European Union Reference Laboratory
for Marine Biotoxins

Monitoring of Toxin-producing Phytoplankton in Bivalve Mollusc Harvesting Areas

Guide to Good Practice: Technical Application

EU Working Group on Toxin-producing Phytoplankton Monitoring in Bivalve Mollusc Harvesting Areas

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DISCLAIMER

This guide is a technical document intended to contribute to a better understanding, and to recommend best practices for the application of EU legislation regulating the official controls in production and relaying areas for live bivalve molluscs. This is intended purely as a guidance tool, and it is necessary to take into account that only the text of the Commission Implementing Regulation (EU) 2019/627 has legal force.

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Monitoring of Toxin-producing Phytoplankton in Bivalve Mollusc Harvesting Areas

Guide to Good Practice

1. General Introduction

Marine phytoplankton, photosynthetic free-living protists and prokaryotes inhabiting the water column, are the key primary producers of the ocean, constitute the base of the oceanic food webs and sustain fishery resources. Some species of marine phytoplankton produce intracellular compounds that are toxic to humans (e.g., Hallegraeff 2004, Alonso et al. 2016). These toxins may accumulate up through the food chains, particularly in filter-feeding edible organisms such as bivalve molluscs. Accumulation of these toxins may be harmless for these organisms but causes serious risks for the consumer's health (e.g., Backer et al. 2004) and has a negative impact on aquaculture and fisheries (e.g., Fernández et al. 2004).

Such a risk is difficult to manage. On the one hand, toxin-producing phytoplankton species are similar to non-toxic ones in terms of morphology and size, but also in their physiological and ecological constraints. Hence the presence of toxin-producing cells within a diverse plankton community that ultimately feeds bivalves, their ingestion by poorly selective filter-feeding organisms, and the ensuing accumulation of biotoxins, are unavoidable natural processes. The rate of ingestion by molluscs of toxin-containing cells depends on their relative abundance within the plankton community, however the processes triggering regional blooms of any particular phytoplankton species, and the rules governing community assembly and succession are poorly known, and bloom dynamics are difficult to predict (e.g., Carstensen et al. 2015). The accumulation of toxins in bivalves also depends on the time of residence of toxin-containing cells in the production area, but the time-varying, patchy distribution of the plankton that is ultimately controlled by rapidly changing hydrodynamics, is also difficult to forecast (e.g., Davidson et al. 2016). Such unpredictable dynamic and heterogeneous distribution makes the direct monitoring of toxin-producing phytoplankton species an essential

instrument to the management of biotoxins-related risks in areas for production of live bivalve molluscs.

Accordingly, to protect consumers, the EU legislation requires that production and relaying areas for live bivalve molluscs are regularly monitored to detect the presence of toxin-producing plankton in the water column. The *Commission Implementing Regulation (EU) 2019/627 of 15 March 2019 laying down uniform practical arrangements for the performance of official controls on products of animal origin intended for human consumption in accordance with Regulation (EU) 2017/625 of the European Parliament and of the Council and amending Commission Regulation (EC) No 2074/2005 as regards official controls Regulation* (Article 59) states:

> "The competent authorities shall periodically monitor production and relaying areas classified in accordance with Article 18(6) of Regulation (EU) 2017/625 in order to check: … (c) for the presence of toxin-producing plankton in production and relaying waters and marine biotoxins in live bivalve molluscs"

Phytoplankton data from these official controls are necessary to assess the spatial and temporal variation of the risk of toxins occurring in molluscs, which is necessary to inform the competent authorities responsible of managing classified production and relaying areas (Regulation (EU) 2019/627, Articles 62 and 63). Such information is also necessary to determine the affected areas most susceptible to contamination, which is a requisite for the design of sampling plans for the official control of biotoxins in live bivalves (Regulation (EU) 2019/627, Article 61).

Given the dynamic and complex nature of the toxin-producing phytoplankton landscape, one fundamental requirement for phytoplankton monitoring in the EU legislation is that sampling plans drawn up by competent authorities ensure that the results of the analyses are representative of the area of interest (Regulation (EU) 2019/627, Article 61). This is a difficult demand that needs to take into consideration regional and seasonal peculiarities in ecological conditions, phytoplankton-derived risks, harvesting or management systems, which may explain some differences in monitoring programmes between Member States. Different sampling plans may be not only justified in distinct hydrographic domains (e.g., the stratified E Mediterranean in summer vs. the mixed English Channel in winter), marine ecosystems (e.g., a coastal upwelling

in a Galician ría, a coastal lagoon in the Adriatic Sea, a tidal flat in the Atlantic coast of France, or a Scottish loch) or types of harvesting (e.g., suspended ropes vs. sea-bed), but may actually be necessary for a harmonised, comparable representation of risks. However, the lack of systematic inter comparative analyses of methods and results was a cause of concern to several competent authorities, and the European Commission requested the European Reference Laboratory for Marine Biotoxins (EURLMB) to coordinate a working group of experts from all interested Member States with the objective to progress towards harmonisation of monitoring protocols for toxin-producing phytoplankton, with the final aim to secure well balanced, comparable analyses of risk under different environmental/managerial scenarios.

