

BS ISO 12878:2012



BSI Standards Publication

# Environmental monitoring of the impacts from marine finfish farms on soft bottom

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**National foreword**

This British Standard is the UK implementation of ISO 12878:2012.

The UK participation in its preparation was entrusted to Technical Committee AW/234, Fisheries and Aquaculture.

A list of organizations represented on this committee can be obtained on request to its secretary.

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ISBN 978 0 580 66579 0

ICS 65.150

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This British Standard was published under the authority of the Standards Policy and Strategy Committee on 31 August 2012.

**Amendments issued since publication**

Date	Text affected
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**Environmental monitoring of the impacts  
from marine finfish farms on soft bottom**

*Surveillance environnementale des impacts sur le fond mou des  
exploitations de pisciculture marine*





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Published in Switzerland

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## Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 12878 was prepared by Technical Committee ISO/TC 234, *Fisheries and aquaculture*.

## Introduction

Particulate effluents from finfish farms can affect environmental conditions on the surrounding seafloor as well as the health of the farmed fish. These effluents consist of excess feed and faecal pellets from the fish, and are released as particles in a variety of sizes, depending on the fish species, feed type, temperature and other conditions in the aquaculture operation. Depending on the hydrodynamic and bathymetric conditions in the area, the particles settle on the seabed at various distances from the finfish cages. This leads to changes in the chemistry and the biology of the sediments, and if the effluent load is high it can even result in sediments depleted of biota.

The aquaculture industry is dependent on favourable environmental conditions to ensure good fish health and optimal growth. Excessive accumulation of organic material in the form of waste feed pellets and fish faeces can change the habitat characteristics of bottom substrates, leading to eutrophication and associated negative changes in biodiversity. Repeated and systematic monitoring can give an overview of changes in bottom conditions, and remedial action can be implemented should the developments be in a negative direction.

All livestock farming has some impact on the environment. It is intended that the environmental impact on the seabed not exceed acceptable and agreed-upon limits established for the local impact zone or farm licence area. Threshold values for environmental impact are expected to be set to prevent unacceptable impact on the seabed in the surrounding area and on its biota. Threshold values are also expected to ensure favourable living conditions for farmed fish such that finfish farm sites can be in use over a longer time period. Pollution control authorities define threshold values for environmental quality. For personnel and organizations using this International Standard, it can be helpful to have a reference to the legal and policy framework of their country or state. It is strongly intended to streamline the environmental monitoring process in a way that involves all institutions responsible for the marine environment.

The main emphasis of this International Standard is on methods for measuring impacts on the bottom conditions at and around finfish farm sites. In certain cases, there can be a need for a broader environmental monitoring programme to highlight a given set of problems or to consider the condition of the receiving environment, as a whole. In this International Standard, examples of monitoring surveys of finfish farms in some countries are presented in Annex A.

Finfish farm sites, which are sited over seabed consisting of bedrock, larger rocks/stones or other hard substrate, can be surveyed following the guidelines given in ISO 19493. This International Standard only gives guidelines for monitoring of effluents from finfish farms sited on soft bottom.





# Environmental monitoring of the impacts from marine finfish farms on soft bottom

## 1 Scope

This International Standard establishes an approach for sampling and empirical measurement of soft-bottom impacts from marine finfish net pen farms, and gives examples of detailed procedures for how environmental impacts from finfish net pen farm sites can be monitored in the field, including guidelines for quality assurance of sampling protocols and safety. The emphasis of the environmental impact in this International Standard is on eutrophication effects on the seabed.

This International Standard identifies ecological objectives, the indicators used, and the methodology and design, and encompasses guidelines for quality assurance of sampling protocols and operational safety.

## 2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 16665, *Water quality — Guidelines for quantitative sampling and sample processing of marine soft-bottom macrofauna*

## 3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

### 3.1

#### **anchoring area**

area delimited by the anchoring points of the cage area

### 3.2

#### **anchor line**

line, cable or chain from the anchor points to the cage area

### 3.3

#### **anchor points**

attachment point of the anchor line

### 3.4

#### **area of influence**

area of seabed where environment is influenced or expected to be influenced, based on the available information or as identified through the use of predictive models

### 3.5

#### **baseline monitoring**

sampling of an area of influence, which previously was not used for finfish production

### 3.6

#### **benthic**

associated with the seafloor

[ISO 16665:2005, definition 2.2]

- 3.7**  
**benthic macrofauna**  
bottom-dwelling animals retained on a mesh screen of 0,5 mm or 1 mm aperture size  
[ISO 16665:2005, definition 2.3]
- 3.8**  
**biological production**  
biomass remaining in the cages(s) at the end of the year, minus the starting biomass at the beginning of the year, plus harvested biomass, mortalities and waste  
  
NOTE The term “waste” includes escapees and sorted-out fish, which are not harvested.
- 3.9**  
**cage**  
floating framework with attached net bag, which encloses the fish, and which forms a part of the fish farm
- 3.10**  
**cage area**  
area of seabed directly below the cage
- 3.11**  
**environmental monitoring**  
systematic observation, measurement and calculation of the condition of the environment, emission of pollutants or populations and species, which are necessary for the assessment of the condition of the environment, the development of environment policies and the planning of environmental protection measures, as well as the control of the effectiveness thereof
- 3.12**  
**fish farm site**  
geographically defined location for aquaculture
- 3.13**  
**hard substrate**  
**hard bottom**  
substrate consisting of bedrock, larger rocks/stones or fixed marine constructions such as wharfs, quays and pipelines
- 3.14**  
**indicator species**  
benthic species that defines a trait or characteristic of the environment or that serves as a measure of the environmental conditions existing in a given location
- 3.15**  
**monitoring level**  
scope of survey required to determine whether or not the environmental impact is retained within specified threshold values
- 3.16**  
**monitoring programme**  
set of routine measurements of parameters which describe environmental effects from finfish farms
- 3.17**  
**operational monitoring**  
sampling conducted during operation of a finfish aquaculture facility
- 3.18**  
**pilot survey**  
survey used for simple rapid assessment and basic information on a site

### **3.19**

#### **production cycle**

period from the time fish are initially stocked into the sea until the cohort is completely removed through harvesting or transfer

### **3.20**

#### **receiving environment**

water body that receives input of natural or anthropogenic origin

### **3.21**

#### **reference station**

sampling station chosen to represent background or natural environmental conditions in a given area on seabed, i.e. free from direct anthropogenic influences

NOTE Adapted from ISO 16665:2005.

### **3.22**

#### **sampling station**

precise location where recording is carried out and any samples are collected

NOTE Adapted from ISO 16665:2005.

### **3.23**

#### **sediment condition**

classification of the observed condition in the sediment

### **3.24**

#### **soft bottom**

areas of seafloor consisting of loose deposited particles, including clay, mud, sand and gravel, shells and maerl, where it is possible to sample with a grab or a corer

NOTE A minimum of three sampling attempts are intended to be carried out. If any one is successful, it is intended that the substrate be treated as soft. It also includes mixed substrata with gravels, small stones and pebbles scattered on a bed of finer material, but excludes cobble.

### **3.25**

#### **soft-bottom fauna**

animals living on or completely/partially buried in soft sediments

### **3.26**

#### **tenure**

total area on seabed that is licensed or otherwise permitted by governmental authority to be utilized for finfish farming

### **3.27**

#### **threshold value**

value of a parameter that divides between defined levels of impact in a monitoring programme

### **3.28**

#### **transect monitoring**

documentation of qualitative and quantitative changes over a distance

## **4 Principles for monitoring**

### **4.1 Aim and principles**

The ecological objective of the monitoring is systematic observation, measurement and calculation of the condition of the environment, emission of pollutants or populations and species, which are necessary for the assessment of the condition of the environment, and the planning of environmental protection measures, as well as the control of the effectiveness thereof.

The effort of environmental monitoring should be proportional to the scale of impact and should focus on long term sustainable use of the seabed in farming areas.

Principles for monitoring of environmental impact on the seabed may be summarized as follows:

- before a site is utilized for aquaculture production, baseline monitoring should be carried out, if possible;
- if baseline monitoring is not possible, a reference station may be utilized for comparison;
- threshold values for environmental impact should be set such that finfish farm sites may be in use over a longer time period.. These values should aim to ensure favourable living conditions for farmed fish as well as to prevent unacceptable impact on the surrounding seabed area. The responsible government may have established threshold values for unacceptable impact and impact categories;
- monitoring of the seabed should be regular; the more impact a finfish farm has on the seabed, the more often the monitoring survey should be performed (see Table 3);
- different monitoring surveys may be used in different areas: where little impact is tolerated by pollution authorities or by society, the survey should be able to detect subtle changes; where more impact is tolerated, a simpler survey may be enough to provide a satisfactory result;
- the monitoring survey used should be suited to the task and the following considered: the aim of the monitoring; how detailed the survey should be to provide a comprehensive result; the level of accuracy needed for the measured variables; practicality, efficiency, time consumption and costs involved in relation to the outcome; transparency;
- surveys comprising multiple parameters are less sensitive to anomalies in individual parameters and may provide a more robust result;
- the variables that make up a monitoring survey may be organized in modules and be replaced or modified, as appropriate, according to new knowledge, techniques or legislations.

