of contaminants in order to be able to determine the achievement of GES.

Report UNEP(DEPI)/MED WG.394/Inf.3 on the development of assessment criteria for hazardous substances in the Mediterranean presents a methodology to develop assessment criteria for the definition of threshold limit values for contaminants, in order to assess the achievement of GES in the Mediterranean marine environment in relation to the Ecological Objective EO9, in the framework of the gradual application of the ecosystem approach for the management of human activities in the Mediterranean, by MAP.

The report follows a relevant methodology developed by OSPAR, which proposes two threshold limits to be defined in sediments and biota: T0 to define the threshold at “pristine” sites and T1 to define the threshold between acceptable (GES) and unacceptable environmental conditions.

Using Mediterranean data from the UNEP/MAP MED POL database and applying the OSPAR methodology, the report presents an evaluation of the background concentrations (BCs) and the background assessment concentrations (BACs)² of trace metals (mercury, cadmium and lead) and organic contaminants (chlorinated hydrocarbons and PAHs) in sediments and biota in the Mediterranean basin.

Regarding the definition of BACs in Mediterranean sediments, the report states that it should be noted that limited data was available and therefore more dated sediment cores from different areas are needed in order to increase the confidence of the proposed values. Additionally, in order to further test if normalization is convenient for sediment particle variability, aluminum (Al) and organic carbon (OC) should be considered as mandatory parameters in the new MAP integrated monitoring programme. There are already evidences from certain regions of the NW Mediterranean where it is well demonstrated that normalization is not convenient as these environmental factors are not well

² Background assessment concentrations” (BACs) are statistical tools defined in relation to the background concentrations (BCs), which enable statistical testing of whether observed concentrations can be considered to be near background concentrations. Observed concentrations are said to be ‘near background’ if the mean concentration is statistically significantly below the corresponding BAC

correlated with contaminant concentrations (León et al. et al, 2014). It will be also necessary to further investigate subregional differences on sedimentation rate and geocomposition of the sediments.

In order to define the relationship between BC and BAC, the report states that a statistical test is required, taking into consideration the data variability of reported data on Certified Reference Materials (sediment and biota) used by Mediterranean laboratories in proficiency tests and in inter-calibration exercises. At this stage a statistical test, as described in the text of the report, on the UNEP/MAP MED POL monitoring programme is not yet available. Alternatively the report states that OSPAR defined relationships between BC and BAC for metals in sediments, fish and shellfish to assess the BACs levels could be adopted. Thus, for sediments and shellfish $BAC = 1.5 \times BC$, for fish $BAC = 2 \times BC$. However, that report states that it is recommended to perform a statistical test to evaluate the precision of UNEP/MAP MED POL monitoring programmes (on a country basis).

Furthermore, the report states that considering the statistical evaluation of the UNEP/MAP MED POL database performed in the report, and the large variability in the concentration levels, it is essential to perform a quality control examination of the datasets in order to better assess BAC values.

As regards the definition of Mediterranean Assessment Criteria for biota using the UNEP/MAP MED POL database, the report underlines that it is biologically inappropriate to evaluate absolute BC, BAC and Environmental Assessment Criteria (EAC) metal levels in one species from the parallel levels of even a close relative species. Therefore, BCs and BACs levels were calculated / assessed in the report generally according to OSPAR procedures.

The report states that in OSPAR assessments, some EACs have not been used mainly because they are less than the OSPAR BACs. The EACs for Cd and Pb in sediment, Hg in mussels and Hg and Cd in fish are below the corresponding BACs. In addition, the BCs and BACs for trace metals in sediments are normalized to 5% aluminum whilst proposed EACs are normalised to 1% organic carbon. It has been concluded by OSPAR that EACs for PAHs or trace metals in sediment and for metals or CBs in biota cannot be used to describe the threshold (T1) between acceptable (GES) and unacceptable environmental conditions. Therefore, in cases where the EACs have not been recommended, alternative approaches to appropriate criteria for the assessment of data on contaminant concentrations in sediment and biota were applied (as shown in Table 9.1.):

- For the Transition (T0) which represents an assessment that concentrations should be at, or close to, background concentrations, BACs are used by OSPAR.
- For the Transitions (T1), the assessment criteria were the ERLs (Effects Range Low³) for PAHs and trace metals in sediment.
- It is a demanding task to determine real EAC levels, generally and also according to OSPAR documents. Therefore, until an appropriate approach becomes available for the assessment criteria for metals in biota, the EC maximum acceptable dietary levels (Commission Regulation (EC) No 1881/2006) were used by OSPAR (QSR 2010 assessment).

In addition, it has to be noted that there are experiences in the Mediterranean according to which ERL has been adopted as threshold for T1 as it was not possible to normalize for TOC in sediment due to low TOC content.

³ Effects range low (ERL) and effects range median (ERM) are specific chemical concentrations that are derived from compiled biological toxicity assays and synoptic sampling of marine sediment. These numerical values are sediment quality guidelines that were developed by Long and Morgan for the National Oceanic and Atmospheric Administration's (NOAA) National Status & Trends programme as informal tools in screening sediment. ERL and ERM are considered guidelines to help categorize the range of concentrations in sediment at which effects are scarcely observed or predicted (below the ERL) and the range above which effects are generally or always observed (above the ERM). These guidelines are used for screening sediments for trace metals and organic contaminants

Table 3. Transition points for assessing contaminants in sediments and biota applied by OSPAR (OSPAR 2009).

Contaminant	Transition Point	Sediment	Biota
Hg, Cd, Pb	T0	BAC	BAC
Hg, Cd, Pb	T1	ERL	EC
PAHs	T0	BAC	BAC
PAHs	T1	ERL	EAC
PCBs (individual congeners)	T0	BAC	BAC
PCBs (individual congeners)	T1	EAC	EAC
Σ 7CBs ICES	To	BAC	-
Σ 7CBs ICES	T1	ERL	-
Lindane	To	BAC	BAC
Lindane	T1	ERL	BAC
HCB	To	BAC	BAC
HCB	T1	ERL	-
pp-DDE	T0	BAC	BAC
pp-DDE	T1	ERL	EAC
α -HCH	T0	-	BAC
α -HCH	T1	ERL	-
Dieldrin	T0	BAC	-
Dieldrin	T1	ERL	EAC

3.1. **Forward procedure for monitoring the achievement of GES for contaminants in the Mediterranean marine environment.**

The recommendations and information presented in the report are proposed to be followed up/ utilized to establish a forward procedure for monitoring the achievement of GES for contaminants. This inter alia would imply further work in separate Contracting Party allocated expert groups, particularly for updating the current BACs and setting EACs for contaminants in biota on a sub-regional level.

Until EACs are defined for the major substances of concern, a two-fold approach could be adopted to support monitoring for the assessment of GES: i) a threshold value for GES (BAC) could be set using concentrations from relatively unpolluted areas on a sub-regional level and ii) a decreasing trend should be observed from values representing the actual level of contaminants concentrations that are above the background assess concentrations (BACs).. Thus, GES could be defined for toxic metals (Hg, Cd, Pb), chlorinated organic compounds and PAHs, for which monitoring data exist as a result of running monitoring programmes.

Temporal trend monitoring

Marine monitoring implies the repetitive observing for defined purposes, of one or more elements of the marine environment, according to prearranged spatial and temporal schedules using comparable methodologies. The temporal trend monitoring starts with the objective to detect trends in concentrations with the aim of monitoring the effectiveness of control measures taken at polluted sites. Trends in pollutant or contaminant levels, in general, are also considered as “state” indicators of

pollution and are included in most of the regional monitoring programmes to provide inputs to the assessments of the state of the marine environment.

Surface sediments and biota can be used for recognizing possible temporal trends of trace metals, organochlorine compounds, PAHS, and those that are accumulated in these matrices in the marine environment and, thus, can be an important tool for the assessment of the effectiveness of control measures taken at the polluted sites and also for state assessment. However, data variability can be influenced by several factors other than contaminant inputs, namely those associated with sampling and the representativeness of the collected samples. In any case, the first requirement is the availability of data series long enough, so that long-term monitoring programmes are maintained in time.

In the 2005 review and analysis of UNEP/MAP MED POL Phase III Monitoring Activities (UNEP (DEC)/MED WG 282/3) consisting of an evaluation of the UNEP/MAP MED POL database for the trend monitoring of contaminants it was concluded that the UNEP/MAP MED POL Phase III programme objectives preliminarily set, were not sufficient to achieve the temporal trend of any selected contaminant for a selected site. The major reason for this was the various difficulties in data analysis, especially when normalization was intended for reducing the variance of the data set by taking into account the differences in morphology (e.g. sediment grain size) or composition (e.g. tissue fat content) of the samples. Both the selected trace metals and the organic contaminants will co-vary strongly with such factors⁴.

A second aspect to be considered is the time span necessary for trends assessment.

In general, the first temporal trend evaluation using sessile marine organisms can be performed with data sets of more than five years ongoing programmes. The use of sediments still require a longer time span (>10yr) for evidencing and assessing significant variations. However, after ten years of the monitoring programme, certain countries still did not have valid and continuous data covering at least five years.

The 2011 analysis of the trend monitoring activities and data for UNEP/MAP MED POL Phase III and IV (UNEP (DEPI) MED365/Inf.5) concluded that though substantially improved after the last trend data evaluation in 2009, some problems were identified mainly dealing with the lack of maintaining the declared sampling strategy. The weakest part of the programme remains the data transfer and manipulation. To overcome these problems, the report states that involved countries are encouraged to write a detailed programme manual where all issues regarding a successful programme achievement would be addressed. Such a manual would include the programme objectives and a detailed methodological approach to successfully maintain the programme over time (positioning, sampling, methods, and data elaboration, exchange and presentation).

From the trend monitoring point of view the report states that the best sampling strategy always leads with attaining the best information on the sampling variance and with that a valuable determination of the underlying trend. While it is advisable to avoid pooling whenever possible, the suggested strategy for smaller organisms, mainly molluscs that are not always sufficient for all analyses, is to use 3-5 samples with 15 pooled specimens or in any case a number of pooled specimens that guarantees the necessary amount of sample to conduct all the chemical analyses. If one sampled organism, mainly fish, provides enough sample for all analyses the use of from 15 to 25 (preferred) samples is suggested if the underlying variances are not known. The sample should be collected in a length stratified manner: divide the size distribution in three or five classes (log scale and depending on size: MG -1 cm; MB - 2 cm.) and sample the central one; the same size class should always be sampled.

4. Monitoring Biological Effects

⁴ A revised manual for sediment sampling and analysis was adopted in 2006 (UNEP (DEC) MED WG.282/Inf.5/Rev.1.)

Biological effects monitoring is considered as an important element in programmes which aim to assess the quality of the marine environment, since such monitoring aims to demonstrate links between contaminants and ecological responses. Biological effects monitoring can thus be used with the intention to indicate the presence of substances, or combinations of substances, not previously identified as being of concern and to identify regions of decreased environmental quality.

Biomarkers include a variety of measures of specific molecular, cellular and physiological responses of key species to contaminant exposure. A response is generally indicative of either contaminant exposure or compromised physiological fitness. The challenge is to integrate individual biomarker responses into a set of tools and indices capable of detecting and monitoring the degradation in health of a particular type of sentinel organism.

The use of biomarkers is relatively new when compared to traditional chemical monitoring. Even today those biomarkers which are considered well understood often still lack historic track records and simple data management adequate for routine risk assessment and monitoring. Some results were produced in the last twenty years through individual research projects national or international programmes in marine waters (BIOMAR, BEEP, IOC-IMO UNEP funded programme of Global Investigation of Pollution of the Marine Environment). Despite the important principle underlying the biomarker concept, that is, response should lead to ecological effects, there are still few examples where biomarker measurements have been directly linked to community level responses. However, many examples revealing environmental problems, that is, acting as warning signals of potential future problems, have been demonstrated in the past decades (Demetrio et al., 2003; Martínez-Gómez et al., 2010; Fernández et al., 2011).

Biological effects monitoring should be coordinated with the monitoring of chemical contaminants in a cost-effective manner, conducting field sampling, whenever possible, within the same time-frame.

The integrated assessment (biological effect and chemical measurements) should comprise only a limited number of stations including at least:

- Reference sites: For reference values and background concentrations
- Areas of concern identified on the basis of the review of the existing information linked to MED POL, WFD and MSFD assessments
- Representative sensitive pollution sites/areas at subregional scale

Strategy for sampling and analysis should include, whenever possible:

- Sampling and analyses of the same tissues and individual/populations than chemical monitoring
- Sampling of individuals for biological effects from the same site/area as that used for chemical analyses at a common time
- Sampling sediments at the same time and location as collecting biota (i.e. fish)

For all stations, biometrics (size/length, age), biological supporting parameters such as condition index (mussels), condition factor, gonadosomatic index, hepatosomatic index (fish) and data on temperature, salinity and oxygen dissolved of the ambient water should be also registered.

For an integrated biomarker data management, an Expert System has been developed at the University of Piemonte Orientale, Italy (**DiSAV**) in the framework of the **BEEP** (Biological Effects of Environmental Pollutants) EU programme. The function of the Expert System is to rank the level of the pollutant-induced stress syndrome by integrating the data obtained from:

- Early warning biomarkers: i.e. sensitive biomarkers of stress, or of exposure, revealing the effects of pollutants at the molecular and/or cellular level.
- Biomarkers of stress, suitable to reveal the development of the stress syndrome at the tissue/organ level: i.e. histological biomarkers, but also biochemical biomarkers such as the GST (Glutathione Transferase) test recently developed (i.e. evaluation of the GST released from the cells and present in molluscan haemolymph).
- Biomarkers of stress at the organism level: i.e. biomarkers able to show that the stress syndrome has decreased the mussel's capacity of survival and/or growth and reproduction (such as stress on stress response, scope for growth, gonad and gamete alterations, survival index).

A good interpretation of the development of the stress syndrome by the expert system depends on the possibility to utilize control samples for each assessment and biomarkers of stress able to integrate the toxic effects of pollutants over a sufficient caging period. Among these, are those biomarkers that show a trend characterized by a continuous increase or decrease in the value of the selected parameter (such as lysosomal membrane stability, lysosomal lipofuscin accumulation, lysosomal neutral lipid accumulation, micronuclei frequency) in relation to an increase in toxicity. Moreover, the expert system takes into account possible interferences among the different biomarkers. However, the representation of the assessment does not maintain all of the supporting information, and it is not easy to identify the causative determinands that may be responsible for the final result on the level of stress syndrome. In addition, different stages of the assessment cannot be readily unpacked to a previous stage to identify either contaminant or effects measurements of potential concern or sites contributing to poor regional assessments.