This good practice guide is a result of such an undertaking, and it was drafted and reviewed under the coordination of the EURLMB together with an advisory group of experts in operational, routine monitoring in toxic phytoplankton, and a working group of experts nominated by EU National Reference Laboratories for Marine Biotoxins from twelve Member States. The objective is to provide technical recommendations within a common framework for a) the design and implementation of similarly representative monitoring programmes for toxin-producing phytoplankton and b) a balanced interpretation of results, all in line with the requirements in the EU legislation.

1.1. References

Alonso, R., Moreira, A., Méndez, S. and Reguera, B. 2016. Introduction. In: B. Reguera, R. Alonso, A. Moreira, S. Méndez and M.-Y. Dechraoui-Bottein (eds). 2016. *Guide for designing and implementing a plan to monitor toxin-producing microalgae*. 2nd ed. Intergovernmental Oceanographic Commission (IOC) of UNESCO and International Atomic Energy Agency (IAEA), Paris and Vienna. IOC Manuals and Guides, no. 59. 66 pages. http://unesdoc.unesco.org/images/0021/002145/214510e.pdf

Backer, L.C., Fleming, L.E., Rowan, A.D. and Baden D.G. 2004. Epidemiology, public health and human diseases associated with harmful marine algae. In: Hallegraeff, G.M., Anderson, D.M., Cembella, A.D. and Enevoldsen, H.O. (eds).

2004. *Manual on Harmful Marine Microalgae*. 2nd revised edition. Paris, France, UNESCO, 793pp. (Monographs on Oceanographic Methodology, 11). http://hdl.handle.net/11329/282

Carstensen, J., Klais, R. and Cloern, J.E. 2015. Phytoplankton blooms in estuarine and coastal waters: Seasonal patterns and key species. *Estuarine, Coastal and Shelf Science* 162, 98-109.

Davidson, K., Anderson, D.M., Mateus, M., Reguera, B., Silke, J., Sourisseau M., Maguire, J. 2016. Forecasting the risk of harmful algal blooms. *Harmful Algae* 53, 1-7.

Fernández, M.L., Shumway, S. and Blanco, J. 2004. Management of shellfish resources. In: Hallegraeff, G.M., Anderson, D.M., Cembella, A.D. and Enevoldsen, H.O. (eds). 2004. *Manual on Harmful Marine Microalgae*. 2nd revised edition. Paris, France, UNESCO, 793pp. (Monographs on Oceanographic Methodology, 11). http://hdl.handle.net/11329/282

Hallegraeff, G.M. 2004. Harmful algal blooms: a global overview. In: Hallegraeff, G.M., Anderson, D.M., Cembella, A.D. and Enevoldsen, H.O. (eds). 2004. *Manual on Harmful Marine Microalgae*. 2nd revised edition. Paris, France, UNESCO, 793pp. (Monographs on Oceanographic Methodology, 11). http://hdl.handle.net/11329/282

2. Sampling Plans

2.1. Introduction

Sampling plans are documents specific to each harvesting area, providing all the necessary details to carry out the collection of samples fulfilling the objectives of monitoring: samples need to be as representative, and results as precise, accurate and free from potential biases as possible; besides, sampling methods need to be practicable, inexpensive and simple to facilitate routine fieldwork. The necessary compromise between scientific rigour and feasibility needs to be resolved for each harvesting area or monitoring network, based upon expert knowledge of the characteristics of the coastal ecosystem under study, and taking into account the nature of the technical means available. This may result in local methodological peculiarities that need to be considered when results from different areas or networks are compared.

The abundance and species composition of phytoplankton in natural ecosystems are highly dynamic and spatially heterogeneous in both the horizontal and vertical axes, over nested scales. Hydrodynamics play a key role in this complex spatial and temporal variability, as they control both the growth and composition of phytoplankton communities, and the transport and dispersion of populations. This scale dependency of phytoplankton observations implies that the location of sampling stations, and the depth, frequency and time of sampling may have significant impacts on the monitoring results, and hence should be carefully detailed in a sampling plan.

Requirements

Commission Implementing Regulation (EU) 2019/627, Title V, Chapter II, Article 59 (Monitoring of classified production and relaying areas):

> The competent authorities shall periodically monitor production and relaying areas classified in accordance with Article 18(6) of Regulation (EU) 2017/625 in order to check:
>
> …
>
> (c) for the presence of toxin-producing plankton in production and relaying waters and marine biotoxins in live bivalve molluscs;

Commission Implementing Regulation (EU) 2019/627, Title V, Chapter II, Article 61 (Sampling plans):

> 1. For the purposes of the checks provided for in points (b), (c) and (d) of Article 59, the competent authorities shall draw up sampling plans providing for such checks to take place at regular intervals, or on a case-by-case basis if harvesting periods are irregular. The geographical distribution of the sampling points and the sampling frequency shall ensure that the results of the analysis are representative of the classified production and relaying area concerned.
>
> …
>
> 3. Sampling plans to check for the presence of toxin-producing plankton in the water in classified production and relaying areas and for marine biotoxins in live bivalve molluscs shall take particular account of possible variations in the presence of plankton containing marine biotoxins. Sampling shall comprise:
>
> - periodic sampling to detect changes in the composition of plankton containing toxins and their geographical distribution. Results suggesting an accumulation of toxins in live bivalve mollusc flesh shall be followed by intensive sampling;
>
> 4. The sampling frequency for toxin analysis in live bivalve molluscs shall be weekly during harvesting periods, except when:
>
> (a) the sampling frequency may be reduced in specific classified relaying or production areas, or for specific types of live bivalve mollusc, if a risk assessment of toxins or phytoplankton occurrence suggests a very low risk of toxic episodes;
>
> (b) the sampling frequency shall be increased where such an assessment suggests that weekly sampling would not be sufficient.
>
> 5. The risk assessment referred to in paragraph 4 shall be reviewed periodically in order to assess the risk of toxins occurring in the live bivalve molluscs from these areas.
>
> …
>
> 7. With regard to the monitoring of plankton, the samples shall be representative of the water column in the classified production or relaying area and provide information on the presence of toxic species and on population trends. If any changes in toxic populations that may

