



## BASELINE SURVEY, BENTHIC - STANDARD

### Introduction

As stated in SEPA's policy and in the Fish Farm Manual (1998), scientific data are required by SEPA in order to assess the existing condition on the site or leased area.

**SEPA reserves the right to request more detailed information and further work if required. The information asked for by SEPA may be subject to change and any requirements should be checked prior to any fieldwork and laboratory analysis.**

The protocols below shall be followed. The completed survey report using the data templates obtainable from the SEPA website shall be returned in duplicate to the local SEPA office.

### SCOPE OF SURVEY

**Sites where there has been no prior on-growing and where the maximum biomass applied for is <1000 tonnes\***

\*For farms of <500T and at which the applicant does not intend to apply for chemotherapeutants a visual sea bed survey may be provided as an alternative. The protocol for this is appended to Annex F.

If the application is for a site which is to be located in an area containing natural heritage concerns (see SEPA Natural Heritage Handbook Section 3.3 and also SEPA Regulation and Monitoring of Marine Cage Fish Farming in Scotland - a procedures manual, Annex C) then additional monitoring may be required, e.g. a visual survey (See Annex F)

**Applicants are strongly advised to discuss these or any alternative proposals in detail with their local SEPA Environmental Protection and Improvement Team or Marine Science staff, before undertaking any survey or analytical work.**

### Strategy and Protocol

#### 1. Timing

The responsible person shall carry out the following survey programme prior to completion and submission of the SEPA Controlled Activities Regulations (CAR) licence application. The time of year for this survey may be dictated by the business requirements of the operator but if it is possible it is recommended that the survey be carried out during summer months, May to October.

## 2. Survey location and strategy

Samples shall be obtained from 2 stations, separated by at least 100m, near the proposed location of the cages. In addition, samples should be obtained from 2 reference stations outwith the proposed (or actual) leased area. The reference stations should be characterised by similar depth, exposure and sediment type as the farm site. They should, ideally, be situated between 500m and 1km from the leased area and not influenced by, e.g. terrestrial discharges or other aquaculture operations.

Samples shall be analysed for benthic infauna and the following physico-chemical parameters: redox (Eh), organic carbon and particle size analysis (PSA). A visual description of the sediments should also be made.

Field notes must accompany any sampling and shall include any observations on weather conditions, position fixing problems, presence of fungus within the samples, anoxic sediments, etc.

**It is recommended that due cognisance be taken of appropriate health and safety procedures during all sampling and analytical processes. Appropriate safety measures must be taken when handling chemicals, biological preservatives and vital stains.**

## Protocols

### 1. Position Fixing

All sampling locations shall have positions recorded. Suitable methods of position fixing include DGPS (Differential Global Position Fixing) and Range Position Fixing Systems; any other method must be agreed with SEPA prior to fieldwork commencing. For more details of this process see Appendix 1.

The position of any sample shall be fixed as near to the exact time the sample is taken (e.g. when the grab hits the bottom). For monitoring purposes, positions must be able to be regained irrespective of cage position and orientation in successive years.

### 2. Benthic sample collection

Samples should be taken using a Van Veen or similar grab with top opening flaps for access and visual examination. The minimum grab size should be 0.02m<sup>2</sup>. At each sampling location, 5 replicate samples shall be collected to provide sediment for biological analysis and a minimum of 2 replicates should be collected for chemical analysis. Where grabs of 0.1m<sup>2</sup> are used, 2 replicate samples for biology will suffice, and 2 for chemistry.

**Samples to be analysed for chemical and physico-chemical determents should be obtained from separate grabs and should not be from sub-sampling of sediment obtained for other uses, e.g. faunal analysis.**

The grab and other sampling equipment should be washed out between the collection of each sample. Sampling personnel must follow any site disinfection policy.

### 3. Sediment Characterisation: Visual Assessment

A report on the condition of the sediment describing: colour (black, brown, etc.), physical consistency (sand, mud, shell gravel, etc.) and texture (soft, firm, etc.), shall be made. The presence of feed pellets and/or the white streaks of *Beggiatoa* on the

sediment surface shall also be noted. The depth of organic waste overlying 'real' sediment should be noted.