Besides of expert system, different indexes have being developed to assess contaminant-related biological responses by combining results from different biomarkers such as Integrated Biomarker Response (IBR) (Belaieff and Burgeot, 2002), the Health Assessment Index (HAI) (Adams et al., 1993), the Bioeffect Assessment index (Broeg at al., 2005), and the Integrative Biomarker Index (Marigómez et al., 2013). Furthermore, different models are becoming available in the Mediterranean region to elaborate various typologies of data with the 5 classes approach, and to aggregate them in a final evaluation, still based on the 5 classes discrimination (Benedetti et al., 2012).

Molluscs (mainly mussels, *Mytilus* sp.) and fish (*Mullus* sp., *Platichthys flesus* L., *Zoarces viviparus*, *Perca* sp.) from natural populations have both been widely employed as sentinel organisms in routine biomonitoring programmes, both at a national and an international level (UNEP/MAP UNEP/MAP MED POL Biomonitoring Programme; OSPAR Convention, RAMOGE, etc.), although some subregional and national research projects have also been conducted in the past years using caged mussels (RINBIO; MYTILOS, MYTIMED Project, etc). Exposure periods lasting several months are generally required to assess bioaccumulation of most persistent organic contaminants and to reveal more subtle chronic effects on organisms. Although caged mussel can be used to assess certain early biological effect responses, they cannot substitute the pollution biomonitoring programmes based on the sampling of mussels from natural populations. As the experience have demonstrated, the use of caged mussels for large-scale biomonitoring programmes involves a higher-cost monitoring strategy than the use of mussels from natural populations because at least, two field sampling campaigns have to be organised, and recovery of the cages is not guaranteed. The use of caged mussels for effect monitoring can however be useful in short-term exploratory environmental studies, e.g. around hot spots.

While the use of fish in biological effects monitoring programmes, building on the key position of these organisms in the trophic chain and their high commercial value is well established, their usage already in the initial stage of the monitoring programme on a regional level would present some problems, including the difficulties encountered in caging experiments with fish as well as more importantly the cost of sampling, caging, transportation. However, field sampling to assess contaminants levels in fish tissues could be integrated and coordinated with sampling of other fish

tissues (liver, blood, gonads, brain, etc) to implement in future the use of biological effects in fish from natural populations instead of caging fish. Their inclusion in the integrated monitoring programme thus is not foreseen in the initial phase, but could be envisioned afterwards..

Molluscs have been taken as the bioindicators of choice on the basis of their wide geographic distribution, their straightforward availability in the field and through aquaculture, and their suitability for caging experiments along coastlines. In the framework of UNEP/MAP MED POL Phase IV, it was decided to apply a 2-tier approach, using caged molluscs:

- the first tier would include a single biomarker, namely, lysosomal membrane stability, and mortality;
- the second tier would include a whole set of biomarkers including acetyl cholinesterase activity, micronuclei frequencies, lipofuscin accumulation, neutral lipid accumulation, , oxidative stress, metallothionein content, , peroxisome proliferation, lysosome to cytoplasm ratio, and stress on stress.

An intercalibration exercise financed by UNEP/MAP MED POL was organised in 2010 by DiSAV with the participation of 11 Mediterranean laboratories from 8 countries (Croatia, Egypt, Greece, Italy, Slovenia, Spain, Syria and Tunisia) and 3 non-Mediterranean laboratories (Norway and UK, from the OSPAR region). The results of the intercalibration exercise showed excellent performance of all laboratories for the measurement of lysosome membrane stability and very good performance for the measurement of metallothionein content. Also a Training course on the measurement of two biomarkers (lysosome membrane stability and micronuclei frequency) was organised in Alessandria, Italy by DiSAV in 2010, with the participation of 15 scientists from 10 countries (Algeria, Croatia, Egypt, Greece, Italy, Morocco, Slovenia, Spain, Tunisia, Turkey) and with the contribution of scientists from ICES-OSPAR (UK).

Based on the work already carried out, the results of the intercalibration exercises and the publication of relevant papers by Mediterranean scientists involved in the UNEP/MAP MED POL programme on biological effects monitoring, there is a network of laboratories in the Mediterranean region with the capacity to carry out biomonitoring activities, in line with the new monitoring requirements to be defined in the framework of the Ecosystem Approach for the management of human activities in the Mediterranean.

Of the second tier biomarkers proposed, only the micronuclei frequency biomarker is able to indicate the presence of genotoxic chemicals in the environment, especially *in sites heavily polluted by polycyclic aromatic hydrocarbons, and in organisms that may also be considered as seafood*. With growing concern over the presence of genotoxins in the sea, the application of cytogenetic assays to ecologically relevant species offers the chance to perform early tests on health in relation to exposure to contaminants. Acetylcholinesterase activity is a cost effective biomarker of neurotoxic effects of pollutants, especially pesticides, applicable with instrumentation available in the Contracting Party laboratories. Its responsiveness has been demonstrated also to various other groups of chemicals present in the marine environment, including heavy metals, and hydrocarbons. Laboratory and field studies have demonstrated the applicability of anoxic/aerial survival as an early warning indicator of contaminant-induced stress. The reduction of survival in air, or stress on stress (SoS), is a simple, low-cost, whole-organism response and can show pollutant-induced alterations in an organism's physiology that render the animal more sensitive to further environmental changes. Bivalve molluscs can survive for a long time in air, but individuals stressed by pre-exposure to pollutants show greater mortality than controls or individuals collected from a reference location. The method for determining SoS in mussels has been applied routinely to both toxicant-exposed mussels in laboratory studies and mussels collected in national monitoring programmes from polluted environments and along pollution gradients. Taking into account the number of samples to be analyzed and available facilities in the Contracting Party laboratories, the best number of further to these biomarkers to be gradually introduced into the biological effects monitoring programme could be determined.

While recognizing that contaminant-specific techniques that cannot guarantee that measuring responses within marine organisms from natural populations are caused to the exposure of single specific contaminants, the most widely used specific technique is the measurement of TBT effects (imposex) on gastropods, where a cause and effect relationship has been established. There is a possibility to use available information for TBT thresholds for GES from other regions (Davies and Vethaak, 2012) in order to propose similar effects thresholds for the Mediterranean.

In general the monitoring of contaminant-related biological effects should be coordinated with the monitoring of chemical contaminants in a cost-effective manner, conducting field sampling, whenever possible, within the same time-frame.

4.1. **Assessing Biological Effects**

In a similar manner to contaminant concentrations, ICES/OSPAR has proposed two/three categories to assess the biological effects observed, by using two assessment criteria: BAC and EAC (Davies et al., 2012). Assessing biomarker responses against BAC and EAC allows establishing if the responses measured are at levels that are not causing deleterious biological effects, at levels where deleterious biological effects are possible or at levels where deleterious biological effects are likely in the long-term. In the case of biomarkers of exposure, only BAC can be estimated, whereas for biomarkers of effects both BAC and EAC can be established. However, unlike contaminant concentrations in environmental matrices, biological responses cannot be assessed against guideline values without consideration of factors such as species, gender, maturation status, season and temperature.

It is expected that in the forthcoming years, the scope of experts groups would be to prepare an adapted manual establishing the BAC and when possible, the formulation of EAC for selected biomarkers in Mediterranean species.

One of the challenges in assessing the health status of organisms using assessment criteria is precisely the strategy by which to integrate the multivariate results obtained. The approach recently developed by ICES was based on an assessment of single responses by assessment criteria, then scoring them in a multi-step process to arrive at a final risk assessment (Davies and Vethaak, 2012).

5. Monitoring acute pollution events for the quantification of acute chemical spills, specifically of oil and its products, but not excluding others (Common Indicator 13 Occurrence, origin and where possible extent of acute pollution events)

The UNEP/MAP-Barcelona Convention and its Prevention and Emergency Protocol aim at the protection of the environment against oil and chemical spills with a coherent coverage and equal level of protection for the entire Mediterranean Sea.. The Regional Marine Pollution Emergency Response Centre for the Mediterranean Sea (**REMPEC**) is responsible for the prevention of, preparedness for and response to marine pollution. In this regard, the Centre's database on alerts and accidents in the Mediterranean Sea contains data on accidents causing or likely to cause pollution of the sea by oil (since 1977) and by other harmful substances (since 1989).⁵

While there should be no overlap or double work with existing provisions, the guidance on integrated monitoring should here ensure that all aspects are being covered under the various frameworks, that monitoring information is exchanged between the networks and that potential for a cost effective integrated monitoring is used.

The operational objective contains two different criteria:

- Occurrence, origin, extent.
 - Impact on biota physically affected.
-

- Monitoring efforts can therefore use the following methods for quantification:
- Quantification of oil and other chemical spills and their size by observation and reporting.
- Satellite radar images, plane observation and imaging approaches.
- Backtracking of oil spills to their source by hind cast modelling.
- Fingerprinting using chemical analysis (GC-MS) and comparison with possible sources.

The organizational framework under which the monitoring of oil and other chemical spills is being dealt with under the UNEP/MAP Barcelona Convention is REMPEC. Mediterranean coastal States, contracting Parties to the 2002 Prevention and Emergency Protocol to the UNEP/MAP Barcelona Convention, committed themselves (Article 9 of the Prevention and Emergency Protocol) to inform each other, either directly or through the Regional Centre (i.e. REMPEC) on:

- all accidents causing or likely to cause pollution of the sea by oil and other harmful substances
- the presence, characteristics and extent of spillages of oil or other harmful substances observed at sea which are likely to present a serious and imminent threat to the marine environment or to the coast or related interests of one or more of the Parties;
- their assessments and any pollution combating actions taken or envisaged to be taken
- the evolution of the situation.

In relation to their obligations under the abovementioned Article 9 of the Prevention and Emergency Protocol, at their Fifth Ordinary Meeting, the Contracting Parties to the UNEP/MAP Barcelona Convention adopted the Guidelines For Co-operation In Combating Marine Oil Pollution In The Mediterranean (UNEP/IG.74/5, UNEP/MAP, 1987) which recommend Parties to report to REMPEC at least all spillages or discharges of oil in excess of 100 cubic metres.⁶

Article 18 of the UNEP/MAP Barcelona Convention Protocol for the Protection of the Mediterranean Sea against Pollution Resulting from Exploration and Exploitation of the Continental Shelf and the Seabed and its Subsoil, states that in cases of emergency the Contracting Parties shall implement mutatis mutandis the provisions of the Emergency Protocol.

While Contracting Parties are under the obligation for the above monitoring, data submitted to REMPEC is still scarce. Thus the main aim during the Initial Phase of the Integrated Monitoring is to strengthen monitoring efforts towards this already existing obligation.

At the same time, for the further development of the Integrated Monitoring and Assessment Programme, it is recommended to analyse closer the links in between acute pollution events and their effects on biota and develop specific assessment criteria for this latter (see Martínez-Gómez et al., 2010).

6. Monitoring of contaminants in fish and other seafood used for human consumption (Common Indicator 14 Actual levels of contaminants that have been detected and number of contaminants which have exceeded maximum regulatory levels in commonly consumed seafood)

Substances to be monitored

Monitoring of contaminants in biota used for human consumption only measures contaminants in fish and other seafood for which regulatory limits have been set in national and international regulations

⁶. <http://www.rempec.org/admin/store/wywigimg/file/News/Forthcoming%20Meetings/MEDEXPOL2013/E-%20Reference%20Documents/E-%20REMPEC%20-%20Guidelines%20for%20co-operation%20in%20combating%20marine%20poll%20in%20the%20med.pdf>

for public health reasons⁷. The significance of an increase for specific contaminants in the marine environment through trend analysis should be regarded as an important element for inclusion in seafood monitoring. Similarly, when results from monitoring of contaminants in the marine environment indicate a very low likelihood for elevated levels in fish and seafood for human consumption, additional monitoring on these commodities is not justified.

Monitoring should at least consider the following contaminants for which regulatory levels have been laid down: Heavy metals (lead, cadmium, and mercury), polycyclic aromatic hydrocarbons, dioxins (including dioxin-like PCBs). Additionally, further contaminants of relevance should be identified.

Species

The selection of the species to be used for monitoring should consider the following criteria:

- Species more prone to biomagnify/bio-accumulate specific classes of contaminants
- Species representative of the different trophic levels or habitats
- Species representative for entire (sub) region
- Species representing consumer habits

Moreover, in order to make monitoring results more comparable between (sub) regions, it would be advisable to select a limited number of target species from the most consumed species of fish and other seafood.

Sample collection

Only unprocessed products should be sampled for this purpose. A key element will be to analyse seafood in the sea from known locations. The monitoring of contaminants in seafood is executed by the responsible authorities in charge, which often are different from the authorities implementing the EcAp and its associated monitoring. Here, cooperation with authorities and environmental institutions in charge of health monitoring is strongly encouraged. Topics for coordination are:

- Providing information on the origin of the samples: Sampling of fish and seafood at retail stage shall only be done when all necessary conditions (e.g. avoid cross contamination, traceability to (sub) region) can be guaranteed
- Exploring synergies in the monitoring of marine top predators
- Exchanging information on data, approaches and methodologies between environmental monitoring institutions and human health risk related monitoring institutions

7. Monitoring microbiological pollution (Common Indicator 15: Percentage of intestinal enterococci concentration measurements within established standards)

Taking into consideration that the Mediterranean Sea continues to attract every year an ever increasing number of international and local tourists that among their activities use the sea for recreational purposes, the issue of monitoring for potential microbiological pollution is of particular importance. Although the general situation has improved considerably in several parts of the region through the establishment of sewage treatment plants and the construction of submarine outfall structures, the matter is still of major concern in a number of areas and the quality of recreational waters needs regular monitoring.

Revised Mediterranean guidelines for bathing waters were formulated in 2007 based on the WHO guidelines for “Safe Recreational Water Environments” and on the EC Directive for “Bathing

⁷ A list of maximum levels for contaminants in foods set by the FAO/WHO Codex Alimentarius Commission can be found at ftp://ftp.fao.org/codex/Meetings/cccf/cccf7/cf07_INFe.pdf

Waters". The proposal was made in an effort to provide updated criteria and standards that can be used in the Mediterranean countries and to harmonize their legislation in order to provide homogenous data.

