Green Book

UK CLEAN SEAS ENVIRONMENTAL MONITORING PROGRAMME

2020

MARINE ASSESSMENT AND REVIEW GROUP

Clean Seas Environmental Monitoring Programme

Green Book

MARINE ASSESSMENT AND REVIEW GROUP (MARG) UK CLEAN SEAS ENVIRONMENTALMONITORING PROGRAMME

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1. Purpose and Background

Purpose of the Green Book

This document is to be used by all parties involved in monitoring and assessment of the Clean Seas Environmental Monitoring Programme (CSEMP) in the United Kingdom. It is designed to ensure a level of consistency of approaches taken by the multiple parties in order to deliver robust data of the required integrity from which reliable assessments can be made.

Background of Clean Seas Environmental Monitoring Programme (CSEMP)

The Clean Seas Environmental Monitoring Programme (CSEMP) is a continuation of a UK-wide marine monitoring activity that has origins in the late 1980's. At that time, it was called the National Monitoring Programme (NMP) and more recently the National Marine Monitoring Programme (NMMP). It is one element of a series of activities coordinated by the Clean, Safe Seas Evidence Group (CSSEG).

Coordination of CSEMP and Active Participation

The Clean, Safe Seas Evidence Group (CSSEG) brings together Agencies responsible for monitoring chemical contaminants, radioactivity, eutrophication, microbiological contaminants, algal toxins, litter and noise. The focus of the CSEMP is on chemical contaminants and eutrophication.

Agencies currently contributing to UK analytical monitoring for CSEMP in the UK are:

- Agri-Food and Biosciences Institute (AFBI)
- Centre for Environment, Fisheries and Aquaculture Science (CEFAS)
- Environment Agency (EA)
- Department of Agriculture, Environment and Rural Affars Northern Ireland (DAERA-NI)
- Marine Scotland Science (MSS)
- Natural Resources Wales (NRW)
- Scottish Environment Protection Agency (SEPA)

Drivers of CSEMP

The drivers of the programme are:

i. <u>OSPAR international agreement:</u>
The OSPAR Convention aims to protect the North East Atlantic marine environment. This requires action to prevent and eliminate pollution of the North East Atlantic and monitoring under the following programmes:
    - OSPAR Joint Assessment and Monitoring Programme (JAMP)
    - Nutrients Monitoring Programme.

ii. <u>Compliance with EC Directives</u>:
This covers the following EC directives:
    - Water Framework Directive (2000/60/EEC)

- Marine Strategy Framework Directive (2008/56/EC)
- Shellfish Directive (2006/113/EC)
- Shellfish Hygiene Directive (91/492/EEC and as amended by 97/61/EC)
- Fisheries Products Directive (91/493/EEC)
- Urban Waste Water Treatment Directive (91/271/EEC)
- Nitrates Directive (91/676/EEC)
- Habitats Directive (92/43/EEC)

iii. To meet research and development needs:
Research and development needs may be driven by OSPAR or nationally to cover new substances or biological effects requiring further investigation. Consideration will follow on whether or not amendments to the monitoring programme are required.

iv. For local monitoring:
There may be environmental events when local marine environmental monitoring undertaken by one or more CSEMP organisations becomes of national interest and intense monitoring may be required. This data can be included in the CSEMP data if quality control requirements are met.

2. Aims of the Clean Seas Environmental Monitoring Programme

The general aims of the programme are:

i. To initiate, co-ordinate and implement UK marine monitoring programmes.
ii. To ensure datasets provide optimum and accurate information for long-term trends in physical, biological and chemical variables at selected estuarine, coastal and offshore sites.
iii. To ensure and maintain a high quality dataset for key chemical and biological variables in the marine environment.
iv. To produce reports providing overviews of the spatial and temporal distributions of target variables and inter-relationships.
v. To support and ensure consistent standards in national and international monitoring programmes for marine environmental quality.
vi. To make recommendations to the Marine and Review Group UK (MARG) as to how new analyses and techniques are best implemented in the United Kingdom.

How CSEMP has Changed and Evolved

The first phase of spatial surveys (NMP) revealed the pattern of marine quality around the UK. The second phase (NMMP2, now CSEMP) started on the detection of long-term temporal trends. The second phase of the programme (1999-2002) was reported long term trends in Charting Progress (2005) which was subsequently followed up in Charting Progress 2 (2010). The programme continuously evolves to incorporate new legislative requirements and improve the power of the programme to detect trends through improved sampling. As trends are established and we are confident that sea areas are not at risk, effort is focused on higher risk areas.

How Changes to the CSEMP Programme are Managed

The programme is reviewed annually, and changes are recorded in this document. To assist, this document and its dependants will be operated as a controlled document operated by CSSEG and through the CSSEG Analytical Quality Control (AQC) groups. The master version of the GreenBook is available on the web at:

https://www.cefas.co.uk/publications/greenbook/greenbookv15.pdf

3. CSEMP Strategy

Integration and Complimenting of CSEMP into Other Monitoring Programmes

The CSEMP seeks to integrate national and international monitoring programmes across UK agencies. It does not represent all the marine monitoring programmes being implemented by these agencies, but it complements existing programmes. CSEMP seeks to ensure :

- consistent standards
- comparability of measurements
- data exchange.

Considerations Required for this Monitoring Programme

The monitoring requirements are outlined in Section 6 for each determinand and matrix combination. Sampling frequencies are designed to detect temporal trends with appropriate statistical accuracy. Sites have been selected to ensure that the maximum amount of information may be gained at each site. Sediment samples, for example, are to be collected at locations suitable for biological measurements. This will ensure maximum co-ordination of information.

Sites are listed, with the organisations responsible for monitoring and details can be found in the MERMAN station dictionary list;

(https://www.bodc.ac.uk/projects/data_management/uk/merman/project_specific/ , accessed 27/02/2020)

Sampling locations were originally based on a fixed-station design, but for sediment sampling was superseded by a mix of fixed-stations for evaluation of long-term trends, combined with stratified-random and stratified fixed designs to provide data representative of wider sea areas, and special survey work for new and emerging contaminants of concern. For regional assessments of biota and sediment ideally 5 representative sites (minimum 3) for sediment and biota (fish and shellfish sites) with good geographic spread are required and must be visited at minimum every 6 years for inclusion in the analysis.

For each element of the sampling programme there is a corresponding appendix which sets out detailed procedural guidance for sampling and analysis of samples. The procedures are intended as standard guidance and take into account published guidance from JAMP, reflecting a UK interpretation of this guidance as well as practical experience of what is realistically achievable with the resources available to Agencies responsible for undertaking the programme.

4. Governance of Programme, Methodology and Quality Control

Governance Bodies and Responsibilities

The following bodies are concerned with the delivery of CSEMP under the Green Book requirements:

i. Clean and Safe Seas Evidence Group (CSSEG):

CSSEG has responsibility for operating the Green Book document.

A number of Analytical Quality Control (AQC) groups report to CSSEG in relation to delivery of CSEMP requirements.

ii. UK National Marine Cemical Advisory Group (NMCAG):

The National Marine Chemical Analytical Quality Control scheme (NMCAQC) was established in 1992, the name and Terms of Reference were updated in 2007 and reviewed in 2016. The aim of the UK NMCAG group is to co-ordinate and advise on both current and future requirements for marine analytical chemistry and supporting monitoring (methodology, techniques, quality assurance and associated research) in the UK marine environment and to quality mark UK marine chemistry analytical data submitted as part of the UK CSEMP. It oversees all aspects of quality control of marine chemistry monitoring for CSEMP. Independent proficiency testing materials are provided by QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring in Europe). The NMCAG group works closely with QUASIMEME through membership to its Scientific Advisory Board (SAB) to ensure that UK CSEMP interests are covered by the scheme. The suitability of contaminant data submitted to CSEMP is judged by means of a data filter (Dobson et al., 1999). The data filter requires laboratories to demonstrate the use of appropriate internal and external Quality Control (QC) procedures to show that their data is fit for purpose. Data is excluded where QC performance does not meet the required standard. Quality Control information required for the data filter is detailed in Table 7.1.

iii. The UK Group for the Biological Effects of Contaminants in the Marine Environment (BECME)

The UK Group for the Biological Effects of Contaminants in the Marine Environment (BECME), formerly the National Marine Ecotoxicological Analytical Quality Control Group (NMEAQC), was established in 1998 to provide methods and quality control procedures for biological effects measurements recommended for CSEMP. The Terms of Reference of the group were revised in 2007 to have a wider remit covering all aspects of the monitoring of biological effects of contaminants in the UK marine environment. This may be carried out to meet the needs of “drivers” such as OSPAR, WFD, etc and

covers for example survey design, sampling protocols, sample analysis including AQC, data assessment and reporting. The group will coordinate with the European project, BEQUALM (Biological Effects Quality Assurance in Monitoring) to ensure that there is minimum duplication of activities. Data quality filters will be developed in this area in line with the chemistry and biology AQC data filters.

Minimum Requirements of Contributing Participant Analytical Capability

All methodologies are outlined in the appendices and references. All laboratories contributing data to the CSEMP are accredited to ISO/IEC 17025 (international standard on general requirements for the competence of testing and calibration laboratories) and must participate in the relevant Analytical Quality Control (AQC) groups outlined below. These groups require laboratories to employ methods which will achieve agreed targets for both chemical or biological data. Data entering the MERMAN database must be of specified quality and for this reason inter-laboratory proficiency testing schemes have been initiated to support the analytical work associated with CSEMP.

Considerable progress has been made in co-ordinating the monitoring activities of the responsible organisations in the United Kingdom through the AQC groups. This ranges from improved dialogue between organisations to collaboration between the individual laboratories responsible for the practical implementation of the work. In parallel with the co-ordination effort, quality control procedures have been developed to ensure comparability of data between laboratories.

Proficiency Testing (PT) Participation

All laboratories contributing data to the CSEMP must participate in a PT scheme (where available) appropriate to CSEMP performance targets. Laboratory PT performance is used in the assessment of the quality of submitted sample results (see Section 6).

i. QUASIMEME PT:

Independent proficiency testing materials for chemical contaminants are provided by QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring in Europe). This inter-laboratory proficiency testing scheme has been initiated to support the analytical work associated with CSEMP.

ii. National Marine Biological Analytical Control Scheme (NMBAQC):

This PT is to monitor all aspects of marine biological quality control for benthic faunal community studies. Format of exercises:

- Ring test identifications - aimed at improving laboratory expertise
- 'Own-sample' exercises - assures quality of marine biological data in the MERMAN data-base (see above).
- A MERMAN data filter, similar to the chemical contaminants system, is currently under development (see Section 7).

iii. BEQUALM:

Intra-laboratory comparison exercies for biological effects of contaminants are provided by BEQUALM (Biological Effects Quality Assurance in Monitoring Programmes).

5. Accuracy Targets and the Power to Detect Trends

The variables and sites to be measured are outlined in Section 6 and in the spreadsheet param_uomval.xls (variables) and the MERMAN station dictionary list. The anticipated power of each aspect of the programme is given within each table where temporal trends are being monitored. It should be noted that where a programme is being carried out to measure compliance with a Directive, the accuracy targets are generally set to a tenth of any environmental quality standard (EQS). The power of the benthic monitoring data is currently being evaluated by the NMBAQC group. There are no long-term biological effects monitoring programmes at this stage to evaluate the power of these programmes. However, as the datasets are gathered, assessments of their power will be made as appropriate.

6. Monitoring requirements

6.1 Temporal Trend Monitoring required in Sediments Strategy

Temporal trend monitoring in sediments is completed to fulfil the requirements of OSPAR Coordinated Environmental Monitoring Programme (CEMP, OSPAR Agreement 2016-01)[5], Theme H (Hazardous substances) mandatory components:

H1	heavy metals cadmium, mercury and lead in biota and sediment
H2	polychlorinated biphenyl (PCB) congeners CB 28, CB 52, CB 101, CB 118, CB 138, CB 153, and CB 180 in biota and sediment
H3	polycyclic aromatic hydrocarbons (PAHs) anthracene, benz[a]anthracene, benzo[ghi]perylene, benzo[a]pyrene, chrysene, fluoranthene, ideno[1,2,3-cd]pyrene, pyrene and phenanthrene in biota and sediment
H4	tributyl tin (TBT)-specific biological effects and TBT in sediment or biota
H5	brominated flame retardants hexabromocyclododecane (HBCD) and polybrominated diphenylethers (PBDEs) BDE 28, BDE 47, BDE 66, BDE 85, BDE 99, BDE 100, BDE 153, BDE 154 and BDE 183 in biota and sediment, and BDE 209 in sediment

The following guidelines are relevant to this part of the programme:

JAMP Eutrophication Monitoring Guidelines: Benthos (OSPAR Agreement 2012-12)[6].

CEMP Guidelines for Monitoring Contaminants in Sediments (OSPAR Agreement 2002-16, Revision 2018) [7].

JAMP Guidelines for General Biological Effects Monitoring. Revised technical annexes 2007 .. (agreement 2007-07)[8].

This part of the programme also meets some of the requirements of the MSFD and the standstill clause of the EC Dangerous Substances Directive.

It is anticipated that the metals programme will at worst have 90% power to detect a 5% per year change over a period of between 15 and 20 years.

Sediment samples will be collected at the designated sampling point within each strata (on a random or fixed basis) and the exact location of each sample recorded. Replicates will be collected for chemistry, benthic analysis and biological effects, as required (see appropriate Appendix). For contaminant analysis time of sampling is not critical. However in order to minimise the effects of seasonal variability in the macrobenthic communitiesICES, sampling should be undertaken within a narrow time window within the broader window of February to June. It is recommended that sampling is undertaken ± 3 weeks of the original sampling date in 1999 or 2000. If sampling is undertaken during May or June then ± 2 weeks is recommended.

Sampling and sample storage

Information on sampling method and sample storage are required for submission of the data to ICES. Relevant codes and field sheets for recording this information are provided in Appendix 3.

Sediments should be analysed for contaminants, macrofauna and biological effects on separate grabs collected from the same location on the same sampling occasion. Details of sample preparation and analysis record requirements are given in Appendix 6, 7, 8, 9, and 10. Parameters to be monitored are detailed in spreadsheet param_uomval.xls and sites to be monitored are listed in the MERMAN station dictionary list.

Table 6.1 Recommended sample holding times

Determinands	Frozen (< -18°C)	Refrigerated (5 ±3°C)	Ambient	Source
PAHs (wet sediment)	2.5 years	<1 week	<48hrs	MSS + CEMP Guidelines for Monitoring Contaminants in Sediments (OSPAR Agreement 2002-16, Revision 2018)
PAHs (dry sediment)			30 days	Cefas
non dl-PCBs (dry sediment)	5 years			MSS
non dl-PCBs		< 1 month	48 hrs	CEMP Guidelines for Monitoring Contaminants in Sediments (OSPAR Agreement 2002-16, Revision 2018)
Dioxin, furans & dl-PCBs	As per non dl-CBs			
PBDEs (dried sediment)			5 years	MSS + CEMP Guidelines for Monitoring Contaminants in Sediments (OSPAR Agreement 2002-16, Revision 2018)
HBCDD	3 months	<1 week	<48hrs	Cefas
Perfluorinated compounds				CEMP Guidelines for Monitoring Contaminants in Sediments (OSPAR Agreement 2002-16, Revision 2018)
Organochlorine pesticides	3 months	<1 week	<48hrs	Cefas
Trace metals (dry sediment)			10 years	MSS MESS CRM certificate + CEMP Guidelines for Monitoring Contaminants in Sediments (OSPAR Agreement 2002-16, Revision 2018)
Trace metals (wet sediment)	6 months			
Organotins (dry sediment)	5 years	<1 week		Ingaki *et al.,* 2007. Certification of butyltins and phenyltins in marine sediment certified reference material... *Anal Bioanal Chem,* 387: 2325–2334.
TOC (freeze dried)			3 years	
PSA	5 years			EA -National Laboratory Services
Macrofaunal/infaunal			1 year	

Cells in green indicate the preferred storage method.

Contaminant record

This record requires information on the methods of preparation and analysis information for the parameters measured (Table 6.2). Samples for trace metal analysis should be sieved to <63 µm however, existing sample preparation methodologies may be maintained where a time series exists.

The sieve size used must be noted in the data returns to the CSEMP database. Parameter codes and reporting units for sediments are listed in the spreadsheet param_uomval.xls. Analytical methods should be designed to achieve detection limits specified in guidelines produced as aprt of the MSFD, WFD or OSPAR CEMP. It is recognised that the targets listed in Table 6.2 are challenging to achieve for some determinands, but achieving these will allow submitting laboratories to achieve a higher score through the MERMAN data filter (Gardner et al. 2002 and Dobson et al. 1999). The targets only contribute to a part of the data filter score with other factors such as accreditation and proficiency testing also contributing. As long as data meet the threshold set in MERMAN then the data are given a STATUS of PASS and will be submitted to ICES for assessments. The value of the score bears no weighting on the data in assessments however, missing metadata or missing associated parameters could still mean the data can be downgraded in assessments i.e. missing CORG in sediment.

Table 6.2 Contaminants in sediments

Determinands	ICES Code	Status	Units	Targets		
				LOD	Bias (%)	Precision (%)
Aluminium	AL	M	% dw	0.1	12.5	6.25
Cadmium	CD	M	µg/kg dw	200	12.5	6.25
Mercury	HG	M	µg/kg dw	10	12.5	6.25
Copper	CU	M	mg/kg dw	1	12.5	6.25
Lead	PB	M	mg/kg dw	2	12.5	6.25
Nickel	NI	M	mg/kg dw	1	12.5	6.25
Zinc	ZN	M	mg/kg dw	2.5	12.5	6.25
Arsenic	AS	M	mg/kg dw	1	12.5	6.25
Chromium	CR	M	mg/kg dw	2	12.5	6.25
Lithium	LI	M	mg/kg dw	0.1	12.5	6.25
Iron	FE	M	% dw	0.1	12.5	6.25
Manganese	MN	M	mg/kg dw	0.1	12.5	6.25
TBT	TBTIN	M	µg/kg dw	1	12.5	6.25
PCB 28	CB28	M	µg/kg dw	0.1	12.5	6.25
PCB 52	CB52	M	µg/kg dw	0.1	12.5	6.25
PCB 101	CB101	M	µg/kg dw	0.1	12.5	6.25
PCB 118	CB118	M	µg/kg dw	0.1	12.5	6.25
PCB 138	CB138	M	µg/kg dw	0.1	12.5	6.25
PCB 153	CB153	M	µg/kg dw	0.1	12.5	6.25
PCB 180	CB180	M	µg/kg dw	0.1	12.5	6.25
Naphthalene	NAP	M	µg/kg dw	10	12.5	6.25
Phenanthrene	PA	M	µg/kg dw	10	12.5	6.25
Anthracene	ANT	M	µg/kg dw	2	12.5	6.25
Fluoranthene	FLU	M	µg/kg dw	2	12.5	6.25
Pyrene	PYR	M	µg/kg dw	2	12.5	6.25
Benzo[a]anthracene	BAA	M	µg/kg dw	2	12.5	6.25
Chrysene/Triphenylene	CHRTR	M	µg/kg dw	2	12.5	6.25
Benzo[a]pyrene	BAP	M	µg/kg dw	2	12.5	6.25
Benzo[ghi]perylene	BGHIP	M	µg/kg dw	10	12.5	6.25
Indeno[123-cd]pyrene	ICDP	M	µg/kg dw	10	12.5	6.25
Acenaphthene	ACNE		µg/kg dw	2	12.5	6.25
Acenaphthylene	ACNLE		µg/kg dw	2	12.5	6.25
Dibenzothiophene	DBT		µg/kg dw	10	12.5	6.25
C1-dibenzothiophenes	DBTC1		µg/kg dw	10	12.5	6.25
C2-dibenzothiophenes	DBTC2		µg/kg dw	10	12.5	6.25
C3-dibenzothiophenes	DBTC3		µg/kg dw	10	12.5	6.25
Fluorene	FLE		µg/kg dw	10	12.5	6.25
1-methylnaphthalene	NAP1M		µg/kg dw	10	12.5	6.25

Determinands	ICES Code	Status	Units	Targets		
				LOD	Bias (%)	Precision (%)
2-methylnapthalene	NAP2M		µg/kg dw	10	12.5	6.25
C1-napthalenes	NAPC1		µg/kg dw	10	12.5	6.25
C2- napthalenes	NAPC2		µg/kg dw	10	12.5	6.25
C3-napthalenes	NAPC3		µg/kg dw	10	12.5	6.25
Benzo[e]pyrene	BEP		µg/kg dw	10	12.5	6.25
Dibenz[a,h]anthracene	DBAHA		µg/kg dw	5	12.5	6.25
Perylene	PER		µg/kg dw	10	12.5	6.25
Triphenylene	TRI		µg/kg dw	20	12.5	6.25
C1-phenanthrenes	PAC1		µg/kg dw	10	12.5	6.25
C2-phenanthrenes	PAC2		µg/kg dw	10	12.5	6.25
C3-phenanthrenes	PAC3		µg/kg dw	10	12.5	6.25
Benzo[c]phenanthrene	PABC		µg/kg dw	10	12.5	6.25
Benzo[b]anthracene	BBA		µg/kg dw	10	12.5	6.25
Benzo[b+j+k]fluoranthene	BBKF		µg/kg dw	10	12.5	6.25
Organic carbon	CORG	M	% dw		12.5	6.25
Hexabromocyclododecane	HBCD	M	µg/kg dw	0.1	12.5	6.25
2,4,4'-tribromodiphenyl ether (PBDE28)	BDE28	M	µg/kg dw	0.1	12.5	6.25
2,2',4,4'-tetrabromodiphenyl ether (PBDE47)	BDE47	M	µg/kg dw	0.1	12.5	6.25
2,3',4,4'-tetrabromodiphenyl ether (PBDE66)	BDE66	M	µg/kg dw	0.1	12.5	6.25
2,2',3,4,4'-pentabromodiphenyl ether (PBDE85)	BDE85	M	µg/kg dw	0.1	12.5	6.25
2,2',4,4',5-pentabromodiphenyl ether (PBDE99)	BDE99	M	µg/kg dw	0.1	12.5	6.25
2,2',4,4',6-pentabromodiphenyl ether (PBDE100)	BDE100	M	µg/kg dw	0.1	12.5	6.25
2,2',4,4',5,5'-hexabromodiphenyl ether (PBDE153)	BDE153	M	µg/kg dw	0.1	12.5	6.25
2,2',4,4',5,6'-hexabromodiphenyl ether (PBDE154)	BDE154	M	µg/kg dw	0.1	12.5	6.25
2,2',3,4,4',5',6-heptabromodiphenyl ether (PBDE183)	BDE183	M	µg/kg dw	0.1	12.5	6.25
Decabromodiphenyl ether (PBDE209)	BDE209	M	µg/kg dw	0.1	12.5	6.25

Key: dw = dry weight; M = Mandatory; LOD = Limit of Detection from RLOD in MERMAN AQCRD.xls ; MU = measurement uncertainty. The precision stated is that used by MERMAN (as standard deviation in % in AQCRD.xls, which in turn was derived from performance criteria derived by Quasimeme in 1993.The precision listed is ½ of that set by Quasimeme.

