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Appendix 1. General Sampling Record Requirements

Sampling Platform

The sampling platform (see 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

Code	Description	Code	Description	Code	Description
74CU	Clupea	74FR	Forth Ranger	74PD	Prince Madog
74CZ	Cirolana	74G1	Coastal Guardian	74S8	Sir John Murray
74E9	CEFAS Endeavour	74GO	Gold Seeker	74S9	Sea Vigil
74!C	Small Craft	74!B	Beach(intertidal)	74!D	Diver
74SC	Scotia	74VC	Vigilance	74WN	Water Guardian
74RY	Corystes	74LG	Lough Foyle		

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

Location

The site code (see list in Table 7) must be recorded together with the Latitude and Longitude (as degrees/minutes/decimal minutes) at which the sample was collected.

Appendix 2: Biological Effects Sampling Procedures

Sampling requirements and analytical protocols

The fundamental assumptions are: that biological effects water samples will, where possible, be taken at the same location and on the same date as samples taken for chemical analysis of the water column; and that biological effects sediment samples will be taken simultaneously with sediment samples obtained for benthic community and chemical analysis. Ideally, samples should be split for the different types of analysis, but if this is not possible, they should be taken sequentially.

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Appendix 2 - Biological Effects Sampling Procedures revised 6/6/07

Sampling requirements and analytical protocols

	Oyster *Crassostrea gigas* embryo bioassay (water)	***Corophium* bioassay (sediment)**	***Arenicola* bioassay (sediment)**	***Tisbe* bioassay (sediment pore-water)**	**Imposex (*Nucella lapillus*)**	**Intersex (*Littorina littorea*)**	**EROD induction (flatfish liver)**		**Fish Disease and Liver Pathology**	
							Flounder (estuary)	**Dab or plaice (open sea)**	**Flounder**	**Dab**
Frequency# per year **-spatial*** **- temporal***	Estuaries and other suspected contaminated areas. Spatial surveys only once per year, optimally 20 sites per area. BECME prioritise sites. If all sites <30 PNR repeat survey after 4 years at prioritised sites. If more than 20% sites are > 30 PNR repeat survey in following year; If one site >50 PNR repeat survey as appropriate in same year and also at same time next year.	Estuaries and other suspected contaminated areas. Once (optimally 20 sites) every 4 years. If any +ve (###) repeat next year with focus on hot spot; if no +ve repeat every 4 years. If +ve tie in with chemistry	Estuaries and other suspected contaminated areas. Once (optimally 20 sites) every 4 years. If any +ve (###) repeat next year with focus on hot spot; if no +ve repeat every 4 years. If +ve tie in with chemistry	Estuaries and other suspected contaminated areas. Used for targeted hot spot sampling and/or when positives are identified with *Corophium* and *Arenicola* assay.	Once both spatial and hot spot Repeat every 3 years	Once both spatial and hot spot Repeat every 3 years	Once Once	Once Once	Once Once	Once Once
Sampling month(s) ## **- spatial*** **- temporal***	Any month Repeat surveys must be at same time of year. Every 4 years	Same time as sediment chemistry and benthos sampling – or as appropriate	Same time as sediment chemistry and benthos sampling – or as appropriate	Same time as sediment chemistry and benthos sampling	Jun-Sep Jun-Sep	Jun-Sep Jun-Sep	May to Dec same time each year avoiding spawning season May to Dec same time each year	May to Dec same time each year avoiding spawning season May to Dec same time each year	May to Dec same time each year avoiding spawning season May to Dec same time each year	May to Dec same time each year avoiding spawning season May to Dec same time each year
State of tide	Low tide (or other worst case) if possible. . ++	NR	NR	NR	Low tide	Low tide	NR	NR	NR	NR
Depth of sample or height on shore	1 m sub-surface or in accordance with local hydrography	Top 10 cm	Top 10 cm	Aerobic surface layer or directly related to whole sediment	Low to mid tide level	Low to mid tide level	NR	NR	NR	NR

Sampling technique	2.5 litre submerged glass bottle	Box core or other method which retains pore water Sample mixed but not seived	Box core or other method which retains pore water. Sample mixed but not seived	Box core or other method which retains pore water Sample mixed but not seived	Hand collect	Hand collect	Trawling	Trawling	Trawling	Trawling
Water or sediment volume	1 litre minimum	5 litre minimum	5 litre minimum	50 ml pore water (minimum)	NR	NR	NR	NR	NR	NR
Organism size	NR	NR	NR	NR	Toothed adult	Young adult	10-35 cm length	10-30 cm length	Greater than 15 cm	Greater than 15 cm
Organism sex	NR	NR	NR	NR	Female & male	Female & male	Male M ; Female optional	Male M ; Female optional	Female or Male	Female or Male
Organisms per station# -spatial -temporal	NR	NR	NR	NR	40 for each station	40 for each station	At least 10 males M 15 R At least 10 males M 15 R	At least 10 males M 15 R At least males 10 M 15 R	50 for histopathology 150 for external	50 for histopathology 150 for external
Numbers of water or sediment samples per station#	2 replicates	Single for spatial and 5 for hotspots	Single for spatial and 5 for hotspots	One sample	NR	NR	NR	NR	NR	NR
Liver sample size	NR	NR	NR	NR	NR	NR	200-500 mg immediately snap frozen	200-500 mg immediately snap frozen	3-5 mm section	3-5 mm section
Sample storage conditions	Glass 4^oC in dark	Plastic bucket 4^oC in dark	Plastic bucket 4^oC in dark	Glass 4^oC in dark	Keep alive 24-72 hrs if doing residue analysis, otherwise within 2-3 weeks	Keep alive 24-72 hrs if doing residue analysis, otherwise within 2-3 weeks	Minus 70^oC	Minus 70^oC	Histological Fixative for Tissues	Histological Fixative for Tissues
Maximum sample storage time	48 hours ideally but 5 days max; if greater store at -20 C	4 weeks at 4^o C	4 weeks at 4^o C	48 hours for extracts	72 hours alive for residue analysis, 2-3 weeks for imposex	72 hours alive for residue analysis, 2-3 weeks for intersex	6 months	6 months	Indefinite	Indefinite
Test protocol reference**	TIMES / EA SCA test guideline, plus salinity correction method (Thain)	ICES TIMES (Roddie and Thain)	ICES TIMES (Thain and Bifield).	ISO/DIS 14669 (1997), plus pore water extraction method (Thain)	OSPAR JAMP guidelines (2002) Tech. Annex 3	OSPAR JAMP guidelines (2002) Tech. Annex 3	ICES TIMES (Stagg et al,1999)	ICES TIMES (Stagg et al, 1999)	ICES TIMES (Feist et al., 2004)	ICES TIMES (Feist et al., 2004))
Contaminant residue measure-ments	For +ve samples only. Standard CSEMP suite on repeat sampling and other contaminants as suspected.	Standard CSEMP sediment suite, if already being done. If not, frozen sub-	Standard CSEMP sediment suite, if already being done. If not, frozen sub-	Archive frozen whole sediment sample for possible future analysis	TBT in soft tissue in females only R for heavily	TBT in soft tissue in females only R for heavily	PCB Mand PAH R in liver 4 pools of 5 fish PAH Bile	PCB M and PAH R in liver R 4 pools of 5 fish	CSEMP biota suite	CSEMP biota suite