The values agreed for the Mediterranean region in COP 17 (Decision IG.20/9 Criteria and Standards for bathing waters quality in the framework of the implementation of Article 7 of the LBS Protocol, (UNEP/MAP, 2012) are presented in Table 4 and could be used to define GES for the indicator on pathogens in bathing waters.

By definition monitoring for the assessment of GES for bathing waters is expected to be close to the shore, but the threshold is valid on a regional level. Therefore, the category A or B values could be defined as a GES threshold for intestinal enterococci in bathing waters in the Mediterranean.

Table 4. Water quality criteria for intestinal enterococci in bathing water

Category	A	B	C	D
Limit values	<100*	101-200*	Up to 185**	>185**(1)
Water quality	Excellent quality	Good quality	Sufficient	Poor quality/ Immediate Action

* 95th percentile intestinal enterococci/100 mL (applying the formula 95th Percentile = antilog ($\mu + 1,65 \sigma$))

** 90th percentile intestinal enterococci/100 mL (90th Percentile=antilog ($\mu + 1,282 \sigma$), μ =calculated arithmetic mean of the log10 values; σ = calculated standard deviation of the log10 values.

8. Quality Assurance and Quality Control of contaminants monitoring

The accuracy and comparability of the data collected is a key requirement for the assessment and description of environmental status and for the assessment of anthropogenic influences and required measures. Quality assurance (QA) and quality control (QC) measures ensure that monitoring results of stated quality are obtained across the Mediterranean Region and at any time.

Much effort has been made by the MAP Secretariat so that the Contracting Parties would be in a position to generate accurate data on marine contaminants. UNEP/MAP MED POL will continue to collaborate with the International Atomic Energy Agency and the specific Marine Environmental Studies Laboratory (MESL), based in Monaco.

The MESL produces Certified Reference Materials (for trace elements and organic compounds in sediment and marine biota) and develops fit-for purpose Recommended Analytical Methods for the analysis of contaminants in marine samples. Also, in collaboration with Regional Organisations and national authorities, MESL organises Proficiency Tests and Training Courses on the analysis of contaminants of concern.

9. Reference methods and guidelines for marine pollution monitoring under UNEP/MAP MED POL

In the framework of the LBS Protocol, UNEP/MAP is assisting Mediterranean Contracting Parties in the assessment of the state of the marine environment and of its resources, of the sources and trends of pollution and the impact of pollution on human health, marine ecosystems and amenities. In order to assist the countries and to ensure that the data obtained through this assessment can be compared on a world-wide basis and thus contributing to the Global Environmental Monitoring System (**GEMS**) of UNEP, a set of reference methods and guidelines for marine pollution studies, covering technical aspects of monitoring, sample selection, preservation and analysis, have been developed and recommended to be adopted by Governments participating in the Regional Seas Programme. The methods and guidelines have been prepared in cooperation with the relevant specialised bodies of the United Nations system (WHO, FAO, IAEA, IOC) as well as other organisations and are tested by competent experts. The Methods and Guidelines are periodically revised taking into account the development of our understanding of the problem, of analytical instrumentation and the actual need of the users. The Marine Environment Laboratory of the International Atomic Energy Agency (**IAEA**) in Monaco is responsible for the technical co-ordination of the development, testing and intercalibration of Reference Methods.

The Reference Methods for the analysis of pollutants in water, sediment and biota, in the framework of the UNEP/MAP-UNEP/MAP MED POL, can be found at www.unepmap.org (Document and publications; Library Resources; Reference Methods). UNEP/MAP is currently in the process of updating selected guidelines and reference methods and it is expected to be submitted to the forthcoming MED POL focal point meeting in June 2015.

ECOLOGICAL OBJECTIVE 09: Contaminants cause no significant impact on coastal and marine ecosystems and human health

Common Indicator description	DESCRIPTION Parameters and/or Elements, matrix	Assessment Method	Monitoring Guidelines, data and existing QA/QC Sampling and Analysis Reference Methods	Recommendations /Additional work, data needed
<p>Common Indicator 11, COP18 indicator number 9.1.1: Concentrations of key harmful contaminants in the relevant matrix (biota, sediment, seawater)</p> <p>With Operational Objective of (9.1. in COP18 Decision): Concentration of priority contaminants (as listed under the UNEP/MAP Barcelona Convention and LBS Protocol) is kept within acceptable levels and does not increase</p> <p>Pressure indicator</p>	<p>Hg, Cd, Pb, PCBs, halogenated pesticides (aldrin, dieldrin, HCB, lindane, ΣDDTs), PAH.</p> <p>In sediment and representative biota (bivalves i.e <i>Mytilus galloprovincialis</i>, fish i.e. <i>Mullus barbatus</i>). PAHs in fish are not considered representative.</p> <p>Aluminum (AL) and Organic Carbon(OC) measurements in sediment for testing normalization purposes</p> <p>pH in seawater to measure acidification</p>	<p>UNEP/MAP MED POL State and Temporal Trend Monitoring Programme</p> <p>At least annually, for biota (for mussels at the pre-spawning period and for fish at the non-spawning period) and every 4-5 years for sediments in low sedimentation areas, (annually for sediments in high sedimentation areas including estuaries and harbours), at the most stable hydrographic conditions. Frequency of monitoring in low sedimentation areas to be further discussed at MEDPOL FP meeting in June 2015.</p>	<p>UNEP/MAP MED POL Programme for the Assessment and Control of Pollution in the Mediterranean Region MAP Technical Reports Series No. 120</p> <p>QA/QC through UNEP/MAP MED POL/IAEA MESL</p> <p>Sampling Analysis Reference Methods are listed in ECAP monitoring guidance, Annex X.</p>	<p>Further contaminants may be added following countries specificities and/or regional importance following a review and assessment of LBS Protocol Priority List of substances [such as another trace metals, TBT, PBDE, etc.]</p> <p>Specification of EAC required for trace metals in sediment and biota and PAH in sediments. Online expert group established to develop BAC and EAC as appropriate</p> <p>First estimates of background concentrations for trace metals in sediments and biota and PAHs in sediments are available from CP National Monitoring Programmes.</p> <p>Common decision needed on whether to develop methodology in order to include monitoring of oil affected seabirds (quantification, aimed at chronic oil pollution events not acute ones).</p> <p>Common decision needed on whether the indicator only covers (a) the period since the cut-off from data used for the UNEP/MAP MED POL initial assessment; (b) only the period from the start of the ECAP monitoring programme; or (c) a</p>

Common Indicator description	DESCRIPTION Parameters and/or Elements, matrix	Assessment Method	Monitoring Guidelines, data and existing QA/QC Sampling and Analysis Reference Methods	Recommendations /Additional work, data needed
	Monitoring of contaminants in seawater presents specific challenges and therefore recommended to be carried out on a country by country decision basis			longer time period, e.g. in view of the interest to show the overall changes in the marine environment
<p>Common Indicator 12, COP18 indicator number 9.2.1: Levels of pollution effects of key contaminants where a cause and effect relationship has been established</p> <p>With Operational Objective of 9.2 Effects of released contaminants are minimized</p> <p>Impact indicator</p>	<p>Lysosomal Membrane Stability (LMS) Tier 1 mandatory biomarker on the basis of the 2-Tier approach</p> <p>Reduction of survival in air or Stress on Stress (SoS) Tier 2 optional biomarker on the basis of the 2-Tier approach.</p> <p>Acetylcholinesterase (AChE) assay as a method for assessing neurotoxic effects in aquatic organisms. Tier 2</p>	<p>UNEP/MAP MED POL State and Temporal Trend Monitoring Programme</p> <p>Sampling minimum annually Or semi-annually in pre-spawning period (case of mussels) and in non-spawning period (in case of fish)</p>	<p>MTS 120 UNEP/MAP MED POL State and Temporal Trend Monitoring Programme</p> <p>Sampling minimum annually or semi-annually in the pre-spawning period in case of mussels.</p> <p>UNEP/RAMOGGE: Manual on the Biomarkers Recommended for the UNEP/MAP MED POL Biomonitoring Programme. UNEP, Athens, 1999.</p> <p>UNEP/MAP, 2005. Fact sheets on Marine Pollution Indicators. WGUNEP(DEC) / MED/ WG.264 / Inf.14.</p> <p>Background document: stress on stress (SoS) in bivalve molluscs. <i>Concepción Martínez-Gómez and John Thain</i>. In ICES Cooperative Research Report No 315.</p>	<p>Further biomarkers may be added following countries specificities and/or regional importance recommendation list established by experts</p> <p>Ache and Micronucleus assay recommended to build the capacity of UNEP/MAP MED POL designated laboratories for a period of 3-4 years after which consideration whether adopted as mandatory components of the UNEP/MAP MED POL ECAP Monitoring Programme.</p> <p>For AChe BAC and EAC should be estimated for different geographical regions and include the differences in seawater T⁰</p> <p>Several studies have demonstrated that Micronuclei baseline frequencies depend on water temperature.</p>

Common Indicator description	DESCRIPTION Parameters and/or Elements, matrix	Assessment Method	Monitoring Guidelines, data and existing QA/QC Sampling and Analysis Reference Methods	Recommendations /Additional work, data needed
	<p>mandatory biomarker on the basis of the 2-Tier approach.</p> <p>Micronucleus assay as a tool for assessing cytogenetic/DNA damage in marine organisms. Tier 2 optional biomarker on the basis of the 2-Tier approach. In bivalves (i.e. mussels <i>Mytilus galloprovincialis</i>)</p>		<p>Background document: Acetylcholinesterase assay as a method for assessing neurotoxic effects in aquatic organisms <i>Thierry Burgeot, Gilles Bocquené, Joelle Forget-Leray, Lúcia Guilhermino, Concepción Martínez-Gómez, and Kari Lehtonen.</i> In ICES Cooperative Research Report No 315.</p> <p>Background document: micronucleus assay as a tool for assessing cytogenetic/DNA damage in marine organisms <i>Janina Baršienė, Brett Lyons, Aleksandras Rybakovas, Concepción Martínez-Gómez, Laura Andreikenaite, Steven Brooks, and Thomas Maes.</i> In ICES Cooperative Research Report No 315.</p> <p>QA/QC through UNEP/MAP MED POL Inter-calibration exercises in agreement with University of Piemonte Orientale Italy (DiSAV)</p> <p>In ICES Cooperative</p>	<p>Common decision needed on whether to develop methodology (including deciding on sentinel species) in order to include monitoring for imposex in gastropods for the effect of TBT. Decision should be taken after a period of several years when imposex data are starting to be available for Mediterranean Region.</p>
<p>Common Indicator 13, COP18 indicator number 9.3.1 Occurrence, origin (where possible, extent of significant acute pollution events (e.g. slicks</p>	<p>All accidents causing or likely to cause pollution of the sea by oil and other harmful substances</p> <p>The presence,</p>	<p>Quantification of oil and other chemical spills and their size by observation and reporting.</p> <p>Optional utilization of:</p> <ul style="list-style-type: none"> Satellite radar images, plane observation and imaging approaches 	<p>UNEP MAP Emergency Protocol Reporting Guidelines available through REMPEC</p> <p>Report available through REMPEC (POL REP) for reporting to REMPEC spills in excess of 100 m³</p> <p>Sampling analysis, reference methods are</p>	<p>Contracting Parties would need to improve reporting of information to REMPEC as part of their commitments under the Emergency and Prevention and Emergency Protocols.</p>

Common Indicator description	DESCRIPTION Parameters and/or Elements, matrix	Assessment Method	Monitoring Guidelines, data and existing QA/QC Sampling and Analysis Reference Methods	Recommendations /Additional work, data needed
<p>from oil, oil products and hazardous substances) and their impact on biota affected by this pollution</p> <p>With Operational Objective 9.3 Acute pollution events are prevented and their impacts are minimized</p> <p>Pressure, Impact indicator</p>	<p>characteristics and extent of spillages of oil or other harmful substances observed at sea which are likely to present a serious and imminent threat to the marine environment or to the coast or related interests of one or more of the Parties;</p> <p>Their assessments and any pollution combating actions taken or envisaged to be taken</p> <p>The evolution of the situation.</p>	<ul style="list-style-type: none"> • Backtracking of oil spills to their source by hind cast modelling; • Fingerprinting using chemical analysis (Gas Chromatography-Mass Spectrometry) and comparison with possible sources 	<p>available through REMPEC/IMO.</p>	
<p>Common indicator 14, COP18 Indicator 9.4.1: Actual levels of contaminants that have been detected and number of contaminants which have exceeded</p>	<p>At least the following contaminants for which regulatory levels have been laid down: Heavy metals (Pb, Cd, Hg), PAH, dioxins including dioxin-like PCBs)</p>	<p>Assessment of the results of monitoring executed/commissioned by the pertinent authorities responsible for health monitoring for cases for which monitoring of contaminants under indicator 9.1.1 (and possibly 9.2.1) show cause for concern</p>	<p>Monitoring executed/commissioned by the authorities responsible for health monitoring, of contaminants in fish and other seafood used for human consumption.</p>	<p>This type of monitoring was not included under UNEP/MAP MED POL Phase IV.</p> <p>It is recommended that to connect the required monitoring data to the UNEP/MAP MED POL Database by the Contracting Parties.</p> <p>In order to make monitoring results more comparable between sub- regions it would</p>

Common Indicator description	DESCRIPTION Parameters and/or Elements, matrix	Assessment Method	Monitoring Guidelines, data and existing QA/QC Sampling and Analysis Reference Methods	Recommendations /Additional work, data needed
<p>maximum regulatory levels in commonly consumed seafood</p> <p>With Operational Objective 9.4: Levels of known harmful contaminants in major types of seafood do not exceed established standards</p> <p>Pressure, Impact indicator</p>				<p>be advisable to select a limited number of target species from the most consumed species of fish and other seafood.</p> <p>A list of maximum levels for contaminants in foods set by the FAO/WHO Codex Alimentarius Commission can be found at: ftp://ftp.fao.org/codex/Meetings/cccf/cccf7/cf07_INFe.pdf</p>
<p>Common indicator 15, COP 18 Indicator 9.5.1: Percentage of intestinal enterococci measurements within established standards</p> <p>With Operational Objective 9.5: Water quality in bathing waters and other recreational</p>	<p>Intestinal enterococci in seawater in bathing and other recreational areas</p>	<p>UNEP/MAP MED POL/WHO</p> <p>Bathing and Recreational Water Monitoring Programme</p> <p>Sampling fortnightly in spring and summer to autumn</p>	<p>Criteria and Standards for Bathing Waters in the Mediterranean Region. COP 17 Decision IG 20/9</p> <p>QA/QC available through UNEP/MAP MED POL/ WHO</p> <p>ISO 7899-2 based on membrane filtration technique or any other approved technique</p>	

Common Indicator description	DESCRIPTION Parameters and/or Elements, matrix	Assessment Method	Monitoring Guidelines, data and existing QA/QC Sampling and Analysis Reference Methods	Recommendations /Additional work, data needed
areas does not undermine human health Pressure, Impact indicaotr				

Chapter III

Marine Litter chapter and related fact sheet

III. MONITORING AND ASSESSMENT METHODOLOGICAL GUIDANCE ON EO10: MARINE LITTER

1. Introduction

In the UNEP/MAP Barcelona Convention/LBS Protocol system, the monitoring of marine litter is regulated both through the Regional Plan on Marine Litter management (herein after referred to as **MLRP**), adopted by COP 18, 2013 and the COP18 EcAp Decision. The latter specified the key relevant marine litter ecological and operational objectives as well as a set of three ML state indicators.