#### 4. Sediment Characterisation: Redox Potential (Eh)

This method is suitable for the determination of Reduction/Oxidation (Redox) potential of marine sediments. The measure of redox potential (Eh) enables comparisons of the intensity of the reducing conditions to be made on a site-to-site basis and hence act as an indication to the degree of organic loading. Eh is a qualitative measurement not quantitative (such as a concentration). In general, the more negative the Eh, the lower the ability of the sediment to exchange electrons, thus impairing chemical reactions vital for the sediment to sustain life.

Redox potential can also be used as an indicator of sulphide in sediments but not a concentration, "Eh (redox potential) is always negative in the presence of sulphide and positive in its absence" Nissenbaum *et al* 1972. This method is suitable for sediments collected by grabs and cores.

To determine Eh values, a portable redox meter should be used with a suitable electrode and reference probe. For practicality, and as they are more robust, a combined electrode may also be used.

Before use, the probe should be tested using a reference solution of known mV value (e.g. commercially prepared Zobells solution). All meter readings should be corrected by the difference between the expected reference solution value and actual meter reading. This accounts for inconsistencies between probes. This reference-solution correction value must be added to a second correction value that is specific to each probe type in order to convert the mV values obtained from the samples to Eh. This second step is essential as Annex A of the SEPA fish farm manual gives Eh quality standards that have been corrected to the Standard Hydrogen Electrode (SHE) and so this same correction must apply to the mV readings obtained from the samples. Typically this has been done by the addition of 198mV to the raw redox measurements but it is advisable to check with the manufacturer of the probe to ensure the correct value for the probe. Note that this value varies subtly with temperature so it is recommended that the temperature of the sediment is measured before readings begin. A range of correction values for some commonly used probes is given below in Table 1. Care must be taken as expected mV readings may already include the SHE correction (refer to manufacturers' specification). Details of all corrections applied must be included in the final report.

Table1: Correction values for some commonly used probes

Temp . C	Silver:Silver chloride ref probe			Calomel ref. probe			Orion 96-78 combi probe
	3M KCl*	3.5M KCl*	4M KCl*	3M KCl*	3.5M KCl*	4M KCl*	
0	226	221	224	265	262	260	260
1	225	220	223	265	261	260	259
2	225	220	222	264	261	259	259
3	224	219	221	264	260	258	258
4	224	219	220	263	260	258	258
5	223	218	219	263	259	257	257
6	222	217	218	262	258	257	257
7	222	217	217	262	258	256	256
8	221	216	216	261	257	255	256
9	221	216	215	261	257	255	255
10	220	215	214	260	256	254	255
11	219	214	213	260	256	253	254
12	218	214	212	259	255	253	253
13	218	213	211	259	255	252	252
14	217	212	210	258	254	251	251
15	216	212	209	258	254	251	250
20	213	208	204	257	252	248	249
25	209	205	199	255	250	244	246
30	205	201	194	253	248	241	242

Samples for redox should not be sub sampled for any other chemo-physical analyses

Samples should be collected by small grab or corer. As a minimum, at each sample location, two profiles of redox measurements should be made. Each profile should be measured in a separate grab/core sample. Where possible, measurements should be made at 1cm intervals from the sediment surface to the bottom of the sampler (i.e. at 0, 1, 2, 3, 4 and 5cm, depending on the depth of sediment in the sampling device) taking care not to allow the probe tip to touch the metal sides of the grab.

Measurements (mV) are taken immediately on collection of the sample whilst still in the sampler. The meter readings should be allowed to stabilise before recording.

#### 5. Sediment Characterisation: Organic Carbon or Organic material (Loss on Ignition)

As a minimum, at each sampling location, one sub-sample (~50ml) of sediment shall be collected from the sample surface (0-2cm). The sample is stored in an airtight container and deep frozen, or frozen then freeze dried, for later analysis.

It is recommended that, as a minimum, the Loss on Ignition (% LOI) method be used following the procedure of Allen *et al* (1974). Total organic carbon can also be measured by combustion (the method used must be specified).