Particle Size Analysis

Sediment structure as determined by particle size analysis is used to support benthic community analysis and contaminants. The sample used to support benthic community analysis should be a representative collected from a separate grab. The full range of parameters detailed in Table 6.3 should be determined on this sample. A separate sample should be collected for particle size analysis to support the contaminants data. The fraction less than 63 µm should be determined on this sample.

Table 6.3 sediment grain size parameters

Code	Description	Interpretation	Unit
GSKURT	Grain size kurtosis	Statistical summary	Scale
GSMEA	Grain size mean	Statistical summary	mm
GSSKEW	Grain size skewness	Statistical summary	Scale
GSSORT	Grain size sorting	Statistical summary	Scale
GSMED	Grain size median	Statistical summary	mm
GSMF>8000	Grain Size Mass Fraction >8000	Phi class which may also be used to derive broader classes (% sand, gravel etc)	%
GSMF>4000<8000	Grain Size Mass Fraction >4000<8000	See above	%
GSMF>2000<4000	Grain Size Mass Fraction >2000<4000	See above	%
GSMF>1000<2000	Grain Size Mass Fraction >1000<2000	See above	%
GSMF>500<1000	Grain Size Mass Fraction >500<1000 µm	See above	%
GSMF>250<500	Grain Size Mass Fraction >250<500 µm	See above	%
GSMF>125<250	Grain Size Mass Fraction >125<250 µm	See above	%
GSMF>63<125	Grain Size Mass Fraction >63<125 µm	See above	%
GSMF63	Grain Size Mass Fraction <63 µm	See above	%
GSMF20	Grain Size Mass Fraction <20 µm	Used for chemistry interpretation	%

Benthic community analysis record

Guidelines for the analysis of macrobenthic samples are given in Appendix 10. Macrobenthic species data should be submitted in conjunction in the coding system used (RUBIN or other agreed list) and information on biological community parameters i.e. biomass and abundance. Information on sediment particle size should also be submitted with macrobenthic species data (see Table 6.4).

Table 6.4 Benthic Macrofauna

DESCRIPTION	CODE
Latin name of species (or aggregated genus/family)	
Reference code list used for species ID	ITLN
Abundance number	ABUNDNR
Biomass-wet weight	BMWETWT
Biomass –dry weight	BMDRYWT
Biomass –ash free dry weight	BMAFDWT

Where toxic effects are observed, toxicity-directed analysis of interstitial water fractions should be carried out.

Biological Effects – Sediments

Biological effects in sediments are not included in any UK monitoring programmes. Whole sediment bioassays are included in the JAMP Guidelines for General Biological Effects Monitoring with revised Technical annexes 2007 (OSPAR Agreement 2007-07). Future monitoring programmes should refer to the guidance within this for sampling and reporting details. Sampling procedures previously used in the UK are detailed in Appendix 2.

6.2 Monitoring required in Shellfish

Strategy

Temporal trend monitoring in biota is completed to fulfil the requirements of OSPAR Coordinated Environmental Monitoring Programme (CEMP, OSPAR Agreement 2016-01)[5], Theme H (Hazardous substances) mandatory components:

H1 heavy metals cadmium, mercury and lead in biota and sediment

H2 polychlorinated biphenyl (PCB) congeners CB 28, CB 52, CB 101, CB 118, CB 138, CB 153, and CB 180 in biota and sediment

H3 polycyclic aromatic hydrocarbons (PAHs) anthracene, benz[a]anthracene, benzo[ghi]perylene, benzo[a]pyrene, chrysene, fluoranthene, ideno[1,2,3-cd]pyrene, pyrene and phenanthrene in biota and sediment

H4 tributyl tin (TBT)-specific biological effects and TBT in sediment or biota

H5 brominated flame retardants hexabromocyclododecane (HBCD or HBCDD) and polybrominated diphenylethers (PBDEs) BDE 28, BDE 47, BDE 66, BDE 85, BDE 99, BDE 100, BDE 153, BDE 154 and BDE 183 in biota and sediment, and BDE 209 in sediment

H11 general biological effects

The following guidelines are relevant to this part of the Programme:

CEMP Guidelines for Monitoring Contaminants in Biota (Agreement 1999-02., Revision 2018)[9].

JAMP Guidelines for General Biological Effects Monitoring. Revised Technical annexes 2007 (OSPAR Agreement 2007-07)[8].

This part of the programme also meets some of the requirements of the MSFD and the standstill clause of the EC Dangerous Substances Directive and the Shellfish Hygiene Directive.

Sample storage

Recommended sample holding times are given below:

Table 6.5 Shellfish Sample Storage Holding Times

Determinands	Frozen (< -70°C)	Frozen (< -18°C)	Ambient	Live animals	Source
Metals		2 years			MSS Hg stability study
non dl-PCBs		1.5 years			MSS
Organochlorine pesticides					
PAHs		5 years			MSS
Organotins					
HBCD or HBCDD					
PBDEs		1.5 years			MSS
Total Lipid					
Moisture Content					
Biological Effects (TBT specific)				2 – 3 weeks	
Biological Effects (General)				< 24 h	

Cells in green indicate the preferred storage method.

Contaminant monitoring

To minimise duplication of effort, Shellfish Hygiene Directive sites should be used for CSEMP purposes where possible. It is anticipated that this programme will have 90% power to detect at least a 10% per year change in metal concentrations and a 20% per year change in organics concentrations over a 20 year period.

The common blue mussel (*Mytilus edulis*) or the Mediterranean mussel (*Mytilus galloprovincialis)* should be used whenever possible. Where these species are not available, the second choice species Pacific oyster (*Crassostrea gigas*) should be used. The same species should be used henceforth for temporal trend monitoring and should be collected at the same time of year on all sampling occasions.

Samples should be collected from the shore at locations avoiding the influence of point source discharges. Samples should be collected between February / March to avoid the spawning period. Sufficient individual mussels in the size range 3-6 cm should be collected to provide sufficient soft tissue for each analysis. To minimise the effects of natural size related variability, the length range of individuals within this broad band should be minimised as much as possible to, for example, 5 mm. This narrower length band should then be fixed from year to year. In selecting the sample, care should be taken that it is representative of the population and that it can be obtained annually. Average data

should be reported with supporting data on species used, mean, maximum and minimum length, % moisture content and % total lipid (wet weight basis).

Appendix 5 details sampling, sample storage and sample preparation procedures and lists appropriate ICES codes. This information must be provided with the data. The Smedes[10] procedure is recommended for measurement of total lipid. Contaminants to be monitored are detailed in Table 6.6 together with required analytical targets. It is recognised that the targets listed in Table 6.6 are challenging to achieve for some determinands, but achieving these will allow submitting laboratories to achieve a higher score through the MERMAN data filter (Gardner et al. 2002 and Dobson et al. 1999). The targets only contribute to a part of the data filter score with other factors such as accreditation and proficiency testing also contributing. As long as data meet the threshold set in MERMAN then the data are given a STATUS of PASS and will be submitted to ICES for assessments. The value of the score bears no weighting on the data in assessments however, missing metadata or missing associated parameters could still mean the data can be downgraded in assessments.

Table 6.6 Contaminants in Shellfish

Determinands	ICES Code	Status	Units	Analytical Targets		
				LOD	Bias (%)	Precision (%)
Mercury	HG	M	µg/kg ww	20 (mussels) 3 (*Fucus*)	12.5	6.25
Cadmium	CD	M	µg/kg ww	50	12.5	6.25
Copper	CU	M	µg/kg ww	100	12.5	6.25
Lead	PB	M	µg/kg ww	50	12.5	6.25
Nickel	NI	M	µg/kg ww	50	12.5	6.25
Zinc	ZN	M	µg/kg ww	2000	12.5	6.25
Arsenic	AS	M	µg/kg ww	300	12.5	6.25
Chromium	CR	M	µg/kg ww	50	12.5	6.25
Silver	AG	M	µg/kg ww	10	12.5	6.25
Selenium	SE	M	µg/kg ww	10	12.5	6.25
PCB 28	CB28	M	µg/kg ww	0.1	12.5	6.25
PCB 52	CB52	M	µg/kg ww	0.1	12.5	6.25
PCB 101	CB101	M	µg/kg ww	0.1	12.5	6.25
PCB 118	CB118	M	µg/kg ww	0.1	12.5	6.25
PCB 138	CB138	M	µg/kg ww	0.1	12.5	6.25
PCB 153	CB153	M	µg/kg ww	0.1	12.5	6.25
PCB 180	CB180	M	µg/kg ww	0.1	12.5	6.25
HCH - alpha	HCHA		µg/kg ww	0.1	12.5	6.25
HCH - beta	HCHB		µg/kg ww	0.1	12.5	6.25
HCH - gamma	HCHG		µg/kg ww	0.1	12.5	6.25
HCH - delta	HCHD		µg/kg ww	0.1	12.5	6.25
op-DDT	DDTOP		µg/kg ww	0.1	12.5	6.25
pp-DDT	DDTPP		µg/kg ww	0.1	12.5	6.25
pp-TDE	TDEPP		µg/kg ww	0.1	12.5	6.25
pp-DDE	DDEPP		µg/kg ww	0.1	12.5	6.25
Dieldrin	DIELD		µg/kg ww	0.1	12.5	6.25
Aldrin	ALD		µg/kg ww	0.1	12.5	6.25
Endrin	END		µg/kg ww	0.1	12.5	6.25
Isodrin	ISOD		µg/kg ww	0.1	12.5	6.25
HCB	HCB		µg/kg ww	0.1	12.5	6.25
HCBD	HCBD		µg/kg ww	0.1	12.5	6.25
Naphthalene	NAP	M	µg/kg ww	1	12.5	6.25
Phenanthrene	PA	M	µg/kg ww	1	12.5	6.25
Anthracene	ANT	M	µg/kg ww	0.5	12.5	6.25
Fluoranthene	FLU	M	µg/kg ww	0.5	12.5	6.25
Pyrene	PYR	M	µg/kg ww	0.5	12.5	6.25
Benzo[a]anthracene	BAA	M	µg/kg ww	0.5	12.5	6.25
Chrysene	CHRTR	M	µg/kg ww	0.5	12.5	6.25
Benzo[a]pyrene	BAP		µg/kg ww	0.5	12.5	6.25

Determinands	ICES Code	Status	Units	Analytical Targets		
				LOD	Bias (%)	Precision (%)
Benzo[ghi]perylene	BGHIP		µg/kg ww	0.5	12.5	6.25
Indeno{123-cd]pyrene	ICDP		µg/kg ww	0.5	12.5	6.25
Acenaphthene	ACNE		µg/kg ww	0.5	12.5	6.25
Acenaphthylene	ACNLE		µg/kg ww	0.5	12.5	6.25
Dibenzothiophene	DBT		µg/kg ww	0.5	12.5	6.25
C1-dibenzothiophenes	DBTC1		µg/kg ww	0.5	12.5	6.25
C2-dibenzothiophenes	DBTC2		µg/kg ww	0.5	12.5	6.25
C3-dibenzothiophenes	DBTC3		µg/kg ww	0.5	12.5	6.25
Fluorene	FLE		µg/kg ww	0.5	12.5	6.25
1-methylnaphthalene	NAP1M		µg/kg ww	0.5	12.5	6.25
2-methylnapthalene	NAP2M		µg/kg ww	0.5	12.5	6.25
C3-napthalenes	NAPC3		µg/kg ww	0.5	12.5	6.25
Benzo[e]pyrene	BEP		µg/kg ww	0.5	12.5	6.25
Dibenz[a,h]anthracene	DBAHA		µg/kg ww	0.5	12.5	6.25
Perylene	PER		µg/kg ww	0.5	12.5	6.25
Triphenylene	TRI		µg/kg ww	0.5	12.5	6.25
C1-phenanthrenes	PAC1		µg/kg ww	0.5	12.5	6.25
C2-phenanthrenes	PAC2		µg/kg ww	0.5	12.5	6.25
C3-phenanthrenes	PAC3		µg/kg ww	0.5	12.5	6.25
Benzo[c]phenanthrene	PABC		µg/kg ww	0.5	12.5	6.25
Benzo[b]anthracene	BBA		µg/kg ww	0.5	12.5	6.25
Benzo[b+j,k]flouranthene	BBKF		µg/kg ww	0.5	12.5	6.25
C1-napthalenes	NAPC1		µg/kg ww	0.5	12.5	6.25
C2- napthalenes	NAPC2		µg/kg ww	0.5	12.5	6.25
Total lipid	LIPIDWT	M	% ww	0.1	12.5	6.25
Dry Weight	DRYWT	M	%		12.5	6.25
Tributyl tin (for imposex only)	TBTIN		µg/kg ww	20	12.5	6.25
Hexabromocyclododecane	HBCD	M	µg/kg ww	0.1	12.5	6.25
2,4,4'-tribromodiphenyl ether (PBDE28)	BDE28	M	µg/kg ww	0.1	12.5	6.25
2,2',4,4'-tetrabromodiphenyl ether (PBDE47)	BDE47	M	µg/kg ww	0.1	12.5	6.25
2,3',4,4'-tetrabromodiphenyl ether (PBDE66)	BDE66	M	µg/kg ww	0.1	12.5	6.25
2,2',3,4,4'-pentabromodiphenyl ether (PBDE85)	BDE85	M	µg/kg ww	0.1	12.5	6.25
2,2',4,4',5-pentabromodiphenyl ether (PBDE99)	BDE99	M	µg/kg ww	0.1	12.5	6.25
2,2',4,4',6-pentabromodiphenyl ether (PBDE100)	BDE100	M	µg/kg ww	0.1	12.5	6.25
2,2',4,4',5,5'-hexabromodiphenyl ether (PBDE153)	BDE153	M	µg/kg ww	0.1	12.5	6.25
2,2',4,4',5,6'-hexabromodiphenyl ether (PBDE154)	BDE154	M	µg/kg ww	0.1	12.5	6.25
2,2',3,4,4',5',6-heptabromodiphenyl ether (PBDE183)	BDE183	M	µg/kg ww	0.1	12.5	6.25

Key: ww = wet weight; M = Mandatory; LOD = Limit of Detection from RLOD in MERMAN AQCRD.xls; MU = measurement uncertainty. The precision stated is that used by MERMAN (as standard deviation in % in AQCRD.xls, which in turn was derived from performance criteria derived by Quasimeme in 1993.The precision listed is ½ of that set by Quasimeme.

Table 6.6cont. Supporting data

Supporting parameters	ICES CODE	Status	UNITS
length (max) (in combination with matrx)	LNMEA	M	Mm
length (mean or individual) (in combination with matrx)	LNMAX	M	Mm
length (min) (in combination with matrx)	LNMIN	M	Mm
Moisture Content	MOCON		%
Dry weight percent	DRYWT		%
weight (max) (in combination with matrx)	WTMAX		g
weight (mean or individual) (in combination with matrx)	WTMEA		g
weight (min) (in combination with matrx)	WTMIN		g
No. Of individuals per batch	NUM	M	
Extractable Lipids	EXLIP		g %
Total lipid	LIPIDWT		%
Species identity	SPECI	M	Full Latin name, (e.g. *Mytilus edulis)*

Biological Effects – Shellfish

Imposex Monitoring

Monitoring of imposex, as a TBT-specific biological effect, is included as a mandatory component of OSPAR Coordinated Environmental Monitoring Programme (CEMP). Sampling should be designed in accordance with the JAMP Guidelines for Contaminant-Specific Biological Effects (OSPAR Agreement 2008-09) which recommend the use of the common dog whelk (*Nucella lapillus*) for the determination of imposex. In areas where dogwhelks are not present in sufficient numbers, imposex can be measured in the other dog whelk species (*Nassarius reticulatus*) or intersex may be measured in periwinkles (*Littorina*). In offshore areas, imposex in whelks *(Buccinum undatum* and *Neptunea antiqua)* can be used for the assessment. The common dog whelk is the species widely used for CSEMP monitoring across the UK. For sampling and reporting details of other species, refer to Technical Annex 3 of the JAMP Guidelines for Contaminant-Specific Biological Effects (OSPAR Agreement 2008-09).

Imposex monitoring in the UK was greatly reduced after 2010 due to the significant decline in imposex across the UK as a result of legislation banning the use of TBT-based antifoulant paints. For design of new sampling programmes, samples should be collected from sites at increasing distances away from point sources (i.e. marinas, ship yards and harbours) to determine gradients of effect. However a risk based sampling approach has mostly been adopted within the UK, focusing on high risk sites but still ensuring sufficient geographic spread. For regional assessment, ideally 5 representative sites (minimum 3) with good geographic spread are required and must be visited at minimum every 6 years for inclusion in the analysis. However, for high risk sites, where VDSI is above the EAC or recorded TBT levels in the water column above the EQS, CMAs may choose to increase sampling frequency.

Dog whelks are collected low tide. Time of year sampled does not effect imposex results. Standard practice is to collect 40 toothed adult dogwhelks, if required juvenile surveys can also be carried out to determine how recently exposure occurred. Dogwhelks should be kept alive and imposex analysed within 2 – 3 weeks. ICES TIMES protocol no. 24 (Gibbs 1999) details the analytical procedure. Laboratories must participate in the QUASIMEME interlaboratory proficiency test exercise for Imposex and report their results to ICES. Both individual and population results should be reported to ICES using the units and codes listed in Table 6.7.

Table 6.7: Imposex parameters required for reporting to ICES

Parameter Description	Code	Unit	Matrix	No. of individuals	Supporting parameters
Shell length (individual)	LNMEA	mm	SH	1	Species; Sex
Length of female penis	LNFPE	mm	SB	1	Species; Sex
Vas Deferens Sequence (stages 0-6)	VDS	st	SB	1	Species; Sex
Length of male penis	LNMPE	mm	SB	1	Species; Sex
Percentage of females in population	%FemalePOP	%	POP	Population e.g. 40	Species
Percentage of sterile females	%STERF	%	WO	Population e.g. 40	Species
Vas deferens sequence index (VDSI)	VDSI	idx	SB	Population e.g. 40	Species
Relative Penis size index (RPSI)	RPSI	idx	SB	Population e.g. 40	Species
Population density*	POPDEN	nr/m2	POP	0	Species

*Population density is only reported if there are no dog whelks present at the site, POPDEN = 0.