		sample taken and archived.	sample taken and archived.		impacted areas with historical data	impacted areas with historical data	metabolites	PAH bile metabolites		
Other supporting measurements	-Salinity (Mandatory) -Temp. (Recommended) -Dissolved oxygen R -Chlorophyll*** R pH R	-Particle size R -Ammonia in overlying water during test (M) -Hydrogen sulphide R -Organic carbon R -Redox potential R (profile with depth)	-Particle size R -Ammonia in overlying water during test (M) -Hydrogen sulphide R -Organic carbon R -Redox potential R (profile with depth)	-Ammonia (in pore water) (M) -Particle size (whole sed) R -Organic carbon (whole sed) R	Shell height Sex ratio	Shell height Sex ratio	-Gonado-somatic index M Hepatic somatic index M -Liver lipid content R -Bottom water temp. R	-Gonado-somatic index M Hepatic somatic index M -Liver lipid content R -Bottom water temp. R	See ICES method document Length, weight, GSI, HSI	See ICES method document Length, weight, GSI, HSI

NOTES

* spatial or temporal monitoring, depending on the objectives of the programme

NR = not relevant

Minima, but subject to statistical advice when a sufficiently large dataset has been collected. + For a given site, sampling should be restricted to a single species for the duration of the programme

++ For a given site, always sample at same state of the tide.

For a given determinand and site, the sampling date(s) each year should if possible be fixed for the duration of the programme

** Controlled copies of the protocols are held by FRS Marine Laboratory, Aberdeen

*** Chlorophyll is used as a marker for the presence of algal blooms which may produce toxins that can give a response with the oyster embryo bioassay, but it should be remembered that not all algal species are toxic.

###Sediment bioassay positive limits are: *Corophium* - >20% sites at >30% adjusted mortality or 1 site >50% adjusted mortality; *Arenicola* - >20% sites >30% adjusted mortality or 1 site >50% adjusted mortality or >20% sites >50% feeding inhibition.

	DNA adducts	**VTG**	**Imposex Off shore**	**Bile metabolites**	**Lysosomal NRR**	**Metallothionein**	**SFG**	**AChE**
	Flounder Dab Plaice	**Flounder Cod**	***Buccinum* and *Neptunea***	**Any fish**	**Mussel (*Mytilus edulis* only)**	**Mussel (*Mytilus edulis* only)**	**Mussel (*Mytilus edulis* only)**	**Fish & Mussel (*Mytilus edulis* only)**
Frequency per year -spatial -temporal	For hotspots only Once Once	Once Once	Once Every 3 years	Once Once	Once Once	Once Once	Once 5 years	Once 5 years
Sampling months -spatial -temporal	May to Dec – avoiding the spawning season	May to Dec – avoiding the spawning season	Any time	May to Dec – avoiding the spawning season	May to Sept – avoiding the spawning season	May to Sept – avoiding the spawning season	May to Sept avoiding the spawning season	Any time
State of tide	NR	NR	NR	NR	Low tide	Low tide	Low tide	NR
Depth of sample or height on shore	NR	NR	NR	NR	Hand pick	Hand pick	Hand pick	Trawling or hand pick mussels
Sampling technique	Trawling	Trawling	Trawling or pots / traps	Trawling	Shore collected or transplanted following shoreline collection guideline to be developed by BECME	Shore collected or transplanted	Shore collected or transplanted	Trawl. Or following shoreline collection guideline to be developed by BECME
Water or sediment volume	NR	NR	NR	NR	NR	NR	NR	NR
Organism size	15 cm plus	10 cm plus	Adults > 5 cm	10 cm plus	40 – 60 mm	40 – 60 mm	40 - 60 mm	10 cm plus fish, 40 – 60 mm mussels
Organism sex	Male or female	Male	Both sexes	Male or female	NR	NR	NR	Male or female
Organism per station -spatial -temporal	10 or 5 pools of 4 using same sex fish	10 or more	100 for Buc. 40 for Nep.	10 or more of same sex	10	At least 20 individuals	10 or more	10 or more
Numbers of water or sediment samples per station	NR	NR	NR	NR	NR	NR	NR	NR
Liver sample size	200mg immediately snap frozen	Blood > 0.25 ml	NR	Any volume of bile present min 10ul	200 ul hlymph	200 mg hepatopancreas derived from pools of 3 animals	NR	200 mg muscle / brain or haemolymph from mussel
Sample storage conditions	-70 C	Plasma at -70 C	Fresh or frozen (-20 C)	-70 C	Must be conducted on live animals ASAP after collection (<24 hours)	-70 C	Must be conducted on live animals	Fresh
Maximum storage time	6 months	6 months	12 months	6 months	NR	6 months	NR	NR
Test protocol reference	ICES TIMES (Reichert)	ICES TIMES (Scott et al)	OSPAR 2002 guidelines	ICES TIMES (Ariese et al.)Fluoresence / GCMS HPLC.	ICES TIMES NRR method document (Lowe et al.)	ICES TIMES (Hylland)	ICES TIMES (Widdows & Staff)	ICES TIMES (Galgani et al)

Contaminant residue measurement	PCB Mand PAH R in liver 4 pools of 5 fish	EDCs eg alkyl phenols and oestrogen mimics R	TBT in soft tissue (not foot) of min 5 females R	None essential	None but all potentially relevant	Metals especially Cd Cu Zn	None but all tpotentially relevant	OPs Carbamates\ etc in biota R
Other supporting measurement	GSI, HSI Sed PAH EROD & PAH Bile metabolites	GSI, M HSI M	TBT in sediments R	GSI, HSI Sed PAH, EROD	Shell length Condition factor	Shell length Condition factor	Shell length Condition factor	Shell length Condition factor

Appendix 3. Procedural Guidelines for subtidal sediment sampling.