## 4.2 Impact zones

Finfish farm effluent consists of large particles (e.g. waste feed pellets and intact faecal pellets), smaller suspended particles (e.g. feed dust and broken faecal pellets) and dissolved material (nutrients, organic compounds, etc.). These types of effluents have different potential dispersal kinetics and affect the water column and seafloor at varying distances from the finfish farm. Normally, a greater impact is accepted under a finfish farm than further out into the receiving environment. Around a finfish farm, various impact zones are formed, which are affected to different degrees (see Table 1). For medium- to high-current sites, it is possible that the maximum impact does not occur under the farm, but rather adjacent and down current to the farm.

**Table 1 — Overview of impact zones**

	Type of impact zone		
	Local impact zone	Intermediate impact zone	Regional impact zone
<b>Definition</b>	Area of seabed under and near a finfish farm where most of the larger particles are deposited, e.g. less than 30 m from cages.	Area of seabed between the local impact zone and the regional impact zone, where sedimentation of smaller particles occurs. At deep, high-current sites, also larger particles can accumulate here.	Area of seabed beyond intermediate zone
<b>Source of impact</b>	Finfish farm	The finfish farm is the main source of impact, but other factors can also contribute.	The finfish farm is one of several potential sources of impact.
<b>Potential impact</b>	Changes in the physical, chemical and biological conditions on the seafloor	Usually less impacts relative to the local impact zone.	Changes in benthic fauna and community structure

The type of survey used depends on the level of impact and on the kind of impact zone which is monitored.

### 4.3 Survey types

Surveys may be divided into three main categories (see Table 2) according to the objectives.

**Table 2 — Overview of main categories of survey type**

Survey type	Objectives
Baseline monitoring	Characterizes conditions in a given area of seabed before operation of a finfish aquaculture facility. Also maps or identifies the impact of other sources. Faunal composition and/or biogeochemical and hydrodynamic parameters are compared with specified assessment criteria or simply with other representative areas of seabed (reference areas).
Operational monitoring of local impact zone	The samples are taken close to the aquaculture facility in the local impact zone for frequent surveillance of impact from finfish farm.
Operational transect monitoring	The samples are taken in a transect from the local, to the intermediate and to the regional impact zone for surveillance of the impact of the finfish farm, impact of other potential sources and for documentation of natural environmental and biological changes.

In addition, a pilot survey may be conducted. A pilot survey gives a general overview of bottom and faunal conditions and is used either for simple rapid assessment or to give basic information for designing more detailed sampling programmes.

For further information on the different survey types, see ISO 16665.

## 5 Methodology

### 5.1 Sampling strategy

#### 5.1.1 Sampling programme and planning

The design of the sampling programme depends on the detailed aims of the survey and the required power of the data. The programme should be developed with regard to local bathymetric and hydrodynamic conditions in the survey seabed area, information on local contamination sources and knowledge from previous surveys, if any. The number of sampling stations, their positions and numbers of replicate samples to be taken at each sampling station, as described in the following subclauses, should be established according to the main prevalent currents within the area and prior to the initiation of the survey.

Baseline monitoring of the seafloor is conducted before operation of a finfish aquaculture facility is started. Based on information about water currents, bathymetry and layout for the planned finfish farm, the amount and pattern of the deposition of effluents from the farm on the seafloor may be predicted. A frequently used term for this deposition is “footprint of deposition” around the cages. There are several ways to estimate the footprint of deposition. A simplified method for prediction is to include the potential site area of seabed, plus the expected deposition downstream of the finfish farm. Recommended computerized tools for prediction of deposition footprints around finfish operations are also available. Based on the predicted footprint of deposition and information about water currents, bathymetry and layout for the planned finfish farm, the geographical coordinates for the sampling stations for baseline and operational monitoring can be set out as described in 5.1.2.

For finfish farms that are already established, positioning makes reference to the positions of cages and containments and, if possible, to geographic position survey locations. It can be set out directly as described in 5.1.2.

For groups of adjacent compact cages or cage arrays, the samples should be taken along the outer edge of, and, if possible, in between the individual cages. The sampling area of seabed should, as far as possible, be representative of the entire area under the finfish farm (local impact zone).

For dispersed cages, the samples should be taken along the outer edge of the cages, and if possible, in between the individual dispersed cages. At least one sample should be taken at each cage, depending on the total number of cages at the finfish farm site, as well as a holistic assessment of how the most representative picture may be achieved.

## 5.1.2 Positioning of sampling stations

Before the positioning of sampling stations, information about water currents, bathymetry, layout and deposition footprint for the finfish farm should be available.

### 5.1.2.1 Sampling stations for operational monitoring of local impact zone

A minimum of four samples should be taken from each finfish farming site. Additional samples should be considered depending on the size of the operation. Sampling should be carried out from the edge of the cages or containments, or in their immediate vicinity. The sampling points should be positioned according to the bathymetry, dominant current direction and the dimensions and layout of the finfish farm, such that they represent as greatly as possible, the entire local impact zone.

All the observations made should be noted in the field log and included in the subsequent evaluation of results. All sampling positions should be shown on a map, preferably geo-referenced.

### 5.1.2.2 Sampling stations for operational transect monitoring

Samples from at least three sampling stations, at least one in each impact zone, should be taken. These three stations shall be considered fixed long-term monitoring stations, provided the cages do not shift in location due to intentional or unintentional changes.

The number of replicates taken on each sampling station should be chosen on the basis of statistical necessity. At least two replicates are recommended at each sampling station:

- at least one set of samples should be taken in the local impact zone downstream as close to the cage area as practicable (sampling station 1) at the perimeter of containment structures;
- at least one set should be taken downstream in the intermediate impact zone (sampling station 2), typically 30 m away from cage area or as defined by the regulator;
- at least one set should be taken downstream in the regional impact zone (sampling station 3), typically, 100 m from cage area or as defined by the regulator;
- if a depression exists in the area of influence where accumulation of waste from the finfish farm is probable, sampling station 3 is positioned in this depression. In this case, sampling station 2 should be taken downstream in the intermediate impact zone, half-way between the cage area and the depression.

If the cages are located over a steep slope without sediment accumulation, sampling station 1 and sampling station 2 should be positioned at the foot of the slope. If it is not possible to obtain samples from near the finfish farm (sampling station 1) or further out into the intermediate impact zone (sampling station 2), all three sampling stations should be positioned in the nearest deep area of seabed.

### 5.1.2.3 Sampling stations for baseline monitoring

The same number of stations for baseline monitoring are positioned, if possible, at the same geographical coordinates or relative positions as stations for operational monitoring of the local impact zone plus stations for operational transect monitoring. Furthermore, one or more reference stations should be chosen between 500 m and 2 000 m beyond the local impact zone. Reference stations are used to determine whether observed changes adjacent to the farm are a result of farm activities or due to changes in the broader receiving environment. They can also be used to indicate changes in the receiving environment associated with carrying capacity. The reference stations should, as far as possible, be representative of conditions unaffected by effluent sources and allow assessment of natural temporal and spatial variations in the soft-bottom faunal communities.

Reference stations should be located in conditions as similar as possible to those at the regular sampling stations, i.e. with similar depth and sediment type as indicated by analyses of sediment grain size distribution. Multiple reference stations are particularly important in heterogeneous areas of seabed. Reference stations should be considered as fixed long-term monitoring stations.

A baseline survey consists of a minimum of eight sampling stations: a minimum of four sampling stations, which during operation of the finfish farm are used as sampling stations for operational monitoring of local impact

zone, plus a minimum of three sampling stations that later will be used for operational transect monitoring, plus minimum one reference station.

Baseline monitoring of the seabed is conducted only once before operation of the farm is started. The parameters, which are monitored during a baseline survey, are described in Table 5. Reference station(s) are, in general, monitored during baseline monitoring and only during operation of a finfish farm if environmental conditions in the area require a comparison with fixed long-term monitoring stations.

#### 5.1.2.4 Positioning of sampling stations for operations with scattered distribution

In special circumstances, sampling stations may be randomly distributed. An example of this application can be where no previous knowledge of the seabed area is available as a guide to appropriate positioning, for instance for baseline monitoring in an area of seabed where it is intended to establish several small finfish farms close to each other in a region. This can also be the case in regions where organic input comes from several independently managed finfish farm operations and where it is not possible to establish a set of sampling stations, which is unaffected by neighbouring farms or other sources of organic input. Under these circumstances, monitoring stations for monitoring of intermediate and regional impact zones may be positioned randomly distributed. Reference stations should still be positioned beyond the affected seabed area. The reference stations should, as far as possible, be representative of conditions unaffected by effluent sources and allow assessment of natural temporal and spatial variations in the soft-bottom faunal communities. Stations for operational monitoring should be positioned along the edge of the cages or containments.