Article 12 of the MLRP provides for a Mediterranean Marine Litter Monitoring Programme, which will be in synergy with the relevant international and regional guidelines including the relevant work carried out under the EU MSFD.

The EcAp CorGest meeting held in February 2014 adopted EcAp marine litter common indicators (common indicators 16-17) and one candidate indicator (candidate common indicator 18).

Special attention was paid to two key relevant documents on marine litter monitoring namely the UNEP Operational Guidelines for Comprehensive Beach Litter Assessment (Cheshire *et al.* 2009) and the “Guidance on Monitoring of Marine Litter in European Seas” produced between 2012 and 2013 by the European Union Task Group on Marine Litter (**TSG ML**). Both aforementioned documents were presented as information documents UNEP DEPI (MED) WG 394. Inf.4 and UNEP DEPI (MED) WG 394. Inf.5 for the EcAp Coordination Group in September 2015.

The recent overviews by UNEP (Cheshire *et al.*, 2009), and by NOAA, (Opfer *et al.* (2012), are the most comprehensive and useful overviews for monitoring methods on the coast. The UNEP overview includes a comprehensive comparison of existing marine litter survey and monitoring methods and protocols in which beach surveys were assessed. Much of the information included in the TSG ML report for the monitoring of beach litter is taken from the UNEP Operational Guidelines for Comprehensive Beach Litter Assessment (Cheshire *et al.*, 2009) and the NOAA Marine Debris Shoreline Survey Field Guide (Opfer *et al.*, 2012).

The objective of the “Guidance on Monitoring of Marine Litter in European Seas” is to provide EU Member States with recommendations and information needed to implement harmonized monitoring programmes for marine litter. The report describes specific protocols and considerations to collect, report and assess data on marine litter, in particular beach litter, floating litter, seafloor litter, litter in biota and micro-litter.

The TSG ML monitoring guidance document was developed through a collaborative programme involving the European Commission, all EU Member States, the Accession Countries and Norway, international organisations, including all the Regional Sea Conventions and other stakeholders and Non-Governmental Organisations. The document should be regarded as presenting an informal consensus position on best practice agreed by all partners. Dealing with a topic under development through research efforts and by fast growing experience this guidance is regarded as a living document to be regularly reviewed.

All the protocols suggested by TSG-ML are aimed mainly at assessing environmental status and environmental targets. All protocols can supply quantitative data, and allow the assessment of trends. The beach litter protocol is also designed to identify sources by using a detailed list of identifiable items, while other protocols can do this to some extent through their lists of items, but also by modifying the sampling strategy (where and when to sample) to match the likely effects of specific measures.

In their analysis of the protocols, the issue of compatibility and coherence has been important. Most of the protocols proposed can be applied across the Regional Seas scale. However, some of the protocols for litter in biota cannot be identical, for the simple reason that the proposed species do not all occur across the Regional Seas.

A complete analysis of risk should ideally include quantitative knowledge of harm. An analysis of harm will be a focus area for future work. In the event of insufficient quantitative data availability on harm, the risk-based approach is chosen to be addressed by an assessment of where the amounts of litter are likely to be highest or the type of litter which has the largest impact (e.g. microplastics). Already in the selections of protocols a degree of risk-based approach is used. For example, it is proposed to measure litter on the sea surface rather than in the whole water column, because pilot studies indicate that litter quantities are higher on the sea surface. Similarly, the protocols for monitoring on the sea floor propose to assess where litter tends to accumulate (e.g. through pilot studies or oceanographic modelling), and then to direct monitoring towards such areas. While there may be problems to generalize the results from this kind of monitoring to other areas, such strategies are in line with a risk-based approach.

As mentioned above in the document, due to lack of experience on marine litter monitoring within the UNEP/MAP MED POL programme, the Secretariat has developed the present working document drawing largely on the above mentioned UNEP Operational Guidelines for Comprehensive Beach Litter Assessment and on the Guidance on Monitoring of Marine Litter in European Seas.

2. Establishing a monitoring framework for marine litter in the Mediterranean

The COP18 EcAp Decision includes definitions of GES and targets for marine litter indicators. These indicators refer to litter washed ashore or deposited on coastlines, litter in the water column, including microplastics, and on the seafloor and litter ingested by or entangling marine organisms, especially marine mammals, seabirds and marine turtles.

Fulfilling the monitoring requirements under the Regional Plan on Marine Litter and under EcAp is a major undertaking, and resources for monitoring can be limited. Contracting Parties are, therefore, faced with the decision of what to monitor, and whether it is essential to assess litter amounts, in all of the environmental compartments mentioned above. It is then important to remember that these different compartments can indicate different pathways and sinks for marine litter, and do not necessarily substitute each other.

Our present understanding of litter in the marine environment, which is based on information for only a subset of these compartments, is not sufficient to draw conclusions about the trends and amounts of litter, in the various size categories, in the total marine environment. Biota indicators have a different, but not less important, function: they give an indication of possible harm. Furthermore, the compartments selected for monitoring should also provide information for the identification of sources, not only in terms of the nature and purpose of the items, but also their original source (which can be related to unsuitable or accidental disposal), and the pathway through which the item entered the marine environment. Again, this may vary among the different environmental compartments. At the same time, it is acknowledged that the protocols/methods such as those listed in the TSG-ML report have different degrees of maturity, i.e. to what extent they are tested in the field, and are in common use.

It is strongly recommended that Contracting Parties, which currently have plans to monitor only in a subset of environmental compartments, to start with small pilot research or development projects in other compartments. This would provide baseline data to make an informed decision about future, full-scale monitoring programmes. Without information on trends and amounts, in all the marine compartments, a risk-based approach to litter monitoring and measures is not possible.

A considerable number of citizens, communities (NGOs, civil society initiatives) and environmental protection associations and institutes across the Mediterranean are already taking part in activities to

tackle marine litter. The aim would be to enable them to participate in a Mediterranean regional attempt to address marine litter issues as envisaged through the MLRP and to empower citizen networks to help improve the evidence base needed to reach the EcAp main objectives.

2.1. Some general considerations on spatial distribution of survey sites: site selection strategies

The strategy used to select sites is partly a statistical/technical issue but foremost it is related to the purpose of monitoring, a decision to be taken when a monitoring strategy is defined. The site selection strategy has fundamental consequences for the monitoring analysis, as has the selection of the survey method. Monitoring programmes are not compatible or comparable if they use the same survey methods, but different site selection strategies (e.g. special site selection on the basis of litter pollution levels, or a randomised selection of sites.)

Sites can be chosen individually because they have certain characteristics and they represent what is needed for the CPs (maritime pollution, characterization of sources, etc.). This may be because they are considered to have certain environmental or societal values. For example, a beach that has a high number of visitors, because the beach is situated in a certain area, or simply because the site has heavy litter loads. Usually, the site is revisited during subsequent surveys to assess trends. The advantage of this approach is that if several sites are chosen for sharing the same characteristics, the litter load they receive is expected to be more similar than those chosen randomly and, therefore, the variation will be less than those chosen randomly. With this in mind, the ability to detect statistically significant trends will be increased. The main disadvantage of the strategy is that, as individual sites are chosen deliberately for special features, they are therefore different from other sites. Hence they may be less suitable for drawing conclusions about average litter levels etc. for a given region. It may add difficulty in interpreting statistical results for technical and philosophical reasons.

Sites may be chosen randomly from a large number of possible sites, meeting certain criteria based upon the method and the monitoring purpose. Sites may be revisited or chosen for each monitoring occasion; the important issue is how they were selected in the first place, e.g. a random selection from many possible sites. The main advantage of this strategy is that results can be extrapolated to other possible sites, i.e. we can use the results to draw conclusions about larger areas. Nevertheless, the variation among sites can be high, making it difficult and costly to find statistically significant trends.

In practice, these two strategies are rarely used in their pure form. Instead a combination is used which is sometimes referred to as, “stratified randomised sampling strategy”. Sites meeting certain criteria are (more or less) randomly chosen. The criteria may include geographic, environmental, societal and other factors. An example would be to choose sites that are close to harbours, to monitor effects of pollution from harbours, and/or sites that are situated in relatively remote areas, to monitor large-scale pollution levels without strong influence from local sources. This is compatible with a risk-based approach. Priority should be given to monitoring programmes that measure environmental status and trends, in sites where the risk of harm is greatest. The criteria for the site selection should then be based on prediction of potential harm. Prediction of potential harm could be based on practical knowledge of which environmental values are most sensitive to harm. However, the current understanding of how different species or biotopes react to litter is insufficient, and should be further researched. Another approach to harm may be based on aspects that are particularly “valuable” to society for other reasons e.g. economically, socially or environmentally. A third approach is to assume that harm is more likely to occur in areas/environments where there is a lot of litter and select sites based on screening monitoring to identify them. While this option may be practical and make sense in terms of societal needs, it is important to remember that we do not know if statistical trends from such sites are representative of other sites (probably not), but represent a “worst case” scenario.

One way to make best use of limited resources is to take advantage of other studies and programmes where litter monitoring can be integrated (what is called “opportunities to reduce costs”). An example is to combine monitoring for litter on the sea floor with scientific trawling for fish stock biomass estimation (such as under the Mediterranean International Trawl Survey, MEDITS). In such a case, the

selection of sites is designed for the original monitoring programme purpose, and representation of other areas are already defined. Where use of such a scheme is made, it is important to analyse the sampling strategy to assess if this is suitable for litter monitoring too.

For marine litter, a stratified, randomised sampling strategy where possible is advocated. Also, that the purposes of the monitoring programmes define the criteria for selecting sites. Simplification is necessary when resources are limited, and concentration of monitoring effort is the logical result.

Monitoring for trend analysis: Statistical power or how many sampling stations are needed to detect a change?

The ability of a monitoring programme to show a statistically significant trend or difference is called statistical power. Statistical power is influenced by the magnitude of the trend, the variation among replicates, and the number of replicates.

The magnitude of the trend is a characteristic of the combined effect of the environment and our (miss-) handling of litter. In that sense, the magnitude of the trend is dependent on the action we take against litter. When designing a monitoring programme an important decision is related to the magnitude of change we wish to detect. It is of course easier to detect a large trend than a small trend. The smaller the magnitude we want to detect, the more comprehensive the monitoring programme needs to be. If the action plans to tackle marine litter aim at reducing litter amounts significantly, then monitoring programmes can detect real changes.

The number of replicates is something that is easy to change given sufficient resources. Replicates, in the case of litter trends, are a combination of monitoring sites and monitoring occasions. Using the same amount of sites, the ability to detect a significant trend increases with time. In monitoring programmes, which often are complex with multiple temporal and spatial layers, the actual number of replicates is less easy to define.

The variation among replicates is a characteristic of the system studied. All biological systems tend to be very variable. To a certain extent, we can influence this by having well defined monitoring protocols and quality assessments, to minimize the added variation due to handling. More important, however, is the ability to decrease variation among sites, by introducing criteria for the sampling, as described in the section on site selection strategies above. This is not cutting corners or cheating, but it is important to realize that the possibility to extrapolate to un-sampled sites decreases.

Common to all three factors influencing statistical power is that they are case specific. It is not possible to give general advice on how many replicates are adequate, except to say the more the better. Firstly, decisions about the purpose of a specific monitoring programme, and what the sites should represent have to be made. Then some estimate of variation is necessary. The data on variation should, ideally, come from a pilot study using the same sites. Otherwise data from similar programmes can be used. Only then can calculations of statistical significance be made, and thus the required number of sites for the monitoring programme be arrived at.

An important and encouraging fact is that it is of value to start a monitoring programme even if the initial resources are limited. The initial data from monitoring can nevertheless be used for subsequent trend analysis (albeit with reduced statistical power), but more importantly, the data collected can be used to refine the design of the programme, including power calculations.

Power calculations for litter monitoring, using methods suggested in this report, have been made for some protocols, e.g. the Sea-bird litter ingestion protocol applied to Fulmars.

A possible challenge in monitoring of time trends of microparticles

Microparticles in the marine environment may enter directly as such from synthetic textile fragments, plastic particles used in cosmetic, or industrial cleansers, etc.), but they can also result from the

progressive fragmentation of larger pieces or items already present in the sea. If the former source is the dominant, conclusions may be drawn from fluctuation of trends. If the latter is the main source it is more problematic. Then it is possible to interpret increasing or decreasing trends as a net input of fragments or microparticles into the marine environment, when the increase may be caused by changes in the rate of breakdown of larger particles, i.e. not caused by a change in the overall amount of marine litter.

2.2. Some general considerations regarding Quality Assessment/Quality Control approaches and requirements

Since important decisions will be taken, based on the results obtained by monitoring programmes, it is important that the data generated is of acceptable quality. In order to ensure the quality and integrity of marine litter monitoring data, investment must be made in the capacity-building of national, regional and local survey coordination and management.

The use of quality control and assurance measures, such as inter-calibrations, use of reference material where appropriate, and training for operators should accompany the implementation of adopted monitoring protocols. These approaches should be developed in the context of dedicated research.

