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Lowestoft,
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Protocol for sampling and transport of water for the purpose of Official Control Monitoring of classified shellfish production areas under EU Regulation 854/2004

England and Wales programme

1. INTRODUCTION

The routine monitoring for the presence of toxin producing plankton in production and relaying areas, and biotoxins in live bivalve molluscs, echinoderms, tunicates and marine gastropods is a requirement of Regulation (EC) No 854/2004 of the European Parliament and of the council of 29th April 2004. These regulations lay down specific rules for the organisation of official controls on products of animal origin intended for human consumption.

Within England and Wales, the official control (OC) monitoring programme for marine biotoxins is divided into two elements: flesh and water monitoring, where samples of commercially harvested shellfish and water samples are collected and tested from each active production and relaying area. Where possible, flesh and water sampling points will correspond with the microbiological sampling points to ensure compatibility between all shellfish hygiene programmes.

The Food Standards Agency (FSA) is the competent authority with overall responsibility for ensuring that the OC monitoring programme for marine biotoxins is effectively carried out. Cefas is the contracted laboratory responsible for the analysis of both water and flesh samples and the co-ordination and integration of both programmes. Local Authorities (LAs) are responsible for collecting water and shellfish samples at the required frequency from the designated sites and for sending these to the relevant testing laboratory.

Water samples are routinely analysed at Cefas Lowestoft for the presence of potentially harmful algal (phytoplankton) species which may be responsible for the production of Amnesic Shellfish Poisoning (ASP) toxins, lipophilic toxins (including Diarrhetic Shellfish Poisons (DSP)) and Paralytic Shellfish Poisoning (PSP) toxins. The presence of these toxins above defined permitted limits may have serious health consequences for the consumers of shellfish.

This protocol relates to the collection, preservation and posting of water samples collected from classified shellfish production and relaying areas in England and Wales for the purpose of identification and enumeration of potentially toxin producing algal

species. Other protocols exist for the specific purpose of shellfish sampling for the OC monitoring programme for marine biotoxins and microbiological contaminants and can be found on the Cefas website. Harvesters, collectors and local authorities can also contact Cefas Weymouth on 01305 206600 for further information on these specific protocols.

This protocol can be read in conjunction with the FSA 'Guide to sampling protocols' DVD[1], copies of which are available from the FSA upon request. *(Please contact Rebecca Watts on 020 7276 8046 to place a request).*

2. SAMPLE SITES

The monitoring programme for harmful phytoplankton is currently implemented on all commercially active, classified shellfish production and relaying areas. The sampling programme is reviewed every year and sampling sites selected to ensure it includes:

- All commercially active production and relaying areas
- All sites with a history of biotoxin occurrence
- Classified sites in the vicinity of areas with known toxin history

Sites are defined by production area, bed name, bed ID and grid reference. A sampling site list is compiled each year and reviewed on a regular basis throughout the year. New or reopening harvesting and relaying areas are included in the programme before harvesting takes place and sites are removed from the list when commercial harvesting is suspended (either permanently or seasonally). This information is gathered from various sources including local authorities, Inshore Fisheries and Conservation Authorities (IFCAs) (or the equivalent in Wales), harvesters, FSA and other monitoring programmes led or co-ordinated by Cefas. Local authorities are kept aware of any changes in the sampling status of sites within their areas, and their co-operation is sought to ensure that any changes in the commercial status of each harvesting area are notified to Cefas and the FSA.

In summary:

1. Water samples must be collected from the identified sampling points at the agreed frequency by the relevant local authority.
2. Sampling points and/or sampling frequency may change depending upon the prevalence of algal toxins and commercial status of the area, but any changes must be agreed beforehand between the local authority and Cefas/FSA.

3. TIME OF SAMPLING

All samples should be collected at the frequency specified by the FSA monitoring plans and policies, unless sampling can be rescheduled by agreement or where circumstances are outside of the sampling officers' control.