General Biological Effects in Shellfish

A general biological effects in shellfish monitoring programme is carried out in Scotland however this is not currently practiced throughout the UK. Mussels are sampled avoiding the spawning season,

within 2 hours of low tide. A consistent size range (within 10 mm) of mussels are collected, ideally between 40 – 60 mm. The Scottish programme samples blue mussel (*Mytilus edulis)* exclusively and includes the following analytical methods:

- Lysosomal membrane stability (LMS) *in vivo*, neutral red retention (NRR) method on live mussel haemocytes. This general indicator is detailed in Technical Annex 6 of JAMP Guidelines for General Biological Effects Monitoring with revised Technical annexes 2007 (OSPAR Agreement 2007-07)[8], the analytical procedure in ICES TIMES 56 (Martinez-Gomez et al. 2015) is followed with a sample size of 10.

- Comet assay as a method to assess DNA damage following ICES TIMES protocol 58 (Bean & Akcha, 2016), sample size of 10.

- Stress on Stress, a general bioindicator of stress, conducted on 40 live animals as soon as possible after collection following Martínez-Gómez & Thain, 2012.

All biological effects methods must be carried out on live animals, for consistency across sampling stations mussels are kept in coolboxes overnight after sampling. Haemolyph (HML) is sampled for the LMS and Comet assays. The stress on stress bioindicator is carried out on live animals. These results are reported to ICES using parameter codes (Table 6.8). Histopathology is also carried out however these results are not reported to ICES.

Table 6.1: General Biological Effects in Shellfish parameters required for reporting to ICES

Parameter Description	Code	Unit	Matrix	No. of individuals	Supporting parameters
% DNA in tail (a measure of the proportion of total DNA present in the comet tail)	%DNAtail	%	HML	1	Species
COMET assay - number of cells screened	CMT-QC-NR	nr	HML	1	Species
Neutral red retention time (mean retention time in minutes)	NRR	mins	HML	1	Species
Survival time	SURVT	d	WO	1	Species

Supporting Determinants

A number of supporting determinants are also reported to ICES with the biological effects data using the parameter codes listed in Table 6.9 These measuremets are made from 30 individuals from the same site, not on the same mussels the biological effects analyses have been carried out on. Species is not submitted as individual determinand but rather submitted as a sample field.

Table 6.9: Supporting determinants for general biological effects in shellfish

Parameter Description	Code	Unit	Matrix	No. of individuals
Dry weight percent	DRYWT%	%	SB	1
Length (individual)	LNMEA	mm	WO	1
Weight (individual)	WTMEA	g	WO	1

6.3 Monitoring required in fish

Strategy

This monitoring is undertaken to fulfil the requirements of the following JAMP Issues.

Temporal trend monitoring in biota is completed to fulfil the requirements of OSPAR Coordinated Environmental Monitoring Programme (CEMP, OSPAR Agreement 2016-01)[5], Theme H (Hazardous substances) mandatory components:

H1	heavy metals cadmium, mercury and lead in biota and sediment
H2	polychlorinated biphenyl (PCB) congeners CB 28, CB 52, CB 101, CB 118, CB 138, CB 153, and CB 180 in biota and sediment
H3	polycyclic aromatic hydrocarbons (PAHs) anthracene, benz[a]anthracene, benzo[ghi]perylene, benzo[a]pyrene, chrysene, fluoranthene, ideno[1,2,3-cd]pyrene, pyrene and phenanthrene in biota and sediment
H4	tributyl tin (TBT)-specific biological effects and TBT in sediment or biota
H5	brominated flame retardants hexabromocyclododecane (HBCD or HBCDD) and polybrominated diphenylethers (PBDEs) BDE 28, BDE 47, BDE 66, BDE 85, BDE 99, BDE 100, BDE 153, BDE 154 and BDE 183 in biota and sediment, and BDE 209 in sediment
H11	general biological effects

The following guidelines are relevant to this part of the Programme:

CEMP Guidelines for Monitoring Contaminants in Biota (Agreement 1999-02., Revision 2018)[9].

JAMP Guidelines for General Biological Effects Monitoring. Revised Technical annexes 2007 (OSPAR Agreement 2007-07)[8].

This part of the programme also meets some of the requirements of the EC Fishery Products Directive, EC Marine Strategy Framework directive and the EC Water Framework Directive.

Contaminant monitoring

It is anticipated that this programme will have 90% power to detect a 2-10% per year change in metal concentrations in fish muscle over a 20 year period. It is anticipated that the programme for monitoring contaminants in fish liver will have 90% power to detect a 3-10% change per year in both metals and organics over a 20 year period.

Preferred species are dab (*Limanda limanda*) or flounder (*Platichthys flesus*). Other acceptable species include plaice, cod and whiting. Whichever species is chosen, it must be analysed throughout the time series dataset, in a consistent strategy, outside the breeding season.

Ideally, about 25 (22 to 28) fish in the size range 18-30 cm (dab), 15-35 cm (flounder), 20-30cm (plaice), 30-45cm (cod) and 20-35cm (whiting) should be collected at a site. Length stratified data are needed from 5 batches of at least 5 fish. If five fish yield insufficient liver tissue for analysis more than five fish

may be collected from one or more catches of the 5 fixed length strata. A minimum of 4 batches are required from the 5 fixed length strata. The number of individual fish pooled in each batch must be the same each year. Visibly damaged fish should not be included. Each batch should correspond to one of the 5 fixed length strata. Data should be reported with supporting data on mean length, % lipid and % wet weight. Both muscle and the liver should be collected with the matrix to be analysed dependant on determinand being measured. Samples should be collected outside the spawning period and at the same time of the year in each year.

Where there is an insufficient range of fish at a site e.g. < 10 cm in size, the sampling strategy may be revised as follows:

Modification 1 Length range 5-10 cm with the fixed length range

Split the length range close to the log mid-point into small and large. Collect a minimum of twenty fish to provide 2 equal replicates of each size group with a minimum of five fish per replicate. Fish should be allocated to replicates before homogenising the tissue.

Modification 2 length range < 5 cm

Collect a random sample of a minimum of twenty fish and randomly allocate them equally to 4 replicates of at least five fish. Again, fish should be allocated to replicates before homogenising the tissue.

An alternative to length stratified sampling may be to minimise natural variability. At least 12 single sex fish, preferably female, age 2-3 years should be caught in a narrow length range (i.e. 26-30cm, 31-35cm etc). The length of the individuals collected should be constant from year to year at each station or should at least fall within a very narrow range, such as within 5cm. In selecting the sample, care should be taken that it is representative of the population and that it can be obtained annually (see JAMP guidelines for more detail on length stratified sampling).

Sample storage

Recommended sample holding times are given below:

Table 6.10 Fish Sample Storage Holding Times

Determinands	Frozen (< -70°C)	Frozen (< -18°C)	Ambient	Live animals	Source
Metals		2 years			MSS Hg stability study
non dl-PCBs (muscle)		1.5 years			MSS
non dl-PCBs (Liver)		3 years			MSS
Organochlorine pesticides					
PAHs (muscle)		1.5 years			MSS
Organotins					
HBCDD					
PBDEs		1.5 years			MSS
Total Lipid					
EROD (liver S9 fraction)	1 year				
PAH metabolites (bile)		6 months			
Micronucleus (fish erythrocytes)			Indefinitely		
AChE (muscle)	< 6 months				
Fish disease (liver pathology)			Indefinitely		
Fish disease (external disease)				< 24 h	

Cells in green indicate the preferred storage method.

Methodology

Fish should be sampled outside the spawning season and contaminants should be measured on the same samples collected for biological effects. Details of sample location and gear used for trawling should be recorded. Samples should be prepared for analysis as soon as possible after collection (see Appendix 4).

Table 6.11 OSPAR CEMP requirements for Contaminants in Fish

Determinands	Status	ICES Code	Matrix	Units	Analytical Targets		
					LOD	Bias (%)	Precision (%)
Mercury		HG	MUSCLE	µg/kg ww	20	12.5	6.25
Arsenic		AS	MUSCLE	µg/kg ww	300	12.5	6.25
Cadmium		CD	LIVER	µg/kg ww	2	12.5	6.25
Lead		PB	LIIVER	µg/kg ww	10	12.5	6.25
PCB 28		CB28	LIVER	µg/kg ww	0.1	12.5	6.25
PCB 52		CB52	LIVER	µg/kg ww	0.1	12.5	6.25
PCB 101		CB101	LIVER	µg/kg ww	0.1	12.5	6.25
PCB 118		CB118	LIVER	µg/kg ww	0.1	12.5	6.25
PCB 138		CB138	LIVER	µg/kg ww	0.1	12.5	6.25
PCB 153		CB153	LIVER	µg/kg ww	0.1	12.5	6.25
PCB 180		CB180	LIVER	µg/kg ww	0.1	12.5	6.25
PBDE 28		BDE-28	LIVER	µg/kg ww	0.1	12.5	6.25
PBDE 47		BDE-47	LIVER	µg/kg ww	0.1	12.5	6.25
PBDE 66		BDE-66	LIVER	µg/kg ww	0.1	12.5	6.25
PBDE 85		BDE-85	LIVER	µg/kg ww	0.1	12.5	6.25
PBDE 99		BDE-99	LIVER	µg/kg ww	0.1	12.5	6.25
PBDE 100		BDE-100	LIVER	µg/kg ww	0.1	12.5	6.25
PBDE 153		BDE-153	LIVER	µg/kg ww	0.1	12.5	6.25
PBDE 154		BDE-154	LIVER	µg/kg ww	0.1	12.5	6.25
PBDE 183		BDE-183	LIVER	µg/kg ww	0.1	12.5	6.25
SUPPORTING DETERMINANDS							
Moisture content	M	MOCON	MUSCLE	% ww			
Dry weight	M	DRYWT	MUSCLE	% dw			
Total lipid	M	LIPIDWT	LIVER	%			
Length (mean)	M	LNMEA	WO	mm			
Length (min)		LNMAX	WO	mm			
Length (max)		LNMIN	WO	mm			
Mean weight		WTMEA	WO	g			
Min weight		WTMIN	WO	g			
Max weight		WTMAX	WO	g			
Species identity	M	SPECI		Full Latin name (e.g.dab is *Limanda limanda*)			
Sex		SEXCO		(M, F,X=mixed, I=immature)			
Liver weight		WTMEA	LIVER	g			
Number in Batch	M	NUM					
Extractable Lipids		EXLIP		%			
Total lipid	M	LIPIDWT		%			

Key: ww = wet weight; dw = dry weight; M = Mandatory; Limit of Detection from RLOD in MERMAN AQCRD.xls; MU = measurement uncertainty. The precision stated is that used by MERMAN (as standard deviation in % in AQCRD.xls, which in turn was derived from performance criteria derived by Quasimeme in 1993.The precision listed is ½ of that set by Quasimeme.

Total lipid should be analysed by the Smedes method.

Table 6.2 WFD priority substances requirements for contaminants in Fish

Determinands	ICES Code	Status	Units	Biota EQS	Analytical Targets	
					LOQ	MU (%)
Mercury	HG	M	µg/kg ww	20	6	50
HCB	HCB		µg/kg ww	10	3	50

Determinands	ICES Code	Status	Units	Biota EQS	Analytical Targets	
					LOQ	MU (%)
HCBD	HCBD		µg/kg ww	55	16.5	50
Fluoranthene	FLU	M	µg/kg ww	30	9	50
Benzo[a]pyrene	BAP		µg/kg ww	5	1.5	50
Hexabromocyclododecane	HBCD	M	µg/kg ww	167	50.1	50
2,4,4'-tribromodiphenyl ether (PBDE28)	BDE28	M	µg/kg ww	0.0085	0.00255	50
2,2',4,4'-tetrabromodiphenyl ether (PBDE47)	BDE47	M	µg/kg ww	0.0085	0.00255	50
2,2',4,4',5-pentabromodiphenyl ether (PBDE99)	BDE99	M	µg/kg ww	0.0085	0.00255	50
2,2',4,4',6-pentabromodiphenyl ether (PBDE100)	BDE100	M	µg/kg ww	0.0085	0.00255	50
2,2',4,4',5,5'-hexabromodiphenyl ether (PBDE153)	BDE153	M	µg/kg ww	0.0085	0.00255	50
2,2',4,4',5,6'-hexabromodiphenyl ether (PBDE154)	BDE154	M	µg/kg ww	0.0085	0.00255	50
Dicofol			µg/kg ww	33	9.9	50
Perflurooctane sulfonic acid and its salts (PFOS)			µg/kg ww	9.1	2.73	50
Dioxin and Dioxin like PCB's			µg/kg ww	0.0065 (TEQ)	1/3 of EQS as TEQ	50
Heptachlor and Heptachlor Epoxide			µg/kg ww	0.0067	0.002	50

Key: ww = wet weight; dw = dry weight; M = Mandatory; LOQ = Limit of Quantitation; MU = measurement uncertainty. The expanded uncertainites above are based on a standard uncertainity multiplied by a coverage factor k=2, providing a coverage probability of approximately of 95%.

Biological Effects – Fish

Monitoring of biological effects in fish is included in OSPAR pre-CEMP, as voluntary components. There are JAMP Guidelines in place for both PAH and metal-specific biological effects and general biological effects (OSPAR Agreement 2008-09 and OSPAR Agreement 2007-7 respectively). Participation in monitoring of biological effects in fish is fairly consistent across the UK between England & Wales and Scotland. Biological effects in fish are currently not included in any monitoring programmes in Northern Ireland. Monitoring laboratories must participate in inter laboratory comparison exercises arranged through the Biological Effects Quality Assurance in Monitoring Programme (BEQUALM).

The sampling requirement are similar for all techniques and based on recommendations in the JAMP guidance and relevant ICES TIMES protocols (Appendix 2). Target species are flounder (*Platichthys flesus*) and dab (*Limanda limanda*) however plaice (*Pleuronectes platessa*) and cod (*Gadus morhua*) are also acceptable species for some analyses as detailed in Appendix 2. Sampling procedures are detailed in Appendix 4. Fish are collected by trawling between May and January to avoid the spawning season. The time between visiting sites varies across the UK however each site is sampled at a consistent time of year. In England & Wales all sites are visited once every two years. In Scotland, a risk-based approach is adopted where sites are visited every 1 – 6 years depending on the results from the previous assessment. For regional assessment, ideally 5 representative sites (minimum 3) with

good geographic spread are required and must be visited at minimum every 6 years for inclusion in the analysis. This can be more challenging than contaminant assessments as this is limited to fishing sites. Technique specifc sample requirements are detailed in Appendix 2. Sites are listed, with the organisations responsible for monitoring details can be found in the MERMAN station dictionary list.

The relevant parameters for reporting are listed in Table 6.13.

Metal-specific biological effects

At present there are only two methods that are reasonably specific for one or more metals and that have been evaluated to any extent for aquatic organisms - metallothionein (MT; Cu, Zn, Cd and inorganic Hg) and δ amino levulinic acid dehydratase (ALA-D; Pb). Neither of these metal-specific biological effects are currently included in any UK monitoring programme. For sampling and reporting details of either of these metal-specific biological effects refer to Technical Annex 1 of the JAMP Guidelines for Contaminant-Specific Biological Effects (OSPAR Agreement 2008-09).

Contaminant specific biological effects

Five PAH-specific biological effects are included in the voluntary pre-CEMP and detailed in Technical Annex 2 of the JAMP Guidelines for Contaminant-Specific Biological Effects (OSPAR Agreement 2008-09) – EROD, DNA adducts, bile PAH metabolites, liver histopathology and macroscopic liver neoplasms. DNA adducts is not curently included in any UK monitoring programme (see JAMP guidelines for details of sampling and reporting), however all other techniques are utilised. These biological effects should supplement the analysis of PCBs in fish liver and PAHs in sediments.

For EROD analysis, the UK use the S9 fraction method following ICES TIMES 57 (Stagg et al. 2016). The bottom water temperature is also reported to ICES (Table 6.13). The molar absorbance of the resorufin; protein assay and standard; and assay conditions used (e.g. temperature, 7-ethoxyresorufin concentration, NADPH concentration, protein concentration in the assay) are all important factors and recorded as part of method but not reported to ICES. For assessment of EROD, males and females are analysed separately as enzyme induction is usually higher in male fish.

Across the UK, a synchronous scanning method with standard addition is used to determine PAH metabolites in fish bile following ICES TIMES 39 (Ariese et al. 2005). Results are reported as 1-hydroxy pyrene equivalent (Table 6.13).

Inspection for gross liver lesions should follow the guidelines set out in ICES TIMES 19 (Bucke et al. 1996). Liver histopathology and confirmation of gross liver lesions is determined following ICES TIMES 38 (Feist et al. 2004).

Table 6.33 Contaminant specific biological effects in fish including liver histopathology

Parameter Description	Code	Unit	Matrix	No. of individuals	Supporting parameters
EROD	EROD	pmol/min/ mgprotein	LIVER S9 fraction (LIS9)	1	species, sex, bottom water temperature, GSI, HSI
1-hydroxy pyrene equivalent	PYR1OHEQ	ng/ml	BILE (BI)	1	species, sex, GSI, HIS, EROD
apoptosis	APOPTS	afnr*	LIVER (LI)	1	species, sex, length, weight, GSI, HSI, age
basophilic foci	BASFC	afnr*	LIVER (LI)	1	species, sex, length, weight, GSI, HSI, age
cholangioma	CHOLA	afnr*	LIVER (LI)	1	species, sex, length, weight, GSI, HSI, age
cholangiocarcinoma	CHOLC	afnr*	LIVER (LI)	1	species, sex, length, weight, GSI, HSI, age
clear cell foci	CLCFC	afnr*	LIVER (LI)	1	species, sex, length, weight, GSI, HSI, age
eosinophilic foci	EOSFC	afnr*	LIVER (LI)	1	species, sex, length, weight, GSI, HSI, age
fibrillar inclusions	FIBINC	afnr*	LIVER (LI)	1	species, sex, length, weight, GSI, HSI, age
fibrosis	FIBROS	afnr*	LIVER (LI)	1	species, sex, length, weight, GSI, HSI, age
variable glycogen content	GLYCCV	afnr*	LIVER (LI)	1	species, sex, length, weight, GSI, HSI, age

granuloma	GRANLM	afnr*	LIVER (LI)	1	species, sex, length, weight, GSI, HSI, age
hemangioma	HEMAGA	afnr*	LIVER (LI)	1	species, sex, length, weight, GSI, HSI, age
hemangiopericytic sarcoma	HEMAPS	afnr*	LIVER (LI)	1	species, sex, length, weight, GSI, HSI, age
hemangiosarcoma	HEMAS	afnr*	LIVER (LI)	1	species, sex, length, weight, GSI, HSI, age
hemosiderosis	HEMOSD	afnr*	LIVER (LI)	1	species, sex, length, weight, GSI, HSI, age
mixed hepatobiliary carcinoma	HEPBCM	afnr*	LIVER (LI)	1	species, sex, length, weight, GSI, HSI, age
hepatocellular adenoma	HEPCA	afnr*	LIVER (LI)	1	species, sex, length, weight, GSI, HSI, age
hepatocellular carcinoma	HEPCC	afnr*	LIVER (LI)	1	species, sex, length, weight, GSI, HSI, age
hepatocellular and nuclear polymorphism	HEPCNP	afnr*	LIVER (LI)	1	species, sex, length, weight, GSI, HSI, age
hydropic vacuolization of biliary epithelial cells and/or hepatocytes	HYVCBE	afnr*	LIVER (LI)	1	species, sex, length, weight, GSI, HSI, age
lymphocytic/monocytic infiltration	LYMCINF	afnr*	LIVER (LI)	1	species, sex, length, weight, GSI, HSI, age
melanomacrophage centres	MELAMC	afnr*	LIVER (LI)	1	species, sex, length, weight, GSI, HSI, age
mixed foci of cellular alteration	MXDFC	afnr*	LIVER (LI)	1	species, sex, length, weight, GSI, HSI, age
no abnormalities identifed	NAD	afnr*	LIVER (LI)	1	species, sex, length, weight, GSI, HSI, age
coagulative necrosis	NECRCG	afnr*	LIVER (LI)	1	species, sex, length, weight, GSI, HSI, age
pancreatic acinar cell adenoma	PANACA	afnr*	LIVER (LI)	1	species, sex, length, weight, GSI, HSI, age
pancreatic acinar carcinoma	PANACC	afnr*	LIVER (LI)	1	species, sex, length, weight, GSI, HSI, age
phospholipidosis	PHOSLD	afnr*	LIVER (LI)	1	species, sex, length, weight, GSI, HSI, age
regeneration	REGNR	afnr*	LIVER (LI)	1	species, sex, length, weight, GSI, HSI, age
spongiosis hepatitis	SPNHEP	afnr*	LIVER (LI)	1	species, sex, length, weight, GSI, HSI, age
macroscopic steatosis	STETMA	afnr*	LIVER (LI)	1	species, sex, length, weight, GSI, HSI, age
microscopic steatosis	STETMI	afnr*	LIVER (LI)	1	species, sex, length, weight, GSI, HSI, age
vacuolated foci	VACFC	afnr*	LIVER (LI)	1	species, sex, length, weight, GSI, HSI, age

* afnr (affected number of individuals)

General Biological Effects

General biological effects of fish monitored in the UK include EROD, liver histopathology, macroscopic liver neoplasms and externally visible disease. EROD, liver histopathology and macroscopic liver neoplasms are also considered organic compound-specific biological effects and detailed in the above section.