General

Samples should be collected from the same time of year at each site to minimise interannual 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. Five replicate samples are collected for contaminants, five for benthic community analysis and five for biological effects[1]. 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.

[1] Estuarine sites only

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%		

Physicochemical sample collection procedure

Particle size analysis sub sampling procedure
Take the sample from the surface to a minimum depth of 5 cm (a core previously used for redox analysis is acceptable). Transfer samples to containers that can withstand freezing, such as plastic bags or pots. Keep samples cool and freeze them as soon as possible. This prevents decomposition from affecting grain size. Record the method of preservation.

Trace Metals sub sampling procedure
Use a polythene scoop to collect the sample. Wash it in clean seawater between samples. Avoid sampling from the edges of the grab. Take the sample from the surface avoiding any anoxic layer and note the thickness of sample. Transfer the sample to a trace metal free (acid wash if necessary) container, cool and freeze it as soon as possible. Mercury samples should be stored in glass or quartz as it can move through the walls of plastic containers. Samples must remain frozen until they are analysed. Record the method of preservation.

Trace Organics sub sampling procedure
Use a metal scoop to collect the sample and wash it in clean sea-water between samples. Avoid sampling from the edges of the grab. Take the sample from the surface to a depth of 1 cm. If an anoxic layer is present within 1 cm of the surface then take a shallower surface scrape to avoid this layer and note the thickness of sample. Transfer the sample to a suitable clean (solvent wash if necessary) glass or metallic container and freeze it as soon as possible. Samples must remain frozen until they are analysed. Record the method of preservation.

Organic carbon sub sampling procedure
Use a metal scoop to collect the sample and wash it with clean sea-water between samples. Avoid sampling from the edges of the grab. Take the sample from the surface to a depth of 1 cm. If an anoxic layer is present within 1 cm of the surface then take a shallower surface scrape to avoid this layer and note the thickness of sample. Transfer the sample to a polythene container and freeze it as soon as possible. Samples must remain frozen until they are analysed. Record the method of preservation.

Method of storage codes:

Code	**Description**	**Code**	**Description**	**Code**	**Description**
FR	Frozen	DF20	Deep freeze -20^{o}C	DF70	Deep freeze -70^{o}C

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.

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. Whichever is chosen at any site it must be used there henceforth for the NMMP2 time series. The fish should be collected outside their spawning period. Fish destined for EROD and Vitellogenin analysis should be collected in September. Samples for contaminants and biological effects analyses should be collected on the same sampling occasion to facilitate impact assessment.

4.2 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 Aggregated Samples
Hauls, sediment cores or grabs taken to comprise sample.

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).

4.4 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.
Note the condition of target species. Note the presence, prevalence and position of any evidence of disease (fin-rot, carcinomas, ulcers, and lymphocystis) according to ICES guidelines (1997). 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^{o}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^{o}C	DF70	Deep freeze -70^{o}C

For each fish record length[2] (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.

[2] Record length to the end of the tail?

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. Samples should be depurated 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^{o}C	DF70	Deep freeze -70^{o}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^{o}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^{o}C
MAN	Manual milling	MMG	Mechanical milling	DRY99	Drying <100^{o}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. Sample preparation for sediment Physico-chemical analysis

6.1. Trace metals

Sediment samples are sieved before analysis for trace metals. Wet sieving (SVW) is the recommended method (QUASH) but dry sieving (SVD) can be used.

Freeze drying is the recommended procedure drying sediments for trace metal analysis for all metals except mercury which is volatile. 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. Samples should be dried below 105^{o}C to avoid loss of mercury which is volatile.

Care must be taken to avoid contamination of the sample during sieving, where possible samples should be sieved in a clean air cabinet and the apparatus thoroughly cleaned between samples.

It is recommended that samples are passed through a nylon 63 um sieve and the < 63 um fraction retained for analysis however a larger sieve can be used where this maintains an existing time series. The size fraction analysed must be reported to ICES.

6.2 Trace Organics

Samples should be air dried or chemically dried as volatile analytes can be lost by oven drying or freeze drying. Samples should not be sieved through a fine mesh as this can result in loss of analyte. Stones are removed from the sample by passing it through a 2mm metal sieve. The size fraction analysed should be reported.

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^{o}C
SVD	Dry sieving	SVW	Wet sieving	DRY99	Drying <100^{o}C
MAN	Manual milling	MMG	Mechanical milling		

Appendix 7. Procedural Guidelines for the analysis of trace metals in sediments and biota.

7.1 Extraction procedure

7.1.1 Sediments
A total digestion procedure is required to allow data to be normalised to a metal that is a constituent of the clay mineral lattice and for which there is little anthropogenic input e.g. aluminium or lithium. This facilitates comparison of levels of contamination between sites where the proportion of silty material (which usually carries the contaminants) is different. A partial extraction method is acceptable for determination of long term trends at sites where this method has traditionally been used and changing the method would disrupt the long term data set. Most laboratories use hydrofluoric acid in a pressured vessel with the addition of boric acid to complex excess HF (method HFB) to extract metals from sediments.

7.1.2 Biota
The acid digestion procedure must be sufficiently vigorous to break down organometallic complexes. Most laboratories use a strong mineral acid in a pressurised container. Samples are usually heated by microwaves and pressure control is used to prevent venting of gases leading to loss of the substance of interest. The digest produced by this procedure can be analysed for the full range of determinands.

Code	Description
AQR	Aqua regia
HF-OV	HF in open vessels, evaporation of HF
HF-C	As HFO but digestion in closed vessels (pressurised decomposition)
HF-CB	As HFC but complexation of excess HF with H3BO3
HNO-CM	Extraction with HNO3, pressure digestion
HNO-OV	Extraction with 1:1 HNO3
ALK	Alkaline fusion digestion

The extraction efficiency of the chosen technique should be determined using a certified reference material (CRM), the matrix and determinands concentration of which resembles that of the environmental samples as closely as possible. Different CRMs should be used, where available, if a single material is not fully representative of all samples.

A blank sample (reagents only) must be included with each batch of samples as a check on contamination. Reagents should be of low trace metal content; extraction vessels must be cleaned between each batch of analyses.