Sample frequency:

a) Water samples should be collected from each agreed sampling point every two weeks from April to September and every four weeks from October to March for subsequent algal analysis. Water samples should be collected as close to the shellfish sampling date as possible to ensure a good correlation between flesh and water results. It is recommended that samples are collected as early as possible in

[1] DVD produced by FSA Scotland but sampling guidance applicable to England & Wales

the week so that subsequent analysis and reporting of results can be completed within the same week. This is to enhance the capability of the algal analysis to provide an early warning mechanism for potential presence of biotoxins in shellfish. For this reason it is suggested that water samples are collected from Mondays to Wednesdays for analysis at Cefas Lowestoft by Friday. Samples arriving on Friday will be analysed the next working day (usually the following Monday) and results reported the same day.

b) Reduced frequency: Sampling requirements may be suspended for sites with no harvesting activity. Local authorities with such sites are asked to contact Cefas to discuss the site status and agree a revised sampling regime before postponing any sampling.

c) Increased frequency: Sampling may be increased (to fortnightly or weekly) for sites with a previous history of toxin occurrence, a short harvesting season, or where toxins or toxin producing phytoplankton have been detected in the area. Increased sampling requirements will be communicated via the FSA.

Local authorities are asked to contact Cefas Weymouth as soon as possible if they are encountering any difficulty in complying with their agreed sampling programme and to advise Cefas of their sampling dates for laboratory planning purposes.

Note: additional samples or samples submitted for analysis too soon after the previous sample will not be analysed unless the samples were requested by the FSA or Cefas, or the revised sampling programme was agreed beforehand with Cefas.

Sampling officers are asked to notify the Cefas programme co-ordinator (via biotoxinmonitoring@cefas.co.uk, see details in section 10) of their **monthly sampling schedule before the start of each month**.

All sampling officers are also requested to note the arrangements agreed with FSA for the submission and testing of samples around bank holidays and Christmas. These will be communicated to all at the start of each calendar year.

4. EQUIPMENT

The following equipment is required for water sampling. Please contact the laboratory (see contact details in section 10) if you are running low on sampling equipment.

a. Tube sampler or Water bottle sampler (pole sampler)
b. Bucket (for mixing of sample)
c. Labelled screw capped plastic sample bottles
d. Packaging
e. Sample collection form
f. Pre-paid special delivery return envelopes
g. In-date Lugol's iodine
h. Pipette
i. Pen

The following equipment should also be available:

j. Device for identification of fixed sampling points (e.g. GPS).
k. Temperature and salinity measuring equipment.
l. Absorbent paper towel
m. Disinfectant (see section 11)

5. PROTOCOLS FOR THE COLLECTION OF WATER SAMPLES

The aim of the water collection method is to obtain samples which are representative of the algal community in the water body being sampled. The water sample should therefore be taken as close to the shellfish bed as possible and at the location from where shellfish samples for flesh testing are taken.

The sampling method used will be dependent on the depth of water at the site. Water samples should ideally be taken at high tide (+/-1h) particularly at inshore sites. Sampling at low tide must be avoided.

Variation in the depth of water sampled means that either of two preferred methods may be employed in order to collect this sample: a tube sampler (taking a depth integrated sample) or a water bottle sampler (there are two types available, which collect discrete samples from shallower water). The traditional use of buckets to collect surface water is discouraged as this method often does not collect samples which are representative of the water mass in which the shellfish are filter feeding. The method to be used at each site will be discussed with each LA and the appropriate water sampler will be provided by Cefas Lowestoft.

Collectors must be aware of interferences caused by disruption of postal services, loss of water sampler, and breakage/leakage of sample bottle or rough weather preventing the safe collection of samples.

If there are obvious signs of contamination at the sampling site e.g. by oils, fuels, sewage, high sediment load etc, then, unless it is possible to avoid the contamination, sampling should be postponed and the Cefas plankton laboratory contacted for further advice (see section 5). **Please note that water samples deemed unsuitable will be rejected and an additional sample will be requested for testing.**

It is preferable for integrated water samples to be collected using a tube sampler as described in paragraph **A (below)**. If this is not possible then discrete water samples can be collected from shallower areas as described in paragraph **B**.

A. Sampling off-shore from a boat (or from a pier or jetty).

A1. You have been supplied with a simple polythene pipe water sampling system which will take an integrated sample from the surface to whatever depth the weighted end is lowered.

A2. If possible take the sample from water depths of at least seven metres or more. If this is not possible, then you may need to shorten the pipe by removing the top plastic valve, cutting the pipe to the maximum length required and refitting the valve. The sample must be taken in the vicinity of the shellfish sampling site.