## 5.2 Frequency of operational monitoring of local impact zone

The effort made for environmental monitoring should be proportional to the scale of impact and should focus on long-term sustainable use of the marine environment. Guidance on the frequency of operational monitoring of local impact zone is given in Table 3. The timing of the survey is determined by the production cycle at the finfish farm site, i.e. surveys should be carried out during periods where the feed consumption is highest. A common component of aquaculture site management is the use of fallow periods, which allow some time for ecosystems to assimilate organic inputs from farm activities. Fallowing may impact the frequency of monitoring required.

**Table 3 — Guidance on the frequency of operational monitoring of local impact zone at finfish farm sites (local impact zone) in relation to impacts at the site (sediment condition)**

Sediment condition	Minimum monitoring frequency
Very good	Every second year or every second production cycle
Good	Every production cycle, alternatively every year
Poor	Every six months
Very poor	In most countries, authorities require that production changes be made.
NOTE Under very poor conditions, it is likely that the sediments are totally anoxic, with production of methane gas and hydrogen sulfide, and with a total absence of burrowing organisms (infauna).	

Threshold values between the various sediment conditions are set by the responsible government.

## 5.3 Frequency of operational transect monitoring

### 5.3.1 General

If the results of operational monitoring indicate declining sediment condition in the local impact zone, operational transect monitoring is triggered. Guidance on the frequency for carrying out operational transect monitoring is given in Table 4. The timing of the survey is determined by the production cycle at the finfish farm site, i.e. surveys should be carried out during periods where the potential maximum impact is likely.

**Table 4 — Guidance on the frequency of operational transect monitoring at finfish farm sites in relation to impacts at the site (sediment condition)**

Sediment condition in local impact zone	Minimum monitoring frequency of transect
Very good	No transect monitoring necessary
Good	Every second year or every second production cycle
Poor	Every production cycle, alternatively every year
Very poor	In most countries, authorities require that production changes be made.

NOTE Under very poor conditions, it is likely that the sediments are totally anoxic, with production of methane gas and hydrogen sulfide, and with a total absence of burrowing organisms (infauna).

Threshold values between the various sediment conditions are set by the responsible government.

### 5.3.2 Parameters

#### 5.3.2.1 General

The decision regarding which parameters to use depends on current legislation, the survey type (baseline or operational monitoring), the impact (high or low) and the size of the impacted seabed area (size of deposition footprint) as well as cost-effectiveness, availability of laboratory or field equipment and trained personnel. Where a set of parameters has been chosen, it should be used repeatedly, thereby ensuring that results can be compared over time. Because rules have to be applied in the same manner to every farm, a set of similar parameters should be selected for finfish farms operating under similar conditions in a region, state or country. Examples of sets of parameters which are applied in monitoring of finfish farms in countries in the temperate zone are given in Annex A.

The aim of monitoring of the seabed is to detect ongoing major changes in the infauna assembly. However, the analysis of changes in fauna communities is time consuming and requires fauna experts. Therefore, the measurement of chemical parameters may be used to reveal the conditions in which the fauna live. These measurements may be used for the prediction of impact on the infauna.

Various parameters have been used to detect changes in the seabed, which result from organic effluents from fish farms. The parameters can be divided into three groups: visual, chemical and biological. A set of parameters within each group can be more informative than a single parameter.

- Visual parameters may be obtained either by underwater photography, videography or visual description of sampled sediments. This requires sampling or video equipment, and trained personnel.
- Chemical parameters require sampling equipment and chemical analysis, either by electrodes or in the laboratory. Personnel should be trained.
- Biological parameters require heavier sampling equipment, fauna identification and highly trained experts.

A survey consists of a set of parameters. The methodology for the analysis of parameters should be standardized as far as possible to allow for comparison between sites and between years.

Threshold levels for individual or combined parameters may be set by the authorities and depend on the degree of acceptable impact.

#### 5.3.2.2 Visual parameters

Visual observations include sediment colour, sediment texture, smell, presence of gas bubbles, presence of mats of white sulfur bacteria (e.g. *Beggiatoa*), presence of fish feed and faecal pellets, and thickness of deposits on top of the original sediment. If it is present and can be seen, the redox potential discontinuity level (the black layer depth) should be reported. These variables are usually not quantitative, but provide useful information about the condition of the sediment.



### 5.3.2.3 Chemical parameters

Commonly used chemical parameters are: redox potential, free sulfide, pH, organic content, total organic carbon, total nitrogen and total phosphorus. Furthermore, zinc and copper content may be measured if relevant, and additional information may be gained by measuring carbonate content (buffer capacity). Chemical parameters are well suited to distinguish between low and high impact.

### 5.3.2.4 Biological parameters

Biological parameters include simple measures for benthic diversity, such as the number of species or indicator species and/or different macro fauna and infauna community analysis, such as Shannon-Wiener Index, Hurlberts Index, Infaunal Trophic Index (ITI) and AMBI (Azti Marine Biotic Index). These indices are calculated from data collected from quantitative analysis and taxonomic identification of animals sampled from the sediments.

The benthic macro fauna and infauna is a better detector of smaller changes than chemical parameters and may be preferred in areas where only low impacts are accepted. However, fauna analysis requires specialists (taxonomists) and heavier equipment and, therefore, is more expensive than measuring of chemical parameters.

Sediment grain size distribution is determined together with biological parameters.

Guidance on the selection of parameters for monitoring programmes is given in Table 5. Sediment grain size distribution is used to determine the type of sediment and the characteristic of the seabed, and may be measured only once as part of the baseline survey. Other additional parameters may be used and the addition of a parameter increases the robustness and accuracy of the survey.

It is recommended that standard methodologies be used whenever possible for all measured variables.

**Table 5 — Monitoring programme for baseline surveys and operational monitoring**

Survey type	Parameters	
	Minimum <sup>a</sup>	Additional <sup>b</sup>
Baseline survey	<p>Visual sediment colour, sediment texture, smell, presence of gas bubbles, presence of mats of white sulfur bacteria</p> <p>Chemical redox potential or free sulfide or total organic carbon or organic content</p> <p>Biological presence or absence of fauna</p> <p>Other sediment grain size distribution</p>	<p>Chemical redox potential, free sulfide, pH, total organic carbon, organic content, total nitrogen, total phosphorus</p> <p>Biological either taxonomic analysis of infauna to family level or infauna community analysis</p>
Operational monitoring for local impact zone	<p>Visual sediment colour, sediment texture, smell, presence of gas bubbles, presence of mats of white sulfur bacteria, presence of fish feed and faecal pellets and thickness of deposits on top of the original sediment</p> <p>Chemical redox potential or free sulfide or total organic carbon or organic content</p> <p>Biological presence or absence of fauna</p>	<p>Chemical redox potential, free sulfide, pH, total organic carbon, organic content, total nitrogen, total phosphorus</p> <p>Biological taxonomic analysis of infauna to family level</p>
Operational transect monitoring	<p>Visual sediment colour, sediment texture, smell, presence of gas bubbles, presence of mats of white sulfur bacteria, presence of fish feed and faecal pellets</p> <p>Chemical redox potential or free sulfide or total organic carbon or organic content</p> <p>Biological taxonomic analysis of macro fauna to family level</p> <p>Other sediment grain size distribution</p>	<p>Chemical redox potential, free sulfide, pH, total organic carbon, organic content, total nitrogen, total phosphorus</p> <p>Biological benthic infauna community analysis</p>
<p><sup>a</sup> Either total organic carbon or electrochemical measurements are strongly recommended. Organic content alone would not be acceptable in most countries.</p> <p><sup>b</sup> This list is not exhaustive.</p>		

#### 5.4 Evaluation of results

The results from measurement of the various parameters should be compared to threshold values for the individual variable or group(s) of variables. The threshold values may be set by the responsible government and reflect the impact which is acceptable. For information on changes in the various parameters as a consequence of organic impact of sediments, see Reference [8]. For examples of threshold levels for various parameters used in different countries, see Annex A.

## 5.5 Maps and charts

At least one map/chart showing the layout of the finfish farm operation and monitoring stations shall be produced. The chart for a fish farm should cover at least the anchoring and the entire monitoring area. The accuracy and presentation should cover all the relevant topographical features of the seabed area. The scale of the chart is adjusted to the size of the area of interest. Sampling points and position of the fish farm should be drawn on to the chart.

A geo-referenced point, preferably, a global positioning system (GPS) noted reference point, associated with the registered place name or equivalent should be marked on the chart of the site, to ensure correct site- and station positioning during monitoring. The reference point should be specified in degrees, minutes and seconds. Examples of suitable chart material are given in Annex B.

## 5.6 Additional data collection — Biological production at finfish farm

The following information provides useful context for interpretation of results. If possible, the following information should be collected:

- the standing biomass at the finfish farm at the time of the survey and the maximum biomass during the year;
- the total production of finfish and total amount of feed used during the production cycle;
- mortality, where it can be a significant organic input.