lead to toxin accumulation are detected, the sampling frequency for live bivalve molluscs shall be increased or precautionary closures of the areas established until results of toxin analysis are obtained.

Rationale

The legislation requires that the spatial and temporal scale and resolution of the sampling points are such that resultant phytoplankton data are representative of the water column and of the areas being monitored. Sampling plans should identify standard quantitative methods to determine the abundance and natural variability of toxin-producing phytoplankton in the water column. These need to be feasible, unbiased, suitable for a range of oceanographic conditions, types of shellfish harvesting, and practical for technical and logistical requirements. Sampling plans should also include all the variables needed to complete the interpretation of phytoplankton abundance data, so that the requirements of the legislation with regard to risk evaluation and early warning can be fulfilled. In this regard, in addition to direct seawater sampling, modelling or historical hydrographic and phytoplankton data may provide the necessary supporting information. Below are recommendations to meet all these goals.

2.2. Location of sampling points

2.2.1. Each classified shellfish harvesting area must contain at least one monitoring station to detect the presence of toxic phytoplankton in the water column. Monitoring stations should represent the conditions and phytoplankton-related risks within the production area, and hence should be located in or adjacent to the production area and taking into account the local hydrography. If an area is split into different management units (e.g. ones that may be subject to different closures), each unit should contain at least one representative sampling point, unless a study has determined that the whole area is characterised by a single phytoplankton-based risk profile, derived from the interaction of population dynamics and advection. As classification of harvesting areas only attends to microbiological risks, several neighbouring classified areas may belong to a larger domain where the phytoplankton-related risk profile is homogeneous. When this is substantiated after pilot, baseline or desk-based studies (see sections 2.2.6 - 2.2.9), a single sampling

station may be valid to represent those conditions and risks in all the harvesting areas.

2.2.2. Accumulation of toxins by filtering molluscs depends on the availability of toxin-containing phytoplankton cells, which can be locally produced at the harvesting area or transported from elsewhere. Representative sampling points should be selected to detect 1) the highest risk of toxin-producing phytoplankton blooms within a production area, and 2) the highest risk of arrival of local or allochthonous toxin-producing phytoplankton cells to filtering bivalves in the production area. This implies that additional sampling points outside the region of harvest (e.g. at the mouth of an estuary) may be necessary to monitor the risk of transport into this region, and additional sampling is recommended where a water discoloration is observed in the area without affecting the standard sampling points.

2.2.3. The number and location of sampling points per harvesting area or management unit will depend upon the spatial variability of these risks. The sampling plan needs to take into consideration the temporal variation (e.g. seasonal, inter-annual) in the spatial distribution of these risks.

2.2.4. Sampling points should be unequivocally geolocated (latitude/longitude) to an accuracy of 10 ± 5 metres. Phytoplankton samples should be taken as close to the shellfish harvesting area as practicable and up-stream, or at a location where the highest risk of arrival of toxic phytoplankton in the production area can be detected, on the same day of shellfish monitoring, to support information on shellfish toxicity attribution.

2.2.5. Both the growth, transport and dispersion of toxic phytoplankton populations are ultimately controlled by hydrodynamics, and hence the above mentioned risks, and the derived optimal location of sampling stations cannot be determined without a knowledge of prevailing hydrodynamics (e.g., currents, fronts, vertical mixing regime, etc.) in and around the production area, and their interaction with phytoplankton dynamics (particularly bloom dynamics).