Routine monitoring of externally visible fish disease is carried out in England & Wales and Scotland following ICES TIMES 19 (Bucke at al 1996). Fish are examined for the diseases and parasites detailed in Table 6.14 and results reported to ICES.

Table 6.44 Externally visible fish disease and parasites

Parameter Description	Code	Unit	Matrix	No. of individuals	Supporting parameters
Epidermal hyperplasia/papilloma	EPID PAP	st (stage)	EP	1	species, sex, length, weight, GSI, HSI, age
Hyperpigmentation	HPIGM	st (stage)	EP	1	species, sex, length, weight, GSI, HSI, age
Lymphocystis	LYMP CYS	st (stage)	EP	1	species, sex, length, weight, GSI, HSI, age
Skin ulcer (acute/healing ulcers)	SKIN ULC	st (stage)	EP	1	species, sex, length, weight, GSI, HSI, age
Fin rot/erosion (acute/healing)	FROT	afnr*	EP	1	species, sex, length, weight, GSI, HSI, age
Acanthochondria sp	ACAN THO	afnr*	GI	1	species, sex, length, weight, GSI, HSI, age
X-cell gill lesions	XGIL LES	afnr*	GI	1	species, sex, length, weight, GSI, HSI, age
Anisakidae	ANISAKIX	afnr*	LI	1	species, sex, length, weight, GSI, HSI, age
Liver disease – nodule/tumour	LIVE NOD	afnr*	LI	1	species, sex, length, weight, GSI, HSI, age
Lateral lipoidosis	LATLIP	afnr*	MU	1	species, sex, length, weight, GSI, HSI, age
Stephanostonum sp.	STEP STO	afnr*	MU	1	species, sex, length, weight, GSI, HSI, age
Glugea sp	GLUG STE	afnr*	VC	1	species, sex, length, weight, GSI, HSI, age
Lepeophtheirus sp.	LEPE OPH	afnr*	WO	1	species, sex, length, weight, GSI, HSI, age
Skeletal deformity	SKEL DEF	afnr*	WO	1	species, sex, length, weight, GSI, HSI, age

* afnr (affected number of individuals)

Other Biomarkers

Other biomarkers included in the UK's biological effects monitoring programme include the micronucleus assay and AChE (acetylcholinesterase) enzyme activity.

The micronucleus assay is used as a tool to assess genotoxicity in fish. There is no ICES TIMES protocol for this method however there are a number of published protocols available (Carrasco et al 1990; Barsiene et al 2012; Bolognesi & Fenech 2012). Different numbers of cells are scored during analysis at Cefas (1,000 cells per fish) and Marine Science Scotland (4,000 cells per fish). A review of the incidence and power to detect micronucleus above the Background Assessment Criteria (dab = 0.5 micronucleus per 1,000 cells) should be undertaken to agree the number of cells to be scored. Results are reported using the parameters listed in Table 6.15, the number of cells counted must also be reported.

Measurement of acetylcholinesterase (AChE) enzyme activity in fish is a suitable method to assess neurotoxic contaminants in the environment. This enzyme is inhibited by some organophosphates and carbamates insecticides. AChE is routinely monitored in England & Wales and the method is being trialled in Scotland (2019). The ICES TIMES 22 protocol for cholinesterase inhibition is not followed as a more specific method for fish AChE has been developed by Sturm et al. (1999). This method is used in both UK laboratories. The relevant parameters for reporting are listed in Table 6.15

Table 6.15 Other biological effects in fish

Parameter Description	Code	Unit	Matrix	No. of individuals	Supporting parameters
Micronuclei	MNC	nr/1000 cells	BL	1	species, sex, length, weight, GSI, HSI
Micronuclei-number of cells counted (QC parameter)	MNC-QC-NR	nr	BL	1	species, sex, length, weight, GSI, HSI
Acetylcholine esterase activity	AChE	nmol/min/mg protein	MU	1	species, sex, length, weight, GSI, HSI

Supporting Determinants

A number of supporting determinants are also determined following ICES TIMES 60 (Hansson et al. 2017) and reported to ICES with the biological effects data using the parameter codes listed in Table 6.16 Some of these determinats are mandatory to support specific biological effects measurements, others are only recommended. Species and sex are not submitted as individual determinands but rather submitted as a sample field.

Table 6.16 Supporting determinants for biological effects in fish

Parameter Description	Code	Unit	Matrix	No. of individuals
Bottom water temperature	BotTemp	degC	SI	1
Length (individual)	LNMEA	mm	WO	1
Weight (individual)	WTMEA	g	WO	1
gonadal somatic index ((gonad weight/whole organism weight) x 100)	GOSOI	idx	GONAD (GO)	1
liver somatic index liver weight/whole organism weight) x 100)	LISOI	idx	LIVER (LI)	1

6.4 Eutrophication monitoring requirements

Strategy

Monitoring to determine the eutrophic status of UK marine waters is required by and follows guidleines as listed :

OSPAR Eutrophication - Common Procedure [x]

The Urban Waste Water Treatment Directive (91/271/EEC)[x]:

The Nitrates Directive (91/676/EEC)[x]:

The Water Framework Directive (2000/60/EEC)x:

The Habitats Directive (92/43/EEC):

Marine Strategy Framework Directive (2008/56/EC)

Revised JAMP Eutrophication Monitoring Guideline: Oxygen (Agreement 2013-05)Revised JAMP Eutrophication Monitoring Guideline: Nutrients (Agreement 2013-04)

JAMP Eutrophication Monitoring Guidelines: Chlorophyll a in Water(OSPAR Agreement 2012-11)

CEMP Guidelines: Phytoplankton monitoring (OSPAR Agreement 2016-06)

JAMP Eutrophication Monitoring Guidelines:Benthos(OSPAR Agreement 2012-12)

Revised CEMP guidelines for coordinated monitoring for eutrophication, CAMP and RID

(OSPAR Agreement 2016-05)

Eutrophication is defined as:

The enrichment of water by nutrients causing an accelerated growth of algae and higher forms of plant life to produce an undesirable disturbance to the balance of organisms present in the water and the quality of water concerned.

Monitoring is designed to determine the presence of:

- Nutrient (nitrogen and phosphorus) enrichment
- Accelerated growth of algae (estimates of biomass)
- An undesirable disturbance (organic enrichment leading to oxygen depletion)

Sample storage

Recommended sample holding times are given in Table 6.17.

Table 6.5 Sample storage and holding times.

Determinand	Frozen (< -18°C)	Frozen (<-70 °C)	Refrigerated (5 ±3°C)	Ambient	Source
Chlorophyll prior to filtration				Preferably in cool place in the dark. Ideally within 4 hours but if not possible as soon as practible	ICES TIMES "Determination of chlorophyll in seawater"
Fluorometric chlorophyll (filter papers)	1 month	1 year			ICES TIMES "Determination of chlorophyll in seawater " & MSS
Chlorophyll pigments	1 month	1 year			ICES TIMES "Determination of chlorophyll in seawater " & MSS
Nutrients (TOxN, Ammonia, silicate, nitrite, phosphate	9 months		< 1 month	10 hrs (in dark)	Grasshoff K, Kremling, K and Ehrardt M . Methods of Seawater Analysis Wiley-VCH Third Edition Hydes, D.J., Aoyama, M., Bakker, K., Becker, S., Coverly, S., Daneils, A., Dickenson, A.G., Gross, O., Kerouels, R., Ouijen, J., Satos, K., Tanhua, T., Woodward, E.M.S and Zhangu, J.Z. 2010. Determination of dissolved nutrients (N, P, SI) in seawater with high precision and inter-comparability using gas-segmented continuous flow analysers . The Go-ship repeat hydrography manual: A collection of expert reports and guidelines. IOCCP report no 14 Avanzino R.J and Kennedy, V.C. 1993. Long-Term Frozen Storage of Stream Water samples for dissolved Orthophosphate, Nitrate plus nitrite and ammonia analysis. Water Resources Research, 29, 3357-3362 Dore, J.E., Houlihan. T., Hebel, D.V., Tein. G., Tupas. L. and Karl. D.M. 1996. Freezing as a method of sample preservation for the analysis of dissolved inorganic nutrients in seawater. Marine Chemistry, 53, 173-1885
Dissolved Oxygen			4 months, when stored under water		Zhang, J-Z., Berberian, G. and Wanninkhof, R. 2002. Long-term storage of natural water samples for dissolved oxygen determination. Water Research, 36, 4165 – 4168.

Cells in green indicate the preferred storage method.

Table 6.6 lists the determinands to be monitored under the Clean Seas programme to provide evidence for eutrophication.

Determinand	Units	ICES	NMCAG Targets		
			LOD	Bias (%)	Precision (%)
Ammonia	µ M	AMON	0.5	6	3
Nitrate	µ M	NTRA			
Nitrite	µ M	NTRI	0.05	6	3
Total oxidised nitrogen	µ M	NTRZ	0.5	6	3
Phosphate	µ M	PHOS	0.05	6	3
Silicate	µ M	SLCA	0.5	6	3
Chlorophyll-a	µg/l	CPHL	0.1	12.5	6.25

Table 6.7 Supporting Determinands

Parameter	Range of Application	Uncertainty	Traceability
Temperature	0 - 29 °C	0.1 °C Constant	ITS90 or ITS68
Salinity	2 - 36	0.2 Constant	IAPSO Standard Sea Water
Dissolved Oxygen	0 - 15 mg/l	5% of reading, proportional	Air Calibration
Turbidity	0.4 - 400 f.t.u	10% of reading, proportional	ISO7027
pH	pH 4 to 9	0.3 pH Constant	Buffers traceable to NIST

Nutrient enrichment

Plant growth is limited by the least abundant nutrient i.e. nitrogen or phosphorus. In coastal waters growth is usually limited by the availability of dissolved inorganic nitrogen (nitrate + nitrite + ammonia) however phosphate should also be measured so that the N:P ratio can be determined to confirm this. Silicate may also be determined as this limits the growth of diatoms which have a silaceous cell wall.

Nutrients are removed from the water column by plant growth during the growing season so monitoring is limited to Winter (November, December, January, February) to obtain a maximum value and allow interannual comparison.

The concentration and ratio of N:P in estuaries changes as nutrient rich river water mixes with seawater. Salinity is used to determine the impact of freshwater inputs in estuaries and regions of freshwater influence in coastal waters.

Accelerated Growth

Spring/ Summer (April - September) chlorophyll concentrations should be determined as an indicator of phytoplankton biomass. Ideally continuous data should be collected as concentrations vary temporally and spatially in response to climatic and physical variables.

The depth of the photic zone should also be determined by measuring the secchi disc depth. This gives an indication of whether phytoplankton growth is inhibited by availability of light.

Undesirable Disturbance

Dissolved oxygen is consumed by the decay of senescent algae at the end of the growing season. The extent of oxygen removal depends on the biomass of algae and flushing rate. Removal of oxygen by organic enrichment is a concern when low oxygen concentrations impact on the fauna. Dissolved oxygen should be measured in bottom waters during the autumn.

Methodology

Procedural guidelines for nutrient and chlorophyll sampling and sample preparation are given in Appendix 12 and 13.

It is recognised that spot water samples for inherently variable determinands such as nutrients, dissolved oxygen and chlorophyll *a* and are of limited value in long term trend monitoring . Continuous monitoring is useful to fill gaps between discrete sampling operations or where discrete samling isn't possible. It should be noted however that any data collected via sensors must be quality controlled by regular calibration.

Data Submission

Winter nutrient, summer chlorophyll and supporting data should be submitted to the MERMAN database. MERMAN can accept data from both fixed and opportunistic sites however there is currently no mechanism for collecting continuous in situ monitoring data and CMA's should list datasets via MEDIN. Quality Control data (both internal and proficiency test data) must be submitted with the environmental data.

The collection year for water submission differs to other determined submissions to account for the winter nutrient collection period. The water collection year runs 1st March through to end of February the following year i.e water samples collected March 2018 and February 2019 would be submitted to MERMAN under the 2018 submission.

6.5 Compliance Monitoring of Contaminants in Water

Strategy

Monitoring for trace metals and organic compounds in water is undertaken to comply with the requirements of the Water Framework Directive (2000/60/EC – "the WFD") and its daughter Directive specifying Environmental Quality Standards (2008/105/EC as amended by 2013/39/EU). Monitoring is required to determine compliance against national and international Environmental Quality Standards which are usually specified as annual average concentrations (see Table 6.20 & 6.21 below). Monitoring frequencies are determined for different contaminants by the WFD, but quarterly monitoring is regarded as the minimum frequency to assess compliance on an annual average basis. Organisations need only submit data collected for their statutory monitoring requirements where there is a known contaminant source. All samples from the water column are to be taken approximately 1 metre below the surface. Sampling should be undertaken at taken at a tidal state, allowing for practicality and safety, which is likely to indicate a worst case contaminant concentration. Every effort should be made thereafter to sample under similar tidal conditions at each site. Biological effects samples for Oyster Embryo Bioassay should be collected in conjunction with contaminants samples at estuarine sites.

Methodology

Care should be taken to avoid contamination of the sample during sampling and sample preparation. Trace metals samples should be filtered to <0.45 µm to allow an assessment of compliance with the EQS values for dissolved metals. Salinity (see table 6.19 and suspended solids samples should also be collected to provide supporting information. Sampling and sample preparation procedures are outlined in Appendix 13.

Metals

Table 6.8 Requirements for metals and supporting determinands in water

Determinands	Units	ICES Code	Target		
DISSOLVED METALS (determined <0.45			**LOD**	**Bias (%)**	**Precision (%)**
LIST I					
Mercury (WFD PHS)	µg/l	HG	0.003	12.5	6.25
Cadmium (WFD PHS)	µg/l	CD	0.04	12.5	6.25
LIST II					
Copper	µg/l	CU	0.2	12.5	6.25
Lead (WFD PS)	µg/l	PB	0.04	12.5	6.25
Nickel (WFD PS)	µg/l	NI	0.25	12.5	6.25
Zinc	µg/l	ZN	0.4	12.5	6.25
Iron	µg/l	FE	100	12.5	6.25
Boron	µg/l	B	700	12.5	6.25
Arsenic	µg/l	AS	2.5	12.5	6.25
Chromium	µg/l	CR	1.5	12.5	6.25
Vanadium	µg/l	V	10	12.5	6.25
SUPPORTING DETERMINANDS					
Suspended solids	mg/l	SUSP	2		
Salinity (see table 6.19)		PSAL			

Table 6.9 WFD compliance requirements for metals

Determinands*	Units	ICES Code	EQS AA Target	EQSD Target	
DISSOLVED METALS (determined <0.45 µm)				**LOQ****	**MU**
LIST I					
Mercury (WFD PHS)	µg/l	HG	0.07	0.010	50
Cadmium (WFD PHS)	µg/l	CD	0.2	0.06	50
LIST II					
Copper***	µg/l	CU	3.76	1.2	50
Lead (WFD PS)***	µg/l	PB	1.3	0.4	50
Nickel (WFD PS)***	µg/l	NI	8.6	2.8	50
Zinc***	µg/l	ZN	6.8	2.2	50
Iron	µg/l	FE	1000	333	50
Arsenic	µg/l	AS	25	8.3	50
SUPPORTING DETERMINANDS					
DOC	mg/l				

*EQS RBMP2 - unchanged and amended (published 2013) EU standards and UKTAG proposed standards for RBMPii and beyond

**LOQ targets based upon 1/3 AA EQS values

***bioavailable

MU = Minimum Measurement of Uncertainty = 2 x %RSD where RSD = relative standard deviation

[1]Member States are to ensure an uncertainty of measurement of 50% or below (k=2) estimated at the level of relevant EQS

(1 taken from the UKTAG Chemistry Task Team Guidance on the Implementation of the QA/QC Directive)

Organics

Organics are to be monitored for WFD purposes. The analysis should be of unfiltered samples. Data must be submitted with supporting salinity and suspended solids data.

Table 6.10 WFD compliance requirements for organics priority substances (PS) and priority hazardous substances (PHS)

Name of substance	PHS *	Units	ICES Code	AA-EQS	MAC-EQS	LOQ	MU (%)
Alachlor		µg/l		0.3	0.7	0.09	50
Anthracene	*	µg/l	ANT	0.1	0.1	0.03	50
Atrazine		µg/l	ATRZ	0.6	2.0	0.18	50
Benzene		µg/l	BENZ	8.0	50.0	2.4	50
Penta BDE 1	*	µg/l		na	0.014	0.0042	50
chloroalkanes, C10-13	*	µg/l		0.4	1.4	0.12	50
Chlorfenvinphos		µg/l	CHLOR	0.1	0.3	0.03	50
Chlorpyrifos		µg/l		0.03	0.1	0.009	50

1 Nine PBDE congeners (BDE28, BDE47, BDE66, BDE100, BDE99, BDE85, BDE154, BDE153, BDE183) have been selected for monitoring by OSPAR, taking into account their occurrence in the environment and their toxicity, to be routinely determined as part of the CEMP (OSPAR Commission, 2007).
For the group of priority substances covered by brominated diphenylethers under the WFD and listed in Decision No 2455/2001/EC, an EQS is established only for (as the sum of) congener numbers 28, 47, 99, 100, 153 and 154.
Hexachlorocylohexane includes the isomers α, β, γ, δ - to be determined to the LOD shown.
Trichlorobenzenes include 124, 123 and 135 isomers - to be determined to the LOD shown.