## 5.7 Report

The report should give a short description of conditions under the finfish farm and a comparison of results from the different samples. The sampling points should be geo-referenced and marked on the map/chart.

If the sediment conditions vary between different parts of the finfish farm, this should be commented upon. Individual samples showing poor and very poor conditions should be noted and assessed independently of the overall conditions at the finfish farm site. The report should include advice on remedial actions to improve the finfish farm sediment conditions, where relevant.

The report should compare the results with previous surveys and explain any changes over time. It should include all data and information necessary to allow others to repeat the survey.

# 6 Quality assurance and quality control

## 6.1 Aim and principles

Quality assurance and quality control measures should be incorporated during all stages of sampling and sample processing programmes. These principles help to guarantee that all data produced are of an acceptable quality, and that all parts of the work are carried out in a standardized and intercomparable manner. All procedures should therefore be clearly described and carried out openly, such that all activities can be audited at any time. The overall aim is to ensure traceability and full documentation of samples, equipment and reporting

It is essential that all participating staff be given the appropriate training and that a minimum level of competence be achieved and documented, for example by a certificate system. This includes all parts of the process, from sample collection and processing to documentation. Staff should participate in appropriate workshops and courses whenever possible. Some countries employ a “qualified person” system, where it is the responsibility of the reporting agency or company to establish competence and qualification, i.e. through a combination of experience and education/relevant body of work.

Staff who survey biological samples and identify taxa shall have a proven level of relevant taxonomic competence. Where available, identifiers should participate in national/international ring tests and other efforts towards taxonomic standardization.

Where available, survey teams and laboratories should participate in internal or external audits and ring tests.

## 6.2 Equipment calibration and operating safety

The technical quality of the equipment used should be verified at appropriate intervals. The most important of these are the following:

- operational safety should comply with health and safety requirements/regulations;
- accuracy of depth and position fixing equipment;
- functionality of electrodes according to manufactures instructions;
- grab or corer for sampling of area;
- sieve mesh apertures (most sieves have manufacturer certification);
- microscope maintenance.

Any other laboratory equipment should also be included in a regular checking system.

## 6.3 Checklists, sample log and anomaly reporting

To ensure sample traceability, a system of checklists for samples should be developed. The worker associated with each stage in the process should be noted. A reporting system for anomalies found or operational errors should also be developed. Immediate action should be taken to reduce the risk of re-occurrence.

## 6.4 Taxa identification

Taxa identification details are specified in ISO 16665. In practice, there are three levels of quality assurance and quality control for taxonomic characteristics:

- the identifications should be scientifically correct and follow updated taxonomic knowledge and faunal nomenclature;
- the identification practices carried out should be consistent at least within a single survey and, in particular, where several identifiers are involved in processing a batch of samples;
- the quantifications should be accurate or within a specified level of accuracy for each individual sample in the case of abundant taxa. For example if results vary by more than approximately 10 % or 10 individuals, all the samples or at least those taxa in the batch should be re-quantified.

## **Annex A** (informative)

### **Examples on environmental monitoring on the seabed impact from marine finfish farms**

#### **A.1 Operational monitoring, local impact zone**

This example is from NS 9410:2007 (Reference [13]), Reference [6], Reference [7] and Reference [12].

Beneath and close to the farm, the impact can be severe, but it is possible that pollution authorities accept more impact there than further away. The survey used is, therefore, composed mainly of chemical variables, which can detect moderate to high impact.

##### **General**

This Norwegian model for operational monitoring in the local impact zone monitors trends in the bottom conditions under a fish farm. Because the survey is repeated regularly, at intervals determined by the extent of the environmental impact, the development of the environmental impact can be followed successively. Both the average sediment conditions at the site and the conditions under different parts of the fish farm are assessed.

The analytical parameters of operational monitoring in the local impact zone are compiled so that they are inexpensive to carry out, and so that results can be made available soon after the survey, without having to wait for laboratory analyses.

The division between acceptable and unacceptable sedimentary conditions is set as the highest level of accumulation within which burrowing benthic macrofauna can survive in the sediment.

Fieldwork is carried out with light equipment deployed from the cage group or boat; and sampling is carried out using a grab or corer.

##### **Parameters**

Operational monitoring in the local impact zone encompasses three groups of sediment parameters:

- faunal assessment (group I): assessment where the presence or absence of animals larger than 1 mm in the sediment is recorded;
- chemical assessment (group II): quantitative assessment where the acidity (pH) and redox potential (Eh) are measured in the sediment;
- sensory assessment (group III): qualitative assessment where sediment outgassing, smell, consistency, colour, grab volume and thickness of the layer of deposits are recorded;
- for each of the parameters, scores are given for the extent to which they are affected by organic material. The higher the score, the more the sediments are impacted.

##### **Equipment**

The following equipment is necessary:

- winch: at deeper sites, it is practical to use a winch to retrieve grab samples. This may be a portable or boat-mounted, winch;

- grab: a grab with a sampling area of at least 250 cm<sup>2</sup>. The grab shall close fully, such that water and sediment do not leak out during hauling to the surface, and it shall be equipped with hinged flaps on the top, to allow assessment of group II parameters;
- pH measuring equipment: both regular combination electrodes and ISFET electrodes may be used. The latter are very robust. Further, a field pH meter is required, together with buffer at pH 4,0 and pH 7,0 and distilled water;
- redox measurement equipment: redox electrode and reference electrode or a combination redox electrode. Furthermore, a field pH meter and redox buffer are required;
- miscellaneous: sieve with round mesh holes of 1 mm in diameter, white plastic bath of a suitable size for receiving the grab contents, volume measure for grab contents, magnifying glass (5X enlargement), electrode holder, disinfectant solution and vessel for disinfecting.

### Preparation of pH and Eh measuring equipment

Upon arrival at the site, the pH and redox electrodes are assembled and calibrated according to the manufacturer's instructions.

- Calibrate the pH electrodes with buffers at pH 4,0 and pH 7,0. The buffer temperature shall be at the ambient seawater temperature. Check the redox- and reference electrodes in a redox buffer. Place the pH, redox and reference electrodes in a container, such that the sensors are at exactly the same height. Direct sunlight should be avoided.
- Immerse the electrodes in a beaker of fresh seawater or seawater buffer. Stir occasionally. The electrodes are ready for use once the readings have stabilized.

### Sampling

A minimum of 10 samples shall be taken from each site, but not more than 20 samples. Sampling shall be carried out from the edge of the cages or in their immediate vicinity. The sampling points shall be positioned according to the topography, dominant current direction and the dimensions and layout of the fish farm, such that they are as representative as possible of the fish farm site. The sampling points shall be positioned such that they, as far as possible, represent the entire local impact zone.

At finfish farms with compact cage groups, the samples shall be taken along the outer edge of, and if possible also in between, the individual cages. The sampling area shall, as far as possible, be representative for the entire area under the fish farm (local impact zone).

At finfish farms with dispersed cages, the samples shall be taken along the edge of the cages and, if possible, between the dispersed cages. At least one sample shall be taken at each cage, depending on the total number of cages at the fish farm site, as well as a holistic assessment of how the most representative picture shall be achieved.

### Evaluation of results

For a detailed description, see NS 9410, which describes the evaluation of results and threshold for allowable impact of the different sediment (site) conditions mentioned in Table 1.

### Report

The report shall give a short description of conditions under the fish farm and a comparison of results from the different samples. The sampling points shall be geo-referenced and marked on the map/chart.

If the sediment conditions vary between different parts of the fish farm, this shall be commented upon. Individual samples showing poor and very poor conditions shall be noted and assessed independently of the overall conditions at the fish farm site. The report shall include advice on remedial actions to improve the fish farm site conditions, where relevant.

The report shall compare the results with previous surveys and explain any changes over time. The report shall include all data and information necessary to allow others to carry out an identical survey. The report shall be presented electronically and a paper version provided, if desired.

### **Survey frequency**

Guidance on the frequency for carrying out operational monitoring in the local impact zone is given in Table 3. The timing of the survey is determined by the production cycle at the fish farm site, i.e. surveys shall be carried out during periods where the feed consumption is highest.

Under very poor conditions, it is likely that there is production of methane gas and hydrogen sulfide in the sediments, and a total absence of burrowing organisms (infauna). Operational monitoring in the local impact zone may then be extended by doubling the number of measurement points to determine if the recorded very poor condition is representative of the entire site. In addition to increasing the number of sampling points, at least five of those points shall be analysed for total organic carbon (TOC) or loss by combustion, total nitrogen, phosphorus, zinc and copper.

The results shall be evaluated continuously and the monitoring level adjusted as necessary.

## **A.2 Operational transect monitoring**

NOTE This example is from NS 9410:2007 (Reference [13]), ISO 5667-19, ISO 16665, ISO 19493 and Reference [10].