A3. Taking the sample:

a) Secure the free end of the line to the boat/jetty to safeguard against losing the pipe.
b) Open the valve at the top of the pipe.
c) Slowly lower the weighted end of the pipe into the water until most of the pipe is immersed (the pipe must remain taut and vertical in order to take an even sample of the whole water column). Care must be taken to ensure that the weighted end of the tube comes no closer than 0.5m from the seabed to prevent contaminating the sample with sediment.
d) Close the valve at the top of the pipe.

e) Retrieve the bottom of the pipe using the attached line. Then empty all the contents of the pipe into a bucket by opening the top valve and if necessary lifting the valve end of the pipe up.
f) Rinse the pipe with fresh water.

See section 5 for fixing and sending the samples.

B. Sampling from the shore.

B1. When sampling from the shore it is preferable that a water bottle (pole) sampler is used. This does enable several samples (at least 3) to be taken from various water depths (max 3m) over the harvesting area. These samples should be mixed in a bucket, using a figure of '8' motion, before taking a 500ml sub-sample from the homogenised water mixture and adding the preservative (Lugols Iodine).

There are two types of pole sampler available. The most commonly used type is a robust, stainless steel version of fixed length (either 1.5m or 2.0m). The second type has recently been introduced, to make transport easier, as it is made from aluminium which is lighter, and it is also extendable to a maximum of 3m. However, it is less robust and does need careful washing after each use, to prevent corrosion.

B2. Taking the sample:

Using the Stainless steel pole sampler.

a) Remove the plastic cap from a 1 litre bottle provided with the sampler. Screw the bottle into the threaded plastic attachment at the base of the sampler. Make sure that it is screwed in fully to create a tight seal.
b) Make sure the plunger in the centre of the handle is pushed down as far as it will go. This prevents anything entering the sample bottle before the sample is taken.
c) If possible, tie the sampler handle to a secure point, to prevent loss of sampler.
d) Grip the sampler by the handle and the top of the shaft in order to support the weight of the sampler and bottle. Place the sample bottle in the water, holding the shaft of the sampler as close to vertical as is feasible from the sampling position.
e) Ensure that the plastic attachment piece at the end of the sampler is completely submerged.
f) While holding the sample bottle under the water, draw the plunger upwards as far as it will go. Bubbles should begin to rise from the holes in the plastic attachment piece. Hold the sampler in position until bubbles are no longer being released from the bottle. This shows that the bottle is full.
g) Push the plunger down as far as it will go to reseal the sample bottle and prevent contamination of the sample. Remove the sampler from the water.
h) Carefully unscrew the sample bottle from the end of the sampler and pour the water collected into a clean bucket.
i) Repeat steps a) to h) above, a minimum of 3 times. At each deployment, try to take water from a different depth (near bottom (>0.5m from bottom), mid water and near surface).
j) Gently mix the water in the bucket using a figure of '8' motion before taking the sample.

k) Rinse the 1 litre sample bottle, bucket and pole in fresh water and dry thoroughly. This will prevent contamination of future samples and reduce corrosion of the sampler.

Using the Aluminium, extendable pole sampler.

a) A 750ml plastic bottle (complete with its lid) should be placed in the holder at the bottom of the pole. There is a hole in the bottle lid and another one in the shoulder of the bottle to enable water to enter and air to escape. The bottle should be held tightly in place by tightening the screw on the plastic banding.
b) If possible, tie the pole sampler to a secure point, to prevent loss of sampler.
c) Adjust the length of the sampler pole according to the depth of water being sampled and adjust the angle of the bottle holder to ensure that the bottle is held vertically in the water as the sample is being taken.
d) Grip the sampler by the handle and the top of the shaft in order to support the weight of the sampler and bottle. Place the sample bottle in the water, to the selected depth. Bubbles should begin to rise from the hole in the bottle lid. Hold the pole in position until bubbles are no longer being released from the bottle.
e) Once the bottle is full, remove the sampler from the water and release the bottle from the holder by unscrewing the plastic banding.
f) Carefully unscrew the bottle lid and pour the water collected into a clean bucket.
g) Repeat steps a) to f) above, 3 times. At each deployment, try to take water from a different depth (near bottom (>0.5m from bottom), mid water and near surface).
h) Gently mix the water in the bucket using a figure of '8' motion before taking the sample.
i) Rinse the 750ml sample bottle, bucket and pole in fresh water and dry thoroughly. This will prevent contamination of future samples and reduce corrosion of the sampler.