1,2-dichloroethane		µg/l	DCE	10.0	na	3	50
Dichloromethane		µg/l		20.0	na	6	50
Di(2-ethylhexyl)phthalate (DEHP)		µg/l		1.3	na	0.39	50
Diuron		µg/l		0.2	1.8	0.06	50
Endosulfan	*	µg/l	END	0.0005	0.004	0.00015	50
Fluoranthene		µg/l	FLU	0.0063	0.12	0.0019	50
Hexachlorobenzene	*	µg/l	HCB	na	0.05	0.015	50
Hexachlorobutadiene	*	µg/l	HCBD	na	0.6	0.18	50
Hexachlorocyclohexane HCH 2	*	µg/l	HCH	0.002	0.02	0.0006	50
Isoproturon		µg/l		0.3	1.0	0.09	50
Naphthalene		µg/l	NAP	2	130	0.6	50
Nonylphenol (NP) (4-nonylphenol)	*	µg/l		0.3	2.0	0.09	50
Octylphenol		µg/l		0.01	na	0.003	50
Pentachlorobenzene	*	µg/l	QCB	0.0007	na	0.00021	50
Pentachlorophenol		µg/l	PCP	0.4	1.0	0.12	50
Benzo(a)pyrene	*	µg/l	BAP	0.00017	0.027	0.00005	50
Benzo(b)fluoranthene	*	µg/l	BBF	0.00017	0.017	0.00005	50
Benzo(k)fluoranthene	*	µg/l	BKF	0.00017	0.017	0.00005	50
Benzo(g,h,I)perylene	*	µg/l	BGHIP	0.00017	0.00082	0.00005	50
Indeno(1,2,4-cd)pyrene	*	µg/l	ICDP	0.00017	na	0.00005	50
Simazine		µg/l	SIMZ	1.0	4.0	0.3	50
tributyltin compounds	*	µg/l	TBT	0.0002	0.0015	0.00006	50
trichlorobenzenes (all isomers)	*	µg/l		0.4	na	0.12	50
Cypermethrin		µg/l		$8*10^{-6}$	$6*10^{-5}$	$2.4*10^{-5}$	50
Dicofol	*	µg/l		3.210-5	na	$9.6*10^{-6}$	50
Perflurooctane sulfonic acid and its salts (PFOS)	*	µg/l		$1.3*10^{-4}$	7.2	$3.9*10^{-5}$	50
Quinoxifen	*	µg/l		0.015	0.54	0.0045	50
Dioxin and dioxin like compounds	*	µg/l		na	na	na	50
Alconifen		µg/l		0.012	0.012	0.0036	50
Bifenox (methyl 5-(2,4-dichlorophenoxy)-2-nitrobenzoate		µg/l		0.0012	0.004	0.00036	50
Heptachlor & hepteachlor epoxide	*			$1*10^{-8}$	$3*10^{-5}$	$3*10^{-9}$	50
Dichlorvos		µg/l	DCV	0.00006	0.00007	0.004	50
Cybutryne (Irgarol)		µg/l		0.0025	0.016	0.00075	50
Hexabromocyclododecane (HBCDD)	*	µg/l		0.0008	0.05	0.00024	50
Terbutryn		µg/l		0.0065	0.034	0.002	50
Trifluralin		µg/l	CHCL3	0.03	na	0.01	50
Other substances listed in (2008/105/EC).							
Trichloromethane (chloroform)		µg/l		2.5	na	0.09	50
DDT isomers		µg/l	TRF	0.025	na	0.0075	50
para-para-DDT		µg/l		0.01	na	0.003	50
Aldrin		µg/l	DDTPP	0.00125	na	0.00038	50
Dieldrin		µg/l	ALD	0.00125	na	0.00038	50
Endrin		µg/l	DIELD	0.00125	na	0.00038	50
Isodrin		µg/l	END	0.00125	na	0.00038	50
Carbontetrachloride		µg/l	ISOD	12	na	3.6	50
Tetrachloroethylene		µg/l		10	na	3	50
Trichloro-ethylene		µg/l		10	na	3	50
Fenitrothion		µg/l	FENT	0.01	na	0.003	50
Malathion		µg/l	MAL	0.02	na	0.006	50
Triphenlytin		µg/l	TPTIN	na	0.008	0.0024	50

2

Xylene (o, m , p – xylene)		µg/l		30	na	9	50
1,1,1 – trichloroethane		µg/l	TCE	100	n/a	30	50
1,1,2 – trichloroethane		µg/l	2TCE	300	n/a	90	50
Bentazone		µg/l		500	n/a	150	50
Biphenyl		µg/l		25	n/a	7.5	50
Specific Pollutants							
2,4 – D (total ester)		µg/l	E240	0.3	1.3	0.09	50
Dimethoate		µg/l	DMT	0.48	4	0.144	50
Linuron		µg/l	LIN	0.5	0.9	0.15	50
Mecoprop		µg/l	MECOP	18	n/a	5.4	50
Toluene		µg/l	TOL	74	370	22.2	50
3,4-dichloroanaline		µg/l		0.2	5.4	0.06	50
Benzyl butyl phthalate		µg/l		0.75	10	0.225	50
Glythosate		µg/l		196	398	58.8	50
Triclosan				0.1	0.28	0.03	50
Diazinon		µg/l	DIAZ	0.01	0.26	0.003	50
4-chloro-3-methyl phenol		µg/l	CMP43	40	na	12	50
2-chlorophenol		µg/l	2CP	50	na	15	50
2,4-dichlorophenol		µg/l	DCP24	0.42	6	0.13	50
Phenol		µg/		7.7	46	2.31	50
4-chloro-2-nitrotoluene		µg/l	4C2N	10	na	3	50
4-chloro-3-nitrotoluene		µg/l	4C3N	10	na	3	50
2-chloro-4-nitrotoluene		µg/l	2C4N	10	na	3	50
2-chloro-5-nitrotoluene		µg/l	2C5N	10	na	3	50
2-chloro-6-nitrotoluene		µg/l	2C6N	10	na	3	50
Permethrin		µg/l	PERM	0.0002	0.001	0.00006	50

*EQS RBMP2 - unchanged and amended (published 2013) EU standards and UKTAG proposed standards for RBMPii and beyond
**LOQ targets based upon 1/3 AA EQS values
***bioavailable
MU = Minimum Measurement of Uncertainty = 2 x %RSD where RSD = relative standard deviation
[1]Member States are to ensure an uncertainty of measurement of 50% or below (k=2) estimated at the level of relevant EQS
(1 taken from the UKTAG Chemistry Task Team Guidance on the Implementation of the QA/QC Directive)

Biological Effects – Water

Biological effects in water are not included in any UK monitoring programmes. Oyster embryo bioassay is included in the JAMP Guidelines for General Biological Effects Monitoring with revised Technical annexes 2007 (OSPAR Agreement 2007-07) and can be used to determine toxicity of waters in estuaries. Future monitoring programmes should refer to the guidance within this for sampling and reporting details. Sampling procedures previously used in the UK are detailed in Appendix 2. In practice samples should be collected in conjunction with contaminant samples.

6.6 Monitoring required for litter (note this section is under review by the litter group)

Strategy

The Marine Strategy Framework Directive (MSFD) requires EU Member States to put in place measures to achieve Good Environmental Status (GES) in their marine waters by 2020.

The definition of GES has not yet been finalised, but Defra has proposed as a draft that GES will be achieved when:

1. Litter and its degradation products currently present in, and entering into, UK waters is reduced over time and does not pose a significant risk to marine life at the population level, either as a result of direct mortality or by way of indirect impacts such as reduced fecundity and bioaccumulation within food chains.

2. Litter currently present in, and entering into, UK waters does not pose a direct or indirect unacceptable risk to human welfare and does not lead to significant detrimental economic impacts for industry and coastal communities.Marine litter is defined as any persistent, manufactured or processed solid material discarded, disposed of or abandoned in the marine and coastal environment, including materials transported into the marine environment from land by rivers, draining or sewage systems or winds.

To take forward the development of GES targets for litter workshops were held at Defra, Nobel House, London. The workshop conclusions are shown in the table below:

Commission Criteria	**Possible Options for UK Targets**
10.1 **Characteristics of litter in the marine and coastal environment**	[x%] overall reduction in the number of visible litter items on coastlines from 2010 levels by 2020. Surveillance indicator to monitor the quantities of litter on the seafloor (number of items). Surveillance indicator to monitor the quantities of micro-plastics.
10.2 **Impacts of litter on marine life**	Surveillance indicator to monitor the amounts of plastic found in the contents of fulmars stomachs (in line with the OSPAR Ecological Quality Objective)

The current state of litter monitoring activities in the UK (Jan 2010):

The MSFD is a significant new piece of legislation, to which the CMAs will have to respond in meeting the implementation requirements within the UK. In order to define GES, a UK marine litter monitoring programme will have to be instated. Currently the UK does not have a defined marine litter monitoring programme in place although different independent ad hoc surveys and research exist which will form the basis for a future defined strategy. These are defined below:

Organisation	**Contact**	**Type of activity**
MSS (CMA)	Lynda webster/ Kelly McIntosh	Benthic trawl surveys

		)
MSS (CMA)	Marie Russell	Microplastic monitoring (water/sediment/biota)
NIEA (CMA)	Michael McAliskey / Matt Service	Benthic trawl surveys
EA (CMA)	Mike Best	Benthic trawl surveys
CEFAS (CMA)	Thomas Maes	Benthic trawl surveys Microplastic monitoring (water/sediment/biota)
MCS	Sue Kinsey	UK Beachwatch surveys
IMARES in the Netherlands coordinate	Jan Andries Van Franeker	UK OSPAR EcoQO "Plastic in Fulmars"

Guidelines/ standardisation:

The current methodologies used can be found in Appendix 18 (Procedural guidelines for sampling and analysis of litter: beach, water column and seabed, and include:

Beach litter monitoring with MCS – 18a

Sea surface monitoring – 18b

Water column monitoring -18c

Collection of fulmars for the monitoring of the OSPAR EcoQO on plastic particles in seabird stomachs is undertaken by volunteers for analysis by IMARES (Netherlands). Further details of the methodology are given in the OSPAR EcoQO Handbook (2009).

It should be noted that these are likely to change as international standards and quality control procedures are further developed and agreed over the next few years.

Data record and storage:

Beach litter – standard procedure and data record templates are provided by the beachwatch programme and all data is held by MCS on main litter database; see MCS web site http://www.mcsuk.org.

Microplastic and seabed litter data is currently held by CSEMP CMAs. Seabed litter is submitted yearly to ICES Datras Database. There is a need for a centralised UK database.

Additional useful information.

Main Drivers

See http://ec.europa.eu/environment/marine/good-environmental-status/descriptor-10/index_en.htm

The 11 Regional Seas around the world are organizing and implementing regional activities on marine litter. These include: North-East Atlantic Region (OSPAR Convention), Baltic Sea (HELCOM Convention), Black Sea and the Mediterranean Sea.

6.7 Ocean Acidfication monitoring requirements

Strategy

Ocean Acidification is the change to the chemistry of the water caused primarily by an uptake in CO_2 from the atmosphere resulting in a decrease in pH of the oceans over time. Ocean acidification was not identified as a descriptor for Good environmental status under the Marine Strategy Framework Directive (2008/56/EC), it is included under Annex iii of the directive – Indicative lists of characteristics, pressures and impacts. OSPAR view ocean acidification as a key pressure on ecosystems in the future. Ocean acidifcation is not currently included in the CEMP and therefore data submission is not mandatory but can be submitted volunterarily by contracting parties.

http://ices.dk/sites/pub/Publication%20Reports/Expert%20Group%20Report/acom/2014/SGOA/sgoa_finalOSPAR_2015.pdf accessed 7/11/19

JAMP Guidelines for Monitoring Chemical Aspects of Ocean Acidification(Agreement 2014-03)x

Sample storage and holding times

Determinands	Ambient	Source
Ocean Acidfication (Total Alkalinity/Dissolved Inorganic Carbon)	Preserved with mercuric chloride. Stored in dark at room temperature. At least 3 years	Dickson, A. G., Sabine, C. L., and Christian, J. R. 2007. Guide to best practices for ocean CO2 measurements, PICES Special Publication, 3. Sidney, Canada. 191 pp. https://www.nodc.noaa.gov/ocads/oceans/Handbook_2007.html (accessed February 2020) Hydes, D. J., McGovern, E., and Walsham, P. (Eds.) 2013. Chemical aspects of ocean acidification monitoring in the ICES marine area. ICES Cooperative Research Report No. 319. 78 pp. ICES 2014. Final Report to OSPAR of the joint OSPAR/ICES Ocean Acidification Study Group (SGOA). ICES CM/2014/ACOM:67.141pp OSPAR 2014. JAMP Guidelines for Monitoring Chemical Aspects of Ocean Acidification https://www.ospar.org/work-areas/cross-cutting-issues/cemp (accessed February 2020) Newton J.A., Feely R. A., Jewett E. B., Williamson P. & Mathis J.,2015. Global Ocean Acidification Observing Network: Requirements and Governance Plan. Second Edition, GOA-ON. http://www.goa-on.org/docs/GOA-ON_plan_print.pdf. OSPAR MIME 2016 Document Reference MIME 16/4/2. Outcomes of the Quasimeme workshop

7. Data Submission

Contaminants, biological effects, fish disease, nutrients, chlorophyll and benthic community data collected for CSEMP are submitted to the the Marine Environmental Monitoring and Assessment National (MERMAN) database which is managed by the British Oceonagraphic Data Centre (BODC) and hosted by Defra. Data is passed through the appropriate AQC filters.

Participants are required to submit all data along with supporting Analytical quality control (AQC) data annually by 1st June of the year following sampling.

The MERMAN database is designed to accommodate all sampling information.

Data entering the MERMAN database must be of specified quality and for this reason inter-laboratory proficiency testing schemes have been initiated to support the analytical work associated with CSEMP.

7.1 Analytical Quality Control Reporting Requirements

The maximum tolerable error associated with contaminant data submitted to the CSEMP is specified in the Tables above in terms of a required limit of detection and a maximum percentage error or measurement uncertainty. Data meeting these requirements are considered to be 'fit for purpose'. The NMCAG group has developed a data quality assessment protocol (the data filter) that is used to screen chemical monitoring data on a fitness for purpose basis. The data filter involves an examination of both within-laboratory and interlaboratory measures of analytical performance. Details of external (QUASIMEME) quality control performance are also required by ICES for their assessments. Internal quality control information should be reported annually with the associated data. External (QUASIMEME) data should be submitted to the MERMAN database when it is received together with a note of the laboratory code.

Laboratories are assigned a score based on the parameters in Table 7.1 and are required to meet a minimum target for the data to be considered fit for purpose. Quality control assessments that fail to satisfy minimum criteria are recorded and associated monitoring data are flagged and excluded from assessments.

Table 7.1. Quality Control Parameters

CODE	DESCRIPTION
CACCRED	Accreditation status of the laboratory for the specified determinand
METCX	Method of chemical extraction
METOA	Method of analysis of parameter/contaminant (user defined)
DETLI	Detection limit value (use reporting units)
CONCH	Control chart basis (CRM, IRM,LRM,SRM)
CRMCO	Control chart reference material code (QUASIMEME samples acceptable here)
CRMMB	Control chart RM mean value - basis
CRMEV	Control chart expected value
CRMMV	Control chart mean value
CRMNM	Control chart reference material - number of measurements
CRMPE	Control chart reference material - period
CRMSD	Control chart reference material - standard deviation

8. Reporting

The 2nd NMMP report (MEMG, 2004) summarised the first 3 years (1999-2001) of data and NMMP2 data was used in the Defra report on the state of the seas (Defra, 2005; Defra, 2010). Data was used

for assessments for the Marine Online Assessment Tool (https://moat.cefas.co.uk, accessed Feb 2020) which were used to assess progress towards Good Environmental Status.

9. References

1 Dobson, J.E., M.J. Gardner, B.S. Miller, M.A. Jessep and R.H. Toft (1999), An Approach to the Assessment of the Quality of Environmental Quality Data. Journal of Environmental Monitoring. 1(1):91-95.

2 Marine Environment Management Group (2004). UK National Marine Monitoring Programme – Second Report (1999 – 2001). ISBN 0 907545 20 3

3 Defra (2005) Charting Progress – an Integrated Assessment of the State of UK Seas

4 Defra (2010) Charting Progress 2 – an assessment of the state of UK seas

5 OSPAR (2019) OSPAR Coordinated Environmental Monitoring Programme (OSPAR Agreement 2016-01) updated 2019. https://www.ospar.org/work-areas/cross-cutting-issues/cemp accessed 05/11/19

6 OSPAR (2012) JAMP Eutrophication Monitoring Guidelines: Benthos (OSPAR Agreement 2012-12) https://www.ospar.org/work-areas/cross-cutting-issues/cemp accessed 05/11/19

7 OSPAR (2018) CEMP Guidelines for Monitoring Contaminants in Sediments (OSPAR Agreement 2002-16, Revision 2018) https://www.ospar.org/work-areas/cross-cutting-issues/cemp accessed 05/11/19

8 OSPAR (2007) JAMP Guidelines for General Biological Effects Monitoring . Revised Technical annexes 2007 (OSPAR Agreement 2007-07) https://www.ospar.org/work-areas/cross-cutting-issues/cemp accessed 06/11/19

9 OSPAR (1999) CEMP Guidelines for Monitoring Contaminants in Biota (Agreement 1999-02., Revision 2018) https://www.ospar.org/work-areas/cross-cutting-issues/cemp accessed 06/11/19

10 Smedes, F. (1999). Determination of total lipid using non-chlorinated solvents. *Analyst*, *124*(11), 1711-1718.

11 OSPAR (2014) JAMP Guidelines for Monitoring Chemical Aspects of Ocean Acidification (Agreement 2014-03) https://www.ospar.org/work-areas/cross-cutting-issues/cemp accessed 06/11/19

12 OSPAR Eutrophication Common Procedure

https://www.ospar.org/work-areas/hasec/eutrophication/common-procedure

13 European Commision (1991) Urban Waste water Directive (91/271/EEC)

https://ec.europa.eu/environment/water/water-urbanwaste/index_en.html

14 European Commision (1991), The Nitrates Directive (91/676/EEC)

https://ec.europa.eu/environment/water/water-nitrates/index_en.html

Appendices

Appendix 1. General Sampling Record Requirements

Sampling Platform

The sampling platform /Ship codes, date, time and position must be recorded on all sampling occasions. The sampling occasion should be identified by a unique code defined by the responsible monitoring authority.

Ship Codes can be access directly from the ICES vocabularies list https://vocab.ices.dk (accessed Feb 2020). If the ship/platform you have used is not listed contact ICES.

Position fixing

Samples should be collected as close to the nominal position as possible using a suitable positioning system (see list). Since 1999 all new issue admiralty charts now use the WGS-84 chart datum and with the widespread use of GPS position fixing, sample locations should be logged using WGS-84 chart datum and the time recorded in GMT. The errors where other chart datum are used could be up to 120 m in some locations. Conversion algorithms are freely available from the Ordinance Survey to allow conversion of existing site locations to WGS-84. Further information on chart datum and conversion formulae can be found on the Ordinance Survey GPS site, http://www.gps.gov.uk/guidecontents.asp

Code	Description
DEC*	DECCA
DGP	Differential Global Positioning System
GPS	Global Positioning System

* Legacy system

Location

Sites are listed, with the organisations responsible for monitoring and details can be found in the MERMAN station dictionary list.

Appendix 2: Biological Effects Sampling Procedures

Sampling requirements and analytical protocols

Details of Biological Effects sampling procedures, requirements and analytical protocols are detailed in the following embedded Table:

Appendix 2.xlsx

Full details of current Biological Effect techniques included in UK monitoring can be found in the relevant ICES Techniques in Marine Environamental Science (TIMES) paper or reference;

Gibbs, P. E. (1999). **ICES TIMES No 24 (**DOI 10.17895/ices.pub.5050)**:** Biological effects of contaminants: Use of imposex in the dogwhelk (*Nucella lapillus*) as a bioindicator of tributyltin pollution. http://ices.dk/sites/pub/Publication%20Reports/Techniques%20in%20Marine%20Environmental%20Sciences%20(TIMES)/TIMES24.pdf accessed 18/12/2019

Martínez-Gómez, C., Bignell, J., & Lowe, D. (2015). **ICES TIMES No 56 (**DOI 10.17895/ices.pub.5084): Lysosomal membrane stability in mussels. http://ices.dk/sites/pub/Publication%20Reports/Techniques%20in%20Marine%20Environmental%20Sciences%20(TIMES)/TIMES56.pdf accessed 18/12/2019

Bean, T. P., & Akcha, F. (2016). **ICES TIMES No 58 (**DOI 10.17895/ices.pub.5086): Biological effects of contaminants: Assessing DNA damage in marine species through single-cell alkaline gel electrophoresis (comet) assay. http://ices.dk/sites/pub/Publication%20Reports/Techniques%20in%20Marine%20Environmental%20Sciences%20(TIMES)/TIMES58.pdf accessed 18/12/2019

Martínez-Gómez, C., and Thain, J. (2012). OSPAR Background Document on Stress on Stress (SoS) in Bivalve Molluscs. In: Davies, I.M. and Vethaak, A.D. (Ed.s). Integrated marine environmental monitoring of chemicals and their effects. ICES Cooperative Research Report no. 315, pp121-123. International Council for the Exploration of the Sea, Copenhagen, Denmark. 277pp.(DOI 10.17895/ices.pub.5403) http://ices.dk/sites/pub/Publication%20Reports/Cooperative%20Research%20Report%20(CRR)/CRR315.pdf accessed 18/12/2019

Stagg, R., McIntosh, A., & Gubbins, M. J. (2016). **ICES TIMES No 57 (DOI 10.17895/ices.pub.5085) :** Determination of CYP1A-dependent mono-oxygenase activity in dab by fluorimetric measurement of EROD activity in S9 or microsomal liver fractions. http://ices.dk/sites/pub/Publication%20Reports/Techniques%20in%20Marine%20Environmental%20Sciences%20(TIMES)/TIMES57.pdf accessed 18/12/2019

Ariese, F., Beyer, J., Jonsson, G., Porte, C., & Krahn, M. M. (2005). **ICES TIMES No 39 (DOI 10.17895/ices.pub.5063) :** Review of analytical methods for determining metabolites of polycyclic aromatic compounds (PACs) in fish bile. http://ices.dk/sites/pub/Publication%20Reports/Techniques%20in%20Marine%20Environmental%20Sciences%20(TIMES)/TIMES39.pdf accessed 18/12/2019

Bucke, D., Vethaak, D., Lang, T., & Mellergaard, S. (1996). **ICES TIMES No 19 (DOI 10.17895/ices.pub.5045) :** Common diseases and parasites of fish in the North Atlantic: Training guide for identification. https://www.ices.dk/sites/pub/Publication%20Reports/Techniques%20in%20Marine%20Environmental%20Sciences%20(TIMES)/TIMES19.pdf accessed 18/12/2019

Feist, S. W., Lang, T., Stentiford, G. D., & Köhler, A. (2004). **ICES TIMES No 38 (DOI 10.17895/ices.pub.5062) :** Biological effects of contaminants: use of liver pathology of the European

flatfish dab (*Limanda limanda* L.) and flounder (*Platichthys flesus* L.) for monitoring. http://ices.dk/sites/pub/Publication%20Reports/Techniques%20in%20Marine%20Environmental%20Sciences%20(TIMES)/TIMES38.pdf accessed 18/12/2019

Sturm, A., De Assis, H. D. S., & Hansen, P. D. (1999). Cholinesterases of marine teleost fish: enzymological characterization and potential use in the monitoring of neurotoxic contamination. *Marine Environmental Research*, *47*(4), 389-398. https://www.sciencedirect.com/science/article/abs/pii/S0141113698001275 accessed 18/12/2019

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Baršienė, J., Lyons, B., Rybakovas, A., Martínez-Gómez, C., Andreikenaite, L., Brooks, S., & Maes, T. (2012). OSPAR Background document on micronucleus assay as a tool for assessing cytogenetic/DNA damage in marine organisms. In: Davies, I.M. and Vethaak, A.D. (Ed.s). Integrated marine environmental monitoring of chemicals and their effects. ICES Cooperative Research Report no. 315, pp121-123. International Council for the Exploration of the Sea, Copenhagen, Denmark. 277pp. http://ices.dk/sites/pub/Publication%20Reports/Cooperative%20Research%20Report%20(CRR)/CRR315.pdf accessed 18/12/2019

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Hansson, T., Thain, J. E., Martínez-Gómez, C., Hylland, K., Gubbins, M. J., & Balk, L. (2017). **ICES TIMES No. 60 (DOI 10.17895/ices.pub.2903)** : Supporting variables for biological effects measurements in fish and blue mussel. http://ices.dk/sites/pub/Publication%20Reports/Techniques%20in%20Marine%20Environmental%20Sciences%20(TIMES)/TIMES60.pdf accessed 18/12/2019

Appendix 3: Procedural Guidelines for subtidal sediment sampling

Sampling Requirements and Analytical protocols

JAMP Eutrophication Monitoring Guidelines: Benthos (OSPAR Agreement 2012-12).