### **General**

Operational transect monitoring is a survey of the bottom conditions of the fish farm (local impact zone) and outwards into the recipient (regional impact zone). The main element is a survey of the bottom faunal communities, carried out according to ISO 5667-19 and ISO 16665. Operational transect monitoring includes information on additional parameters, which can be used to determine whether organic material arises from the fish farm or from other sources in the area. In the case of hard-bottom substrates, the guidelines in ISO 19493 should be followed.

### **Aim**

Operational transect monitoring shall give information on the environmental conditions in the regional and intermediate impact zones and towards the local impact zone of the fish farm.

### **Personnel**

For personnel, see requirements specified in ISO 16665.

### **Analytical parameters**

The following analytical parameters are obligatory:

- fauna: quantitative and qualitative investigations of benthic macrofauna (animals retained on a 1 mm mesh screen);
- chemical parameters: total organic carbon (TOC). Analyses shall be in accordance with ISO 16665;
- elemental analyses: phosphorus (P), zinc (Zn) and copper (Cu). The elements may be analysed from the same sample after total dissolution in nitric acid. The detection limit shall be  $\leq 10 \mu\text{g/g}$  dry sediment for P,  $\leq 1 \mu\text{g/g}$  for Zn and  $\leq 0,5 \mu\text{g/g}$  for Cu;
- sediment grain size distribution: measurement of the relative proportion of clay, silt, sand and gravel in the sediment, analysed in accordance with ISO 16665;
- oxygen content: measurement of the oxygen content of the water masses during sampling;

- salinity and temperature. measurements of salinity and temperature in the water column, close to the bottom, in connection with sampling;
- parameters in operational monitoring of local impact zone according to A.1 should be included.

### Sampling

Samples come from at least three stations in the local, intermediate and regional impact zones. One set of samples is taken downstream as close to the cage area as practically possible (sample 1), one set is taken half-way between the cage area and the deepest part of the area (sample 2), and one set is taken in the deepest part of the area (sample 3). If the cages are located over a steep slope without sediment accumulation, samples 1 and 2 shall be taken from the foot of the slope. If it is not possible to obtain samples from near the fish farm (sample 1) or further out into the intermediate impact zone (sample 2), all three samples shall be taken in the nearest deep area.

Two parallel grab samples shall be taken at each station. If the grab is recovered empty, another attempt shall be made. Should the second attempt also be unsuccessful, it is likely that the bottom is rocky, without accumulation of organic material. If the bottom is severely affected, with a strong smell of hydrogen sulfide and devoid of benthic macrofauna, only one grab sample replicate is taken. All the observations made shall be noted in the field log, and included in the subsequent evaluation of results. All sampling positions shall be geo-referenced and shown on a map.

It is not necessary to analyse samples taken from the middle of the fish farm and the deepest part, if the analyses of both the other samples show conditions to be “good” or better for all parameters, and the environmental conditions in the recipient as a whole are unchanged over time.

### Sample processing

The samples shall be processed in accordance with ISO 16665.

### Evaluation of results

The following are the threshold values in the intermediate impact zone.

Where there are relatively few species with an even distribution of individuals, as often is the case close to cage areas, faunal diversity indices are less suitable to define environmental conditions. Therefore, close to the fish farm, evaluations shall be carried out, based on the number of species and the species composition.

- Environmental condition 1 (very good):
  - at least 20 species of benthic macrofauna (>1 mm), excluding nematodes within a sampling area of 0,2 m<sup>2</sup>;
  - none of the species contribute more than 65 % of the total number of individuals.
- Environmental condition 2 (good):
  - five to nineteen species of benthic macrofauna (>1 mm), excluding nematodes within a sampling area of 0,2 m<sup>2</sup>;
  - more than 20 individuals, excluding nematodes within a sampling area of 0,2 m<sup>2</sup>;
  - none of the species contribute more than 90 % of the total number of individuals.
- Environmental condition 3 (poor):
  - 1 to 4 species of benthic macrofauna (>1 mm) excluding nematodes within a sampling area of 0,2 m<sup>2</sup>.
- Environmental condition 4 (very poor):
  - no benthic macrofauna (>1 mm) excluding nematodes within a sampling area of 0,2 m<sup>2</sup>.



## Reporting

A report containing all original data and a concluding assessment shall be compiled. If results are available from a previous survey carried out within the recipient, these are compared with the new results using multivariate analyses. Similarly, any changes over time should be outlined and the previous results compared with the present ones. If considerable impacts from the fish farm are shown, the report shall include advice on remedial measures which should be implemented.

Sampling positions shall be geo-referenced and marked on a map. The report shall include all the necessary information required for others to carry out the survey in an identical manner. The report shall be presented in both electronic and paper formats.

### A.3 Operational transect monitoring

NOTE This example is from British Columbia (see Reference [11]).

#### Equipment

- a) Acceptable sampling devices for chemical/ physical sampling include Petite Ponar, Ponar, Smith-MacIntyre, VanVeen or other appropriate equipment.
- b) For biological sampling, use a Smith-MacIntyre, VanVeen or other appropriate large-volume sediment sampling device with a 0,1 m<sup>2</sup> footprint.
- c) Various probes and chemicals, described fully in Clauses 4 and 5, are to be used.

#### Procedures

##### a) Baseline monitoring:

- within each of the probable footprints, at least three grab samples should be taken for each sediment type. If only one sediment type predominates, at least five grab samples should be taken;
- two reference stations should be selected as described in Clause 5 for video surveys; at least three grabs should be taken at each reference station.

##### b) Operational monitoring:

- ensure the transect is parallel to the predominant current direction;
- use at least one transect for each dominant current direction or alternate design, provided extent and magnitude of effects are represented;
- sample at least three stations on each transect: at perimeter of the containment structure, at 30 m from zero metre station, and at perimeter of tenure.

##### c) Selection of reference stations:

- 1) sample at least two reference stations for each facility;
- 2) ensure the stations are within 0,5 km to 2,0 km of the facility tenure, if possible;
- 3) ensure reference stations are at least 0,5 km apart, if possible;
- 4) ensure the mean depth is within 20 % of the mean depth of facility stations;
- 5) ensure the SGS fractions are within 15 % of the facility stations's SGS fractions;
- 6) ensure that characteristics, such as topography, current and tidal regimes, amount of freshwater run-off, are similar to that of the facility stations;

- 7) if the facility stations appear to be influenced by anthropogenic activity, ensure that the reference stations have similar characteristics to that of the facility stations (e.g. log dumps).

**d) Sampling preparation:**

- 1) prepare a sulfide stock solution and EDTA/NaOH solution in advance. Note that 10,000  $\mu\text{M}$  sulfide stock solution is stable for up to 5 d (5 days), if it is kept cool with limited head space. EDTA/NaOH solution is stable for up to 7 d (7 days), if kept cool;
- 2) check tidal conditions. If possible, do not sample during maximum flood and ebb tides or strong wind conditions;
- 3) obtain latitude/ longitude using DGPS, with a minimum accuracy of  $\pm 5$  m at each station;
- 4) where sampling on a transect, use polypropylene rope pre-marked in 1 m increments to ensure accurate measurements;
- 5) where sampling on a transect, note bearing. Report the true-north bearing as well as the magnetic north reading and correction factor;
- 6) report water depths in metres;
- 7) check the Eh electrode against standard (re-check every 4 h and whenever recalibrating the sulfide meter);
- 8) calibrate the sulfide electrode, and recalibrate it at least every 3h to 4 h;
- 9) drift: before recalibration and hourly during sampling or at a minimum at the completion of each sampling station, check and record the drift by measuring the sulfide concentration against each of the standard concentrations originally used to calibrate the electrode. Always use fresh standards (1 000  $\mu\text{M}$ , 100  $\mu\text{M}$ , 10  $\mu\text{M}$ ) by serial dilution from the stock solution whenever recalibrating or checking drift. Do not attempt to correct the data for any observed drift. A drift of up to 20 % is acceptable.

**e) Collect and describe samples:**

- 1) deploy and retrieve sampling device at a maximum rate of 0,3 m/s. Rinse all equipment with ambient seawater between grab deployments. Take care that second and third grab samples are not taken from the crater formed by the first grab sample. Typically, this is only of concern where the sampling vessel is moored at the edge of the containment structure;
- 2) check for these indicators of an acceptable sample:
  - overlying water present: indicating minimal leakage;
  - overlying water not excessively turbid: indicating minimal sample disturbance;
  - sediment surface relatively flat: indicating minimal sample disturbance or washing;
  - desired penetration depth achieved: at least 4 cm to 5 cm for characterizing surficial sediments;
  - overfilled sampling device: if occurring routinely, some or all of the detachable mass can need to be removed;
- 3) do not make more than four deployments of the grab to obtain a suitable sample. If unsuccessful, provide a video of the station as an alternative (see Clause 2);
- 4) siphon the overlying water from the sample. Retain it for sieving if biological samples are required;
- 5) examine the sediment sample and record the following:
  - sediment texture;
  - colour;
  - odour;

- presence or absence of gas bubbles;
  - white sulfur bacteria;
  - fish feed;
  - fish;
  - faeces;
  - flocculent organic material;
  - macrophytes;
  - terrigenous material and;
  - farm litter;
- 6) take a colour photo of the sample or score sediment colour by comparing with colour charts;
  - 7) record the penetration depth of the sampler in centimetres.