7.2 Analytical technique

The use of a single analytical technique is not mandatory. Techniques may be chosen according to the expertise and instrumentation available. Most laboratories now use ICP/MS for trace metals determination. The addition of a collision cell facilitates the determination of refractory elements such as chromium. Mercury can be determined by atomic fluorescence or cold vapour atomic absorption spectrometry

Code	Description
AAS-AA	Atomic absorption spectrometry-air acetylene
AAS-CV	Atomic absorption spectrometry- cold vapour
AAS-GF	Atomic absorption spectrometry – graphite furnace
AAS-NO	Atomic absorption spectrometry – nitrous oxide
ICP-MS-CC	ICP/MS with collision cell
ICP-MS-WC	ICP/MS without collision cell
AFS	Atomic fluorescence spectrometry
ICP-OES	Optical emission spectrometry

XRF	X-ray fluorescence analysis (total method)
NAA	Neutron activation analysis

System suitability checks such as periodic examination of linearity and sensitivity are recommended as a valuable supplement to the separate (and independent) analysis of quality control samples (CRMs).

7.3 References

JAMP Guidelines for monitoring contaminants in sediments and biota:

http://www.ices.dk/committe/acom/comwork/report/2008/Special%20Requests/OSPAR%20JAMP%20Guidelines%20for%20monitoring%20contaminants%20in%20biota%20and%20sediments.pdf

EU discussion document on sediments:
http://www.sednet.org/download/AMPS_sediment_monitoring_discussion_doc_v2.pdf

Appendix 8. Procedural guidelines for analysis of trace organics in sediments and biota

8.1 Extraction

Most laboratories are moving to Accelerated Solvent Extraction (ASE) for extraction of PCBs and PAH as this increases extraction efficiency and reduces solvent use compared to soxhlet extraction. Recovery standards should be added prior to extraction. When using a Soxhlet a combination of polar and apolar solvents is recommended. Apolar solvents are normally not able to extract all CBs (and OCPs) from mussel or fish tissue. When using chemical drying (*e.g.* with sodium sulphate) several hours are required between grinding and extraction, to allow complete binding of the water present in the sample. A time-span between grinding and extraction which is too short normally results in extraction efficiencies which are too low. Alternatively, saponification may be used (recommended for PAH).

Code	Description
ACD	Acetone/dichloromethane
SOX	Soxhlet extraction method
MHX	Methanol/hexane mixture in acetic acid environment
EXP	Extraction of organic contaminants by shaking with polar solvent
EXN	Extraction of organic contaminants by shaking with non polar solvent
EXO	Other principles of extraction/separation of organic contaminants
SON	Sonicate

The extract must be cleaned up to remove substances that would interfere in the subsequent analysis.

8.1.1 Biota

Determinands may be separated from lipids by column extraction using *e.g.* Florisil or performing gel permeation chromatography (GPC) which is of advantage for the determination of compounds unstable during sulphuric acid clean-up. Fractionation of CBs and OCPs is recommended to prevent coelution. Additionally treating the OCP fraction with concentrated H_2SO_4 could improve the quality of the chromatograms. However, this treatment is not to be used if decomposable target-analytes are to be analysed, *e.g.* those of the dieldrin type or heptachloroepoxides.

8.1.2 Sediments

Removal of sulphur and sulphur-containing compounds

An aqueous saturated Na_2SO_3 solution is added to a hexane extract. In order to allow the transfer of the HSO_3^- ions to the organic phase, tetrabutylammonium salts (TBA) and isopropanol are then added to the mixture. Water is subsequently added to remove the isopropanol. The aqueous phase must then be quantitatively extracted with hexane (Jensen *et al.*, 1977). If the extraction was performed by a polar solvent miscible with water, then a Na_2SO_3 solution can be added directly after extraction. If the extraction mixture also contains an apolar solvent, then depending on the ratio of the solvents, the addition of TBA and isopropanol may or may not be necessary. Any excess Na_2SO_3 and reaction products can be removed by the addition of water and thus partitioning between apolar solvent and water.

Mercury, activated copper powder, wire or gauze (Smedes and de Boer, 1994 and in press; Wade and Cantillo, 1996) remove the sulphur directly from an organic solvent. Although mercury is appropriate for removing sulphur, it should be avoided for environmental reasons. Copper can be applied during or after Soxhlet extraction. Ultrasonic treatment might improve the removal of sulphur. If sulphur appears to be present in the final extract the amount of copper or mercury used was insufficient and the clean-up procedure must be repeated.

Further clean up

As CBs are apolar, clean-up using normal-phase chromatography is the most appropriate technique for the separation from other compounds. Using an apolar solvent (*e.g.* hexane or isooctane) as an eluent, CBs normally elute very rapidly. All polar solvents used in the

extraction or sulphur removal step should be removed before further clean-up. The last concentration step is usually performed by evaporation with a gentle stream of nitrogen. Evaporation to dryness should always be avoided.

Deactivated Al_2O_3 (5-10% water) is often used as a primary clean-up. Provided that sulphur has been removed, Al_2O_3 sometimes gives a sufficiently clean extract for a GC-ECD analysis of the sample. Al_2O_3 removes lipid compounds from the extracts (although samples with very high lipid content and low CB concentrations may require additional clean-up).

Deactivated silica (1-5% water) does not retain CBs (including planar CBs) and only slightly retains polycyclic aromatic hydrocarbons (PAHs) when eluted with hexane or isooctane. When organochlorine pesticides are also to be determined in the same extract, deactivation of the silica with a few percent of water is necessary.

For high activity silica (overnight at 180°C) the retention of CBs is negligible, while PAHs are more strongly retained. The CBs and a few other organochlorine compounds are eluted with apolar solvents. More polar solvents (*e.g.* hexane/acetone) should be avoided as some interfering organochlorine pesticides would be eluted.

When GPC is used for removing sulphur, the removal of high molecular weight material can also be incorporated into the procedure. Separation is obtained on polystyrene-divinylbenzene copolymer columns (*e.g.* Biobeads). GPC does not separate CBs from other compounds in the same molecular range such as organochlorine pesticides and so additional clean-up may be required.