CEMP Guidelines for Monitoring Contaminants in Sediments (OSPAR Agreement 2002-16, Revision 2018) .

JAMP Guidelines for General Biological Effects Monitoring. Revised technical annexes 2007 (agreement 2007-07)

Webster,L., Roose, P., Bersuder, P., Kotterman, M., Haarich, M. and Vorkamp (2013) DOI 10.17895/ices.pub.5078 : DETERMINATION OF POLYCHLORINATED BIPHENYLS (PCB s) IN SEDIMENT AND BIOTA
http://ices.dk/sites/pub/Publication%20Reports/Techniques%20in%20Marine%20Environmental%20Sciences%20(TIMES)/TIMES53.pdf, accessed March 2020

Webster,L., Tronczynski, J., Bersuader, P., Vorkamp, K. & Lepom, P. (2009) DOI 10.17895/ices.pub.5071 : Determination of polybrominated diphenyl ethers (PBDEs) in sediment and biota.
http://ices.dk/sites/pub/Publication%20Reports/Techniques%20in%20Marine%20Environmental%20Sciences%20(TIMES)/TIMES46.pdf, accessed March 2020

Webster, L., Tronczynski, J., Korytar, P., BooiJ, K. & Law, R. (2009) DOI 10.17895/ices.pub.5070: Determination of parent and alkylated polycyclic aromatic hydrocarbons (PAHs) in biota and sediment.

http://ices.dk/sites/pub/Publication%20Reports/Techniques%20in%20Marine%20Environmental%20Sciences%20(TIMES)/TIMES45.pdf, accessed March 2020

Webster, L., Bersuader, P., Tronczynski, J., Vorkamp,K. & Lepom, P. (2009) DOI 10.17895/ices.pub.5069: Determination of Hexabromocyclododecane (HBCD) in sediment and biota.

http://ices.dk/sites/pub/Publication%20Reports/Techniques%20in%20Marine%20Environmental%20Sciences%20(TIMES)/TIMES44.pdf, accessed March 2020.

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http://ices.dk/sites/pub/Publication%20Reports/Techniques%20in%20Marine%20Environmental%20Sciences%20(TIMES)/TIMES48.pdf, accessed March 2020.

General

Samples should be collected from the same time of year at each site to minimise inter-annual variability due to seasonal fluctuations in the benthic community. It is recommended that samples are collected in late Winter/early Spring (Feb-May) to avoid juvenile recruitment, but this may vary between CMA's. Five replicate samples are collected for contaminants, five for benthic community

analysis and five for biological effects. Record the date of sampling, the location of each individual grab and sampling platform used (see Appendix 1).

Sampling equipment

A 0.1m^2 stainless steel Day Grab or Van Veen grab is recommended although alternative methods of sampling are acceptable (see list on ices web site www.ices.dk). The type of sampler and its diameter must be recorded.

Sediment Sampler Code

Code	Description	Code	Description	Code	Description
DA	Day grab	VV	Van Veen grab	OS	Other sampling device

Sample collection

Set the grab down on the seabed and close it as gently as possible to reduce the shock wave and sediment loss by premature rising. Keep the winch wire as vertical as possible to guarantee that the grab is set down and lifted vertically. Record the thickness of material at the centre of the grab to the nearest centimetre. Reject samples less than 7cm thick in mud and 5 cm in hard packed sands. Note the surface colour and the colour change with depth (as a possible indicator of redox state). Also note any smell (hydrogen sulphide, oil residues). Note a description of the sediment, to include important observations such as concretions, surface features, algae etc. Photographs can assist in this. Separate samples are required for macrobenthos, contaminants and biological effects analyses.

Macrobenthos sample collection procedure.

Take care not to spill the sample once the grab is on board.

Each grab should be sieved, stored and documented separately.

Empty the grab into a container, ensuring the interior of the grab is rinsed thoroughly into the same container to avoid loss of sample. Transfer portion by portion into the sieve as a water-sediment suspension.

Sprinklers or douches to suspend the sample from beneath the sieve are recommended to prevent clogging of the mesh.

Do not sieve the sample with a direct jet of water against the mesh to avoid damaging fragile animals.

Pick out fragile animals by hand during sieving to minimise damage. Also, pick out stones and large shells to avoid grinding effects on organisms and the sieve.

Flush off all material retained on the sieve into an appropriate receptacle, with water from below. Avoid the use of spoons and other tools.

Clean the sieve after each sample, to prevent clogging and ensure an equal mesh size throughout the entire sieving procedure.

Sieve samples to 0.5mm or 1mm according to the following requirements:

For estuarine sites use layered sieves to provide separate 1 mm and 0.5 mm fraction in the field or laboratory and analyse separately. Whether separated in the field or lab, the sieving method employed should remain consistent from year to year. Check to ensure the 1mm sieve is always on top. For intermediate and offshore sites, sieve samples to 1 mm in the field or laboratory. The >1mm fraction should then be reported. Whether separated in the field or laboratory, the sieving method employed should remain consistent from year to year.

The laboratories which have used a 0.5mm sieve in the past should continue to analyse the 0.5mm fraction where these data form part of a time series for temporal trend analysis.

All sieves should conform to BS 410 and be replaced at the first signs of any damage to the mesh.

Fix all material retained on the sieves in buffered 4% formaldehyde solution. In very organic mud, increase this concentration to 10% or more.

Stain may be added to the sample to increase sorting accuracy, especially for small animals, although this is left to the personal preference of the laboratory.

Record the method of sample preservation:

Code	Description	Code	Description
F4B	4% buffered formaldehyde (pH7-8)	FET	10% neutral buffered formalin + ethanol
FOR	formaldehyde	IMS	Industrial methylated spirit
NBF	Neutral buffered formalin 10%		

Physico-chemical sample collection procedure - *See JAMP Guidelines for monitoring contaminats in sediment*

Redox analysis (to be done in situ)

Redox should be measured as soon as possible after sample collection to avoid changes in condition of the sediment. For convenience a core (min 5cm) can be taken from the centre of the grab for redox analysis.

Redox is measured using a platinum electrode calibrated in Zobel's solution. Redox (mV) is measured at 0.5cm intervals by gently pushing the electrode through the sediment. The physical disturbance caused by this procedure changes the redox environment and makes the readings unstable so measurements should be taken after a standard period (1 min) or when the readings stabilise, whichever is sooner.

Appendix 4: Procedural Guidelines for the Collection and Processing of Fish Tissues for contaminants and biological effects.

JAMP Guidelines for General Biological Effects Monitoring. Revised technical annexes 2007 (agreement 2007-07)

CEMP Guidelines for monitoring contaminats in biota (Agreement 1999-02) Revision 2018.

Webster,L., Roose, P., Bersuder, P., Kotterman, M., Haarich, M. and Vorkamp (2013) DOI 10.17895/ices.pub.5078: DETERMINATION OF POLYCHLORINATED BIPHENYLS (PCB s) IN SEDIMENT AND BIOTA
http://ices.dk/sites/pub/Publication%20Reports/Techniques%20in%20Marine%20Environmental%20Sciences%20(TIMES)/TIMES53.pdf, accessed March 2020

Webster,L., Tronczynski, J., Bersuader, P., Vorkamp, K. & Lepom, P. (2009) DOI 10.17895/ices.pub.5071 : Determination of polybrominated diphenyl ethers (PBDEs) in sediment and biota.
http://ices.dk/sites/pub/Publication%20Reports/Techniques%20in%20Marine%20Environmental%20Sciences%20(TIMES)/TIMES46.pdf, accessed March 2020

Webster, L., Tronczynski, J., Korytar, P., BooiJ, K. & Law, R. (2009) DOI 10.17895/ices.pub.5070: Determination of parent and alkylated polycyclic aromatic hydrocarbons (PAHs) in biota and sediment.

http://ices.dk/sites/pub/Publication%20Reports/Techniques%20in%20Marine%20Environmental%20Sciences%20(TIMES)/TIMES45.pdf, accessed March 2020

Webster, L., Bersuader, P., Tronczynski, J., Vorkamp,K. & Lepom, P. (2009) DOI 10.17895/ices.pub.5069: Determination of Hexabromocyclododecane (HBCD) in sediment and biota.

http://ices.dk/sites/pub/Publication%20Reports/Techniques%20in%20Marine%20Environmental%20Sciences%20(TIMES)/TIMES44.pdf, accessed March 2020.

Ahrens, L., Vorkamp, K., Lepom , P., Bersuader, P., Theobald, N., Ebinghaus, R., Bossi, R., Barber, J.L. & McGovern, E. (2010) DOI 10.17895/ices.pub.5073. Determination of perfluoroalkyl compounds in water, sediment, and biota.

http://ices.dk/sites/pub/Publication%20Reports/Techniques%20in%20Marine%20Environmental%20Sciences%20(TIMES)/TIMES48.pdf, accessed March 2020.

4.1 General

The sampling platform, date, plus time and position at the beginning and the end of the trawl must be recorded (see Appendix 1).

Common dab (*Limanda limanda*) or flounder (*Platichthys flesus*) are the preferred species. Other acceptable species include plaice, cod and whiting. The fish should be collected outside their spawning period. Samples for contaminants and biological effects analyses should be collected on the same sampling occasion to facilitate impact assessment.

Sampling Equipment

Note the equipment used (see list).

If a 2m Beam Trawl is used:

Shoes should weigh a minimum of 25kg each to keep the trawl on the sea-floor and should to be wide enough not to cut into the sediment by more than 15cm during trawling.

Weight the footrope to keep the mouth open in use.

Use 40 mm mesh size with 10mm inner mesh size in the cod end.

The length of the net should be in excess of 4 metres.

Code	Description	Code	Description	Code	Description
AGT	Agassiz Trawl	BMT	Beam Trawl	BT15	1.5m Beam Trawl
GIL	Gill net	GOV	GOV Trawl	BT2	2m Beam Trawl
PEL	Pelagic Trawl	PRS	Purse Seines	SRN	Seines and ring net

Sample collection

Various equipment may be used. In the particular case of a beam trawl:

Shoot the trawl while the vessel is moving.

When shooting the trawl, introduce the net into the water cod end first and allow it to stream behind the vessel before the beam is lowered into the water. This avoids entanglement of the net around the beam.

For effective trawling the deployed trawl warp length needs to be 2.5-3 times water depth.

Tow in a direction contrary to the current. To maximise catch efficiency in estuaries, trawl on the ebb tide. Ideally fish should be collected from more than one tow.

Trawl at 3 knots over a distance of at least 1km.

Haul the trawl when the vessel reaches the end of the station and is steaming away from the site.

Number of Individuals in sub-sample

(i.e. 1 individual or number in pool), must be recorded

Stage of Development

Code	Description

AD	Adult
IM	Immature/sub-adult
JV	Juvenile
MX	Mixed for pooled specimens
NS	Not Specified

Condition of Specimen

Code	Description
G	Specimen Damage by Gear
M	Maturing gonads
N	Not ripe (stage of gonad development not known)
R	Ripe, about to spawn
U	Undeveloped Gonads

Number of diseases examined for

For checking program to check that non-diseased fish are reported correctly

Bulk Identification

(For individuals only)

If an individual (or parts thereof) has been analysed in one or more bulks, insert the SUBNO identification(s) of the bulk(s).

Sample processing

On retrieval of the trawl, open the cod end and deposit the catch in an appropriate container (sieve or tray). Take care that the container is large enough to lose none of the catch.

Sort the catch for target species. Return non-target species immediately. Ideally, dab collected are 20 – 30 cm and flounder 20 – 35 cm in length however there is no upper limit in length for analysis of fish disease and liver pathology.

Note the condition of target species. Note the presence, prevalence and position of any evidence of external disease according to ICES TIMES No. 19 (Bucke et al 1996). However, take care not to confuse damage inflicted during trawling with the effects of disease. Visibly damaged or fish in poor condition must not be selected for analysis.

Personnel must wear clean gloves when samples are taken from the net. The samples should be rinsed with clean seawater to remove any material adhering to the surface.

Samples should be dissected immediately after collection if possible. Where this is not possible samples of ungutted fish should be preserved by deep freezing, preferably shock freezing to -20°C or lower as soon as practicable after collection.

Samples for EROD analysis must be processed immediately to prevent degradation of the analyte. Remove the livers from the fresh fish and immediately freeze using liquid nitrogen.

Record method of storage (see list).

Code	Description	Code	Description	Code	Description
FR	Frozen	DF20	Deep freeze -20°C	DF70	Deep freeze -70°C

For each fish record length (mm) and weight (g) prior to dissection. Remove and weigh the liver and remove a fillet of muscle. When pooling samples an equivalent quantity of tissue must be taken from each fish e.g. 10% of the whole fish for muscle. Combine the livers and muscle tissue from 5 fish for analysis of contaminants.

Physico-chemical sample collection procedure - *See CEMP Guidelines for monitoring contaminats in biota*

Appendix 5 Procedural Guidelines for the Collection and Processing of Shellfish and Algal Tissues for Body Burden Analysis.

5.1 General

The blue mussel *Mytilus edulis* is the preferred tissue type for assessment of bioaccumulation. Where mussels are not present, brown seaweed *Fucus sp.* can be used however, seaweed is not a suitable matrix for assessment of bioaccumulation of trace organic compounds due to the low fat content. The size of mussel collected, time and location of sampling should be standardised to reduce variability. The JAMP specifies mussels should be the size range 3-6cm. It is recommended that the size of mussels collected at individual sites should be restricted to a narrow band within this size range. Samples should be collected are collected prior to spawning (usually late February – early March). Brown seaweed should be collected during the same period to avoid the reproductive cycle. Samples should be collected at the same time every year.

5.2 Sampling

Samples are normally collected from the shore by hand, if samples are dredged from the sea bed the type of dredge used should be recorded (see list)

Code	Description	Code	Description	Code	Description
BO	Boillet dredge	CH	Charcot dredge	EN	Endoume dredge
HAN	Hand collection	HM	Hamon grab	LS	Lister dredge
ND	Nodules dredge	NT	Naturalists dredge	RA	Rallier dredge
RD	Rock dredge	WD	Warren dredge		

Personnel collecting samples by hand should wear gloves.

5.2.1 Mussels

Samples should be free of fouling and bored shells. Collect 3 pooled samples each of at least 20 individual (50 is recommended to provide sufficient tissue for analysis of metals, organochlorines and PAH). At each station, the length of the collected individuals should be the same from year to year within a very narrow range (5mm). Transport samples back to the laboratory in clean containers, keep samples cool and damp using ice packs and seaweed. Samples should be depurated for 20 – 24 hrs to remove their gut content within 24 hrs of collection.

5.2.2 Macroalgae (Fucus sp.):

In the absence of mussels collect sufficient plant material to provide 5g wet weight of thallus for analysis (25-30 plants).

5.3 Sample processing

5.3.1 Mussels:

Scrape off extraneous material from the shells and scrub them clean using de-ionised water. Depurate mussels in clean seawater for 24 hrs to remove sediment from the gut or mantle prior to the analysis. Keep depurating mussels cool and aerate the water. Whole animals are best analysed immediately but may be deep-frozen.

5.3.2 Macroalgae (Fucus sp):

Split the sample into 3 replicates and dissect only thallus representative of the last years growth for analysis.

Scrub and wash the plants in de-ionised water. Whole plants can be refrigerated up to 10 days. Whole plants may be deep-frozen.

Record the method of sample storage

Code	Description	Code	Description	Code	Description
FR	Frozen	DF20	Deep freeze -20°C	DF70	Deep freeze -70°C

5.4 Sample preparation

Sort the mussels into pools of sufficient size to provide enough material for subsequent analysis (50 is recommended to provide sufficient material for trace metal, organochlorine and PAH analyses). Measure and record the length (mm) of each individual using calibrated callipers. Calculate the maximum, minimum and mean length of individuals in each pool.

Defrost frozen samples, open the shells and allow the body fluids to drain out. Remove the soft tissue from the shells taking care not to contaminate the sample (use ceramic scalpel or equivalent) and combine the tissue for a predetermined number of individuals (usually 50). Homogenise the soft tissue using either an agate ball mill or other contaminant free equipment. Do not allow samples to over heat during homogenisation as this may result in the loss of volatile contaminants. Clean the homogeniser between samples.

Dry and weigh the shells.

5.4.2 Trace metal samples

Freeze drying is the recommended procedure drying sediments for trace metal analysis for all metals except mercury which is volatile. Samples should be oven dried below 105°C to avoid loss of mercury. Freeze drying provides a free flowing powder whereas samples must be ground to a powder if they are oven dried. Samples should be ground using an agate pestle and mortar where necessary.

5.4.3 Trace organics

Trace organics samples should be chemically dried as other procedures can result in loss of analyte.

ICES sample preparation codes

Code	Description	Code	Description	Code	Description
DAIR	Air drying	DCHEM	Chemical drying	DFRZ	Freeze drying
DNO	Not dried	DOVN	Oven dried	DRY100	Drying >100°C
MAN	Manual milling	MMG	Mechanical milling	DRY99	Drying <100°C

5.5 Moisture Content

Determine the moisture content of the sample by weighing the sample wet and again when it is dry. Ensure the sample is completely dry before reweighing (the moisture content of mussels is typically around 80%).

5.6 Total Lipid

The total lipid content of shellfish samples should be determined using the Foppes Smedes or other suitable method.

Appendix 6. Guidelines for the analysis of macrobenthos in sediment samples

6.1. Macrobenthos

The basic premise of all macrobenthic sample analysis in the laboratory is that all specimens extracted from the samples are to be identified to the lowest possible taxonomic level and counted.