The value of the monitoring programmes results can be enhanced where a standard list of litter items is used as a basis for preparing assessment protocols. A master-list of categories of litter items has been prepared by TSG-ML. The use of appropriate field guides with examples of each litter type will assist survey team members (particularly volunteers) to be consistent in litter characterization. Such field guides should be coupled to the master list of litter items, and be made available over the web to increase consistency between survey teams working at remote locations.

The use of standard lists and definitions of items will enable the comparison of results between regions and environmental compartments. Items can be attributed to a given source e.g. fisheries, shipping etc. or a given form of harm e.g. entanglement, ingestion etc. The value of monitoring results can be increased further by identifying the main sources of marine litter pollution, and the potential level of harm that marine litter may inflict. This will enable a more target-orientated implementation of measures. Throughout the period 2013-2014, the TSG-ML will further elaborate on approaches to link detailed categories of items to the most probable source, and to other important strategic parameters which can help design and monitor measures and UNEP/MAP may also benefit from this work.

3. Monitoring of litter washed ashore and/or deposited on coastlines (Common indicator 16, Trends in the amount of litter washed ashore and/or deposited on coastlines, ie Beach Litter)

3.1. Introduction to Beach Litter

The recent overviews by UNEP, in Cheshire et al. (2009), and by NOAA, in Opfer et al. (2012), are the most comprehensive and useful overviews for monitoring methods on the coast. The UNEP overview includes a comprehensive comparison of existing marine litter survey and monitoring methods and protocols in which beach surveys were assessed (Cheshire et al., 2009).

Much of the information included in the Final Report of TSG ML is taken from the UNEP Operational Guidelines for Comprehensive Beach Litter Assessment (Cheshire et al., 2009) and the NOAA Marine Debris Shoreline Survey Field Guide (Opfer et al., 2012).

When designing marine litter surveys it is necessary to differentiate between standing-stock surveys, where the total load of litter is assessed during a one-off count, and the assessment of accumulation and loading rates during regularly repeated surveys of the same stretch of beach with initial and subsequent removal of litter.

Both types of survey provide information on the amount and types of litter, however, only the accumulation surveys provide information on the rate of deposition of litter and trends in litter pollution. As the ECAP requires an assessment of trends in marine litter recorded on coastlines only methods for the assessment of accumulation would be recommended.

The type of survey selected depends on the objectives of the assessment and on the magnitude of the pollution on the coastline. A single survey method has been recommended by TSG-ML with different spatial parameters for light to moderately polluted coastline and for heavily polluted coastlines

3.2. Requirements of a harmonised protocol

The comparison of beach litter data between assessment programmes is the primary aim of a harmonised protocol. Comparison is difficult if different methods, different spatial and temporal scales, different size scales of litter items and different lists or categorisation of litter items recorded on beaches are used within the Regional Seas

The type of survey selected depends on the objectives of the assessment and on the magnitude of the pollution on the coastline. A single survey method is recommended by the TSG-ML, with different spatial parameters for light to moderately polluted coastline and for heavily polluted coastlines.

Amounts of litter on the shore can be relatively easily assessed during surveys carried out by non-scientists using unsophisticated equipment. Coastal surveys are thus a cost effective way of obtaining large amounts of information. The litter deposited on the coastline can vary greatly between sites and seasons, affected by hydrographical and geomorphological characteristics of the area (e.g. prevailing winds and currents, exposure of the beach to the sea) but also depending on the use of the coast (e.g. larger amounts can be deposited during the tourist season or during special events). Therefore, coastal surveys should focus on fixed sites, which fulfil the requirements of the protocol, and the timing of the survey (i.e. season) should take into account the potential sources of litter to the site (e.g. flooding in rainy seasons may increase the amounts). Sites can be placed far from known sources, in order to better reflect reference values for background litter pollution levels, or close to potential sources. By using temporal trends for assessments, both of the survey strategies give important information for managers.

3.2.1 Amounts, composition, distribution and sources of Beach Litter

Amounts of litter on the shore can be relatively easily assessed during surveys carried out by non-scientists using unsophisticated equipment. Coastal surveys are thus a cost effective way of obtaining large amounts of information. The litter deposited on the coastline can vary greatly between sites and seasons, affected by hydrographical and geomorphological characteristics of the area (e.g. prevailing winds and currents, exposure of the beach to the sea) but also depending on the use of the coast (e.g. larger amounts can be deposited during the tourist season or during special events). Therefore, coastal surveys should focus on fixed sites, which fulfil the requirements of the monitoring protocol, and the timing of the survey (i.e. season) should take into account the potential sources of litter to the site (e.g. flooding in rainy seasons may increase the amounts). Sites can be placed far from known sources, in order to better reflect reference values for background litter pollution levels, or close to potential sources. By using temporal trends for assessments, both of the survey strategies give important information for managers.

Trends in amounts of litter

The variation in the amount of litter present on a given beach between surveys and the variation between beaches, even in the same region, can be extremely large. This makes the identification of trends difficult, especially taking into account seasonal variations. Moreover, as litter accumulates on

beaches, surveys should be carried out at regular intervals in time so that the accumulation periods are approximately of the same length.

Composition of litter

The assessment of composition of litter is one of the great strengths of coastal assessments. A detailed assessment of litter composition provides information on potential harm to the environment and in some cases on the source of the litter found. The assessment of composition must follow commonly agreed categories in order to provide results which are comparable over larger regions.

Spatial distribution

Amount and composition of marine litter varies over geographical scales and reflects hydrographical (e.g. currents, wave exposure, wind directions) and geomorphological (e.g. steepness of a shore, amounts of inlets islands) characteristics of the coast. Hydrographical characteristics determine the amount of litter accumulating in waters adjacent to the coast, whereas geomorphological characteristics determine how much of this litter becomes washed ashore.

Sources of marine litter

The source of litter found on the coast can be clearly identified for some litter items. These are mostly items which originate from fisheries, or debris flushed down sewerage systems. Even with these items some caution is needed e.g. a fish box may originate from a fishing vessel or from a fishing port.

A comprehensive master list of items and categories has been developed within the TSG-ML. The sources for some items need to be designated at a regional level, because initial assessments of litter on coastlines show that sources for a given item can be different between regions.

The master list will enable at least a rough estimate of the sources of litter found on coastlines, but it should be evaluated in survey sites against known local sources. If detailed information is required it will, be necessary to carry out detailed research into the sources involved e.g. to identify between litter deposited directly on the beach by tourists and litter arriving on the beach from adjacent waters. In addition drift analysis of litter in adjacent waters could provide valuable information on its geographical origin.

3.2.2 Strategy for monitoring beach litter

Selection of survey sites

Ideally the selected sites should represent litter abundance and composition for a given region. Not any given coastal site may be appropriate, as they may be limited in terms of accessibility, suitability to sampling (sand or rocks/boulders) and beach cleaning activities. If possible the criteria below should be used:

- A minimum length of 100m;
- Clear access to the sea (not blocked by breakwaters or jetties) such that marine litter is not screened by anthropogenic structures;
- Accessible to survey teams year round, although some consideration needs to be;
- Ideally the site should not be subject to any other litter collection activities, although it is recognized that in many parts of Europe large scale maintenance cleaning is carried out periodically; in such cases the timing of non-survey related beach cleaning must be known such that litter flux rates (the amount of litter accumulation per unit time) can be determined.
- Survey activities should be conducted so as not to impact on any endangered or protected species such as sea turtles, sea birds or shore birds, marine mammals or sensitive beach vegetation; in many cases this would exclude national parks but this may vary depending on local management arrangements.

Within the above constraints, the location of sampling sites within each zone should be stratified such that samples are obtained from beaches subject to different litter exposures, including:

- Urban coasts may better reflect the contribution of land-based inputs;
- Rural coasts may better reflect background values for litter pollution levels
- Coasts close to major rivers, if downstream from the prevailing drift, may better reflect the contribution of riverine input to coastal litter pollution.

Number of sites

At present there is no agreed statistical method for recommending a minimum number of sites that may be representative for a certain length of coast. This depends greatly on the purpose of the monitoring, on the geomorphology of the coast and how many sites that meet the criteria described above are available. The representativeness of survey sites should be assessed in pilot studies, where initially a large numbers of beaches are surveyed. Subsequently, selection of representative beaches from these sites should be made on the basis of a statistical analysis.

Frequency and timing of surveys

At least two surveys per year in spring and autumn are recommended and ideally 4 surveys in spring, summer, autumn and winter. However, because of the large seasonal variation in amounts of litter washed ashore, initially a higher frequency of surveys may be necessary in order to identify significant seasonal patterns, which can then be considered when treating raw data for long-term trend analyses.

Preferably, the surveys for all participating beaches in a given region should be carried out within the shortest timeframe possible within a survey period. Coordinators within these regions should try and coordinate the survey dates between beaches. Furthermore a given beach should be surveyed on roughly the same day each year if possible.

It should be kept in mind that circumstances may lead to inaccessible and unsafe situations for surveyors: heavy winds, slippery rocks and hazards such as rain, snow or ice, etc. The safety of the surveyors must always come first. Dangerous or suspicious looking items, such as ammunition, chemicals and medicine should not be removed. Inform the police or authorities responsible. If working on remote beaches it is recommended to work with a minimum of two people.

Documentation and characterisation of sites

It is very important to document and characterise the survey sites. As surveys should be repeated on exactly the same site the coordinates of the site should be documented.

Sampling unit

Once a beach is chosen sampling units can be identified. A sampling unit is a fixed section of beach covering the whole area between the water edges (where possible and safe) or from the strandline to the back of the beach.

- At least 1 section of 100m on the same beach, optimum 2 sections, are recommended for monitoring purposes on lightly to moderately littered beaches
- At least 2 sections of 100 m for heavily littered beaches (exceptionally 50m section with a normalisation factor of up to 100m to ensure coherence)

Permanent reference points must be used to ensure that exactly the same site will be monitored for all surveys. The start and end points of each sampling unit can be identified by different methods. For example numbered beach poles could be installed at the site or easily identifiable landmarks could be used. Coordinates obtained by GPS are useful for identifying the reference beaches especially where easily identifiable landmarks are lacking.

Units (quantification) of litter

Counts of items are recommended as the standard unit of litter to be assessed on the **coastline**.

Collection and identification of litter items

All items found on the sampling unit should be entered on survey forms. On the survey forms, each item is given a unique identification number. Data should ideally be entered on the survey form while picking up the litter. Collecting the litter first and identifying it later may alter numbers as collected litter tends to get more entangled or broken.

Unknown litter or items that are not on the survey form should be noted in an appropriate “other item box”. A short description of the item should then be included on the survey form. If possible, digital photos should be taken of unknown items so that they can be identified later and, if necessary, be added to the survey form.

A master list of litter categories and items is included in the TSG-ML Final Report. This master list includes a list of categories and items to be recorded during beach litter surveys.

It has been strongly recommended to produce regional photo guides including pictures of all litter items on the regional survey protocol. This will assist in the correct identification and allocation of recorded items.

Size limits and classes of items to be surveyed

There are no upper size limits to litter recorded on beaches.

The lower limit of detection, when walking a beach, is probably somewhere around 0.5 cm (plastic pellets), however, it is doubtful that such small items can be monitored effectively and in a repeatable fashion during beach surveys.

A lower limit of 2.5 cm in the longest dimension is recommended for litter items monitored during beach surveys. This would ensure the inclusion of caps & lids and cigarette butts in any counts.

Removal and disposal of litter

Removal of litter should be carried out at the same time as monitoring the litter. Coupling removal with monitoring ensures better accuracy of reporting and enables comparison of litter accumulation over time; It also has the added advantage of leaving a clean beach. It is important to note that only the 100m ref section(s) need to be monitored and cleaned. Further areas of a beach can be cleaned without monitoring if surveyors/volunteers wish to do so.

The litter collected should be disposed of properly. Regional or national regulations and arrangements should be followed. If these do not exist local municipalities should be informed.

Larger items that cannot be removed (safely) by the surveyors should be marked, with for example paint spray (for marking trees) so they will not be counted again at the next survey.

Many municipalities will have their own cleaning programme, sometimes regularly, sometimes seasonal or incident related. Arrangements should be made with the local municipalities so that they either exclude the reference beach from their cleaning scheme or they provide their cleaning schedule so surveying can be carried out a few days before the municipality will clean the beach.

Preferably a set time should be established for each beach between the date when the beach was last cleaned and the date when the survey is carried out. It is advisable to contact the municipality before

starting a survey to obtain the latest information on beach cleaning activities. Sometimes an incident, for example a storm, will alter their cleaning programme.

3.3. Quality Assessment /Quality Control for beach litter

Based on the UNEP Guidelines (Cheshire et al., 2009), any long-term marine litter assessment programme will require a specific and focussed effort to recruit and train field staff and volunteers. Consistent, high quality training is essential to ensure data quality and needs to explicitly include the development of operational (field based) skills. Staff education programmes should incorporate specific information on the results and outcomes from the work so that staff and volunteers can understand the context of the litter assessment programme.

Quality assurance and quality control should be primarily targeted at education of the field teams to ensure that litter collection and characterization is consistent across surveys. Investment in communication and the training of the country/regional and local survey coordinators and managers is thus critical to survey integrity.

The quality assurance protocol of Ocean Conservancy's National Marine Debris Monitoring Program (USA) required a percentage of all locations to be independently re-surveyed immediately following the scheduled assessment of litter (Sheavly, 2007). The collected litter from the follow-up survey could then be added to that of the main collection and could be used to provide an estimate of the error level associated with the survey. This approach should be employed as a component of beach litter surveys.

3.4. Conclusion

In order to enable temporal and spatial comparisons within and across regions, standard litter survey methods should, where possible, be applied at all levels (local to regional) and the assessment of its composition follows agreed categories of items.

4. Monitoring of litter at sea (Common Indicator 17 Trends in the amount of litter in the water column including microplastics and on the seafloor, so-called Floating Litter)

Note: Because of the low occurrence of litter in midwater, it is recommended that the indicator focus on surface and seafloor litter

4.1. Introduction to floating litter

There exists early documentation of the occurrence of man-made objects, mainly plastic, floating at sea (Venrick 1972, Morris, 1980). While significant actions in waste management and disposal have been taken, floating litter is still a concern. It poses a direct threat to fish, marine mammals, reptiles and birds. Harm can occur through ingestion of whole items or pieces or by feeding on larger litter items. Entanglement can occur by floating bags, nets and other fishing gear. It can be assumed that marine macro litter is a precursor of marine micro litter.