For the separation of CBs from lipids or oil components, reversed-phase HPLC can be used. In reversed-phase chromatography CBs elute during a solvent gradient of 80 to 90% methanol together with numerous other compounds of the same polarity. Most of the above mentioned extraction methods and clean-up procedures yield an extract containing an apolar solvent. These cannot be injected directly for reversed-phase chromatography, and so compounds must be transferred between solvents several times *e.g.* before injection and after elution. When using polar solvents for extraction (*e.g.* for wet sediments) reversed-phase columns could be used directly for clean-up. When eluting an acetonitrile extract from a C_{18} solid phase extraction (SPE) column with acetonitrile, high molecular hydrocarbons are strongly retained while CBs elute in the first few column volumes.

The above mentioned normal-phase chromatographic procedures on silica and Al_2O_3 can be transferred to HPLC having the advantages of higher resolution and better reproducibility.

In summary, the most commonly used methods are Al_2O_3 with 5-10% water as a rigid primary clean-up followed by elution from silica with an apolar solvent. GPC is also regularly applied as primary clean-up. A drawback of GPC and reversed-phase chromatography is that CBs must be transferred to an apolar solvent for further clean-up. The use of Florisil is not recommended.

8.2 Analytical technique

Code	Description
GC-AED	Gas chromatography – atomic emission detection
GC-ECD	Gas chromatography – electron capture detection
GC-EI	Gas chromatography – electron impact ionisation
GC-FPD	GC – flame photometric detector
GC-MS	Gas chromatography – mass spectrometry
GC-MSD	Gas chromatography – mass selective detector
HPLC	High performance liquid chromatography
HPLC-FD	HPLC – fluorescence detector
HPLC-MS	HPLC – mass spectrometry
HPLC-UV	HPLC – UV detector

The chosen analytical technique depends on available resources however it must achieve the specified targets for limit of detection, accuracy and precision.

GC/MS is recommended for both PCBs and PAH. The method provides positive identification of the compound.

GC/ECD can be used for PCBs but the presence of the analyte must be confirmed on a second column.

HPLC with a fluorescence detector can be used for PAH but it is only suitable for a limited range of compounds.

Two columns with stationary phases of different polarity should be used, as column-specific coelution of the target CBs with other CBs or organochlorine compounds occurs. The temperature programme must be optimised for each column to achieve sufficient separation of the CB congeners to be determined. An isothermal period in the programme around 200-220°C of approximately 30 minutes is recommended. Care should be taken that CBs of interest do not coelute with other CB congeners (for example CB28 and CB31). When using GC-ECD, compounds are identified by their retention time in relation to the standard solutions under the same conditions. Therefore GC conditions should be constant. Shifts in retention times should be checked for different parts of the spectrum with the help of characteristic, unmistakable peaks (*e.g.* originating from the internal standard or higher concentrated CBs such as CB153 and CB138+). Using a GC/MS system, the molecular mass or characteristic mass fragments or the ratio of two ion masses can be used to confirm the identity of separated CBs. Since calibration curves of most CBs and OCPs are normally non-linear using a GC-ECD or GC-MS system, a multilevel calibration of at least five concentrations is recommended. The calibration curve must be controlled and the best fit must be applied for the relevant concentration range. Otherwise, the linear range of the detector must be identified. Analysis of the calibration solutions should be carried out in a mode encompassing the concentrations of the sample solutions (or alternatively by injecting matrix-containing sample solutions and matrix-free standard solutions distributed regularly over the series). When the chromatogram is processed with the help of automated integrators the baseline is not always set unambiguously and always needs to be inspected visually. Peak height is preferable to peak area for quantification purposes. From the two columns of different polarity the more reliable result should be reported.

References

JAMP Guidelines for monitoring contaminants in biota
JAMP Guidelines for monitoring Contaminants in sediments

Appendix 9. Procedural Guidelines for analysis of sediment supporting determinands

9.1 Organic carbon in sediments

Organic carbon is a supporting determinand for trace organic compounds. Sediments are analysed dry and freeze drying is recommended as this produces a free flowing powder. Samples are sieved as for trace organics.

The organic carbon content of the sample is determined using an elemental analyser either by removal of the inorganic fraction or the organic fraction and comparing this to the total.

Inorganic carbon is removed by treatment with acid, alternatively organic carbon can be removed by heating the sample to 500^{o}C in a muffle furnace

9.2 Particle size analysis of sediments

Particle size analysis is a supporting determinand for biological community interpretation as well as contaminant concentrations. Sediments are analysed without drying by laser diffraction for the fraction <1mm (sand (except very coarse and coarse sand), and mud). The fraction >1mm (coarse sand, very coarse sand and gravel) sieved at 0.5phi intervals. Details of exact methodology are given in Mason, C. 2011. NMBAQC's Best Practice Guidance. Particle Size Analysis (PSA) for Supporting Biological Analysis. National Marine Biological AQC Coordinating Committee, 72pp,

http://www.nmbaqcs.org/scheme-components/particle-size-analysis/reports.aspx

Appendix 10. Guidelines for the analysis of macrobenthos in sediment samples

10.1. 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.

10.2 Biomass Measurement

Biomass can be expressed in a variety of ways (eg 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 (eg 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:	
NMMP Station code & Sample/rep no:	
Sample Date:	
Date of Weighing:	
Biologist:	

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

Appendix 11. Procedural Guidelines for sampling and sample preparation of waters for nutrient analysis

11.1 Background.

Nutrient samples are collected in Winter (Dec- Jan) 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 NMMP 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.

11.2 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.

11.3 Sample containers.

Sample containers should be clean and sterile. There are currently no clear recommendations for bottle types or material. Security of closures is paramount if sample integrity is to be maintained.

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.

11.4 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.

11.5 Pretreatment.

Prior to freezing, removal of suspended matter that has potential to bias the determination in some way should be removed. Phosphate for example can be leached from particulate matter once exposed to the chemicals used for the analysis.

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.

11.6 Preservation.

There is little doubt that 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^{o}C	DF70	Deep freeze -70^{o}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.

11.7 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.

11.8 Quality Control.

To quantify uncertainty resulting from the sampling and sample handling procedures, organizations are encouraged to incorporate duplicate samples and fields blanks into their sampling procedures and then submit this as supporting QA Data.

11.9 References

QUASH Quality Assurance of Sampling and Sample handling. Final Report Quasimeme Project Office.

Appendix 12. Procedural Guidelines for sampling and sample preparation of waters for Chlorophyll analysis.

12.1 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 (June, July, August). 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.

12.2 Sampling

Water column samples can be collected using opaque containers and should be protected from excess heat and light. Filtration should be performed as soon as possible after sampling and preferably within one hour of sampling. Zooplankton, where present, may continue consume chlorophyll.