6.2 Biomass Measurement

Biomass can be expressed in a variety of ways (e.g. wet weight, dry weight, and ash-free dry weight). As the evaluation of ash-free dry weight (AFDW) ignores the contribution of inorganic material, water content and all non-living parts to the mass of an organism, it is considered as the most appropriate measure of living biological matter. However, as the determination of AFDW requires combusting specimens, thus removing any possibility for further taxonomic analysis, it is recommended that a non-destructive method be employed. This can be done by measuring wet weight, from which AFDW can be estimated by applying conversion factors, many of which can be obtained from the literature (e.g. Rumohr *et al*, 1987), backed up by local calibration where necessary.

This procedure applies to identified and enumerated invertebrate fauna extracted from marine and estuarine benthic samples. It is recommended that specimens are stored in preservative (70% IMS, 10% glycerol, 20% water) for a minimum of three months to allow for weight loss stabilisation prior to weighing (Rees *et al*, 1990).

It may not be possible to weigh each species separately; therefore it is recommended that species be weighed to family, or in some other appropriate grouping. However, as each project is inherently different, it will be necessary to change these groups according to the species present. For the temporal trend programme once a method has been established then biomass should follow this methodology consistently thereafter.

Record all appropriate information on a Biomass Data Sheet (see sheet at end of this Appendix as a guide).

Place weighing boat or crucible on balance pan and tare. The balance should be accurate to 0.0001 g.

Using forceps, remove all specimens to be weighed from specimen tube (tube dwelling species will need to be removed from their tubes) and rinsed in water. It is important to remove as much preservative as possible, otherwise problems may be experienced during weighing.

Subsequently place specimens on dry piece of high absorbency paper and move them around until no wet patch is left behind, ensuring that undue pressure is not applied. Use further paper if necessary. A new piece of paper should be used for each taxon.

When dry, immediately transfer specimens to tared container on the balance, (the water will stop further evaporation of preservative from the specimens).

Follow operating instructions for balance and record the weight on the data sheet after 30 seconds has elapsed. Record the weight of animals to 0.0001 g. However, where a taxon weighs less than this, record the weight as 0.0001 g.

Remove specimens from container and return to specimen tube. Re-tare the container and water before weighing the next taxon.

Guideline Biomass Data Sheet

NB Complete in ink and initial and date any corrections.

Survey:	
CSEMP Station code & Sample/rep no:	
Sample Date:	
Date of Weighing:	
Biologist:	

Taxon	Blotted Wet Weight (mg)	AFDW (mg)	Comments
General Comments:			

Appendix 7. Procedural Guidelines for sampling and sample preparation of waters for nutrient analysis

Revised JAMP Eutrophication Monitoring Guideline: Oxygen (Agreement 2013-05)Revised JAMP Eutrophication Monitoring Guideline: Nutrients (Agreement 2013-04)

CEMP Guidelines: Phytoplankton monitoring (OSPAR Agreement 2016-06)

JAMP Eutrophication Monitoring Guidelines:Benthos(OSPAR Agreement 2012-12)

Revised CEMP guidelines for coordinated monitoring for eutrophication, CAMP and RID

(OSPAR Agreement 2016-05)

https://www.go-ship.org/HydroMan.html, accessed March 2020

7.1 Background.

Nutrient samples should be collected in Winter (November – end February) to minimise variability due to uptake by algal growth and remineralisation of senescent algae. Continuous monitoring data has shown high variability in nutrient concentrations due to fluctuations in freshwater runoff, seawater temperature, current patterns and insolation. Ideally continuous data should be collected at CSEMP sites or at least monthly samples. Axial transects should be collected in estuaries and coastal locations influenced by freshwater inputs to allow normalisation of the data to salinity.

7.2 Sample location.

The sample location should be tested for or be of known homogeneity by collecting replicate samples, studies by some authors have suggested that up to 50% of the overall uncertainty may be due to the initial sampling carried out on site. Depth profiles should be collected at stratified sites.

7.3 Sample containers.

Sample containers should be clean. Glass bottles may leach silicate and phosphate into samples and therefore it is recommend that Polyethylene or polypropylene bottles be used for these. Glass bottles are recommended for the storage of water samples for ammonia analysis. The sampling bottles and storage containers should always be rinsed with sample water before filling.
Tests should be performed on new containers to verify that the material and construction of the container does not give rise to unacceptable changes in the sample over the stated storage time and under actual storage conditions.

7.4 Sampling.

Reversing bottles or air displacement samplers should be used to collect samples from discrete depths in stratified waters and where depth profiles are required; water samples can also be pumped on board for a specified depth. Record the sampler type (see list).

Code	Description	Code	Description	Code	Description
AZT	Azlon-type sampler	GFL	Go-Flo sampler	LW	Limnos sampler
NAN	Nansen sampler	NSK	Niskin bottle	PMP	pump
ROS	Rosette sampler				

All sampling equipment should have well documented cleaning procedures. Where sampling is carried out from survey vessels, procedures should take account of the risks of potential contamination by the vessels overboard discharges.

7.5 Pre-treatment.

Pre-treatment prior to storage may be required to remove suspended matter that has potential to bias the determination, this will be dependant on the water body and individual CMAs should determine whether pre-treatment is required. Phosphate for example can be leached from particulate matter once exposed to the chemicals used for the analysis. However, unnessary pre-treatment of samples should be avoided in areas where there is limited suspended material to prevent advantageous sample contamination.

There are currently no clear recommendations for filter types or material. Encapsulated filters having cellulose nitrate membrane and glass fiber pre-filter can offer advantages where a high particulate loading is present in the samples.

Record the method of filtration (see list)

Code	**Description**	**Code**	**Description**	**Code**	**Description**
FCN	Cellulose nitrate filter	GFC	Glass fibre cartridge	GFF	Glass fibre filter
MF120	1.2 um membrane Filter	MF20	0.20um membrane filter	MF45	0.45um membrane filter
MF80	0.80um membrane filter	N40	0.40um nucleopore filter	SAR	Sartorious filter

Excess pressure or vacuum should be avoided to reduce the risk of cell rupture and release of nutrients from biological material.

Tests should be performed on a regular basis to verify that the material and construction of the filter medium does not give rise to unacceptable contamination of the sample as a result of filtration.

Because filtration removes nearly all of the particulate matter, some of which may breakdown or leach nutrients once exposed to the chemicals used during analysis, hard criteria should be used to determine the need for filtration. It would not be acceptable to filter a sample on one occasion but not another purely as a result of visual appearance. Filtration at the time of sampling is therefore the preferred method.

7.6 Preservation.

Analysis immediately after sampling is the preferred procedure for determining dissolved nutrients in sea water however, due to operational constraints, samples must sometimes have to be stored and transported pending analysis.

Historically chemical preservatives, mercuric chloride or chloroform have been used to inhibit biological activity, but due to the environmental unacceptability, their use has almost ceased. Deep freezing is now the favoured option. Record the method of sample preservation

Code	Description	Code	Description	Code	Description
FR	Frozen	DF20	Deep freeze -20°C	DF70	Deep freeze -70°C
CHL	chloroform				

Due to the expansion of sea water during freezing, sample containers should not be completely filled to allow room for expansion and prevent subsequent loss of the analyte, from the sample.

7.7 Transport

Where freezing is employed bottles should not become brittle and a large as possible container is preferred to ensure that there is sufficient thermal mass to maintain the sample in a frozen state during transport. Miniature data loggers are now easily affordable, and tests can be performed to verify the integrity of samples during transport and storage, particularly where third parties are involved.

Appendix 8. Procedural Guidelines for sampling and sample preparation of waters for Chlorophyll analysis.

JAMP Eutrophication Monitoring Guidelines: Chlorophyll a in Water(OSPAR Agreement 2012-11)

ICES TIMES Determination of chlorophyll in seawater in peer review a draft is available on request via pamela.walsham@gov.scot

8.1 Background.

Chlorophyll a is the most frequently measured parameter in water column samples that is used as an indicator of biomass. Samples should be collected as frequently as possible during Summer months (May, June, July, August, September). Sampling strategies should take account of the heterogeneous distribution of chlorophyll in the water column. Although this guide focuses on discrete samples that will be subject to laboratory analysis, most of the guidance can be used where discrete samples are taken for other methods of analysis.

8.2 Sample Transport

Where transport of filter papers are necessary, steps should be taken to maintain the filters in a frozen state since the thermal mass of these samples alone may not be sufficient.

Samples can be frozen in blocks of ice using sea water or sandwiched between freezer packs [1] of sufficient size.

Portable low voltage freezers offer a suitable alternative.

[1]. Quasimeme Laboratory performance studies Round 17/19 DE-6 Chlorophyll a in sea water.

Appendix 9. Procedural Guidelines for sampling and sample preparation of waters for the determination of trace contaminants

Principles

Concentrations of contaminants in estuarine and coastal waters are commonly in the low µg/l to ng/l range. Therefore great care must be taken when sampling to avoid contamination of the sample. Contamination can arise from a number of sources including the sampling platform, the sampling equipment and the surface microlayer.

The guiding principles of handling samples for trace analysis are as follows:

Define the sampling / sample handling procedure – it is essential to include in the sampling procedure details of all steps that might involve a risk of contamination of the sample, or loss of determinand from it. These details include the type – manufacturer, specification and model - of all items of equipment used. If any changes in equipment are introduced, the sampling procedure should be considered to have been changed and further suitability tests on it should be carried out. Issues of particular potential importance are the type of filters used and the cleaning procedure adopted for sampling and filtration equipment;

Test the procedure to demonstrate that it is fit for purpose – tests to show adequate control over contamination during sampling are an essential part of the evidence that monitoring data are valid for their intended use. Such tests include the use of field blanks and field spikes3;

Always follow the procedure - and always include ongoing tests (field blanks) to demonstrate continued satisfactory operation;

Record any changes and review fitness for purpose.

It is not desirable to specify the exact details of suitable procedures for sampling, sample pretreatment and preservation – many options are possible and their suitability depends on a number of factors, including:

- The determinand - different determinands are more or less liable to contamination, according to the extent to which they are present in dust etc;
- The concentration level of interest – ultra trace analysis (<1 ng/l) might required more rigorous contamination control than determinations at the µg/l level;
- The nature of the sample – some types of waters (eg those high in organic matter are less stable than less contaminated waters);
- The sampling location and circumstances – contamination might be less likely if the local environment is clean and well controlled (we might compare sample handling in the laboratory with the same operation carried out on a boat);
- The skill and awareness of contamination risk of the sampling staff.

3 http://water.usgs.gov/owq/FieldManual/chapter5/pdf/5.3.2.pdf (accessed 29/4/2010)

In the end, whatever procedure is adopted, its acceptability must rely on the provision of data to demonstrate adequate control over contamination and sample stability. Without this, no sampling procedure, however elaborate, can be regarded as satisfactory.

Some basic recommendations.

Contaminant concentrations in saline waters are assessed against national and international Environmental Quality Standards (EQSs). EQSs for metals are specified in terms of "dissolved" concentrations. Dissolved metal is operationally defined as the portion of the total metal in the sample that remains after the sample has been filtered through a 0.45µm pore size filter membrane or equivalent. Samples should be filtered as soon as possible after collection, prior to preservation by acidification and subsequent analysis. No filtration is required for trace organic substances because their EQSs are based on total contaminant content. Samples for the determination of trace metals samples are usually collected in acid washed bottles; samples for the determination of trace organic substances are collected in solvent washed or baked glass bottles or other bottles that have been shown to be appropriate by testing.

Trace metal samples can be collected by hand (wearing gloves) or pumped on board ship using peristaltic or bellows system so that the sample avoids contact with metal. Samples must be collected from beneath the surface microlayer, preferably from a depth of 1m or beneath the ship's hull.

Filtration apparatus for trace metals should be plastic and is usually cleaned with acid (eg 10% v/v nitric acid) solution prior to use; the equipment should be triple rinsed with ultra pure deionised water. Once washed, filtration equipment should be double bagged in zip sealed polythene bags. Samples are usually filtered through acid (10% nitric acid) washed, triple rinsed 0.45 µm (min) filters (fluorocarbon, polycarbonate or cellulose nitrate). Filtration of samples for the determination of mercury using glass or quartz fibre filters, although it has been used previously, it is not recommended because such filters do not meet the WFD specification for separation of dissolved metal. The careful application of cleaning procedures to plastic filters has been shown to achieve blank mercury concentrations of less than 0.5 ng/l 4 5.

Procedural filter blanks should be included with each batch of analyses to check contamination arising from the filtration procedure. Filtration should take place in an area that is as clean and dust free as possible.

Preparation of bottles

500 ml polyethylene bottles are generally used for all metals except mercury. Samples for the determination of mercury should be collected in bottles that prevent ingress or egress of mercury vapour. Suitable bottle materials include glass, Teflon® or polyethylene terephthalate copolyester, glycol-modified (PETG). Bottles are stored filled with 1% nitric acid. Bottles are emptied before use and rinsed three times with ultra pure water. Trace organic samples are collected in solvent rinsed

4U.S. Geological Survey, National field manual for the collection of water-quality data: U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chaps. A1-A9, available online at http://pubs.water.usgs.gov/twri9A.

http://water.usgs.gov/owq/FieldManual/chapter5/pdf/5.6.4.B_v1.0.pdf (accessed 29/4/2010)

5 http://www.swrcb.ca.gov/water_issues/programs/swamp/docs/qamp/appxd_ultracleansamplefiltration.pdf

glass bottles with PTFE inserts in the caps. Rinsing of cleaned bottles or other sampling apparatus with the water sample to be collected is not recommended.

Sampling

Samples can be collected by hand from small boats where practical, but where larger hulled boats are used trace metal samples are usually pumped aboard; trace organic samples are collected using a weighted metal sampling apparatus.

Trace metals - Clean plastic powder free gloves should be worn when collecting the samples. The tubing used to pump the sample on board must be metal free and not come into contact with any metal surfaces. Peristaltic or PTFE bellows pumps are suitable for this purpose. The tubing is weighted and deployed over the side of the boat below the surface of the water. Samples must be collected from water that the vessel has not contaminated, i.e down wind of the boat. When using a pumping system it is important to ensure that the tubing is well flushed before collecting the metals samples. Trace metal samples should be filtered as soon as possible after collection to avoid changes in metal partitioning within the sample bottle.

Trace organics - Sampling for the determination of trace organic substances should be taken at approximately 1m below the surface – generally using a proprietary sampling device. Sample bottles are usually filled to the shoulder if solvent is to be added for the purpose of for preservation/extraction. Samples collected for determination of volatile substances should be filled to the brim, avoiding entrainment of air bubbles.

Sample preservation

Trace metals - Samples are preserved by acidification to a pH value of less than 2 (0.1% v/v nitric acid). Mercury is an exception, where other methods of preservation are required. In the case of seawater samples of low natural organic matter content acidification to pH less than 1 has been shown to be satisfactory[6]. For less “clean” samples, for example those from estuaries, preservation by addition of an oxidant (acid dichromate or acid bromate/bromide) might be required[7,8]. Samples can be stored at room temperature.

Trace organics - Samples may be preserved by the addition of an appropriate volume of solvent if this is compatible with the analytical method to be used; storage at reduced temperature (ca 4 ◦C) is advisable.

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6 Stability of Mercury in Seawater Samples. Gardner, M.J. and Gunn, A.M. Analytical Communications, September 1997, 34 (245–246).

7 Feldman., C., (1974) Preservation of dilute mercury solutions. Anal. Chem., 46 (1), pp 99–102.

8 Defra (1996) General Principles of Sampling Water and Associated Materials (second edition) 1996 HMSO ; ISBN 011752364X

Appendix 10: Data Submission

The Marine Environment Monitoring and Assessment National Database (MERMAN) is an application supporting the Clean and Safe Seas Evidence Group (CSSEG) in monitoring the UK waters. It integrates chemistry, biology and biological effects data from the participating agencies and is used for national and international reporting.

The database is accessible to all via a portal from the Defra intranet. From the portal the users submit data using templates and can extract data using a software tool 'Business Objects'.

The data are submitted to the MERMAN database using standardised MS Excel spreadsheets where the users collate their annual submission. They then use the MERMAN portal to load the data into the database. The data are automatically validated before they are loaded into the database giving users the benefit of knowing that their data fulfils the set criteria. The data submitter receives an email confirmation of the submission status and if there are any errors in the data, these are detailed in an error report.

Business Objects is a web based reporting package that allows users to run standard reports as well as easily build their own queries which allow them to extract the quality assured data from the database. One of the key standard reports is the UK's annual data submission of OSPAR data to the ICES database; the system compiles the data according to the ICES reporting requirements (v3.2) and the data are submitted to ICES without any manual processing. A number of customised reports have also been developed to allow users to extract raw data and interpreted information easily.

Access to MERMAN is by recommendation of the Responsible Officer of each CMA. The user will then be sent user names and passwords to submit and extract data.

Contaminants in water, sediment and biota and associated AQC data from the previous monitoring year must be submitted to MERMAN by the 1st June each year. Following the submissions there are a series of checks completed by BODC and the data submitted to ICES by the 1st September.

The following documents are available from the BODC MERMAN webpages to help users http://www.bodc.ac.uk/projects/uk/merman/

- A MERMAN user guide which gives further details of the submission process, data extraction and obligations
- A report on how to use and build queries in Business Objects using MERMAN data as examples
- A report detailing customized reports for MERMAN which can be extracted using Business Objects
- The MERMAN station dictionary

For further information contact the MERMAN management team on merman@bodc.ac.uk or 0151 7954861

Appendix 11 The CSEMP Station Dictionary

Background

MERMAN is a national database which holds and provides access to data collected under the Clean Safe Seas Environmental Monitoring Programme (CSEMP) — formerly the National Marine Monitoring Programme (NMMP). CSEMP itself provides a coordinated approach to environmental monitoring in the UK's coastal and estuarine areas. The programme fulfils the UK's commitment to European directives including its mandatory monitoring requirements under the Oslo and Paris Convention (OSPAR) Joint Assessment Monitoring Programme (JAMP).

The general aims of CSEMP are to

1. Detect long-term spatial and temporal trends in physical, biological and chemical variables at selected estuarine and coastal sites
2. Support consistent standards in national and international monitoring programmes for marine environmental quality
3. Establish appropriate protective regulatory measures
4. Coordinate and optimise marine monitoring in the UK
5. Provide a high quality chemical and biological data set from the UK's marine environment

Station Naming Protocol

Historically, most data in the CSEMP (or NMMP) have come from a *fixed station* monitoring design, in which several samples are taken from a small fixed area at the same time each year. However, the last NMMP report showed that the power of this programme for detecting temporal trends was often poor. Further, the programme could only support very localised environmental assessments.

The ensuing redesign proposed the monitoring of larger regions. This would lead to greater power, as local spatial variation would be controlled, and would support more meaningful (less localised) environmental assessments. Larger regions would be monitored by stratified sampling, either random sampling within strata (sub-regions), or sampling a network of fixed stations representative of the strata within a region.

This document proposes a protocol for naming monitoring stations in MERMAN. Ideally, the protocol should have a clear connection between sampling design, data storage, and data analysis. However, the protocol also needs to cope with drivers such as the Water Framework Directive (WFD) and Charting Progress, and is thus something of a compromise. It will be suitable for assessing our monitoring data, but will require some post-processing of data.

