UK-NRL Phyto 1 SOP v3

TITLE: Standard operating procedure for the collection of water samples for analysis of potential toxin producing phytoplankton cells in compliance with EU reg. 2019/627

Production Summary

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History of Procedure

Issue	Date Issued	Changes
1	24 April 2006	Version 1 (final)
2	August 2012	Clarification of wording and reformatting of text throughout.
3.	January 2021	Legislation updated 8.1 use of tube sampler permitted when sampling from pier/ jetty in water depths ≤3m. 8.3.2 Amount of Lugol's iodine which can be added to samples amended.

1. Introduction and Scope

This SOP details the methods employed in the collection of seawater samples for the identification and enumeration of potentially toxin producing phytoplankton species in fulfilment of EU Regulation 2019/627 The method of collection (depending on the depth of water at the site involved) and preservation of the samples are described.

2. Principle of the Method

The aim of this method is to collect samples of phytoplankton that are representative of the phytoplankton community in the water body being sampled. The water sample should be taken as close to the shellfish bed as possible and at the location from where shellfish flesh samples are taken for biotoxin analysis. The sampling method used will be dependent on the depth of water at the site. Water samples should be taken at high tide (+/- 1 hour) particularly at inshore sites to reduce the risk of samples containing large amounts of re-suspended sediment or detritus. Sampling at low tide is discouraged and should be avoided if at all possible.

The depth of water at shellfish production areas, at the time of sampling, varies from site to site. Therefore, one of three different methods is employed to collect a representative water sample (Table 1). The method and water sampler to be used will be provided by the relevant analytical laboratory. Details of each of the sampling methods are given in section 8.

Table 1: Methods employed to collect seawater samples to determine the presence of potentially toxin producing phytoplankton in UK coastal waters.

Depth	Method
≤ 3m	Pole sampler, or bucket.
>3m	Tube sampler of appropriate length

The seawater collected should be mixed in a clean bucket, by stirring in a ‘figure of 8’ motion before a 500 ml subsample is taken using a brown Nalgene bottle. This subsample should be preserved in acidic or neutral Lugol’s iodine. The bottle should be externally labelled with the site name and/or reference. A sample information sheet must also be completed detailing the following information:

Site location
Collector
Date collected
Mode of collection (bucket/pole sampler, tube sampler)
Time
Tidal state (ideally within +/- 1h of high water)

Sample depth

The sub-sample and the completed information sheet must be returned to the relevant laboratory for analysis as soon as possible, preferably the day of collection.

Samplers are requested to collect samples early in the week to allow for delivery, sample preparation, analysis and reporting of results within the same week. This is to enhance the capability of the phytoplankton monitoring programme to provide an early warning of the occurrence of phytoplankton species that have the potential for producing biotoxins.

3. Reference Material

N/A

4. Reagents

Acidic or neutral Lugol's iodine solution (as described by Hallegraeff, 2003), is supplied by the analytical laboratory.

5. Equipment

For seawater sample collection: either a Bucket; or Pole sampler; or Tube sampler is required together with the following additional equipment:

Bucket (for mixing of the sample, as described above. This can be the same as the bucket used to collect the sample at shallow water sites).
500 ml brown, screw capped Nalgene bottles.
Packaging (including boxes for the sample bottles and special delivery bags for sample return).
Sample information sheet.
Return address labels
Bottle containing acidic or neutral Lugol's iodine
Graduated disposable pipettes if required.
Pen.

All equipment should be checked before use to ensure it is clean. After use all equipment should be washed in freshwater, dried and stored in a clean, dry place.

6. Environmental Control

N/A

7. Interferences

Disruption of, or access to, the postal service

Loss of water sampler

Breakage/leakage of sample bottle

Rough weather preventing collection of seawater sample from a boat or remote / exposed areas.

Unsuitable tides e.g. high tides coincides with times of darkness

8 Sampling and Sample Preparation

The sampling method varies with the depth of water sampled. A different method is used if the depth of water is greater or less than 3m.

8.1 Water Depth ≤ 3m

At those sites where the water depth is less than 3m and the water column is well mixed, a pole sampler should be used to collect the water sample. The equipment should be well rinsed with seawater from the sampling site just prior to sampling. If a pole sampler is deemed unsuitable, or health and safety reasons preclude the use of a pole sampler, then a surface water sample should be collected using a bucket.

If access is impractical for surface collection, samples may be collected from a pier/ jetty using a tube sampler.

8.1.1 If a bucket is to be used, take a near surface sample of sea water using a bucket from as far from the shore as practicable, but as close to the shellfish sampling location as possible.

8.1.2 If a pole sampler is to be used, this should be lowered below the surface before the sample is taken. It is recommended that seawater samples are taken from near surface, mid-water and near to, but not on, the bottom. The depths at which the samples are taken should be recorded.