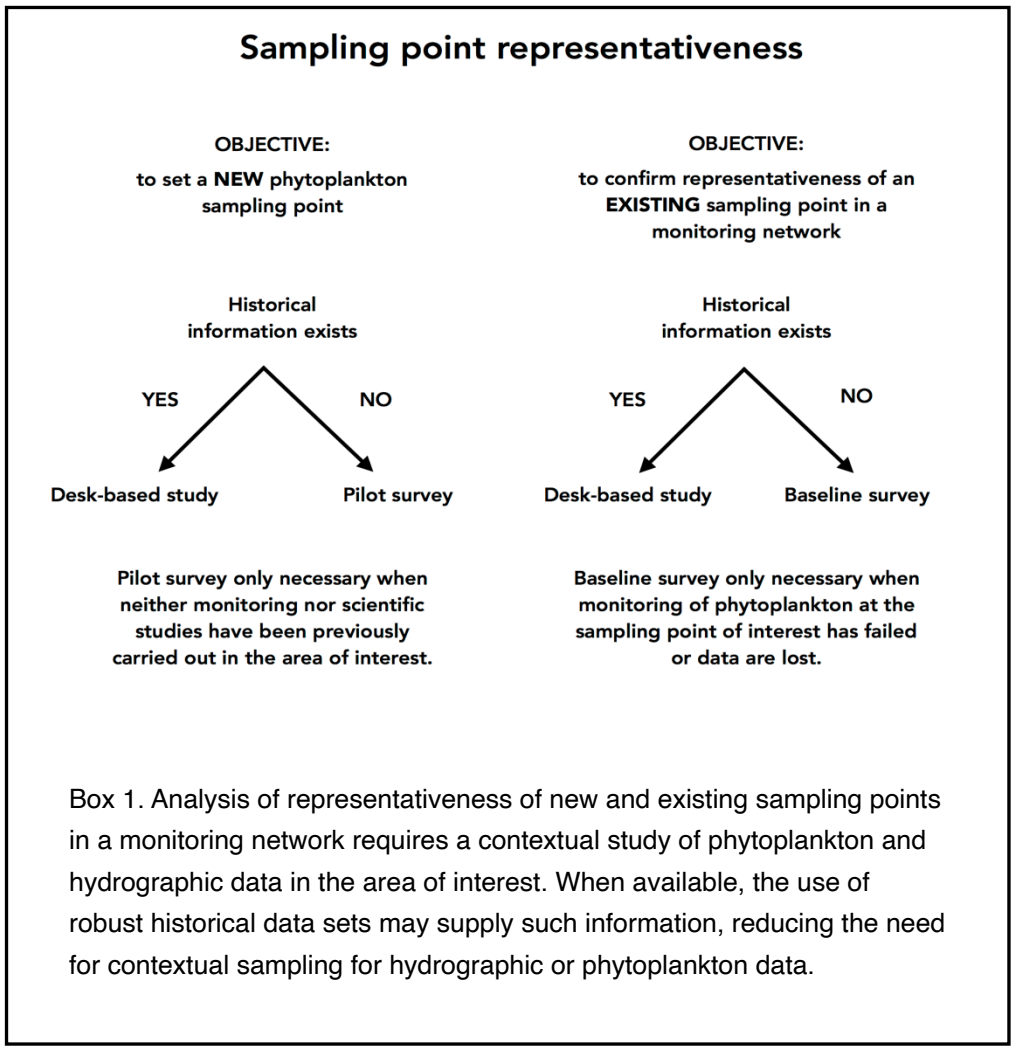
2.2.6. To comply with the requirements above, and to secure that sampling points are, and remain representative of the variability within the production area, spatial and temporal (e.g. seasonal) surveys of phytoplankton abundance and hydrodynamic conditions are necessary before establishing a new phytoplankton sampling point (pilot surveys) and periodically (baseline surveys)

(see also section 2.3. Sampling frequency). The use of hydrodynamic models is recommended.

2.2.7. When available, hydrographic or phytoplankton data from scientific studies or environmental monitoring may replace the actual sampling for pilot or baseline surveys. Information on hydrodynamics from microbiological sanitary surveys used to classify bivalve production and relaying areas (see European Commission 2017. Community Guide to the Principles of Good Practice for the Microbiological Classification and Monitoring of Bivalve Mollusc Production and Relaying Areas with Reference to Regulation 854/2004. Issue 3: January 2017) may be of particular value.

2.2.8. Once a representative phytoplankton monitoring network is operative, its own results may feed baseline studies. That is, a periodical desk-based analysis of spatial variability and statistical covariation of physical and biological data collected through the whole area by the monitoring network can substitute the field sampling for baseline surveys (see Box 1). However, field-based baseline surveys are recommended whenever new risks or changing conditions are observed after standard monitoring (e.g., new toxin-producing species, changes in seasonality, frequency or intensity of toxic events, changes in nutrient loads, or hydrographic features such as stratification, upwelling, currents, fronts, etc.) (see also section 2.3. Sampling frequency).

2.2.9. These surveys, or the surrogate desk-based studies, should contain hydrographic information to infer the characteristics of the seawater circulation (e.g., transport velocities, retention times) and the potential for advection of particles into the production area, which may include in situ measurements, hydrodynamic models or remote sensing. These surveys, or the surrogate desk-based studies of spatial variability based on historical data, should also include information on abundance of toxin-producing phytoplankton species enough to determine the representativeness of each sampling point, and to infer how spatial and temporal changes in the physical conditions of the water column and circulation patterns relate to changes in the abundance and distribution of phytoplankton in and around the harvesting area. These surveys should provide information on the frequency and distribution of toxic events in the area. To secure representativeness, a minimum 3 years of weekly-fortnightly (see 2.3) data of cell abundance and shellfish toxicity are recommended.



Box 1. Analysis of representativeness of new and existing sampling points in a monitoring network requires a contextual study of phytoplankton and hydrographic data in the area of interest. When available, the use of robust historical data sets may supply such information, reducing the need for contextual sampling for hydrographic or phytoplankton data.

2.3. Sampling frequency

2.3.1. During periods when harvesting is allowed, the sampling frequency at monitoring stations should be at least weekly. Phytoplankton samples should be taken on the same day as shellfish samples at monitoring stations. A regular (not randomised) weekly sampling under standardised tidal conditions is recommended. Whenever tides significantly influence water column properties, sampling should be carried out at high tide ±1h, although, considering logistical constraints, two hours before high tide may still be acceptable.