**f) Measure S = and Eh levels:**

- 1) extract two subsamples by removing the top 2 cm of sediment from the centre of each side of the sampling device. Limit the volume of each subsample to what is needed for the required tests as summarized in Annex B (i.e. 50 mL required for Eh potential and sulfide concentration). Place the two subsamples in a suitable container and homogenize by gently stirring with a flat-tipped steel spatula. Sulfide and Eh analyses should be done within 60 min of sampling to avoid sample degradation. Gloves should be worn if the sediment is being touched;
- 2) measure sulfide:
  - a. rinse the electrode with distilled water and blot it dry. Then insert it into sample;
  - b. once the initial sample is obtained and accepted, add 8,75 mg·L ascorbic acid to 250 mL of previously prepared EDTA/NaOHbuffer and thoroughly mix to create SAOB buffer. (Various amounts of SAOB can be made, provided the 8,75 g·L ascorbic acid: 250 mL EDTA/NaOHratio is maintained.);
  - c. combine equal volumes of sediment and SAOB in a suitable container (5 mL of each is typically sufficient for this analysis). The sediment from the sample can be extracted using a cut-off syringe or spatula. Do not include material more than 0,5 cm in diameter;
  - d. homogenize the mixture with a spatula;
  - e. insert the sulfide electrode into the solution and gently swirl it until the meter reads READY (typically 2 min to 5 min);
  - f. gently wipe the probe before the next sample. If an oily residue is observed on the probe, wash it with detergent before taking another sample;
- 3) obtain an Eh measurement. Insert the probe into the homogenized sample described above, and wait until either the meter reads READY or the drift is 3 mV or less over a 2 s period. Gently wipe excess sediment from the probe between sample measurements;
- 4) record the temperature in the sediment sample;
- 5) correct the Eh measurements using sediment temperature and the correction factor for the filling solution in the probe supplied by the manufacturer;
- 6) perform any additional measurement or analysis using the sediment subsamples collected in A.3.2 c, step 1. Do not collect additional subsamples for these analyses. Remove all unrepresentative material (e.g. shells, large worms, wood waste and rock) before filling the sampling receptacle. For sample frequency and location, see Annex A;

7) store all laboratory samples at 4 °C.

**g) Biological sampling:**

- 1) where collecting biological samples, scrape and rinse sediments from the grab into precleaned containers. Save the rinse water\* for infaunal sampling (prior to use in sieving biological samples, rinse water should be filtered through a minimum 250 µm screen);
- 2) where sieving biological samples in the field: Sieve each sediment sample, associated overlying water and rinse water through a 1,0 mm screen. Count, identify and record megafauna (e.g. large cnidarians, echinoderms and tube worms) then return them to the sea. Photograph specimens that need to be identified by taxonomists. Fix the remaining organisms in 10 % buffered formalin. After 4 d (4 days), rinse over 0,5 mm screen and preserve in 70 % isopropyl alcohol or ethyl alcohol. Retain all coarse gravel and cobble less than 2,5 cm in diameter. Remove epifauna adhering to rocks and other material, which are greater than 2,5 cm diameter and include them in sieved sample. Prior to use in sieving biological samples, rinse water should be filtered through a minimum 250 µm screen;
- 3) for samples not sieved during the day they were obtained in the field, use a 10 % buffered formalin solution for preservation.

**Reporting**

Submit monitoring data by filling out templates supplied by WLAP.

## Annex B (informative)

### Example — Base map

The maps/charts should cover the receiving area, site and fish farm.

Maps of the cage area should be made to the scale of 1:50,000 or lower, using marine charts as bases. The anchoring area, cage area and anchor lines should be drawn on to these charts.

The following are the technical terms:

- anchoring area: area delimited by the anchoring points of the cage area;
- cage area: area where the individual cages are located;
- anchor line: line (cable or chain, etc.) from the anchor points to the cage area;
- anchor points: attachment point of the anchor line; a difference is made between BOTTOM and LAND BOLT.