## 6. Sediment Characterisation: Particle Size Analysis

As a minimum, at each sampling location, one sub-sample (100ml) of sediment should be collected from the surface (0-2cm) and stored in suitable containers.

Assessment of the particle size distribution of sediments is generally determined by dry sieving or laser granulometry, though other methods are possible. The method used must be quoted in any final report.

For laser analyses, fresh samples may be used or samples can be stored overnight in a fridge at 4°C or longer term in a deep freeze at -18°C. Laser granulometry results are reported graphically and in table format.

For dry sieving, storage at -18° C, prior to drying, is recommended. Samples may be freeze or oven dried. Sieved sediments pass through each sieve in a stack. The size fractions are weighed and the results are expressed as a percentage of the total.

## 7. Benthic Biology: Sample preparation

Each replicate sample must be processed separately. Samples are usually sieved on site, with each sample being washed with seawater, through a 1mm metal mesh sieve. The material retained on the sieve is then transferred to a clearly labelled, airtight container, taking care not to leave animals on the sieve. Alternatively in the field, samples may be potted whole and separately into labelled sealed pots or double plastic bags.

Samples are subsequently preserved using buffered formalin (50g sodium tetraborate in 2.5litres of 40% formaldehyde solution, diluted with seawater to 4% solution), as soon after collection as practicable.

## 8. Benthic Biology: Sample Analysis

Prior to sorting and identification, formaldehyde is removed from the sample by thorough rinsing with fresh water through a 1mm sieve. To further aid sorting, light organic matter and fauna may be elutriated off and sorted separately.

The use of a vital stain, e.g. Rose Bengal, is not essential but is recommended to aid sorting. If the sample is to be stained, the sieve and its contents are placed in a white sorting tray, containing 5ml of rose bengal solution and enough water to cover the contents of the sieve; this is left to stain for at least 20mins. The sample is then thoroughly rinsed again.

The sample retained on the sieve (sieve residue) is then washed into a white sorting tray, with enough water to cover the sample. The sieve is checked to ensure no animals are left in the mesh, and then cleaned to ensure no cross-contamination. The sample identification label must remain with the sample at all times.

All the fauna is picked by hand, using forceps and/or pipette, under suitable illumination and magnification, and transferred into suitable labelled containers. Each tray should be sorted in a methodical manner to minimise the risk of missing any fauna. The specimens should be stored in 70% IMS/water preservative which can be made viscous to prevent evaporation by the addition of glycerol.

The analysis must be carried out by a competent benthic taxonomist. Before submission of survey data, SEPA requires evidence of training and continuing competency of analysts.

SEPA also requires evidence of participation in external AQC schemes, such as National Marine Biological AQC Scheme (see also Annex B regarding Quality assurance and control).

The fauna should be identified and enumerated to the lowest taxonomic level, with the use of stereo/compound microscopes, and standard identification keys and references (a comprehensive list of these works is available from SEPA on request). Nomenclature should follow the MCS species directory as far as is possible, or the most recent taxonomic publication on that group. The level of identification expected for major taxonomic groups is species level.

Analysts should note the following:

- The anterior ends of specimens are counted to determine the abundances of each taxon. Where a species does not have an obvious head, then it is counted if more than half of the specimen is present,
- Nematodes <1cm in length are considered to be meiofauna – their presence may be noted, but only nematodes >1cm are to be counted,
- Estimated counts will only be acceptable for common taxa, and where their abundance in a sample is >200 individuals,
- Certain taxa (e.g. colonial, encrusting) are not included as quantitative taxa but are simply recorded as present (qualitative taxa), and
- A note will also be made of any fauna that are listed on the UK Biodiversity Action Plan list, which may be found on JNCC's website ([www.ukbap.org.uk](http://www.ukbap.org.uk)).

**Sub-sampling of taxa is not recommended. If considered necessary, it must be agreed with SEPA in advance.**

Following analysis, the containers of preserved identified specimens must be retained for a period of at least 12 months and must be made available on request by SEPA for auditing purposes.

The raw data shall be reported as a species/abundance matrix, both on a by-replicate and by-station basis. Faunal indices including diversity (Shannon-Weiner  $H'$   $\log_2$ ), evenness indices (Pielou 1975) and Infaunal Trophic Index (Cotling and Ashley 1992) should be derived.