2.3.2. The standard sampling frequency at monitoring stations may be reduced to fortnightly if a risk assessment based on robust data of the dynamics of toxicity and phytoplankton suggests a very low risk of toxic episodes. This

should take into account that not all species of toxic phytoplankton have an annual period of appearance, and some bloom after several years of absence.

Before sampling frequency at any point may be reduced to fortnightly for a certain period of the year, at least three (3) consecutive years of absence of blooms with toxification impact, of any species of toxin-producing phytoplankton, must be observed during such period. However, weekly sampling should always be maintained throughout the period at least at one (1) sentinel station per water-body or region characterised by homogeneous hydrodynamics, similar chemical-physical characteristics and phytoplankton dynamics or phytoplankton-related risks, which may include more than one harvesting area. The representativeness of sentinel stations needs to be assessed according to the criteria for monitoring stations (sections 2.2.6 – 2.2.9). A sentinel station may coincide with a monitoring station in the area. Weekly sampling at all monitoring stations in a region should be resumed if the phytoplankton analysis of samples collected from their representative sentinel station indicates an increased risk.

Exceptionally, monitoring frequency may be further reduced at high latitudes during winter periods when the light conditions preclude the growth of any phytoplankton, if this is supported by a risk assessment based on historic evidence.

2.4. Water column sampling

2.4.1. Shallow stations (depth < 5m): a water sample taken from the top 2 metres (using a bucket, oceanographic bottle or a pole sampler) is considered representative of the water column. Sampling has to avoid the disturbance of the bottom sediment.

2.4.2. “Deep” stations (> 5 m depth): sampling should be integrated, including the range of depths where shellfish are grown, by means of a hose made of any flexible, inert and clean material (e.g., polythene, polypropylene, silicone) and with a minimum inner diameter of 2 cm. A thorough description of the hose design and handling can be found in Lindahl (1986) and in Mendez et al. (2017). At shallow stations the hose can be substituted with a PVC tube with a stopper at the top end (Mendez et al. 2017). Integrated sampling, especially at stations deeper than 30 m, may be carried out by pooling discrete samples or

arithmetically integrating data from oceanographic bottles (e.g., Niskin). Net sampling may complement the integrated hose sampling by detecting species in very low densities. The methodology for hose and bottle sampling, and net hauls is described in the European Standard EN 15972:2011, Water quality - Guidance on quantitative and qualitative investigations of marine phytoplankton.

2.4.3. The hose must be lowered with all stopcocks open at a speed not exceeding 20 m/min, and with the help of a weight attached to its lower end, to ensure a vertical descent. Once fully lowered, the top stopcock is closed and the hose can be pulled back to deck gently. To obtain subsamples representative of the entire water column, the hose should be gently emptied into a carboy or bucket by opening the stopcock. Subsamples are taken immediately into 100-1000 mL bottles, or the sample needs to be homogenised by stirring the carboy gently prior to subsampling. Subsamples are left as they are, or fixed with Lugol's solution as described in the chapter "Sampling, sample transport and Storage" and exhaustively in the standard EN 15972:2011.

2.4.4. When information on the vertical distribution of fluorescence is available, collection of additional samples at the depth of the maximum of fluorescence is recommended.

2.4.5. Details on sampling equipment, bottles, fixatives and preservatives, labelling, transport and storage can be found in the chapter "Sampling, sample transport and Storage" and exhaustively in the standard EN 15972:2011.

2.5. References

EN 15972:2011, Water quality - Guidance on quantitative and qualitative investigations of marine phytoplankton.

European Commission 2017. Community Guide to the Principles of Good Practice for the Microbiological Classification and Monitoring of Bivalve Mollusc Production and Relaying Areas with Reference to Regulation 854/2004. Issue 3: January 2017.

Lindahl, O. 1986. A dividable hose for phytoplankton sampling. In: *Report of the Working Group on phytoplankton and management of their effects*. International Council for the Exploration of the Sea, C.M. 1986/L:26, Annex 3.

Méndez, S., Alonso, R., Moreira, A., Reguera, B. 2016. Designing a plan to monitor potentially toxic microalgae: General guidelines and methods. In: Reguera, B., Alonso, R., Moreira, A., Méndez, S., Dechraoui-Bottein, M.-Y. (Eds). 2016. *Guide for designing and implementing a plan to monitor toxin-producing microalgae*. 2nd Ed. Intergovernmental Oceanographic Commission (IOC) of UNESCO and International Atomic Energy Agency (IAEA), Paris and Vienna. IOC Manuals and Guides, no. 59. 66 pages. http://unesdoc.unesco.org/images/0021/002145/214510e.pdf

3. Sampling, sample transport and storage

3.1. Introduction

Seawater samples containing representative collections of natural plankton communities need to be transported to the laboratory in order to be processed according to chapter 4 -“Counting method”. This requires that the composition of the community remains unchanged, and that the phytoplankton cells maintain a condition that allows their accurate identification and counting under the light microscope. Comprehensive descriptions of the materials and techniques necessary to meet these objectives may be found in the European standards EN15972:2011 and EN 15204:2006. Here we summarise the recommendations applicable to the monitoring of toxin-producing phytoplankton.