8.13 Follow the instructions for sample preservation, data recording and delivery to the analytical laboratory as described in section 8.3 below.

8.2 Water Depth >3m

A tube water sampler will be supplied by the analytical laboratory when water depths at a shellfish production area collection point are >3m. This tube sampler will allow the collection of an integrated sample from the surface to a depth defined by the depth of water at the site and the length of the tube. The length of the tube sampler supplied is appropriate to the depth of water expected at high water, at the site to be sampled. The tube sampler will be marked at 1m intervals with insulating tape so the depth over which the tube sample is taken can be estimated.

8.2.1 Prior to sampling ensure all equipment to be used is well rinsed, ideally with freshwater.

8.2.2 A line is attached to the bottom of the tube sampler to help with recovery of the weighted end.

8.2.3 Open the valve at the top of the tube.

8.2.4 Slowly lower the weighted end of the tube into the water until most of the tube is immersed or until the weight is approximately 1m from the seabed. The tube sampler must remain taut and vertical to take an even sample of the whole water column. If the weight touches the bottom, retrieve and then re-deploy the tube sampler, as disturbed sediment will affect the quality of the seawater sample collected.

8.2.5 Once the tube is hanging vertically in the water, close the valve at the top of the tube sampler.

8.2.6 Retrieve the bottom of the tube sampler using the attached line and empty ALL the contents of the tube sampler into a bucket by opening the top valve. If necessary lift the valve end of the tube sampler up to allow the water to drain into the bucket.

8.2.7 The depth of water sampled should be recorded. For example, if the tube sampler is lowered to a depth of 5m, then the depth sampled should be recorded as 0 – 5m.

8.2.8 Wash the tube sampler and bucket in fresh water as soon as possible after sampling. Allow to dry.

8.2.9 Follow the instructions for sample preservation, data recording and delivery to the analytical laboratory as described in section 8.3 below.

8.3 Sample preservation, data recording and delivery to analytical laboratory

8.3.1 Mix the contents of the bucket by stirring in a ‘figure of 8’ motion and immediately fill a 500 ml Nalgene sample bottle to the ‘shoulder’ by immersing the bottle in the bucket. Be careful to leave an airspace at the top of the Nalgene bottle to allow for mixing of the sample at the analytical laboratory.

8.3.2 Check the Lugol’s solution provided is within the use-by date specified on the bottle before adding 2.5 – 5.0 ml of Lugol’s Iodine to the sample bottle.

8.3.3 Tightly screw on the lid of the Nalgene sample bottle and gently invert the sample bottle 3 times to ensure complete mixing of the preservative and seawater.

8.3.4 Label the Nalgene sample bottle with the site reference, before completing the sample information sheet.

8.3.5 The sample information sheet should contain the following information

Site location

Date collected

Mode of collection (bucket/ pole sampler, tube)

Time

Tidal state (ideally within +/- 1h of high water)

Sample depth

8.3.6 Place the Nalgene sample bottle and information sheet in the pre-labelled cardboard box (if posting) or return to the laboratory for analysis.

8.3.7 It is recommended that seawater samples are collected early in 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 phytoplankton analysis to act as an early warning mechanism for the potential occurrence of biotoxins in shellfish.

9. Analytical Procedure

N/A

10. Results

N/A

11. Precision, Bias and Limit of Determination

Inaccuracies in obtaining representative cell concentrations will be introduced if the water samples collected are NOT well mixed, using a 'figure of 8' motion, before being sub-sampled.

Similar inaccuracies will be introduced if the tube sampler is not vertical when the seawater sample is taken, or if the top valve of the tube sampler is opened after the tube is lowered into the water.

12. Reports

N/A

13. Safety

Provision of a COSHH assessment for use of Lugol's iodine is the responsibility of the Local Authorities in England & Wales, Scotland and N. Ireland. In addition, the analytical laboratories will supply their own COSHH assessments for the collectors to follow, if required.

It is the responsibility of each Local Authority in England & Wales and N. Ireland to assess ALL risks likely to be incurred by the collectors during the collection of seawater samples for the phytoplankton monitoring programme. The Scottish laboratory will provide relevant documentation as guidance for collectors to make their own assessment of risk. The analytical laboratories can take NO responsibility for assessing the risks for collectors at any monitoring site.

14. Uncertainty of Measurement

To ensure representative samples of phytoplankton are collected, the collectors must be familiar, and comply with the sampling SOPs provided by each analytical laboratory and

the equipment supplied. To assist with this, it is recommended that the analytical laboratories provide regular training to the collectors.

15. Literature

Hallegraeff, G.M., Anderson, D.M., Cembella, A.D. (2003) "*Manual on Harmful Marine Microalgae*", Unesco Publishing , 793pp..

Sournia, A. (ed.)(1979) "*Phytoplankton Manual*" Unesco Publishing, 337pp.