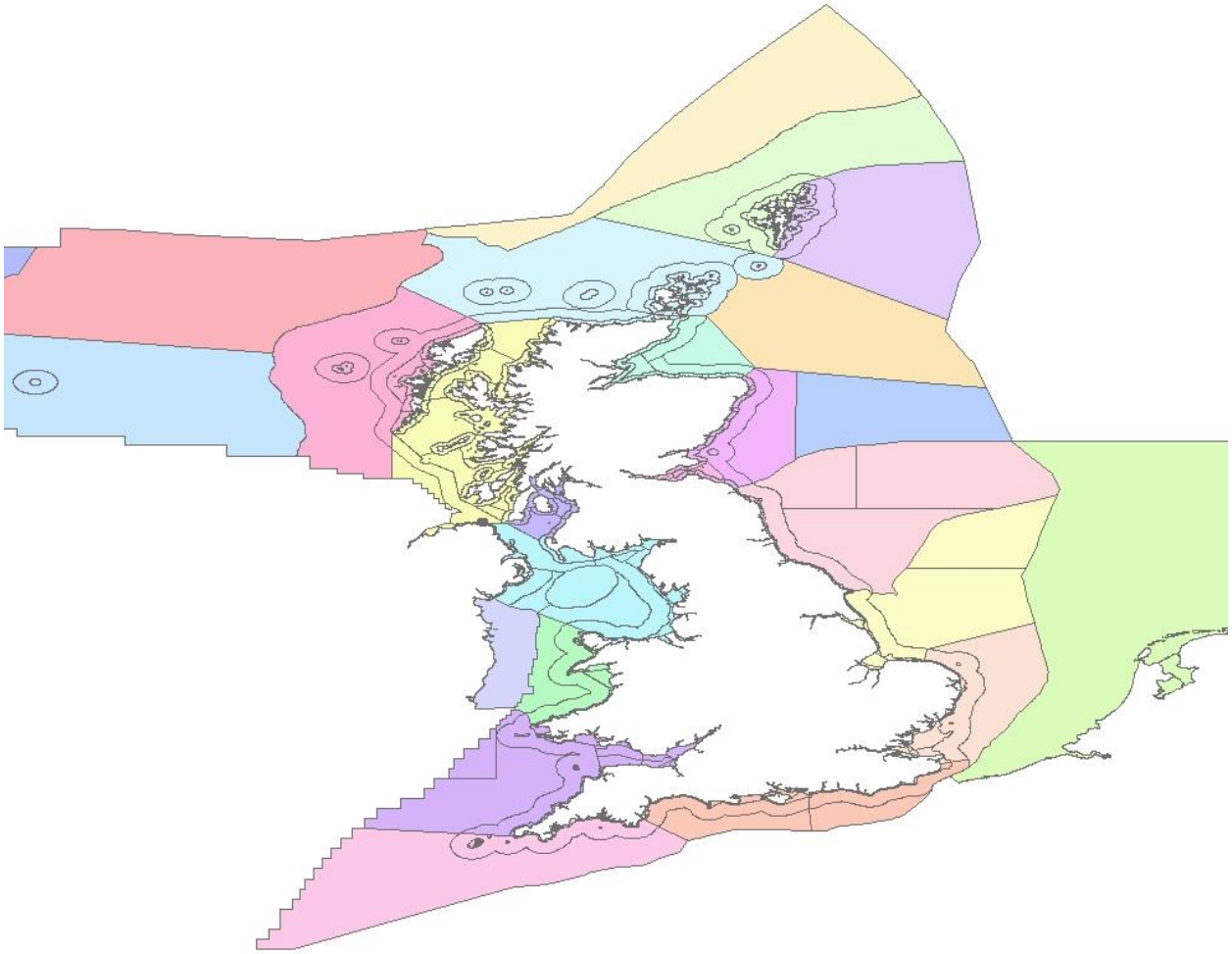
There are three main elements to the protocol:

1 UK territorial waters will be divided into *regions* and *strata*. Regions are aligned with the regional seas used for Charting Progress and strata are aligned with WFD water bodies. Thus, all CSEMP data can be allocated to a region and a stratum.

2 All samples will be allocated to a *sampling strategy* that describes the method of data collection. Four main sampling strategies are recognised in the CSEMP described below

3 Each station name must be unique for ICES reporting purposes (there is no concept of a region of stratum in the ICES database). The station field is a character string with at most 20 characters.

The regions used for CSEMP are shown in the figure below shaded differently. The stratum are npot shaded but can be identified by boundaries.



The four sampling strategies are:

- fixed (FI) a sample taken at random from a fixed station (a pre-defined, usually small, area within a strata)
- stratified random (SR) a sample taken at random within a strata

- stratified fixed (SF) a sample from one of a network of fixed stations that give 'good coverage' and are representative of a strata
- opportunistic (OP) a catch-all for other sampling strategies

Historically, most CSEMP data would come from fixed stations, based on the same time, same place monitoring mantra. Stratified random and stratified fixed will be reserved for data that come from core CSEMP monitoring activities and have been designed accordingly. At present, this will be restricted to contaminants, biology, and effects in sediment, and maybe some fish and nutrient monitoring. Examples of opportunistic sampling might include nutrient measurements taken along a cruise track, or one-off surveys. At the analysis stage, the opportunistic tag provides due warning that the data do not come from a standard design and detailed scrutiny is required to make sense of them!

It is important that the sample design field is completed correctly for submission of data to MERMAN else it is possible that the data will be misinterpreted.

Station names

A meaningful unique station code is constructed by concatenating (and abbreviating) the region and stratum name and appending the matrix and a number.

Adding new fixed stations to the MERMAN station Dictionary.

Only new fixed stations need to be added to the Station Dictionary. A template for adding new stations is available as the last sheet in the station dictionary available at http://www.bodc.ac.uk/projects/uk/merman/project_specific/ . This must be completed and sent to merman@bodc.ac.uk who will then generate the station codes for you.

Generating opportunistic station codes

BODC hold the master GIS file for strata and regions from which opportunistic station names can be generated from lat and long pairs. CMAs should send a xls or csv file to BODC who will complete the analysis and will aim to send it back with the opportunistic station names within a few days.

The station dictionary is available for download from http://www.bodc.ac.uk/projects/uk/merman/project_specific/

Appendix 12: Procedural Guidelines for sampling and analysis of litter; beach, water column and seabed (Note this section is under review by the litter group)

18a: Protocol for volunteer Beach Litter Surveys

For insurance purposes, groups must be registered with the Marine Conservation Society (MCS) and follow MCS guidelines before carrying out any surveys.

https://www.mcsuk.org/get-active/beachcleans

Finding a beach

- Ensure the beach is not already surveyed by an existing group – go to www.mcsuk.org to find out existing beaches
- Get permission from the beach owner to carry out the survey
- Register with MCS and carry out a risk assessment on the beach

Organising volunteers

- Responsibility – you must remind everyone taking part that they are involved at their own risk and that they should have read the risk assessment.
- Young volunteers (Under 18) should have provided a completed Parental Consent form and identified the adult who will be responsible for them.
- Safety guidelines – go through safety guidelines thoroughly and check everyone has come prepared.
- Risk assessment – run through the risk assessment. Identify and point out any hazards, e.g. mudflats, potential for rock falls from cliffs.
- Make sure they know who to go to in an emergency – where to get First Aid and where the emergency telephone is.
- You should be carrying out your Beachwatch on a falling tide - ideally an hour after high tide. Ensure everyone is aware of the low-tide time so they are not caught out when it begins to rise. Also let them know the weather forecast for the day, so they know what to expect.
- Divide them into teams of between 2 to 5 people. Adults responsible for accompanying young volunteers must be teamed with them.

- Allocate roles – one person should record the data on the sheet, one person hold the bag, whilst others collect rubbish.

- Allocate a section beach to each survey team. Divide your marked out area between the groups to ensure it is all covered

The Survey

- Survey area – the survey should be carried out between the current high water mark (the strand-line) and the upper edge of the usable part of the beach (e.g. up to the edge of the dunes or promenade).

- Survey length - the survey should be carried out along a stretch of beach a 100m in length (unless the beach is less than 100m). It is essential that you record the total length and width of beach surveyed.

- Every item of litter within the survey area should be collected and recorded. We can't accept estimates or averages for data analysis.

- Identifying litter - the survey form is divided into sections based on material, litter type and origin. Ensure that volunteers recording the data understand what every item on the volunteer survey sheet is, for example, strapping bands are the strong strips of plastic used for securing boxes and containers; cotton buds washed up on the beach often look like plastic lolly sticks; remains of condoms often look like elastic bands.

- Tally counting – using a tally to count items in fives (i.e. ~~IIII~~) for the items you collect. The total for each category should be noted in the right-hand column (see volunteer survey sheet).

- 'Other' items - items that do not fit into any of the categories listed on the volunteer survey sheet should be entered into the 'other' category and a brief description of the item given.

- Unusual litter - volunteers should note any particularly unusual items, to be entered onto the survey summary form by the Organiser.

- Foreign litter - volunteers should note any litter obviously originating from abroad. Organisers should transfer these details to the survey summary form.

- Stranded, entangled or dead marine animals - volunteers should note any injured or dead marine mammals found.

- Other pollutants - volunteers should note the presence of oil or tar on the volunteer survey sheet, and any other pollutants on a separate sheet of paper. If you encounter a pollution event or an algal bloom, contact the Environment Agency hotline on 0800 807060.

- Traceable litter - note any litter items that are directly traceable to an individual or company. If you do come across a form of pollution that can be traced to a specific source - such as a cup from a cruise ship, a balloon with a company logo, or a chemical container with a codename on it you should: 1. Take down the serial number, company logo or any other indication of who the polluter

was. If possible, photograph the item found. 2. Send copies of any photographs, together with information on where and when the item was found and any further details, to MCS.

- At the end of the clean send the survey summary form to MCS where it will be entered onto the Litter database. Groups receive a feedback form detailing their clean – amounts, sources etc

18b: Protocol for monitoring offshore water column / seabed litter

These are guidelines for monitoring litter using beam trawls or otter trawls. Samples are usually taken opportunistically alongside sampling for other purposes e.g. benthos or fish. There are several variables which must be considered and must be regarded as essential for data interpretation (see below). In general, the amounts of offshore litter collected are small and as such it is recommended that all items are described and measured and in addition, observations made on colonising organisms. The effort involved is small – typically 5-10 minutes after each trawl deployment.

For opportunistic sampling the following information should be recorded:

1 Date:
2 Location name: e.g. CSEMP name or Tyne estuary, Liverpool Bay
3 Gear used: e.g. 2m beam, Granton, GOV trawl
Width of trawl; ie distance between shoes on beam or across headline
Height of trawl; ie height of beam or maximum height of headline
4 Mesh size; knot to next knot in cm
If liner used; what was the size of the liner
5 Time of sampling: GMT
Time shot
Time hauled
6 Position:
Position of shooting: ideally when trawl hits the bottom - Lat Long
Position of hauling: ideally when trawl comes off the bottom
7 The tow:
Time of tow (when started and when finished)
With the tide – across the tide – against the tide
Velocity of tide in knots
Speed of tow (or ship) in knots
Water depth
8 General information:
Are there known gyres in the sampling area
Prevailing weather at time of sampling and prior to sampling if available
Wind and wave heights
9 Litter record:
Record all pieces of litter using the tables for codes for litter type and size.

Table XXX Litter Type

A: Plastic	B: Metals	C: Rubber
A1. Bottle	B1. Cans (food)	C1. Boots
A2. Sheet	B2. Cans (beverage)	C2. Balloons
A3. Bag	B3. Fishing related	C3. bobbins (fishing)
A4. Caps/ lids	B4. Drums	C4. tyre
A5. Fishing line (monofilament)	B5. appliances	C5. Glove
A6. Fishing line (entangled)	B6. car parts	C6. other
A7. Synthetic rope	B7. cables	
A8. Fishing net	B8. other	
A9. Cable ties		
A10. Strapping band		
A11. crates and containers		
A12. diapers		
A13. sanitary towel/tampon		
A14. other		
D: Glass/ Ceramics	**E: Natural products**	**F: Miscellaneous**
D1. Jar	E1. Wood (processed)	F1. Clothing/ rags
D2. Bottle	E2. Rope	F2. Shoes
D3. piece	E3. Paper/ cardboard	F3. other
D4. other	E4. pallets	
	E5. other	

10 Related Size Category

Table XXX Litter Size

A: <5*5 cm= 25 cm^2
B: <10*10 cm= 100 cm^2

C: <20*20 cm= 400 cm^2

D: <50*50 cm= 2500 cm^2

E: <100*100 cm= 10000 cm^2= 1 m^2

F: >100*100 cm = 10000 cm^2= 1 m^2

11 Where possible identify origin of litter – e.g. :

The Household/Consumer class is composed of the subcategories plastic bottles, sheeting and bags. The Fishing/Shipping class comprises the subcategories fishing net, fishing line (monofilament/entangled), synthetic rope, cable ties, strapping band, crates and containers.

12 Record of colonising organisms:

For each piece of litter record generically the colonising organisms either as individuals or as percentage coverage e.g. when species can be identified these should be recorded as % coverage of the item.

- Bryozoans
- Hydroids
- Barnacles
- Sea anemones
- Algae
- Other as appropriate

13 Assessment of data:

Basic requirement for reporting is: - "number of items per km^2 of seabed"

Other data collected above is used for interpretation.

Colonisation data could be used for indicating residence times, behaviour of plastic and identifying non-indigenous species.

Example of record sheet for water column / seabed litter

TO be added

18c: Protocol for monitoring microplastic litter

1. **At the sea surface: manta trawl or similar (CEFAS)**
2. **At the sea surface: neuston net with a PVC filtering cod end (MSS)**
3. **In sediments: grab sampling**

1. At the sea surface: surface trawl eg manta trawl or similar

A. Sample collection and storage

Rinse trawl net from outside to the inside through the net with hose or bucket to make sure the complete sample is concentrated in the cod end. Don't rinse the sample from the top through the opening of the net.

a) Equipment:-

Large bowl, squirt bottles, sample jar, spoon, tweezers, pens, pencils, labels and a preservative. Samples will be stored frozen.

b) Processing steps

i.) Remove the cod end over a bucket, as a precaution to catch any spillage.
ii.) You will need a We freeze the samples to store them.
iii.) Pour sample into a large bowl.
iv.) Invert the cod end and scrape left over sample from the inside of the cod end into the large bowl using the spoon. Rinse the spoon into the bowl.
v.) Using very little water, squirt the surface of the cod end so any leftover sample falls into the bowl.
vi.) Pour entire sample into the sample jar, then freeze. A funnel may make it easier to pour sample into jar. More than one jar may be needed for a sample.
vii.) Label the outside of the sample jar and the lid with the sample number, date, latitude and longitude. Make sure the jars are labelled to include how many jars make up the sample. For example, if the sample needs to be stored in three jars, the labels should include 1 of 3, 2 of 3, and 3 of 3. Use waterproof marker for labels.

Include label in sample. The internal label should be a strip of waterproof paper with information written in #2 pencil. This label will contain the same information as the external labels.

B. sample analysis

a) Equipment:

i.) One 5mm sieve to divide sample in two size classes (>5mm<)
ii.) Two large bowls to hold each size class (2 liter size)
iii.) Two large Petri dishes for sorting samples.
iv.) Tweezers and a fresh water wash bottle full of water to separate plastic from other material
v.) Two jars or vials for holding two size classes of separated plastic
vi.) Two trays for drying each size class separately
vii.) Gram scale for weighing plastic content from each size class
viii.) Copy of data sheet to record all information

b) Sample Preparation

i.) Drain sample through 5 mm sieve into one large bowl.
ii.) Use fresh water wash bottle to rinse off plastic particles adhering to the cod end or inside of the sample jar.
iii.) Rinse sample inside sieve in order to separate plastics thoroughly.
iv.) Now that the sample is separated into two size classes, transfer each size class to a different large Petri dish.
v.) Rinse equipment gently with the wash bottle so that no plastic particles are left in the bowl or in the sieve.
vi.) If above process does not result in adequate liquid in the Petri dishes for sorting, then add sufficient water to float all plastic bits – do not overfill

NOTE: If the sample is too large to perform steps i) thru vi) for the complete sample, then split is carefully, sort separately, and combine data later.

c. Separating sample into both size classes >5mm and <5mm

i.) Place each Petri dish under a lighted microscope or use magnifying glass to see all particles.
ii.) Using tweezers (forceps), remove all recognizable pieces of plastic that are floating.
iii.) Rinse off plastic bits with fresh water wash bottle to make sure smaller particles or plankton are not sticking to them.
iv.) Place rinsed bits of plastic in separate labelled jar or vial and set aside for later drying, typing, counting and weighing. This jar or vial should be empty, except for fragments of plastic.
v.) For size class <5mm, use a spoon to remove all remaining plastic from Petri dish if necessary. There may be more there, so start looking at center of Petri dish and move out to the sides. If you have a dissecting microscope use it to conduct a more thorough check of the sample.

d. Drying of separated plastic

i.) If you have a drying oven, set it to 60°F.
ii.) Spread sample onto Petri dishes.
iii.) Place sample in oven, but if you do not have an oven, leave sample in a secure dry location, like a bookshelf or cabinet.
iv.) dry samples at 60° for about 30 minutes. If the samples are still wet after 30 minutes, leave them in the oven and check every 15 minutes to see if they have dried. If they are left on a shelf, then check every few hours.

e. Sorting plastic to determine type, count and weight

i.) With each size class dried in its own Petri dish, use forceps to sort sample into different types of plastic as they are categorized on the data sheet.
ii.) Count number of plastics for each type for each size category.
iii.) Prepare gram scale for weighing sample. Tare the scale with Petri dish.
iv.) Transfer sorted plastic to tared Petri dish to obtain weight in grams
v.) Record this weight, next to the count, on the data sheet
vi.) Transfer sorted and weighed plastic to a vial or jar that is labelled appropriately.
vii.) Continue with this procedure until all the sorted and counted plastic is weighed and recorded on the data sheet

2. At the sea surface: surface trawl - neuston net with a PVC filtering cod end

A. Sample collection and storage

Rinse neuston net from outside to the inside through the net with hose or bucket to make sure the complete sample is concentrated in the cod end. Do not rinse the sample from the top through the opening of the net. Allow water to drain out of the mesh side window before removing the cod end.

a) Equipment

5 mm and 125 µm sieves and base
Squeeze bottle of fresh water
Aluminium foil
Tweezers
Sample storage jars
Lighted magnifying glass

Each sample will require 2 glass jars (<5 mm particles, >5 mm fragments) – each sample should be labeled with a unique sample number which is linked to the ship's electronic data system, recording lat/long of start and end trawl amongst other relevant information.

Scientific staff carrying out this procedure should wear cotton coveralls to prevent transfer of clothes fibres to sample.

b) Processing steps
i) rinse sieves with fresh water, ensure no damage to mesh, stack them together with base
ii) empty the contents of the cod end, including any material >5 mm such as seaweed, into the sieve stack, rinsing cod end out with freshwater and paying particular attention to the side window mesh
iii) rinse sieve stack and contents with fresh water, allow the sieves and contents to drip dry for a short time before the beginning examination using the lighted magnifying glass
iv) carefully examine the contents of the 5 mm sieve, picking off any particles <5mm and placing them temporarily in the 125 µm sieve. Litter >5 mm should be recorded using the same categories as for the <5 mm – i.e. fibres, cosmetic beads, polystyrene, plastic films, plastic fragments, nurdles and other (some categories will not be found). The >5 mm portion should be placed in a glass jar labeled as UNIQUE ID No. >5 mm.
v) repeat the procedure with the next sieve placing suspect particles in a glass jar labeled UNIQUE ID No. <5 mm>125 µm.


MSS Sea Surface Sampling Microplas

3. In sediments: grab sampling

Number of Micro litter particles/ kg DW

Size: 100 - 300, 300-1000 and 1000- 5000 µm

(more size classes may be used, but these should be additive into these three basic size classes)

Shape: spheres, fragments, fibres, film, pellets, polystyrene, other

Colour [Optional]: transparent/translucid, grey, white, black, blue, green, orange, yellow, red

For validation purposes: select 5% of micro litter or a maximum of 20 micro litter items per filter and validate if they are plastics or another material and analyse the polymer type: PE, PP, PS, PVC, PET, PC, PU, acrylic or other polymer type. 80% similarity match, report false positive

The sediment sampling would be undertaken alongside the existing CEMP monitoring for contaminants, however there may need to be further specification of the sampling procedure for micro litter, e.g. amount collected, number of subsamples, etc.

Sampling, in line with CEMP monitoring e.g.:

- at least 3 grab samples per station

- top surface layer of sediment – homogenised, metal spoon in glass jars

- collect 5 times 500g in separate pots, homogenise and take 5g triplicates from every 500g

OR

- 1 mixed sample from different grabs on a station, collecting up to 8 L_in containers. Followed by thorough homogenisation and subsampling into smaller appropriate volumes. Subsequently samples are frozen till extraction of plastics from 25-50 grams of sediment.

Analysis

Determination of dry weight

Density (extraction) separation = 1.47g/l or more

Repeat extraction 3 times, and combine the extracts

Glass jars and frozen

Amount: e.g. 15 * 5g, 1 * 100g - To be defined later

Minimum Visual, optional fluorescence, 5-10% Cross referencing FTIR/Raman [Max 20 particles]

Storage of dried samples

In glass jars

Guidelines from contaminant monitoring, see CEMP, would also be relevant for this indicator

Standard Operating Procedure (SOP) = Analytical methods and lab capacity

QA-QC would need to be further developed, including the use of blanks in analysis, ring testing of laboratories, etc.

Useful Links:

- http://publications.jrc.ec.europa.eu/repository/bitstream/JRC83985/lb-na-26113-en-n.pdf
- https://www.ospar.org/ospar-data/10-02e_beachlitter%20guideline_english%20only.pdf