3.2. Equipment and reagents

3.2.1. Wide mouth glass or plastic bottles with tight seal to minimise evaporation. Standard volumes are 100-500 mL, although different volumes may be used, e.g., 25-50 mL are typically used for high phytoplankton abundances and up to 5000 mL in oligotrophic areas. For long-term storage, bottles should be made of materials that do not affect the phytoplankton, non-reactive to the fixatives and UV resistant (e.g., LPDE), and be stored in the dark and at stable low temperature.

3.2.2. Preservatives.

Lugol’s solution is the most commonly used preservative. The solution can be:

- Weakly alkalized Lugol’s solution: dissolve 20 g potassium iodide (KI) in 140 mL distilled or demineralised water, then add 10 g iodine (I_2, crystalline) and 10 g sodium acetate.

- Acid Lugol's solution: dissolve 20g potassium iodide (KI) in 200 mL distilled or demineralised water; then add 10g iodine (I_2, crystalline), shake until it is dissolved and add 20g glacial acetic acid.
- Neutral Lugol's solution: dissolve 20g potassium iodide (KI) in 200 mL distilled or demineralised water; then add 10g iodine (I_2, crystalline) and shake until it is dissolved.

All the solutions are near saturation; hence any possible precipitate should be removed by decanting the solution before use. For fixation of marine phytoplankton, acid Lugol's solution is usually used. The neutral solution is recommended for epifluorescence microscopy, as it prevents the formation of precipitates. Advantages and disadvantages of each preservative, and derived specific recommendations, can be found in Annex D of EN15972:2011.

Calcofluor white solution (0.1–100 $\mu g\ mL^{-1}$) is used for epifluorescence microscopy.

3.3. Sampling and fixation

3.3.1. The sampling bottle is filled with seawater to approx. 80% of its volume to facilitate sample homogenisation. Each fixed sample is recommended to be accompanied by a non fixed sample for live material inspection (see the European Standard EN 15204:2006, *Water quality - Guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermöhl technique)*).

3.3.2. The fixative should be added immediately after sample collection, typically 0.2 to 1.0 mL of Lugol's solution to 100 mL of sample, or until the sample turns to a cognac colour. When phytoplankton abundance is high, the volume of Lugol's solution may need to be increased up to 1 mL per 100 ml of sample, until the required colour is attained.

3.4. Transport and storage

3.4.1. The fixed samples will be transported to the laboratory, and may be stored up to 12 months, in a dark, cool place. If duplicated unfixed samples are also collected, these should be transported in the dark at approx. 10 ºC, or a temperature similar to the ambient seawater temperature, and should be examined within 36 h.

3.5. References

EN 15204:2006, Water quality - Guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermöhl technique).

EN 15972:2011, Water quality - Guidance on quantitative and qualitative investigations of marine phytoplankton.

4. Counting method

4.1. Introduction

Accurate identification of toxin-producing species and determination of their cell abundance are critical steps in phytoplankton monitoring. Although there is no specific method required by EU legislation for quantification of toxin-producing phytoplankton, the procedure based on inverted microscopy after sedimentation of fixed samples (Utermöhl technique, Utermöhl 1958) remains a standard both in current practice across toxic phytoplankton control networks and as uniform procedure for phytoplankton monitoring [EN 15204:2006. *Water quality - Guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermöhl technique)*].

It is an uncomplicated and reliable method, suitable for identification and enumeration of most phytoplankton species causing toxicity in molluscs, and has been used for decades, thus producing extensive datasets that support most of our knowledge on the environmental control of the dynamics of toxin-producing phytoplankton species, as well as on shellfish toxicity attribution. This method remains the reference to which new methods should be compared, and although it is not free from important drawbacks, particularly its dependence on the observer, its inability to discriminate some toxic species, and its high demand of personal effort, it is the core method recommended here. However, national standards as well as EN15972 and national authorities needs (e.g. detection of all species in the national list of toxin-producing species, detection limit below national thresholds, time set between sampling and reporting), should be taken into consideration when selecting enumeration methods for HAB monitoring programs. Moreover, the development of molecular methods bodes well for the incorporation of these techniques into standard monitoring, although these highly specific methods are not currently available for all toxin-producing phytoplankton species nor laboratories, and do not always conform to the intensive, continuous and rapid nature of

monitoring. Routine monitoring is progressing towards an interdisciplinary approach of complementary techniques targeting specific groups of species, such as the very small ones, the morphologically cryptic and those that are modified by the preserver.

The Utermöhl method is based on the concentration of fixed phytoplankton cells of a representative subsample through sedimentation, and subsequent optical identification and counting of organisms using an inverted optical microscope. For quantification, the method assumes that cells in the sedimentation chamber follow a Poisson distribution, i.e. the sedimentation of each cell is independent over time, depending on its size and morphology.

The detailed procedure is thoroughly described in the European Standard EN 15204:2006, and is not repeated here. Below are specific recommendations for identification and enumeration of toxic phytoplankton cells and some suggestions to overcome intrinsic drawbacks of this method. State of the art equipment and well-trained personnel are necessary, and regular instruction in taxonomic identification of toxin-producing phytoplankton is recommended.

4.2. Equipment

4.2.1. Calibrated sedimentation chambers (Utermöhl chambers) of several volumes, according to the concentration of plankton in the seawater. A detailed description of the chambers is in EN 15204:2006.