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.

Where pumped systems are employed, cell rupture and subsequent loss of pigment should be a consideration. Where there may be doubt as to the suitability of a pumped supply, comparative studies should be carried out to verify that sample integrity is not being compromised.

Record the method of sample collection

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				

12.3 Sample Pretreatment

Glass fibre or membrane filter papers are mostly used and the final choice of filter media will depend on the subsequent analytical methods to be used.

Either pressure or vacuum the extent of which should be limited to avoid cell rupture usually assists filtration.
The pore size of the filter media should be small enough to capture picoplankton and GF/F (0.7 um pore size) is recommended. [1]

The actual size and type of the filter media should be chosen prior to testing of analytical methods since the amount of water retention can significantly reduce the attack strength of the solvent used during the analytical stage. Once the amount of water retained is known for the media and filtration method used, the concentration of solvent added can be adjusted to compensate and to ensure that the attack strength of the solvent is at the optimum.

Removal of zooplankton is desirable because they can contain chlorophyll, they can be removed by pre filtering through a 100-150 um mesh or can be picked off filters using tweezers.

Care should be exercised where large colonial phytoplankton are present as these may also fall victim to the pre filtering above.

Record the method of filtration

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

12.4 Sample storage.

Storage conditions will have the largest impact on the end result, Deep freezing to –18C or below is both practical and the favoured option[1],[2] where the objective is to estimate total biomass. Where pigment information is required, more rigorous storage conditions may be needed.

Storage up to one month[1] [3] is possible when considering only chlorophyll a since early degradation products have spectral properties close to those of chlorophyll a .

Filters can be folded and wrapped in aluminium foil to reduce storage space needed.

Record the method of sample storage

Code	Description	Code	Description	Code	Description
FR	Frozen	DF20	Deep freeze -20^{o}C	DF70	Deep freeze -70^{o}C

12.5 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 [2] of sufficient size.

Portable low voltage freezers offer a suitable alternative.

12.6 Quality Control.

Information on the performance of laboratories carrying out the analysis of nutrients in sea water is already a criteria used within the NMP data filter, but this falls short on providing information on the overall uncertainty of the submitted data. To quantify uncertainty resulting from the sampling and sample handling procedures, organizations are encouraged to incorporate duplicate samples and fields blanks into their sampling procedures and then submit this as supporting QA Data.

12.7 References

[1]. ICES Techniques in Marine Environmental Sciences. Standard Procedure for the determination of chlorophyll a by spectroscopic methods ISSN 0903-2606

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

[3]. Eds. Jeffrey S.W., Mantoura R.F.C. , Wright S.W. . 1997. Phytoplankton Pigments in Oceanography:Guidelines to modern methods. UNESCO.

Appendix 13. 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 spikes[3];
>
> **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.

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.

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

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 (fluoro- carbon, 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 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.

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[4]U.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

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.

[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 14: References for Analytical Methods

Subject	Publication
Biological Effects	
Benthos	Marine Pollution Monitoring Management Group (1990). Procedures for the Monitoring of Marine Benthic Communities at UK Sewage Sludge Disposal Sites. Scottish Fisheries Information Pamphlet Number 18.
	Rumohr, H. (Ed.) (1990). Soft-bottom Macrofauna: Collection and Treatment of Samples. ICES Techniques in Marine Environmental Sciences, No 27. 19pp.
	Rees, H. L., Heip, C., Vincx, M. and M. Parker. 1991 Benthic Communities: Use in Monitoring Point-Source Discharges. ICES Techniques in Marine Environmental Sciences, No 16. 70pp.
Oyster Embryo Bioassay	Environment Agency (1998). Short-term Ecotoxicological Method Guidelines for effluent and receiving water monitoring (Draft). Environment Agency Technical Report E83 ISBN 1 85705 115 7 - pages 6-1 to 6-34 plus note.
	Thain, J.E. (1998) Salinity correction method (unpublished B in draft).
Tisbe battagliai bioassay (sediment pore water)	ISO (1997). Water quality - determination of acute lethal toxicity to marine copepods (Copepoda, Crustacea), Draft International Standard ISO/DIS 14669, International Organisation for Standardization, Geneva, 20 pp.[3]
	Thain, J.E. (1998). Pore water extraction method (unpublished B in draft).
Corophium bioassays (whole sediment)	Roddie, B.D. and J.E. Thain (in press). Biological effects of sediment-bound contaminants: *Corophium* sp. Sediment bioassay and toxicity test. ICES Techniques in Marine Environmental Sciences, International Council for the Exploration of the Sea, Copenhagen.[2]
Arenicola bioassays (whole sediment)	Thain, J.E. and S. Bifield (in press). A sediment bioassay using the polychaete *Arenicola marina*. ICES Techniques in Marine Environmental Sciences, International Council for the Exploration of the Sea, Copenhagen.[2]
Imposex / intersex (dogwhelk / periwinkle)	OSPAR (1998). Technical Annex 3. TBT-specific biological effects monitoring. In JAMP Guidelines for Contaminant-specific Biological Effects Monitoring, Oslo and Paris Commissions, London, pp. 14-25.
EROD (fish liver)	Stagg, R. & A. McIntosh (1999). Biological effects of contaminants: determination of CYP1A-dependent mono-oxygenase activity in the liver of dab (*Limanda limanda*) by the fluorimetric measurement of 7-ethoxyresorufin-o-deethylase (EROD) activity. ICES Techniques in Marine Environmental Sciences, No. 23. International Council for the Exploration of the Sea, Copenhagen.[2]