4.2. Scope and key questions to be addressed

Monitoring of litter at open sea and on long transects, is not currently addressed as this requires different approaches, in particular regarding the observation conditions provided by the ships used for the surveys and regarding the possibility to monitor smaller items.

The fraction of litter under discussion, includes floating items in the water column close to the surface, as caused e.g. by the temporary mixing of floating particles under the water surface due to wave action. Litter in the deeper water column is currently not recommended for routine monitoring and should be subject of research efforts.

4.3. Existing approaches for visual ship-based observation of floating litter

HELMEPA (Hellenic Mediterranean Protection Association) uses a fleet of ocean going member vessels on a voluntary basis to obtain monitoring data through a reporting sheet. The EcoOcéan Institut is performing monitoring of floating litter in parallel with monitoring of marine mammals in the north-western Mediterranean Sea. UNEP guidance considers both sampling of an area through a dedicated observation pattern and transect sampling for monitoring of surface floating litter (UNEP, 2009).

4.3.1. Discussion of observation protocol elements

The observation of floating marine litter from ships is subject to numerous variables in the observation conditions. They can be divided into operational parameters, related to the ship properties and observation location.

The processing of the collected information, starting from the documentation on board, its compilation, elaboration and further use should be part of a protocol in order to derive comparable final results. The format should allow a compilation across different observing institutes and areas or regions. This would allow a plotting of floating litter distribution over time and thus finally allow the coupling with oceanographic current models.

4.4. Strategy for monitoring of floating litter

4.4.1 Source attribution of floating marine litter

Due to the observation methodology, the source attribution for floating litter is challenging. The type of marine litter objects can only be noted during very short visual observation. Therefore, in difference to beach litter, it is likely that only rough litter categories can be determined.

The spatial distribution of floating marine litter instead gives, in combination about currents, and river information indications about the physical source, i.e. the litter input zone and its pathway, which is very valuable information about source strength and may help to design appropriate measures and check their efficiency.

The monitoring of floating litter is very likely to be an iterative process during which in an initial phase hot spots and pathways are determined, while in an evolving monitoring programme selected transects help with the quantification of trends.

4.4.2. Spatial distribution of monitoring

The monitoring of floating marine litter by human observers is a methodology indicated for short transects in selected areas. In a region with little or no information about floating marine litter abundance it might be advisable to start by surveys in different areas in order to understand the variability of litter distribution. The selected areas should include expected low density areas (e.g. open sea) as well as expected high density areas (e.g. close to ports). This will help to obtain maximum/minimum conditions and train the observers. Other selected areas (e.g. in estuaries), in the vicinity of cities, in local areas of touristic or commercial traffic, incoming currents from neighbouring areas or outgoing currents should be considered.

Based on the experience obtained in this initial phase, a routing programme including areas of interest should then be established.

4.4.3. Timing of floating marine litter monitoring

The observation of floating marine litter is much depending on the observation conditions, in particular on the sea state and wind speed. The organization of monitoring must be flexible enough to take this into account and to re-schedule observations in order to meet appropriate conditions. Ideally the observation should be performed after a minimum duration of calm sea, so that there is no bias by litter objects which have been mixed into the water column by recent storms or heavy sea.

The initial, investigative monitoring should be performed with a higher frequency in order to understand the variability of litter quantities in time. Even burst sampling, *i.e.* high sampling frequency over short period, might be appropriate in order to understand the variability of floating marine litter occurrence.

For trend monitoring the timing will depend on the assumed sources of the litter, this can be *e.g.* monitoring an estuary after a rain period in the river basin, monitoring a touristic area after a holiday period.

The timing of the surveys will also depend on the schedule of the observation platforms. Regular patrols of coast guard ships, ferry tracks or touristic trips may offer frequent opportunities which thus also allow the use during the needed calm weather conditions.

4.5. Visual monitoring of floating litter

The reporting of monitoring results requires the grouping into categories of material, type and size of litter object. The approach for categories of floating litter is linked with the development of a “master list” with the categories for other environmental compartments such as the “master list” prepared by the TSG-ML. This allows cross comparisons.

The categories of items for floating litter should be, as far as practical, consistent with the categories selected for beach litter, seafloor litter and others. There are limitations to this, but in principal the derived data should allow a comparison across different environmental compartments, in particular between beach and surface floating litter. Therefore the list of item categories that should be adopted for floating litter corresponds to the Master List of items. For the practical use during the monitoring the list has to be arranged by object occurrence frequency so that the data acquisition can be done in the required short time. Tablet computer applications for facilitating the data documentation are under development.

As floating litter items will be observed but not collected, the size is the only indicative parameter of the amount of plastic material that it contains. The size of an object is defined here as its largest dimension, width or length, as visible during the observation.

The lower size limit for the observations is determined by the observation conditions. These should be harmonized so that a lower limit of 2.5 cm can be achieved. That size appears to be reasonable for observation from “ships-of-opportunity” and is in line with the size for beach litter surveys. This denotes that observations not achieving this minimum size limit cannot be recommended.

For reporting purposes size range classes must be introduced as visual observation will not permit the correct measuring of object sizes. Only the estimation of size classes is feasible.

The size determination/reporting scheme should enclose the following classes:

- 2.5 – 5 cm
- 5 - 10 cm
- 10 – 20 cm
- 20 – 30 cm
- 30 – 50 cm

While also wider size range classes (e.g. 2.5–10cm, 10–30cm, 30–50 cm) could be utilized, it will be important that a common approach is used, as the data will be combined in common data bases. The test phase of implementing a monitoring protocol should allow the determination of overall accepted and final size range classes. The upper size limit will have to be determined by statistical calculations regarding the density of the object occurrence in comparison to transect width, length and frequency. In coherence with the beach litter surveys an upper limit of 50 cm is here provisionally proposed. It has to be evaluated in experiments and from initial data sets if items larger than 50 cm should be reported, as their relevance in the statistical evaluation of data from short and narrow coastal transects might be questionable.

4.6. Visual monitoring of floating litter

A harmonized approach for the quantification of floating marine litter by ship-based observers has been developed by the TSG-ML. It has the scope to harmonize the monitoring of floating marine litter:

- In the size range from 2.5 to 50 cm,
- Observation width needs to be determined according to observation set-up,
- It is planned for use from ships of opportunity,
- It is based on transect sampling,
- It should cover short transects, and
- Also record necessary metadata.

4.6.1. Observation

The observation from ships-of-opportunity should ensure the detection of litter items at 2.5 cm size. The observation transect width will therefore depend on the elevation above the sea, the ship speed and the observation conditions. Typically a transect width of 10 m can be expected, but a verification should be made and the width of the observation corridor chosen in a way that all items in that transect and within the target size range, can be seen. Table 10.1 below provides a preliminary indication of the observation corridor width, with varying observation elevation and speed of vessel (kn = knot = nautical mile/h). The parameters need to be verified prior to data acquisition.

The ideal location for observation will often be in the bow area of the ships. If that area is not accessible, the observation point should be selected so that the target size range can be observed, eventually reducing the observation corridor, as ship induced waves might interfere with the observations. An inclinometer can be used to measure distances at sea (Doyle, 2007).

Table 5: Width of “observation corridor” based on observation height and ship speed (to be reviewed)

Observation elevation above sea	Ship speed 2 knots = 3.7 km/h	6 knots = 11.1 km/h	10 knots = 18.5 km/h
1 m	6m	4m	3m
3m	8m	6m	4m
6m	10m	8m	6m
10m	15m	10m	5m

The protocol will have to go through an experimental implementation phase during which it is applied in different sea regions by different institutions, its practicality is tested and feedback for definition of observation parameters is provided.

The observation, quantification and identification of floating litter items must be made by a dedicated observer who does not have other duties contemporaneously. Observation for small items and surveying intensively the sea surface leads to fatigue and consequently to observation errors. The transect lengths should therefore be selected in a way that observation times are not too long. Times of 1 h for one observer could be reasonable, corresponding to a length of a few kilometres.

4.6.2. Reporting of monitoring results

A harmonized reporting of monitoring results is crucial for the comparison of data. The data output from the application of the protocol, when using a computer interface, is a list of geo-referenced objects according to a list of categories. The use of a portable computer device for documenting marine floating litter has clear advantage over paper documents. A specific application, based on the TSG -ML protocol for the monitoring of floating macro litter will be developed by JRC and field tested within the PERSEUS project.

It is not uncommon that floating litter items appear grouped, either because they have been released together or because they accumulate on oceanographic fronts. The reporting system should acknowledge this and foresee a way to report such groups. The occurrence of such accumulation areas needs to be considered when evaluating the data.

For floating marine litter the unit of reporting will be: items/km². The data will be available for the different categories and size classes. They can then be aggregated at different levels for providing overview data.

Along with the litter occurrence data, a series of metadata should be recorded, including geo-referencing (coordinates) and wind speed (m/s). This accompanying data shall allow the evaluation of the data in the correct context.

4.6.3. Quality assessment/Quality control

The widespread acquisition of monitoring data will need some kind of inter-comparison or calibration in order to ensure comparability of data between different areas and over time, for trend assessments. Approaches for this should be developed and implemented. This can be hands (eyes)-on training courses with comparisons of observations. Such events should be organized at Regional level with further implementation at national scale.

A methodology for calibrating observation quality by artificial targets may be devised through research efforts.

4.6.4. Equipment

The equipment used for the monitoring of floating litter is very limited. Besides the transportation platform some instruments may facilitate the work:

- A system for visually marking the observation area,
- GPS for determination of ship speed and geographical coordinates,
- A tablet PC (with GPS) for documenting the results (including a dedicated application/program),
- A system for training and calibrating size classification.

4.6.5. Implementation of the TSG-ML Protocol

The finalization and wide acceptance of the protocol proposed by TSG –ML will require an experimental testing period during which observation parameters and reporting approaches are being studied on a wide range of ships and conditions, covering different regional seas. This can be achieved through the ECAP implementation process and through dedicated activities in research projects, such as PERSEUS. Resulting data can be used for adjusting the protocol. Once the protocol parameters, such as standardized size ranges, categories and observation conditions are confirmed, a final version can be prepared. The final protocol should be widely disseminated and accompanied by activities for its implementation. Training courses and workshops can contribute to the harmonized acquisition of comparable datasets.

4.7. Other methodologies

Open sea surveys

While the proposed protocol is aiming at coastal surveys, there are also approaches for monitoring of litter from large, seagoing vessels. While covering large areas, these surveys face considerably different observation conditions and therefore different observation protocols.

Aerial surveys

The opportunistic use of aerial surveys (e.g. for marine mammal observation/monitoring) has been considered. The minimum size of observed objects is at ca. 30 cm, therefore this approach might be adequate to the size fraction above 30 cm considered by the TSG-ML.

Net tow surveys for macro litter

Physical sampling of floating macro litter requires large net openings operated at the sea surface. Given the density of larger macro litter items occurrence this would require significant dedicated ship time and specific equipment. This method is applicable for floating micro litter. There should be methodological research on how to cover the size range between 5 mm and 2.5 cm, which is very relevant to ingestion by marine biota.

Riverine litter monitoring

While not envisaged in the current litter monitoring framework, the TSG –ML protocol is equally well applicable for the monitoring of floating litter on rivers as an indication of a potential source of loads of litter to the marine environment, by observation from bridges or similar platforms.

New methodologies

Closely related to the monitoring by human visual observation is the monitoring through image acquisition by digital camera systems and their subsequent analysis by image recognition techniques. Such is the Sealittercamera, which is being developed by the EC JRC, a system being temporarily deployed on Costa Crociere cruise ships in the Western Mediterranean Sea (Hanke, 2011, publication in preparation).

4.8. Conclusions

Key messages to the ECAP implementation process:

- The monitoring of floating marine litter in selected coastal transects is recommended
- Monitoring Marine Litter suspended in the middle water column is not recommended
- Monitored size categories should include a range covering relevant small items
- Monitoring of floating litter should follow a specific protocol agreed on a Regional scale within the ECAP/UNEP/MAP MED POL monitoring implementation process

5. Seafloor Litter (Common Indicator 17, Trends in the amount of litter in the water column including microplastics and on the seafloor)

5.1. Introduction to seafloor litter

The most common approaches to evaluate sea-floor litter distributions use opportunistic sampling. This type of sampling is usually coupled with regular fisheries surveys (marine reserve, offshore platforms, etc.) and programmes on biodiversity, since methods for determining seafloor litter distributions (e.g. trawling, diving, video) are similar to those used for benthic and biodiversity assessments. The use of submersibles or Remotely Operated Vehicles (ROVs) is a possible approach for deep sea areas although this requires expensive equipment. Monitoring programmes for demersal fish stocks, undertaken as part of the Mediterranean International Bottom Trawl Surveys (MEDITS), operate at large regional scale and provide data using a harmonized protocol, which may provide a consistent support for monitoring litter at Regional scale on a regular basis and within the ECAP requirements.

5.2. Scope and key questions to be addressed

For shallow waters, the monitoring of litter on the seafloor may not be considered for all coastal areas because of limited resources. In these areas the strategy is to be determined by each Contracting Party at national level, depending on the priority areas to be monitored. Opportunistic approaches may be used to minimize costs. Valuable information can be obtained from on-going monitoring of benthic species in protected areas, during pipeline camera surveys, cleaning of harbours and through diving activities. Additional monitoring might have to be put in place to cover all areas creating a consistent monitoring network. The sampling strategy should enable the generation of good detail of data, in order to assess most likely sources, the evaluation of trends and the possibility of evaluating the effectiveness of measures. The TSG-ML proposes simple protocols based on existing trawling surveys and two alternative protocols based on diving and video imagery which fit with the ECAP requirements and support harmonisation at Regional level, if applied trans-nationally.

Trawling (otter or beam trawl) is an efficient method for large scale evaluation and monitoring of sea-floor litter. The monitoring strategy for sea-floor can efficiently be based on on-going monitoring already developed at Regional level. It must be noted, however, that the geomorphology may impact the accumulation of litter in the seafloor and some sampling restrictions in rocky areas (incompatible with trawling) may lead to underestimation of the quantities present. Designing and developing an adequate monitoring programme will have to take account of these limits. Existing fisheries stock assessment programmes are covering most Regional Seas.