4.2.2. Inverted light microscope equipped with a digital camera that may be connected to a computer. Epifluorescence microscope equipped with a UV light source (330 – 380 nm) to excite Calcofluor White. Emission is viewed at 420 nm. Details of the essential optical and mechanical characteristics of inverted and epifluorescence microscopes can be found in EN 15204:2006 and in Andersen and Kristensen (1995).

4.2.3. List of toxin-producing species. The target species are those producing regulated biotoxins listed in the IOC-UNESCO taxonomic reference list (http://www.marinespecies.org/HAB/index.php).

4.2.4. Recommended taxonomic guides are:

Al-Yamani, F.Y., Saburova M.A. 2010. Illustrated guide on the flagellates of Kuwait's intertidal soft sediments. Kuwait Institute for Scientific Research, Kuwait, 197 pp.

Balech, E. 1995. The Genus *Alexandrium* Halim (Dinoflagellata). Sherkin Island, Co. Cork, Ireland: Sherkin Island Marine Station, 151pp.

Berard-Therriault, L., Poulin, M., Bosse, L. 1999. “Guide d’Identification du Phtyoplancton Marin de l’Estuaire et du Golfe du Saint -Laurent”. CNRC_NRC. Publication special canadienne des sciences halieutiques et aquatiques 128, Canada, 387 pp.

Cronberg, G., Annadotter, H. 2006. Manual on aquatic cyanobacteria. A photo guide and synopsis of their toxicology. IOC UNESCO-ISSHA, 106pp.

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4.3. Sample preparation and analytical procedure

4.3.1. When using inverted light microscopy, sample processing, taxonomic identification procedure and counting strategy for both live and fixed material should follow the recommendations in EN15204:2006. Results should be expressed in cells per litre.

4.3.2. Many thecate dinoflagellates that are difficult to be characterized under the inverted microscope after Lugols´s staining, can be actually identified using Calcofluor White and epifluorescence microscopy. Procedures for identification

on filters and in Utermöhl chambers can be found in Fritz and Triemer (1985), Andersen and Kristensen (1995) and Andersen and Throndsen (2003).

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5. Interpretation of data

5.1. Introduction

Representative data of toxin-producing phytoplankton abundance in or near a production area can inform on the potential for biotoxins accumulation in bivalve molluscs. Interpretation of those data is hence an intrinsic component in biotoxins control in live bivalve molluscs, particularly in determining the sampling frequency. Defining thresholds for an impending bloom formation, or for the point at which the concentration of cells has a toxification impact on humans through mollusc ingestion, entails knowing what causes a toxic phytoplankton episode to occur and what affects the timing, spatial extent and intensity of such an episode, a challenge to forecasters.

Requirements

Commission Implementing Regulation (EU) 2019/627, Title V, Chapter II, Article 61 (Sampling plans):

> …
>
> 3. Sampling plans to check for the presence of toxin-producing plankton in the water in classified production and relaying areas and for marine biotoxins in live bivalve molluscs shall take particular account of possible variations in the presence of plankton containing marine biotoxins. Sampling shall comprise:
>
> (a) periodic sampling to detect changes in the composition of plankton containing toxins and their geographical distribution. Results suggesting an accumulation of toxins in live bivalve mollusc flesh shall be followed by intensive sampling;
>
> 4. The sampling frequency for toxin analysis in live bivalve molluscs shall be weekly during harvesting periods, except when:
>
> (a) the sampling frequency may be reduced in specific classified relaying or production areas, or for specific types of live bivalve mollusc, if a risk assessment of toxins or phytoplankton occurrence suggests a very low risk of toxic episodes;

(b) the sampling frequency shall be increased where such an assessment suggests that weekly sampling would not be sufficient.

5. The risk assessment referred to in paragraph 4 shall be reviewed periodically in order to assess the risk of toxins occurring in the live bivalve molluscs from these areas.

…

7. With regard to the monitoring of plankton, the samples shall be representative of the water column in the classified production or relaying area and provide information on the presence of toxic species and on population trends. If any changes in toxic populations that may lead to toxin accumulation are detected, the sampling frequency for live bivalve molluscs shall be increased or precautionary closures of the areas established until results of toxin analysis are obtained.

Commission Implementing Regulation (EU) 2019/627, Title V, Chapter III, Article 62 (Decisions following monitoring):

1. Where the results of the monitoring provided for in Article 59 indicate that the health standards for live bivalve molluscs are not met or that there may otherwise be a risk to human health, the competent authorities shall close the classified production or relaying area concerned, preventing the harvesting of live bivalve molluscs. However, they may reclassify a production or relaying area as being of Class B or C if it meets the relevant criteria set out in Articles 54 and 55 and presents no other risk to human health.

Commission Implementing Regulation (EU) 2019/627, Title V, Chapter III, Article 63 Re-opening of production areas):

1. The competent authorities may re-open a closed production or relaying area only if the health standards for live bivalve molluscs comply once again with the relevant requirements of Chapter V of Section VII of Annex III to Regulation (EC) No 853/2004 and present no other risk to human health.

2. Where the competent authorities have closed a production or relaying area because of the presence of plankton or levels of toxins in live

bivalve molluscs that exceed the regulatory limit for marine biotoxins laid down in point 2 of Chapter V of Section VII of Annex III to Regulation (EC) No 853/2004, they may re-open it only if at least two consecutive analytical results separated by at least 48 hours are below the regulatory limit.