Note: It may not be possible under all circumstances to use any of the above methods at every site. The reasons for this must be clearly explained to Cefas and either an alternative sampling site will be selected, where the preferred equipment could be used, or the use of a bucket may be permissible, in which case:-

Take a near surface sample of sea water using a bucket from as far from the shore line as practicable but in the same location as the shellfish sample is taken, or in the vicinity of the shellfish harvesting bed.

6 FIXING AND RETURNING THE SAMPLES

a) Take a 500ml, brown plastic bottle (supplied) and fill in the site name, bed ID and date on the label.
b) Use a figure of eight motion to mix the sample within the bucket then immerse the brown bottle to take the sample, filling it just to the shoulder. Under no circumstances should the sample be allowed to settle within the bucket before filling the brown bottle, as the sample will no longer be representative of the algal content of the water.
c) Using a graduated pipette (supplied), add 2.0ml of Lugols Iodine (preservative) to the sample. Ensure that there is still an air gap at the top of the bottle to enable mixing.

d) Secure the bottle lid **tightly,** and gently invert the sample bottle three times to ensure complete mixing of the preservative and seawater and place it in the box supplied.

e) Record the sample details - location, date, time, sampled depth (the real or average depth of the sample taken, not the depth of the water body), sampling method, bed ID etc on the sample collection form provided. Please complete ALL sections of the sample collection form and if possible please use Celsius and metres when completing the water temperature and depth fields.

f) If the water samples are to be collected by a sub-contractor then these **must** first be approved by the FSA and verified as competent by the LA. The contact details for the sub-contractor must be provided in the 'Additional comments' box at the bottom of the sample collection form. The Local Authority should also contact the Biotoxin co-ordinator at Cefas Weymouth (see section 10) with the contact details of any regular sub-contractor, in advance of any sampling commencing.

g) When back at your laboratory or office, ensure that the lid on the bottle is securely tightened and place the sample bottle in a return box and close securely. It is not necessary to tape the lid or to place the bottle in a plastic bag as these can cause the sample to 'sweat' on route, making the label difficult to read. Place the box (again it is not necessary to tape the box closed) and the water sample collection form in a special delivery envelope and send to the following address (which is also printed on the special delivery return labels provided):

The Plankton Laboratory
CEFAS - Lowestoft Laboratory,
Pakefield Rd,
Lowestoft,
Suffolk, NR33 OHT
Tel: **01502 524432**
Fax: **01502 513865**
E-mail: **Planktonlab@cefas.co.uk**

7. SAMPLE COLLECTION FORM

An individual sample collection form must accompany each sample to the laboratory. The form must be completed in full and accurately. Incomplete or inaccurate collection forms may lead to the rejection of samples.

In addition to the information requested, sampling officers are asked to report unusual observations (e.g. weather, boating activity, dredging, animals in water, plankton bloom, etc.) which can help target investigations and possible remedial actions.

8. REPORTING

Unsuitable samples: The condition of each sample is assessed on arrival at the laboratory. Samples which have leaked, are not preserved properly, or are contaminated, or contain high amounts of sediment or are collected outside specifications (duplicate samples, additional samples not agreed with Cefas or the FSA) will be rejected as unsuitable for analysis. Unsuitable samples will be reported to the FSA without delay and where applicable, the local authority will be contacted by the Biotoxin co-ordinator to arrange for another sample to be collected.

Results:

- Routine sample results are reported by Cefas daily to the FSA. In addition, full weekly analysis results are compiled and reported to the FSA every Monday. Results are made available to local authorities via the FSA website. (**http://www.food.gov.uk/enforcement/monitoring/shellfish/ewbiotoxin/**)
- Results exceeding agreed **trigger** levels are reported to the FSA without delay and local authorities are contacted by the FSA immediately if follow up samples are to be taken.

9. RESAMPLING

Where shellfish testing is not performed weekly, additional water samples may be requested by FSA if algae concentrations reach the trigger levels agreed within the statutory testing programme (see below). Additional shellfish samples will also be requested and the testing undertaken on the flesh resample will be guided by the species of algae which have breached the trigger level.