Further notes to aid statistical analysis are given in Appendix 2. Additionally, extensive descriptions of the methodologies can be found in Rees, Moore, Pearson et al (1990), and Pielou (1975).

### **Reporting**

The data and accompanying text shall be reported in the Standard Baseline template. The details of this system can be found in Attachment XII. These are spreadsheet-based templates and the preferred format for submission to SEPA shall be CD or DVD. If the operator has difficulties in completing this survey template then they should contact SEPA to seek advice. It is not recommended that operators submit paper records as an alternative without prior discussion.

Completed benthic survey reports shall be submitted to SEPA within 16 weeks. Should additional surveys be required e.g. seabed video, the submission period is 12 weeks. If difficulty in achieving this is experienced, then the local SEPA team must be notified as soon as possible.

### **Auditing of Results**

SEPA may require at any time to seek evidence of quality assurance and control on any procedures or processes being undertaken by the responsible person or their agents, or may require independent audit of any resulting data.

**References**

Allen, S.F., Grimshaw, H.M., Parkinson, J.A. & Quimby, C. (1974) Chemical Analysis of Ecological Material, 1st edn. Blackwell Scientific Publications, Oxford, UK

Codling, I. D. and Ashley, S. J. (1992) Development of a biotic index for the assessment of pollution status of marine benthic communities. Final report to SNIFFER and NRA. NR 3102/1

Pielou, E.C. (1975) Ecological diversity. Pub. Wiley, New York 165pp

Rees, H. L., Moore, D.C., Pearson, T.H., Elliot, M., Service, M., Pomfret, J. and Johnson, D. (1990) Procedures for the Monitoring of Marine Benthic Communities at UK Sewage Sludge Disposal Sites. Dept. of Agriculture and Fisheries for Scotland. Scottish Fisheries Information Pamphlet No. 18. 79p.

## Appendix 1

### Notes to Aid Position Fixing

#### Position Fixing

The position of any point on a surface can be fixed using a two-dimensional co-ordinate system (X and Y). Two principle systems of X and Y co-ordinates are used within the UK:

- **National Grid References (NGR).** A full NGR consists of two six-digit numbers, an Easting and a Northing and is accurate to 1m. In practice many locations are not known (or required) this accurately and a position is more usually given by an alphanumeric, e.g. NS 300 710. Such an NGR is only accurate to 100m; where possible SEPA will record a 10-digit alphanumeric NGR that is accurate to 10m.
- **Latitude/longitude position fixing** is routinely used for navigational purposes and is usually invoked as a marine/coastal site descriptor once the location is identified by other means, e.g. GPS, Range Position Fixing. The angle west or east from the meridian is given in degrees, minutes and seconds.

There are several reference systems against which the three-dimensional position in space may be recorded. Although these may use the same reference units, i.e. degrees of latitude and longitude, there are differences between datums and the idealised reference shapes (geoids) used to approximate the surface of the earth. Thus any one location may have significantly different co-ordinates under different systems or conversely one co-ordinate pair may refer to positions that may be up to 1km apart when different datums are used. So, it is important to include the name of the reference datum or co-ordinate system when quoting positions.

GPS receivers are commonly set to WGS84 (equivalent to ETRS89) as a default. Positions on OS maps and Admiralty charts for British waters, and National Grid References are specified with respect to OSGB36, which is often provided as an alternative datum option on GPS receivers sold in the UK. A software tool for conversion between the WGS84 and OSGB36 and further information about geodesy is available from [www.gps.gov.uk](http://www.gps.gov.uk).

#### Methods of position fixing

- **GPS (Global Position System) and DGPS (Differential GPS)** are satellite navigation systems. Transmissions from satellites are detected by a receiver and calculated into positional data. GPS accuracy is around 10m, depending on the receiver, the number of satellites in view and other factors. High accuracy (<1m) may be achieved by using a differential correction system. It is important to be aware of the datum against which a receiver is referencing the positions it produces (see above).
- **Range Position Fixing Systems.** These are normally microwave devices that display the distance from a master transmitter to a set of onshore 'slaves' at precisely known locations. The accuracy depends on the accuracy of the position of these slaves. Accuracy of 25m - <1m can be achieved.