Only some countries will have to consider deep sea areas in terms of monitoring of sea-floor litter. The strategy is to be determined by each Contracting Party at national level, depending on affected areas but previous results indicate that priority should be given to coastal canyons. Protocols based on video imagery are the only approaches to monitor deep sea areas. These protocols are based on the use of (ROVs)/submersibles. As litter accumulates and degrades slowly in deep sea waters, a multiyear evaluation will be sufficient.

5.3 Monitoring the shallow sea-floor (<20m)

The most commonly used method to estimate marine litter density in shallow coastal areas is to conduct underwater visual surveys with SCUBA/snorkelling. These surveys are best based on line transect surveys of litter on the sea-floor, which is derived from UNEP (Cheshire, 2009). The protocol is actually in use for evaluation of benthic fauna. It requires SCUBA equipment and trained observers. Only litter items above 2.5 cm are considered, between 0 and 20 m (to 40 meters with skilled divers).

5.3.1. Technical requirements

Frequency

The minimum sampling frequency for any site should be annually. Ideally it is recommended that locations are surveyed every three months (allowing an interpretation in terms of seasonal changes).

Transects

Surveys are conducted through 2 line transects for each site. Unbiased design-based inference requires allocating the transects randomly in the study area or on a grid of systematically spaced lines randomly superimposed. However, with a model-based approach like density surface modelling (DSM), it is not required that the line transects are located according to a formal and restrictive survey sampling scheme, although good spatial coverage of the study area is desirable. Line transect are defined with a nylon line, marked every 5 meters with resistant paints, that is deployed using a diving reel while SCUBA diving.

Individual litter within 4 m of the line (half of the width – W_t - of the line transects) are recorded. For each observed litter item, when possible, the corresponding line segment of occurrence and its perpendicular distance from the line (y_i - for the estimation of detection probability, measured with the use of a 2 m plastic rod), and litter size category (w_i) are recorded. The nature of the bottom/habitat is also recorded. The length of the line transects vary between 20 and 200 m, depending on the depth, the depth gradient, the turbidity, the habitat complexity and the litter density (Katsavenakis, 2009). Results are expressed in litter density (items/m² or items/ 100 m²).

Detectability

In distance sampling surveys, detectability is used to correct abundance estimations (Katsavenakis, 2009). The standard software for modelling detectability and estimating density/abundance, based on distance sampling surveys, is DISTANCE (Thomas et al., 2006).

5.3.2. Use of volunteers in shallow waters surveys

Recreational and professional scuba divers can provide valuable information on litter they see underwater and they are uniquely positioned to support benthic litter monitoring efforts. They can access, have the skills and the equipment needed to collect, record, and share information about litter they encounter underwater. Many dive clubs and dive shops organize underwater clean-ups, often in partnerships with NGOs or local governments. Many of these events, when managed, can be a valuable source of information and possibly be a part of a regular survey, monitoring or even assessment efforts while using volunteers.

For some Contracting Parties use of volunteer divers might be a good opportunity for shallow-water litter monitoring but standardization and conformity with common methodologies and tools such as those propose by TSG-ML should be achieved. Fixed sites, common frequency and sampling methodology can be easily established by each Contracting Party and training, material distribution etc. can be achieved relatively easily when partner NGOs or research institutions are involved.

5.4 Monitoring the Sea-floor (20-800m)

From all the methods assessed, trawling (otter trawl) has been shown to be the most suitable for large scale evaluation and monitoring (Goldberg, 1995, Galgani et al., 1995, 1996, 2000). Nevertheless there are some restrictions in rocky areas and in soft sediments, as the method may be restricted and/or underestimate the quantities present. This approach is however reliable, reproducible, allowing statistical processing and comparison of sites. As recommended by UNEP (Cheshire, 2009), sites should be selected to ensure that they (i) Comprise areas with uniform substrate (ideally sand/silt bottom); (ii) consider areas generating/accumulating litter, (iii) avoid areas of risk (presence of munitions), sensitive or protected areas; (iv) do not impact on any endangered or protected species.

Sampling units should be stratified relative to sources (urban, rural, close to riverine inputs) and impacted offshore areas (major currents, shipping lanes, fisheries areas, etc.).

General strategies to investigate seabed litter are similar to methodology for benthic ecology and place more emphasis on the abundance and nature of items (e.g. bags, bottles, pieces of plastics) rather than their mass. The occurrence of international bottom trawls surveys such as MEDITS (Mediterranean/Black Sea) provide useful and valuable means for monitoring marine litter. These are using common gears depending on region (MEDITS net in the Mediterranean) and provide some harmonized and common conditions of sampling (20 mm mesh, 30-60 min tows, large sampling surface covered) and hydrographical and environmental information (surface & bottom temperature, surface & bottom salinity, surface & bottom current direction & speed, wind direction & speed, swell direction and height). More than 20 sampling units are sampled within each region as recommended by UNEP (Cheshire, 2009).

Therefore, the TSG-ML strongly recommends using these on-going and continuous programmes to collect data on marine litter in the sea-floor. This will enable to compare data from one country to another and to evaluate transnational transportation.

5.5 Technical Requirements

The protocol of the TSG-ML for sampling and trawling margins (20-800m) has been standardized for each region:

Mediterranean and Black Seas

For the Mediterranean Region, the protocol is derived from the MEDITS protocol (see the protocol manual, Bertran et al., 2007). The hauls are positioned following a depth stratified sampling scheme with random drawing of the positions within each stratum. The number of positions in each stratum is proportional to the surface of these strata and the hauls are made in the same position from year to year. The following depths (10 – 50; 50 – 100; 100 – 200; 200 – 500; 500 - 800 m) are fixed in all areas as strata limits. The total number of hauls for the Mediterranean Sea is 1385; covering the shelves and slopes from 11 countries in the Mediterranean.

The haul duration is fixed at 30 minutes on depths less than 200m and at 60 minutes at depths over 200m (defined as the moment when the vertical net opening and door spread are stable), using the same GOC 73 trawl with 20 mm mesh nets (Bertran et al, 2007) and sampling between May and July, at 3 knots between 20 and 800 m depth.

Detecting trends

Consistency of results is based on sampling strategy and monitoring efforts. Long term monitoring of litter on the sea floor has been performed in Spain and France. In some cases such as the margins of gulf of Lion (France), trends studies (70 Stations, depth 40-800m,) indicated a statistically significant decrease [Abundance (10-4) = 0.038 x (Year) + 1.062 (R2 =0.36)] enabling the measurement of 15% decrease in 15 years.

However, Power Analysis of IBTS related sampling by Cefas indicates that detection of a 10% change over 5 or 10 years is unlikely without massive sample sizes. However, 50% changes over 5 or 10 years look to be readily detectable with current designs based on fish stock surveys such as IBTS.

Data recording and Management

Templates for data recording have been integrated in MEDITS Manuals . Data on litter should be collected on these templates using items categories such as those listed for Sea-floor prepared by TSG-ML. Other elements from the haul operations should be also recorded – See MEDITS for the Mediterranean/Black Sea.

Data on litter should be reported as items/ha or items/km² before further processing and reporting.

5.6 Litter categories for Sea-floor

As marine litter degradation is affected by light, oxygen and wave action, the persistence of marine litter on the sea floor and deep sea floor is increased with notable outcomes on the nature of litter found. Another important factor influencing the composition of benthic litter is related to the type of activity. Typically, the analysis of sources indicated the importance and differences between ship based litter, as in the Southern North Sea, and land based litter such as in the Mediterranean. The definition of categories will have to take this in account when defining a protocol. Although marine litter is strongly affected by transportation, fishing has been shown as a main source of litter in some fishing or aquaculture grounds. Similarly specific types of marine litter were also found in areas affected by tourism, around beaches, as in the Mediterranean Sea. This may affect the strategy for monitoring selected areas, such as shallow waters.

A standardized litter classification system has been defined for monitoring the sea floor by TSG-ML. The categories were defined in accordance with types of litter found at regional level, enabling common main categories for all regions. The main categories have a hierarchical system including sub categories. It considers 4 main categories of material for the Mediterranean (wood, paper/cardboard, other, unspecified). There are various subcategories for a more detailed description of litter items. Other specific categories may be added by Contracting Parties and additional description of the item may provide added-value, as long as the main categories and sub-categories are maintained. Furthermore, the weight, picture and note of potential attached organisms may further complement the classification of items.

Other parameters

Site information and trawling sampling characteristics such as date, position, type of trawl, speed, distance, sampled area, depth, hydrographical and meteorological conditions should be recorded

Data-sheets should be filled out for each trawl and compiled by survey. If multiple counts (transects/observers) are run at any given site then a new sheet should be used for each trawl shot. After each survey data must be aggregated for analysis and reporting.

5.7. Complementary sea-floor monitoring – Video camera

Large-scale evaluations of marine litter in the deep sea-floor are scarce because of available resources to collect data. Special equipment is necessary including ROVs and/or submersibles that may be very expensive to operate, especially in deep sea areas.

Towed video camera for shallow waters (Lundqvist, 2013) or ROVs for deeper areas are simpler and generally cheaper and must be recommended for litter surveys. There are some available protocols where litter is counted on routes and expressed as item/km, especially when using submersibles/ROVs at variable depths above the deep sea floor (Galgani et al., 1996) however technology enables the evaluation of densities through video-imagery using a standardized approach especially for shallow waters.

5.8. Quality Assessment /Quality Control for sea-floor litter

Several Contracting Parties from UNEP/MAP MED POL have indicated they will use their fish stock surveys for benthic litter monitoring. This is considered to be an adequate approach although quantities of litter might be underestimated, given restrictions in some areas. The adoption of a common protocol will lead to a significant level of standardization among the Contracting Parties countries that apply this type of sampling strategy.

Data on litter in shallow sea-floor are collected through protocols already validated for benthic species.

Until now, no quality assurance programme has been considered for litter monitoring on the sea-floor. For MEDITS, sampling data are collected in the DATRAS database and participate in data quality checking for hydrographical and environmental conditions. This process may also support quality insurance for data on litter. Currently, there are on-going discussions on how to organize and harmonize a specific system to collect, validate and organize data through a common platform, enabling the review and validation of data. MEDITS has included litter data to be analysed within a specific sub-group.

5.9. Conclusions

Considering opportunities to couple monitoring efforts may be the best approach to monitor litter on the sea-floor.

There may be other opportunities to couple marine litter surveys with other regular surveys (monitoring in marine reserves, offshore platforms, etc.) or programmes on biodiversity.

6. Litter ingested by or entangling marine organisms, especially mammals, marine birds and turtles (Litter in Biota, Candidate Common Indicator 18, Trends in the amount of litter ingested by or entangling marine organisms focusing on selected mammals, marine birds and marine turtles)

Note: Due to the availability of protocols and the state of knowledge, it is recommended that the indicator focus on the sea turtle *Caretta caretta*

6.1. Scope and 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 proposed by TSG-ML which can be used for seabirds in general, e.g. to be applied in regular monitoring for shearwaters in parts of the Mediterranean.

However alternative tools are needed for the Mediterranean Sea. On the basis of available information and expertise, a monitoring protocol for marine litter in sea turtles with focus on relevant parameters for application in the Mediterranean is proposed by TSG-ML. 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 harmonized protocol for the assessment of the use of plastic litter as nesting material and associated entanglement mortality in birds breeding colonies including shearwater is proposed by the TSG-ML for immediate application.

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 are reflected in the final report of the TSG-ML. However only ingestion of and entanglement in marine litter by marine mammals are considered by the TSG-ML 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.

6.2. Seabirds

The methodology of the tool proposed by the TSG-ML 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., 2011a, b). 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 categories, and this categorisation is followed for marine biota monitoring ingestion in seabirds, marine turtles and fish.

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 as master list, such as the “Master List” developed by the TSG ML group.

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.

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.

Maturity of the tool

The method is mature and in use.

Regional applicability of the tool

The tool is applicable to the regions where fulmars occur; for similar seabird species such as any of the family of the tubenoses, the methodology can follow this approach. This could for example be applied to shearwater species occurring in the Mediterranean Sea.

6.2.2. 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 and implemented on Northern Fulmars *Fulmarus glacialis*, including those from Canadian Arctic and northern Pacific areas. 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 southern 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.

6.3. **Sea turtles**

The stomach contents of stranded Loggerhead sea turtles *Caretta caretta* (Linnaeus, 1758) are used to measure trends and regional differences in marine litter. A recent 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.

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.

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 on an “Identification Data” Sheet. 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 and recorded on an appropriate Data Sheet. External examination of the animal should be conducted, including inspecting the oral cavity for possible presence of foreign material. The methodology suggested in the TSG ML report could be followed to carry out a dissection of the animal to expose the gastrointestinal system (GI).

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 adequate size, pre-weighed on electronic balance (accuracy of ± 1 g). 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, note of the compound and the percentage of dilution should be noted and communicated to the staff in charge of 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 (Van Franeker et al., 2005; 2011b; Matiddi et al., 2011) and using a Standard Data-Sheet.

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 be 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.

An optional methodology for application for sampling litter excreted by live sea-turtles (faecal pellet analysis) in case of finding a specimen alive is recommended by the TSG-ML.

Extraction of data

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

Data entry is carried out using a Standard Form.

Litter Categories - source related information

For turtle analyses, stomach contents are sorted into the same categories as for birds. Following the method for seabirds, abundance by mass (weight in grams, accurate to 3th decimal) is the main information useful for the monitoring programme. Other information such as the 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.

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 (by catch) 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

The tool is not considered mature at this stage. Specific monitoring programmes are required.

Regional applicability of the tool

The tool is applicable to the Mediterranean Sea region.

6.3.2. Quality assurance/quality control

There is a lack of quality assurance/quality control (QA/QC) due to lack of long-term monitoring programmes. More publications in peer reviewed scientific literature are required.

Trend assessment

Specific long-term monitoring programmes are required.

Target definitions

Specific long monitoring programmes are required.

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

6.4.1. 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. 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 (by-catch). Nevertheless it is possible to differentiate between animals that have died quickly due to 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. The possible use of entangled beached birds as an indication of mortality due to litter will be further investigated by the TSG-ML.

In marine mammals, numbers of beached animals and especially cetaceans are often high and many have body marks suggesting entanglement, although remains of ropes or nets on the corpses are mostly rare. Given that in a number 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.