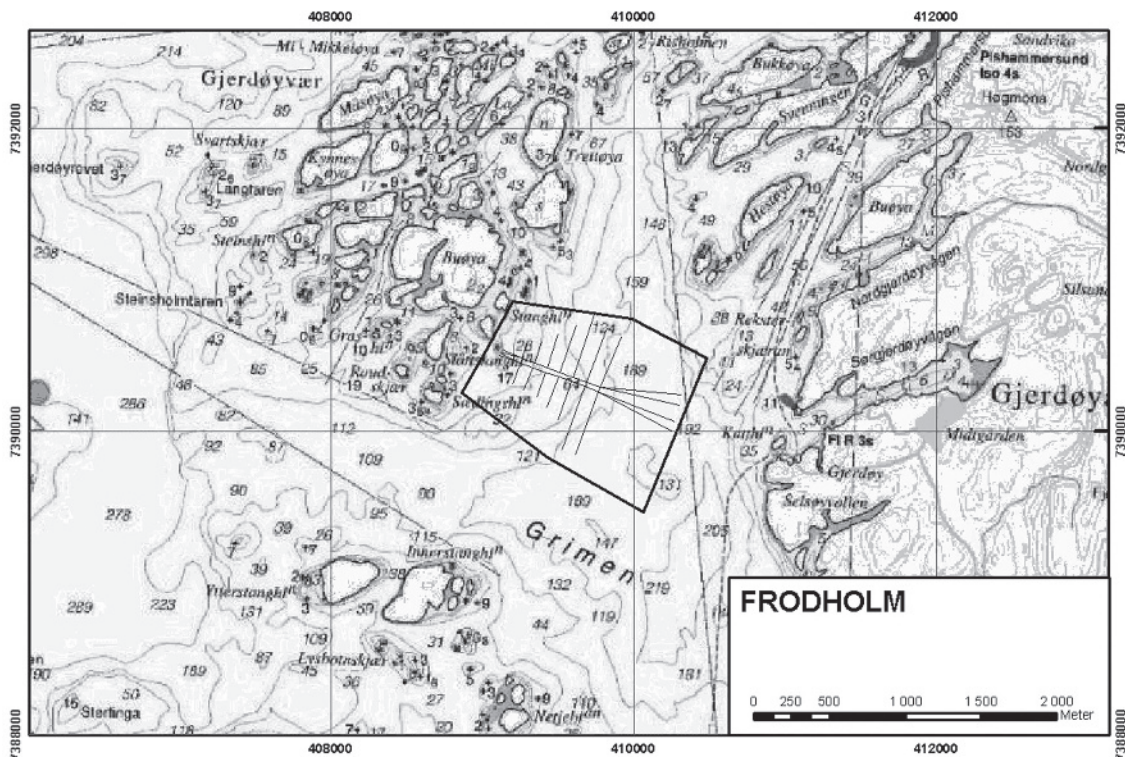


Figure B.1 — Fish farm with anchoring area drawn in on marine chart

## **Annex C** (informative)

### **Example of monitoring report format and some sample content**

#### **C.1 Format**

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Statistical Analysis

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## C.2 Field sampling

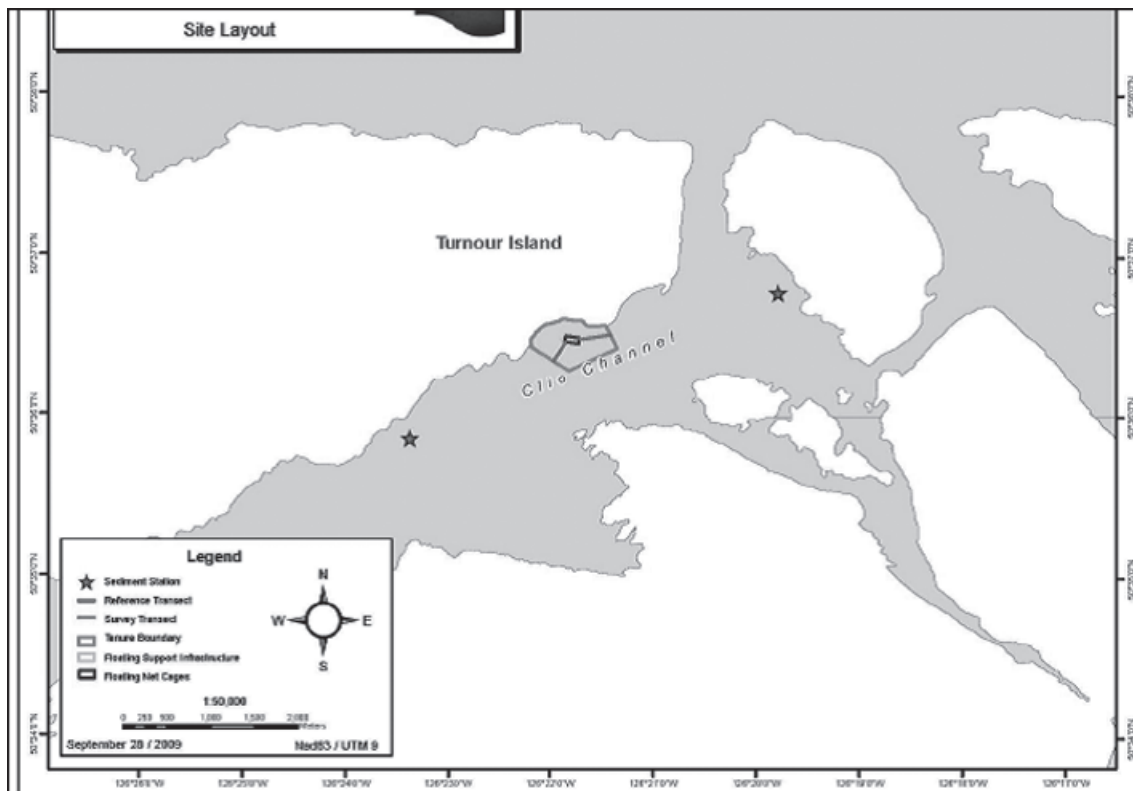


Figure 1. The location of the reference stations in relation to the pen system and tenure boundary at the finfish aquaculture system on September 10, 2009

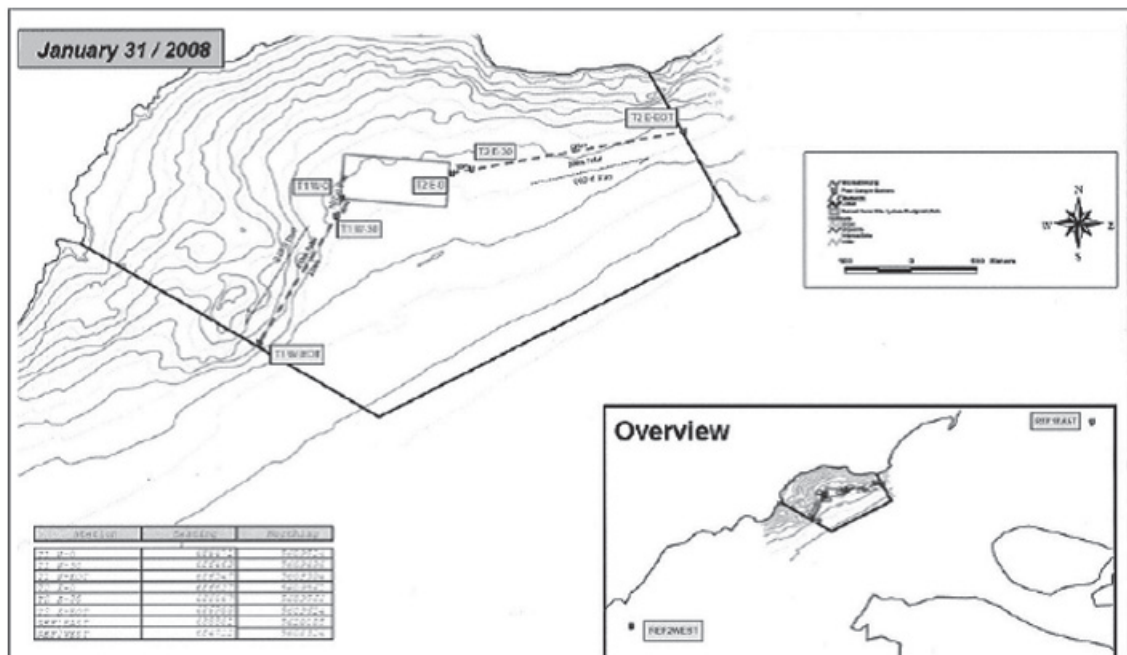


Figure 2. The location of the pen system in relation to transect monitoring stations at the finfish aquaculture system on September 10, 2009

**Table 1. The results of further analysis with respect to regulatory requirements for sulfide levels in sediment samples collected at the T1-TE-W tenure boundary on 2009-09-10 at the site, located in Clio Channel, BC**

Sampling station	Test 1			Test 2 (substitution)		
	T1-TE-W	Ref 1	Ref 2	T1-TE-W	Ref 1	Ref 2
Sulfide levels (µM)	49.2	88.7	128	100	100	128
	62.2	81.4	90.6	100	100	100
	34.1	87.4	65.8	100	100	100
Result of analysis:						
Mean	48.5	85.8	94.8	100	100	109
SD	14.1	3.89	31.3	0	0	16.2
t-value		-5.36			-0.577	
P value (1 tail)		0.059			0.333	
Biological trigger?		No			No	

**Table 2. The results of analysis with respect to regulatory requirements for sulfide levels in sediment samples collected at the T2-TE-E tenure boundary on 2009-09-10 at the site located in Clio Channel, BC**

Sampling station	Test 1			Test 2 (substitution)		
	T2-TE-E	Ref 1	Ref 2	T2-TE-E	Ref 1	Ref 2
Sulfide levels (µM)	27.6	88.7	128	100	100	128
	36.4	81.4	90.6	100	100	100
	41.5	87.4	65.8	100	100	100
Result of analysis:						
Mean	35.2	85.8	94.8	100	100	109
SD	7.0	3.89	31.3	0	0	16.2
t-value		-7.07			-0.577	
P value (1 tail)		0.045			0.333	
Biological trigger?		Yes			No	

**Operational transect monitoring data summary**

Operational transect monitoring	Data summary sheet
Company:	Sample date: 2009-09-10
Farm site:	Field crew:
Licence number:	Analysis:
<b>Data summary:</b>	
By: K. Len (name)	
Date: 2009-30-10	



SGS	Field	% Mud	Field duplicate
T1-0-W		34.3	
T2-0-E		34.3	34.3 (A)
Ref 1		69.9	
Ref 2		95.5	

LAB	Copper	Zinc	TVS %	Mud %
Duplicate 1	166	288	10.4	34.3
Duplicate 1	137	276	9.7	31.2
% Diff	19.1	4.3	7.0	9.5

Sulfides: Performance standard: 6 000 µmoles

Station	Rep A	Rep B	Rep C	Field duplicate	n	Mean	SD	Max
T1-0-W	364	315	252		3	310	56.1	364
T1-30-W	702	645	596		3	648	53.1	702
T1-TE-W	(49.2)100	(62.2)100	(34.1)100		3	(48.5)100	(14.1)0	62.2
T2-0-E	840	1200	7740	890 (A)	3	3260	3884	7740
T2-30-E	583	487	993	606 (A)	3	688	269	993
T2-TE-E	(27.6)100	(36.4)100	(41.5)100		3	(35.2)100	(7.0)0	41.3
Ref 1	(88.7)100	(81.4)100	(87.4)100		3	(85.8)100	(3.9)0	88,7
Ref 2	128	(90.6)100	(65.8)100		3	(94.8)109	(31,3)16.2	128

TVS (%) Performance standard: None

Station	Rep A	Rep B	Rep C	Field duplicate	n	Mean	SD	Max
T1-0-W	4.3	4.1	4.0	11.7 (A)	3	4.1	0.2	4.3
T2-0-E	10.8	9.7	9.7		3	10.1	0.6	10.8
Ref 1	5.4	4.6	5.9		3	5.3	0.7	5.9
Ref 2	9.0	9.3	9.0		3	9.4	0.4	9.0

COPPER (µg/g) Performance standard: None

Station	Rep A	Rep B	Rep C	Field duplicate	n	Mean	SD	Max
T1-0-W	59.3	58.5	49.3	166 (B)	3	55.7	5.6	59.3
T2-0-E	129	137	166		3	144	19.5	166
Ref 1	36.0	42.2	30.6		3	36.3	5.8	42.2
Ref 2	43.6	41.0	41.4		3	42.0	1.4	43.6

ZINK (µg/g) Performance standard: None

Station	Rep A	Rep B	Rep C	Field duplicate	n	Mean	SD	Max
T1-0-W	125	11.5	109	288 (B)	3	116	8.1	125
T2-0-E	285	276	310		3	290	17.6	310
Ref 1	81.3	81.2	64.9		3	75.8	9.4	81.3
Ref 2	88.5	88.0	88.0		3	88.2	0.3	88.5

Statistical comparison with

Standard	Reference	
One-sample t-test (1-tail) Prob (value)	Two-sample t-test (1-tail) Prob (value)	Adjusted two-sample t-test (1-tail) Prob (value)
Mean < 6 000		
Mean < 6 000	Mean < refs	N/A
Mean < 6 000		
Mean < 6 000	Mean < refs	N/A
...		
<b>Biological trigger</b>		
Yes No X		
Stations: N/A	Number of grabs:	
Fieldwork before: N/A		