Sampling will return to its normal frequency when algae and toxin concentrations fall below agreed levels. The affected local authorities will be contacted by FSA to discuss the status of the sampling programme in the affected area.

The table below shows the trigger levels which may initiate more frequent (weekly) sampling of water and will initiate additional shellfish monitoring due to increased risk of toxin accumulation in commercially harvested shellfish.

Algal species	Toxins produced	Trigger level (cells/L)
Alexandrium species	PSP	40
Dinophysis species	Lipophilic toxins	100
Prorocentrum lima	Lipophilic toxins	100
Pseudo-nitzschia species	ASP	150,000

10. CONTACT INFORMATION

Enquiries relating to the FSA monitoring programmes (including monitoring points, frequency of sampling, actions in case of breach of pre-defined levels, availability of sampling DVD) should be referred to the following FSA contacts:

England: **Becky Watts**, Tel. 020 7276 8046
rebecca.watts@foodstandards.gsi.gov.uk

Wales: **Daniel Morelli**, Tel. 029 2067 8902
Daniel.Morelli@foodstandards.gsi.gov.uk

Sampling schedules, general queries or problems relating to sampling should be referred to Cefas Programme Co-ordinators:

Lewis Coates, Tel. 01305 206744, Fax. 01305 206601
lewis.coates@cefas.co.uk or biotoxinmonitoring@cefas.co.uk
Deputy: Ben Stubbs, Tel. 01305 206713, Fax. 01305 206601
ben.stubbs@cefas.co.uk or biotoxinmonitoring@cefas.co.uk

For specific enquires related to sample collection/delivery, request for further packaging/postage or other specific laboratory queries, please contact the

Plankton Laboratory on 01502 524432, Fax 01502 513865 or e-mail planktonlab@cefas.co.uk.

11. HEALTH, SAFETY & BIOSECURITY ADVICE

Sampling officers are asked to comply with the Health and Safety policies of their respective organisation. This includes compliance with all safety measures prescribed in risk assessments relevant to their travelling to the agreed sampling locations and the collection and handling of water samples from such areas for the purpose of the FSA monitoring programmes. The risks associated with the use and handling of Lugol's iodine should be covered by a COSHH assessment. A copy of the Cefas COSHH assessment is sent to each local authority with the sampling equipment, to assist each LA when producing its own COSHH assessment. The drafting, implementation and review of all relevant H&S documentation are the responsibility of sampling contractors.

When undertaking sampling duties, sampling officers must be mindful of the risks of introduction or transfer of aquatic pathogens and invasive species to the areas being visited, through their sampling activities. Officers are asked to comply with biosecurity measures such as cleaning and disinfection of instruments, equipment and shoes/boots between sites and not driving/parking onto beaches or in close proximity to shellfish beds. All disposable items should be treated as clinical waste. Advice on suitable disinfectant and disinfection procedures are available from the fish health inspectorate (see details below). As a minimum, Cefas recommends the use of Virkon S or Virkon Aquatic S at 1% and with a minimum contact time of 15 min (or spray onto clean surface and leave to dry). A list of other suitable disinfectants is available at: http://www.defra.gov.uk/aahm/guidance/disinfectant/list/. Disinfection regimes should include all sampling equipment however it is recognised that this is difficult to achieve for pole and tube samplers. As a minimum, these should be rinsed with freshwater.

Sampling officers should also be mindful of the health status of the sites that they visit and schedule their visits to ensure that the risk of transfer of pathogens and invasive species from site to site is minimised. Details of sites under specific designations and for which specific movement controls do apply are available from the Fish Health Inspectorate (see below) and up to date lists and maps of designated areas are published on the aquatic animal health and movements page of the Defra website

It is recommended that sampling officers familiarise themselves with biosecurity plans operated by the farmers in the harvesting areas and with rules that apply to site visitors.

Where new risks of transfer of specific fish or shellfish pathogens are identified, the requirement for implementation of additional biosecurity measures will be discussed between the programme co-ordinators and the sampling officers as soon as reasonably practicable following notification by the relevant competent authorities for shellfish health.

For further advice on biosecurity measures, please contact:

Fish Health Inspectorate
Cefas
Barrack Road
The Nothe
Weymouth
Dorset DT4 8UB

Tel: 01305 206700
Fax: 01305 206602
Email: fhi@cefas.co.uk