## Appendix 2

### Biological Data Analysis

#### Primary measures and Univariate methods

Primary measures are simply the number of taxa and number of individuals at each site, whereas univariate methods refer to the calculation of diversity indices. A variety of indices have been proposed but the widely used Shannon-Wiener index ( $H'$ , using logs to the base 2) is recommended by Rees *et al* (1990) and Pearson and Rosenberg (1978). Diversity is considered to have two components; species richness and equitability. Equitability can be measured using Pielou's evenness index (Pielou 1975).

These single figure measures represent a severe simplification of the biological data and consequently, if used without other complementary methods, may lead to misinterpretation. They can be extremely useful, however, if a gradient of effect is apparent or expected. In addition their simplicity suggests that the derivation of biological standards may be practical.

#### Multivariate Methods

These methods have the advantage that the maximum amount of information in the original 'species at sites' data matrix is maintained because species identity is not lost during the analysis. Multivariate techniques are known to be very sensitive methods for analysing changes in community structure (Warwick and Clark, 1991).

There are two main types of multivariate methods as follows:

- a) Ordination, which condenses the biological information down to a two dimensional graph of the relationship between sites. The widely used techniques are: Multi-Dimensional Scaling (MDS) and Dendrograms available in the statistics software package PRIMER and De-trended Correspondence Analysis (DECORANA) available in the Cornell Ecology Programme (CEP).
- b) Classification, where the sites are sorted into groups on the basis of the similarity of the biological information between replicates and/or stations. Two widely used classification techniques are Two-way Indicator Species Analysis available in the CEP package as TWINSPAN and Group Average Sorting of Bray-Curtis Similarity Coefficients available in PRIMER.

All four of these methods are described by Rees *et al* (1990).

Before any multivariate analysis of the biological data takes place, it is common practice to transform the data. The purpose of transformation is to alter the balance between the contribution from common and rare species in the analysis. Effectively all transformations increase the contribution of rare species and decrease the contribution of common species (see Rees *et al*, 1990, for further details).

Multivariate methods are particularly useful in examining the relationship between environmental data and the biological data, using for example the BIOENV programme in PRIMER. BIOENV selects environmental variables, which best explain the community pattern by "maximising a rank correlation between their respective similarity matrices" (Carr,1996). Spearman Rank Correlation coefficients (Siegel and Castellan, 1988) can be used in

conjunction with DECORANA to elucidate the relationship between environmental variables and sites. Data on the environmental variables in question must be available for each sampling station before any relationship can be established. Furthermore multivariate analysis cannot prove a relationship exists but it is strong evidence that one may exist.

The application of standards to multivariate methods is complex but there is no reason why a predictive system similar to the RIVPACS Scheme used in freshwater (Moss *et al*, 1987) could not be developed for marine systems. Rees *et al* (1990) have outlined the approach necessary. Multivariate methods form an essential part of the consideration of benthic data due to their sensitivity and must be used to confirm the conclusions of the univariate methods applied.

**Infaunal Trophic Index**

The Infaunal Trophic Index (ITI) is a biotic index, which has been developed by the Water Research Centre (WRc). It relies on the assessment of the changes in the feeding (trophic) mode of benthic organisms in areas subject to elevated levels of organic enrichment. Details of the index and its use can be found in WRc (Codling and Ashley 1992). The index was developed from a system originally devised for use in California (Word, 1979). This index differs from the preceding essentially numerical methods because knowledge of the ecology of the taxa involved is required. Thus it forms a useful complement to the numerical methods in that the data are considered from an alternative perspective. The index was found to respond satisfactorily to pollution gradients from a variety of sources including sewage and industrial discharges.

The ITI has great potential with regard to the setting of standards. The index has values that range from 0 to 100 and results can be interpreted as follows:

<u>Index Value</u>	<u>Assessment</u>
60 to 100	Community 'Normal'
30 to 60	Community 'Changed'
< 30	Community 'Degraded'