Biomass Protocol	Rees, H. L., Moore, D. C., Pearson, T. H., Elliott, M.,Service, M., Pomfret, J. and Johnston, D. (1990). Procedures for the Monitoring of Marine Benthic Communities at UK Sewage Sludge Disposal Sites. Scottish Fisheries Information Pamphlet No 18 DAFS, 79 pp.
	Rumohr, H., Brey, T. and Ankar, F. (1987). Compilation of Biometric Conversion Factors for Benthic Invertebrates of the Baltic Sea. Baltic Marine Biologists Publication No. 9.
Sample Preparation	Gardner, M.J. and S.D.W. Comber (1997) Sample filtration as a source of error in the Determination of Trace Metals in Marine Waters. Analyst. 122, 1029-1032.
Quality Assurance	QUASIMEME Annual Scheme for Laboratory Performance Studies. Available on the QUASIMEME web site (http://www.QUASIMEME.marlab.ac.uk)
Fish Disease	
Liver Neoplasia/Hyperplasia	ICES (1997) Report on the Special Meeting on the use of Liver Pathology of Flatfish for monitoring Biological Effects of Contaminants. ICES CM 1997/F:2. 75pp.
Liver Nodules and Externally Visible Fish Diseases	Bucke, D., Vethaak, A.D., Lang, T. and S. Mellergaard (1996) Common Diseases and Parasites of Fish in the North Altantic: Training Guide for Identification. ICES Techniques in Marine Environmentasl Sciences, No 19. 27pp.[2]
Coal	Eagle, R.A., P.A. Hardman, M.G. Norton, R.S. Nunny, and M.S. Rolfe(1980), The field assessment of dumping wastes at sea: 5. Fisheries Research Technical report No. 51. MAFF, Pages 1-17.
Contaminants	JAMP Guidelines for monitoring contaminants in sediments. OSPAR Commission Monitoring Guidelines No. 2002-16
	JAMP Guidelines for monitoring contaminants in biota. OSPAR Commission Monitoring Guidelines
Other Useful References [1]	9th Report of the Benthos Ecology Working Group - ICES CM 1990/1.95.
	Proudfoot, R.K., Elliott, M.E., Dyer, M.F., Barnett, B.E., Allen, J., Proctor, N., Hemmingway, C. and N. Cutts (in preparation). National Marine Biological Analytical Quality Control (NMBAQC)
	Benthic Field Methods Workshop: Collection and processing of macrobenthic samples from soft sediments; best practice review. Hull University 17-21 March 1997.
	ICES publication in the 'Techniques in Marine Environmental Sciences' series : Biological effects of contaminants: Oyster (Crassostrea gigas) embryo assay.
	ICES Environmental Data Reporting Formats. TF 6/INFO.2.1-E, Sixth Meeting of the North Sea Task Force.
	JAMP Monitoring Guidelines (1997) Oslo and Paris Commission (9/6/97).
	ICES (1997) Report of the ICES Advisory Committee on the Marine Environment, 1997. ICES Cooperative Research Report, 222.

Appendix 15: Reference to Relevant Web Sites

OSPAR	http://www.ospar.org
ICES	http://www.ices.dk
DEFRA	http://www.defra.gov.uk
SERAD Marine Laboratory	http://www.marlab.ac.uk
CEFAS	http://www.cefas.co.uk
EA	http://www.environment-agency.gov.uk
EHS	http://www.ehsni.gov.uk
SEPA	http://www.sepa.org.uk

Appendix 16: 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 17: 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

Monitoring stations

There are approximately 80 core stations, as illustrated in the map on the right, monitored to determine long-term trends around the UK coastline. Data are also collected from a number of opportunistic stations increasing the spatial coverage of the monitoring network. Contaminants are measured in waters, sediments and biota to assess their distribution and fate in the environment. Biological effects are also measured to determine the response of organisms to contaminants.

Data are quality assured using internal and external programmes. The participating laboratories subscribe to the Quality Assurance of Information for Marine Environmental Monitoring in Europe (QUASIMEME) or the Biological Effects Quality Assurance in Monitoring Programmes (BEQUALM) inter-laboratory proficiency-testing schemes and perform internal quality assurance.

The principal output from the coordinated monitoring is an annual submission of quality assured data to the International Council for the Exploration of the Seas (ICES).

A map showing the core stations which are sampled under the CSEMP programme ©

A map showing the total spatial coverage of monitoring, including opportunistic stations ©

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.

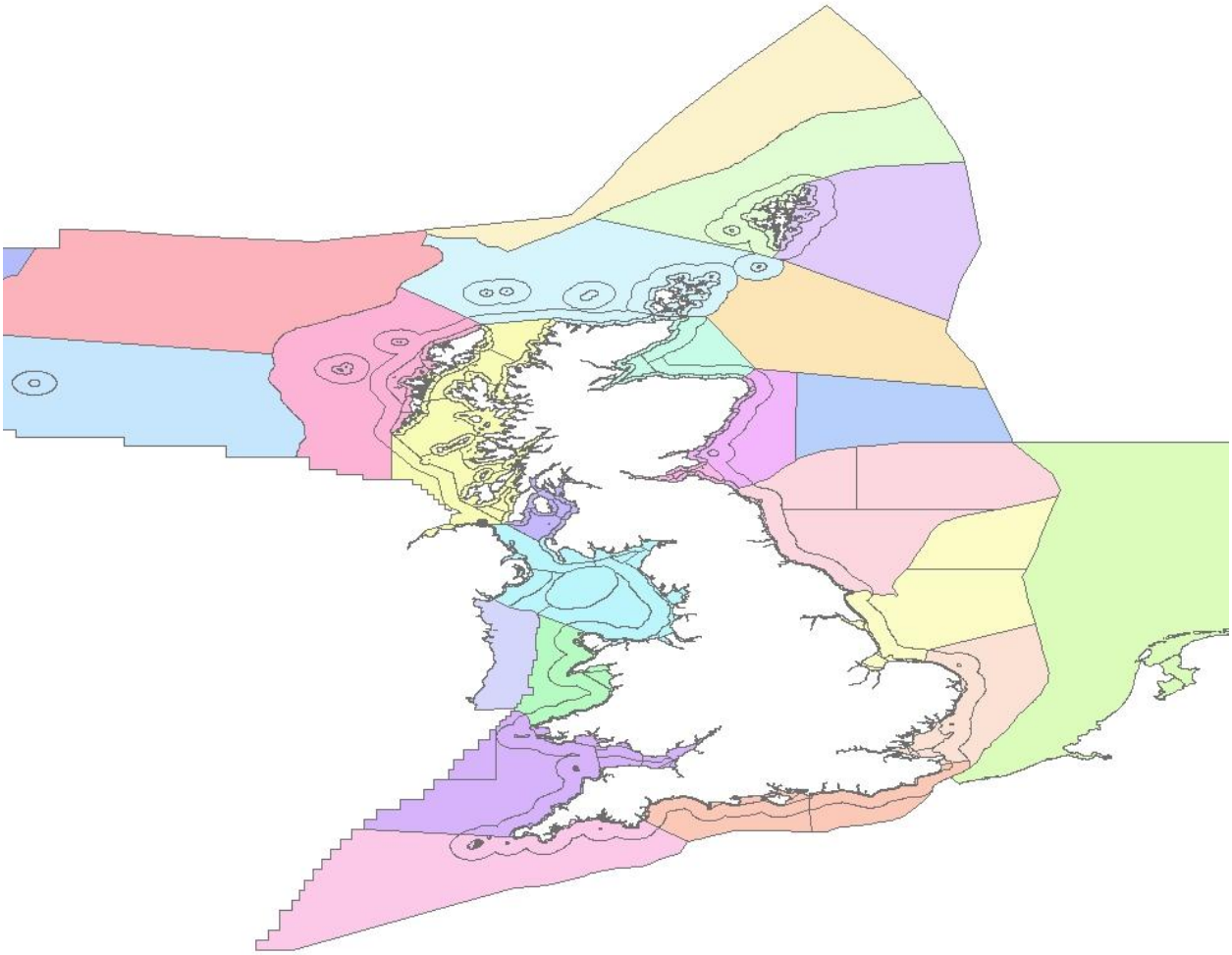
There are three main elements to the protocol:

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 nee 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 18: Procedural Guidelines for sampling and analysis of litter; beach, water column and seabed.

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.

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 strandline) 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. ~~𝍸~~) 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

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These are guidelines for monitoring litter using beam trawls or "granton type" trawls. Samples are usually taken opportunistically alongside sampling for other purposes e.g. benthos or fish.
(Note there are UNEP/IOC Guidelines for site specific programmes not easily transferable to opportunistic sampling currently undertaken for much of the current offshore fish and sediment monitoring programme of CSEMP.)
There are several variables which must be taken into account such as type of gear used, length of tow, water depth etc and e must be regarded as essential for data interpretation.
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: eg 2m beam, Granton trawl
Width of trawl; ie dsistance 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
Prevailing weather for one week prior to sampling (eg three days before sampling there were SW gales for one day)

9 Litter record:
Record all pieces of litter descriptively as follows:- eg
Polythene sheet / bags – length x breadth in cm
Plastic pieces – length x breadth in cm, or plastic drinks contaners by size e.g. 1Litre
Polypropylene –
Net eg old trawl – length x breadth in cm
Rope – length in cm
Strands – length in cm
Balls of polypropylene strands – these are common – sized as golf ball / tennis ball / 2 x tennis ball / football / n x football
Wood – length x breadth x height where applicable in cm
Metal – rod in length cm or piece in area length x breadth x height where applicable in cm
Cloth – describe item and size
Rubber – describe item and size where applicable eg rubber mat 50 x 70 cm or work-mans glove, car tyre
11 Other + + + +

12 Where possible identify origin of litter – e.g. :
Fishing
Sewage
Industrial
Domestic
Other + + +

SEE EXAMPLE OF RECORD SHEET below.
13 Record of colonising organisms:
For each piece of litter record generically the colonising organisms either as individuals or as percentage coverage eg. When species can be identified these should be recorded
Bryozoans 20%
Hydroids 35%
Barnacles 6 individuals or as %
Sea anenomies 7
Algae 5%
Other as appropriate
14. Assessment of data:
Basic requirement for reporting is: - "no of items per hectare of seabed"
Other data collected above is used for interpretation eg for polythene this is dependent on water flow through the net as it is buoyant whereas rigid heavier plastic may be confined to the sea bed.

Colonisation data is used for monitoring trends and behaviour of plastic and identifying non indigenous species..
Example of record sheet for water column / seabed litter

18c: Protocol for monitoring surface litter using a mantatrawl

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Protocol for sample storage

Rinse manta 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. Steps for processing:

a) Remove the cod end over a bucket, as a precaution to catch any spillage.

b) You will need a large bowl, squirt bottles, sample jar, spoon, tweezers, and a preservative. 4% Formalin is ideal, because it "fixes" tissues. If the sample will be stored for a short period of time (approximately 1 month) ethanol can also be used. Isopropyl alcohol could also be used as a short term substitute because of its accessibility and ease of use.

c) Pour sample into a large bowl.

d) 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.

e) Using very little water, squirt the surface of the cod end so any left over sample falls into the bowl.

f) Pour entire sample into the sample jar, then add preservative. A funnel may make it easier to pour sample into jar. More than one jar may be needed for a sample.

g) 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 or 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.

Protocol for sample analysis

1. YOU WILL NEED:

a.) One 5mm sieve to divide sample in two size classes (>5mm<)

b.) Two large bowls to hold each size class (2 liter size)

c.) Two large Petri dishes for sorting samples.

d.) Tweezers and a fresh water wash bottle full of water to separate plastic from other material

e.) Two jars or vials for holding two size classes of separated plastic

f.) Two trays for drying each size class separately

g.) Gram scale for weighing plastic content from each size class

h.) Copy of data sheet to record all information

2. Sample Preparation

a.) Drain sample through 5 mm sieve into one large bowl.

b.) Use fresh water wash bottle to rinse off plastic particles adhering to the cod end or inside of the sample jar.

c.) Rinse sample inside sieve in order to separate plastics thoroughly.

d.) Now that the sample is separated into two size classes, transfer each size class to a different large Petri dish.

e.) Rinse equipment gently with the wash bottle so that no plastic particles are left in the bowl or in the sieve.

f.) 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 #a thru #f for the complete sample, then split is carefully, sort separately, and combine data later.

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

a.) Place each Petri dish under a lighted microscope or use magnifying glass to see all particles.

b.) Using tweezers (forceps), remove all recognizable pieces of plastic that are floating.

c.) Rinse off plastic bits with fresh water wash bottle to make sure smaller particles or plankton are not sticking to them.

d.) Place rinsed bits of plastic in separate 46abelled 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.

e.) 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.

4. Drying of separated plastic

a.) If you have a drying oven, set it to 60°F.

b.) Spread sample onto Petri dishes.

c.) Place sample in oven, but if you do not have an oven, leave sample in a secure dry location, like a bookshelf or cabinet.

d.) 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.

5. Sorting plastic to determine type, count and weight

a.) 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.

b.) Count number of plastics for each type for each size category.

c.) Prepare gram scale for weighing sample. Tare the scale with Petri dish.

d.) Transfer sorted plastic to tared Petri dish to obtain weight in grams

e.) Record this weight, next to the count, on the data sheet

f.) Transfer sorted and weighed plastic to a vial or jar that is 46abelled appropriately.

g.) Continue with this procedure until all the sorted and counted plastic is weighed and recorded on the data sheet

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