Appendix 1 Data Sheets

Station Number	Latitude (deg/min)	Longitude (deg/min)	Transect Number	Transect Direction (deg)	Distance from pens (m)	Sampling device	Sampling Area (m <sup>2</sup> )	Sampling depth	Penetration Depth (m)	Replicate Number	S <sup>2</sup> (micro-M)	Temperature (deg C)
T1-0-W	60.36.480	126.21.682	1	82	0	Petite Ponar	0.023	86.6	6	A	364	10.4
T1-0-W	60.36.480	126.21.681	1	82	0	Petite Ponar	0.023	86.7	7	B	315	10.4
T1-0-W	60.36.480	126.21.681	1	82	0	Petite Ponar	0.023	86.4	7	C	252	10.5
T1-30-W	60.36.479	126.21.659	1	82	30	Petite Ponar	0.023	86.0	6	A	702	10.4
T1-30-W	60.36.479	126.21.659	1	82	30	Petite Ponar	0.023	86.1	7	B	645	10.3
T1-30-W	60.36.479	126.21.660	1	82	30	Petite Ponar	0.023	86.8	6	C	596	10.4
T1-TE-W	60.36.510	126.21.437	1	82	293	Petite Ponar	0.023	84.5	6	A	49.2	11.2
T1-TE-W	60.36.510	126.21.433	1	82	297	Petite Ponar	0.023	84.9	6	B	62.2	11.1
T1-TE-W	60.36.510	126.21.431	1	82	301	Petite Ponar	0.023	84.3	6	C	34.1	11.1
Reference 1	60.36.768	126.19.813			2300	Petite Ponar	0.023	99.1	Full	A	88.7	10.1
Reference 1	61.36.767	126.19.811			2300	Petite Ponar	0.023	99.4	Full	B	81.4	10.1
Reference 1	62.36.766	126.19.812			2300	Petite Ponar	0.023	101.6	Full	C	87.4	10.0
T2-0-E	60.36.468	126.21.873	2	211	0	Van Veen	0.1	83.0	8	A	840	9.6
T2-0-E	60.36.468	126.21.873	2	211	0	Van Veen	0.1	83.0	8	A (Dup)	890	9.6
T2-0-E	60.36.468	126.21.874	2	211	0	Van Veen	0.1	83.4	8	B	1200	9.7
T2-0-E	60.36.468	126.21.874	2	211	0	Van Veen	0.1	83.4	12	C	7740	9.6
T2-30-E	60.36.453	126.21.877	2	211	30	Petite Ponar	0.023	86.1	6	A	583	10.3
T2-30-E	60.36.453	126.21.877	2	211	30	Petite Ponar	0.023	86.1	6	A (Dup)	606	10.4
T2-30-E	60.36.453	126.21.877	2	211	30	Petite Ponar	0.023	84.7	6	B	487	10.3
T2-30-E	60.36.453	126.21.877	2	211	30	Petite Ponar	0.023	84.6	6	C	993	10.2
T2-TE-E	60.36.448	126.21.986	2	211	255	Petite Ponar	0.023	71.3	6	A	27.6	11.1
T2-TE-E	60.36.349	126.21.984	2	211	252	Petite Ponar	0.023	70.8	Full	B	36.4	11.1
T2-TE-E	60.36.352	126.21.985	2	211	250	Petite Ponar	0.023	67.1	6	C	41.5	10.9
Reference 2	60.36.853	126.23.402			2100	Petite Ponar	0.023	100.6	Full	A	128.0	10.4
Reference 2	60.36.853	126.23.000			2100	Petite Ponar	0.023	104.3	Full	B	90.6	10.4
Reference 2	60.36.853	126.23.000			2100	Petite Ponar	0.023	100.6	Full	C	65.8	10.4

Station Number	Eh Uncorrected (mV)	Eh Corrected (mV)	TVS (%)	Cu (micro-g/g)	Zn (micro-g/g)	Black Sediment	Sediment Odour	Gas Bubbles	Jelly-like Sediments	Fish Feed	Fish Feces	Fluorescent Organic Material	Oily Sediment	Farm Litter
T1-0-W	-347.4	-133.4	4.3	59.3	125	1	1	0	0	1	1	0	0	0
T1-0-W	-341.2	-127.2	4.1	68.6	115	1	1	0	0	0	1	0	0	0
T1-0-W	-343.5	-130.5	4.0	49.3	109	1	1	0	0	1	1	0	0	0
T1-30-W	-385.9	-171.9				2	2	0	0	1	1	0	0	0
T1-30-W	-377.6	-163.6				2	2	0	0	0	1	0	0	0
T1-30-W	-378.8	-164.8				2	2	0	0	1	1	0	0	0
T1-TE-W	-279.6	-66.6				0	0	0	0	0	0	0	0	0
T1-TE-W	-264.7	51.7				0	0	0	0	0	0	0	0	0
T1-TE-W	-266.7	-53.7				0	0	0	0	0	0	0	0	0
Reference 1	-323.7	-109.7	5.4	36.0	81.3	0	0	0	0	0	0	0	0	0
Reference 1	-323.9	-109.9	4.6	42.2	81.2	0	0	0	0	0	0	0	0	0
Reference 1	-297.3	-83.3	5.9	30.6	64.9	0	0	0	0	0	0	0	0	0
T2-0-E	-285.1	-71.1	10.8	129	286	2	2	0	0	1	1	0	0	0
T2-0-E	-280.3	-66.3				2	2	0	0	1	1	0	0	0
T2-0-E	-293.0	-79.0	9.7	137	276	2	2	0	0	1	1	0	0	0
T2-0-E	-451.4	-237.4	9.7	166	310	2	3	0	0	1	0	0	0	1
T2-30-E	-352.7	-138.7				1	1	0	0	1	0	0	0	0
T2-30-E	-367.7	-153.7				1	1	0	0	1	0	0	0	0
T2-30-E	-335.8	-121.8				1	1	0	0	1	0	0	0	0
T2-30-E	-385.3	-171.3				1	1	0	0	1	0	0	0	0
T2-TE-E	50.1	263.1				0	0	0	0		0	0	0	0
T2-TE-E	57.4	270.4				0	0	0	0	0	0	0	0	0
T2-TE-E	108.2	321.2				0	0	0	0	0	0	0	0	0
Reference 2	-351.2	-137.2	8.9	43.6	88.5	0	0	0	0	0	0	0	0	0
Reference 2	-263.1	-49.1	8.3	41.0	88.0	0	0	0	0	0	0	0	0	0
Reference 2	-265.6	-51.5	8.0	41.4	88.0	0	0	0	0	0	0	0	0	0

Table notes:

- Black sediment odour: ranked on a scale of 0 (no black or no odour) to 4 (all black or very strong sulphide odour)
- Gas bubbles, jelly-like sediments, fish fed, fish feces, flocculent, oily sediment, farm litter: 0 = not present, 1 = present

Station Number	Mud (%)	Sand (%)	Gravel (%)	Terregeous Material	Shell Hash	Macrophytes	Beggiatoa	Comments
T1-0-W	34.3	65.5	0.2	0	1	0	1	
T1-0-W				0	1	1	0	Kelp
T1-0-W				0	1	0	1	
T1-30-W				1	0	0	1	Trace feed and hemlock needles
T1-30-W				0	1	0	1	
T1-30-W				0	1	0	1	Trace feed
T1-TE-W				0	0	0	0	Hydroides and small gravel
T1-TE-W				0	0	0	0	
T1-TE-W				0	1	0	0	
Reference 1	69.9	30.1	0.0	0	1	0	0	Green mud with trace amounts of shell hash
Reference 1				0	1	0	0	Green mud with trace amounts of shell hash
Reference 1				0	1	0	0	Green mud with trace amounts of shell hash
T2-0-E	34.2	65.8	0.0	0	1	0	0	Abundant fish feed and feces
T2-0-E				0	1	0	0	Abundant fish feed and feces
T2-0-E				1	1	0	0	Abundant fish feed and feces
T2-0-E				0	1	0	0	Lots of barnacles and a 10cm long piece of 1 inch rubber
T2-30-E				0	1	0	1	Trace fish feed
T2-30-E				0	1	0	1	Trace fish feed
T2-30-E				1	0	0	1	Trace fish feed
T2-30-E				0	1	0	1	Trace fish feed
T2-TE-E				0	0	0	0	Green mud with bits of dead sponge
T2-TE-E				0	0	0	0	Green mud with bits of dead sponge
T2-TE-E				0	0	0	0	Green mud with bits of dead sponge
Reference 2	95.5	4.5	0.0	0	0	0	0	Green mud
Reference 2				0	0	0	0	Green mud
Reference 2				0	0	0	0	Green mud

Table note:

- Terregeous material, shell hash, macrophytes, *Beggiatoa*: 0 = not present, 1 = present

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