6.4.2. Ingestion of litter by marine mammals and entanglement.

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 ECAP 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.

The development of a monitoring protocol for the ingestion of marine litter in the different size categories by marine mammals will therefore be considered in the next report of the TSG ML. Opportunistic monitoring of marine mammals is envisaged under the population demographic characteristics component of the EcAp biodiversity common indicators.

7. Microlitter (with special reference to microplastics)

7.1. Introduction to microlitter

In effect microparticles consist of similar materials to other types of litter; they are merely pieces of litter at the very small end of the size spectrum. Microparticles of a range of common material types including glass, metal, plastic and paper litter are undoubtedly present in the environment. The focus is on microplastics, implying that they are considered to be the most significant component of the microlitter in the environment. This statement is partly based on the frequency of reports of microplastics (Hidalgo-Ruz et al. 2012, but relative proportions of material types will be influenced by the physical conditions of the habitat sampled, for example metal and glass microlitter is not likely to be found at the sea surface.

When first described the term microplastic was used to refer to truly microscopic particles in the region of 20 µm diameter (Thompson et al. 2004). The definition has since been broadened to include all particles < 5 mm (Arthur et al. 2009). Microplastics are widely dispersed in the environment and are present in the water column, on beaches and on the seabed.

Under EcAp, it is considered that in order to achieve GES that the quantities of microplastics in the environment should not result in harm. When defining methodological criteria it is essential to recognise that our understanding of the potential impacts of microplastic on organisms and the environment (i.e. the 'harm' that they might pose from the perspective of EcAp) is still not fully understood.

An upper size bound of 5mm has been widely (but not exclusively) adopted and for the purpose of EcAp it is suggested that the upper bound to be taken to as items <5mm in their largest dimension as recommended by the TSG-ML. Current definitions do not explicitly state a lower size limit and lower size limits have seldom been reported for microplastic concentrations in the environment. The lower size limit is perhaps assumed to be the mesh size of the net or sieve through which the sample passed during the sampling, sample preparation or extraction. The size limits of microplastic particles that can be reported are also dependent on the method of detection, in many cases microscope-aided visual inspection. When identifying microparticles there are also size limits imposed by the analytical techniques employed (e.g. minimum sample intake requirements for detection and analysis). Hence an important part of establishing standard methods and protocols within EcAp will first be to define the appropriate size range, and this aspect is considered in the report of the TSG-ML.

After an initial period of discovery, microplastics research now finds itself at a stage of development where there is a lack of quality assurance/quality control (QA/QC) instruments available: e.g. no organisations yet offer proficiency training or testing, there have been no inter-laboratory studies, no certified reference materials are available, no standardized sampling and analysis protocols have been published, no accreditation certificates have been issued and some procedures in use have not yet been validated. Approaches for QA/QC will therefore be very useful for evaluating sources of variability and error and increasing confidence in the data collected.

Microplastics comprise a very heterogeneous assemblage of pieces that vary in size, shape, colour, specific density, polymer type, and other characteristics. For meaningful comparisons and to answer the specific questions and to test hypotheses through monitoring, it is important to define methodological criteria to quantify such metrics as for e.g. the abundance, distribution and composition of microplastics and to ensure sampling effort is sufficient to detect the effects of interest. Protocols to monitor microplastic in sediments, sea surface, and biota have been prepared by the TSG-ML. At present our understanding of the sources, distribution and fate of microplastics in the environment are very limited, as is our understanding of any associated effects on wildlife. As a consequence it is not possible to present fully validated standard operating procedures. Instead the TSG-ML presents recommendations for monitoring supported by a discussion of considerations and limitations according to the knowledge base at the time of writing. It considers monitoring design,

sampling, analysis, reporting. The aim of the TSG-ML text is to maximise consistency and comparability of future data collection by recommending approaches.

7.2. General Sampling Methods

Sampling of microplastics in different main marine environments (sea surface, water column, sediment and biota) has been approached using a variety of methods: samples can be selective, bulk, or pre-treated to reduce their volume (Hidalgo-Ruz et al., 2012).

Most studies use a combination of these steps after which a purification step is required to sort the micro litter from natural particulates. Visual characterisation is the most commonly used method for the identification of microplastics (using type, shape, degradation stage, and colour as criteria). Chemical and physical characteristics (e.g., specific density) can also be used. However, the most reliable method is to identify the chemical composition of microplastics by infrared spectroscopy (Hidalgo-Ruz et al., 2012). This approach requires equipment that may be considered relatively costly compared to sampling of large items of debris.

In all four compartments (sea surface, water column, sediment and biota) the TSG-ML recommends quantifying microplastics in the size range 20 μ m to 5mm. Since the lower size limit is perhaps assumed to be the mesh size of the net or sieve through which the sample passed during the sampling, sample preparation or extraction, for sampling purposes this could in the majority of cases taken to be 330 μ m. Microplastics should be categorised according to their physical characteristics including size, shape and colour. Categories used to describe microplastics appearance are available in the TSG M-L report. To achieve the greatest efficiency regarding sampling frequency it is recommended that microparticles be sampled alongside other routine sampling programmes. Sampling of the sea surface could be incorporated into routine monitoring programmes.

Sampling seawater for microplastics

Seawater samples have mostly taken by nets, the main advantage being that large volumes of water can be sampled quickly, retaining the material of interest. Most studies from surface waters have used Neuston nets and from the water column, zooplankton nets. Another instrument, that is deployed on a global scale and that has also been used for microplastic sampling is the continuous plankton recorder (CPR). The most relevant characteristics of the sampling nets are mesh size and the opening area of the net. Mesh sizes used for microplastic sampling range from 0.053 to 3 mm, with a majority of the studies (rather than individual samples collected) ranging from 0.30 to 0.39 mm. The net aperture for rectangular openings of neuston nets (sea surface) ranged from 0.03 to 2.0 m². For circular-bongo nets (water column) the net aperture ranged from 0.79 to 1.58 m². The length of the net for sea surface samples has varied from 1.0 to 8.5 m, with most nets being 3.0 to 4.5 m long. Techniques using apparatus to collect seawater and pass it through a filter on-board ship are being developed where the ship water inlet is used, collecting seawater from the side at specified depths, mostly ranging between 4m and 1m depth. The seawater is passed through sieves or nets in closed containers after which these can be removed and analysed for microplastics.

A key consideration in collecting seawater samples is the cost of ship time. Hence the advantage to sample during existing cruises or from existing monitoring programmes such as the Continuous Plankton Recorder. Manta and bongo nets have been used at the sea surface. With nets it is important to deploy the trawl out of the wake zone as turbulence inside the wake zone does not allow for a representative sample to be collected. A spinnaker boom or 'A' frame may be used to deploy the trawl away from the side of the vessel. A close eye on the net while trawling would need to be kept to observe its performance and adjust speed and cable length if necessary. Sampling at the peak of plankton blooms should be avoided as this may clog the net.

Since most plastics are buoyant they are likely to accumulate at the sea surface. Surface sampling techniques can be used close inshore, but are restricted to calmer weather conditions, whereas CPR and other sub surface approaches can be used in rougher weather. High speed Manta trawls can be deployed in a range of sea states, but CPR is the least sensitive to sea state and samples at an average depth of around 6m. Manta trawls can be used to sample large volumes of surface water, but are relatively insensitive to smaller size fractions (< 1mm) which can be difficult to separate or sort from the large surface area of the net. CPR has a very much smaller aperture (around 1.6cm²) and hence samples smaller quantities of water per km but can be deployed for much longer periods (distances) than the Manta trawl without clogging. With the CPR the entire filter is sealed automatically and then transferred to the laboratory for examination under the microscope. Preliminary data indicate CPR and Manta nets collect similar quantities of debris per unit volume of water sampled; however because of the larger aperture of nets such as Manta the quantity of debris collected per distance towed is substantially greater than CPR. During trawls it is important to maintain a steady linear course at a constant speed. A hi-speed manta trawl can be deployed up to 8 knots, building up the speed slowly towards maximum speed. Higher speeds reduce the ability to sieve seawater, creating a bow wake in front of the trawl.

At present it is not appropriate to recommend one approach over all others. Each approach has advantages and disadvantages and may be preferable according to local availability / sampling opportunities, the characteristics of the area to be sampled. The recommendation of the TSG-ML is to obtain samples from sea water and to ensure the following details are recorded to accompany each sample: type of net, aperture, mesh size (preferably 333 µm mesh, 6m length for greatest inter-comparability among sampling programmes). It is not possible to specify standard haul duration as at some times of year, for example during a plankton bloom, nets may readily become clogged with natural material rendering them inefficient – a duration of 30 min is suggested and the duration of the trawl and the estimated water volume must be recorded. Samples from nets should be stored in glass jars taking care to rinse material as thoroughly as possible from the sides of the net using filtered sea water. Microparticles are recorded as the total quantity of such captured by the net during the period it is deployed.

The TSG-ML report provides detailed information on Laboratory analyses of microplastics samples collected in the field and detailed protocol for sampling surface waters.

ECOLOGICAL OBJECTIVE 10: Marine and coastal litter do not adversely affect the coastal and marine environment

Common Indicator description	DESCRIPTION Parameters and/or Elements, matrix	Assessment Method	Guidelines Reference Methods QA/QC	Recommendations /Additional Data needed
<p>Common indicator 16, COP 18 Indicator 10.1.1.:</p> <p>Trends in the amount of litter washed ashore and/or deposited on coastlines, including analysis of its composition, spatial distribution and, where possible, source.</p> <p>With Operational Objective 10.1.: The impacts related to properties and quantities of marine litter in the marine and</p>	<p>Counts of litter items minimum lower limit 2.5 cm in the longest dimension on at least 1 section of coastline of 100m on lightly to moderately littered beaches (optimum 2 sections) and 2 sections of 100m on heavily littered beaches (exceptionally 50m section with a normalization factor of up to 100m to ensure coherence)</p>	<p>UNEP/MAP MED POL Trend Monitoring Programme</p> <p>At least 2 surveys per year in spring and autumn (Ideally 4 surveys per year in spring, summer, autumn and winter)</p>	<p>As Guideline, with reference methods: UNEP DEPI (MED) WG 394. Inf.5</p> <p>QA according to recommended Quality Assurance Protocols (i.e. Ocean Conservancy National Marine Debris Monitoring Programme (Sheavly, 2007, see text of ECAP monitoring guidelines)</p>	

<p>coastal environment are minimized</p> <p>Pressure, Impact indicator</p>				
<p>Common indicator 17, COP 18 Inmdicaotr 10.1.2: Trends in amounts of litter at sea, including micro-plastics* and on the seafloor</p> <p>With Operational Objective of 10.1: The impacts related to properties and quantities of marine litter in the marine and coastal environment are minimized</p>	<p>Litter in the water column: Items of floating litter, 2.5 to 50cm, per km²</p> <p>Litter on the seafloor shallow coastal waters(0-20m): visually surveyed litter items size above 2.5cm</p> <p>Litter on the seafloor 20-800m: items/ha or items/km² of litter collected in bottom trawl surveys</p>	<p>For floating litter visual ship-based monitoring of floating litter 2.5cm to 50cm as items/km²</p> <p>For litter on the seafloor shallow coastal waters (0-20m): minimum annual, maximum quarterly underwater visual surveys with SCUBA/snorkelling</p> <p>For seafloor 20-800m collection of litter data through on-going and continuous bottom trawl fish stock</p>	<p>For Guideline and reference methods: UNEP DEPI (MED) WG 394. Inf.5</p> <p>For floating litter: approaches for inter-comparison and calibration are to be developed at regional level and implemented</p> <p>For shallow seafloor: Data on litter in shallow sea-floor are collected through protocols already validated for benthic species.</p> <p>For Litter on the seafloor 20-800m, the adoption of a common fish stock survey benthic- trawl protocol will lead to a</p>	<p>It is recommended to focus on surface and sea floor litter</p> <p>*For microplastics at the surface, samples taken by zooplankton nets (333µm mesh, 6m length, sampling for 30 minutes) or by Continuous Plankton Recorder (CPR). Minimum size 330 µm</p> <p>Collection of data on microplastics is costly and it will be critical to identify monitoring approaches (and associated metadata such as QA/QC) that directly support the aims of the</p>

<p>ensure, Impact</p>		<p>survey programmes (such as MEDITS)</p>	<p>significant level of standardization among the countries that apply it as their benthic litter sampling strategy.</p>	<p>indicator. Because of the relative infancy of microplastics research it is essential that existing proposed approaches would need to be re-evaluated and refined as new information emerges.</p>
<p>Candidate Indicator 18, COP18 Indicator 10.2.1.: Trends in the amount of litter ingested by or entangling marine organisms, especially mammals, marine birds and turtles With Operational</p>	<p>Quantities of ingested litter (minimum size 1mm), by mass (weight in grams) from stomach contents of seabirds (any of the family of the tubenoses - Procellariiformes i.e. shearwater species) Quantities of ingested litter (minimum size 1mm) by mass (weight in grams) in the stomach contents of stranded Loggerhead sea turtles (<i>Caretta caretta</i>)</p>	<p>Continuous sampling of dead birds collected from beaches or accidental mortalities such as long line victims, fledgling road-kills etc., to obtain a sample size of 40 birds or more for a reliable annual average for a particular area or lower sample sizes for the analysis of trends based on individual birds</p>	<p>For Guidelines and reference methods: UNEP DEPI (MED) WG 394. Inf.5 For seabirds the methodology is based on OSPAR methodology which has received full quality assurance by publication in peer reviewed scientific literature. For sea turtles there is a lack of QA/QC due to the lack of long-term monitoring</p>	<p>For seabirds, the tool works only locally For sea turtles the tool require a validation (long term data, QA/QC). Specific monitoring programmes are required to commence as pilots, to establish minimum sample population size for year and period of sampling, for</p>

<p>Objective: 10.2. Impacts of litter on marine life are controlled to the maximum extent practicable Impact</p>		<p>Continuous sampling of dead sea turtles collected from beaches or at sea from accidental mortalities such as victims of long-line fishing (by-catch) or of boat collisions.</p>	<p>programmes</p>	<p>reliable conclusions on change or stability in ingested litter quantities.</p> <p>This issue of entanglement requires further investigation for the development of a dedicated monitoring protocol for the entanglement of marine organisms in marine litter</p>
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