3. When deciding whether to re-open a production or relaying area, the competent authorities may take account of information on phytoplankton trends.

Rationale

The growth and dominance of a harmful population of a given species are ruled by complex biogeochemical processes, hydrographic and meteorological conditions favourable to the accumulation of organisms. The definition of specific thresholds or ranges of abundance should rely heavily on expert interpretation of existing data on HAB occurrence, correlated environmental parameters measured *in situ* or determined by remote sensing or modelling (e.g., wind, water temperature, salinity, currents, radiation…) and on historical data on biotoxins in shellfish and associated harvesting closure.

Analysis of time-series of a minimum of 3 years would provide information on i) how to identify (species specific) blooms and whether or not they might be harmful, ii) how to define a HAB occurrence and iii) how meaningful quantitative methods are for establishing spatial extent. Addressing these questions are a first step towards developing a predictive understanding of a bloom dynamics, important to define trigger thresholds for an impending bloom formation (warning threshold) or for harvesting closure, in the absence of biotoxins (closure threshold). In general, the presence of cells in water above warning levels is desynchronized or precedes biotoxins detection in bivalves, hence the definition of thresholds, in particular those that anticipate the potential toxicity of bivalves, is a recognisable harvesting management tool.

5.2. Interpretation and thresholds

5.2.1. Defining threshold values or ranges.

Thresholds, individual values or ranges of cell concentrations in water should act as guidelines for harvest management. This management may include increasing the frequency and intensity of sampling for phytoplankton and biotoxins in bivalves, implementing tracing measures for cells in the water, or proposing precautionary closures. The potential for bivalves toxification is different between different groups of toxin-producing phytoplankton, and differs between bivalve species, consequently a harmonised assessment of the risk requires that algal abundance thresholds are species- or genus-specific. Habitat-related differences in toxicity of any phytoplankton species (e.g. connected to changes in seawater temperature) imply that different regional thresholds for the same species may be similarly rigorous. Thresholds should hence be flexible and primarily defined by species ecology and toxicity potential, regardless the toxic syndrome. Reference concentrations should be reviewed at least every five years based on sound empirical evidence. Since species have a set of environmental conditions within which they can best survive and grow, and since the definition of thresholds comes from the combined interpretation of parameters, a range of cell concentrations could make sense as threshold in aquatic systems were the variability of harmful species and conditions are high. Such a threshold range could also be preferable to one based on a unique cell concentration when monitoring for: i) species within the same genus with distinct specific toxification impact levels (e.g. *Dinophysis acuta* has a toxic effect on shellfish in lower concentrations than *D. acuminata*), ii) species whose individual toxicity is undetermined but belong to the same genus that is regarded as toxic (e.g *Alexandrium* species), iii) species from distinct genera with distinct living strategies but responsible for the same toxification syndrome (e.g *Alexandrium* species, *Gymnodium catenatum* and *Pyrodinium bahamense* are all associated with PSP, and DSP syndrome is either caused by planktonic *Dinophysis* species and by some

benthic *Prorocentrum* species, such as *Prorocentrum lima*).
Table 5.1 presents current threshold levels for different toxin-producing phytoplankton species in different European regions.

5.2.2. Interpretation.

When the concentration of a harmful species is above the **warning threshold**, phytoplankton and bivalve sampling should be intensified in the harvesting area, and samples should be re-collected after 2-4 days. The routine sampling in the following week would remain unaltered.
When the abundance of a toxin-producing species exceeds the **closure threshold**, before a precautionary closure for harvesting in the production area is activated in absence of biotoxin data in molluscs, it is recommended to analyse:

i. whether the species was already in the water in the previous two weeks,

ii. if biotoxins have already been detected in the previous two weeks in concentrations below ban level,

iii. if the forecasts for wind and hydrodynamic conditions in the following three days, including seawater circulation and renewal rate, are favourable to the species growth, to population dispersal or accumulation and to toxin build-up in filtering bivalves,

iv. the likelihood of consequential presence of the species for the specific time of the year; this should be based on a time-series of at least 3 years of cells abundance in the area, in relation to prevailing environmental conditions, together with historical data on biotoxins in shellfish and associated harvesting closure.

All these parameters taken together could determine a protective closure of the harvesting area, or management measures including the increase of sampling frequency and intensity, or the implementation of tracking and tracing measures, before any toxin measurement is taken in shellfish. In case of closure for the excessive presence of cells in the water and

favourable conditions for its further proliferation and toxification impact, water and bivalve sampling should be intensified in the harvesting production area, and samples re-collected after 2-3 days. The routine sampling in the following week remains unaltered. If in the following week or two the level of cells in the water remains high but no biotoxins are still detected in bivalves, the ban should be reconsidered due to the social and economic implications for the bivalve harvesting sector. This decision requires knowledge of the species ecology and toxicity in the specific area, and an expert analysis of historical data regarding shellfish toxicity due to the specific microalgal species, together with a reliable forecast of environmental conditions, cell abundance and shellfish toxicity.

Box 2. Simplified flowchart representing the knowledge-based process leading to the establishment or release of precautionary measures in a harvesting area, based on the analysis of phytoplankton abundance and associated risks.

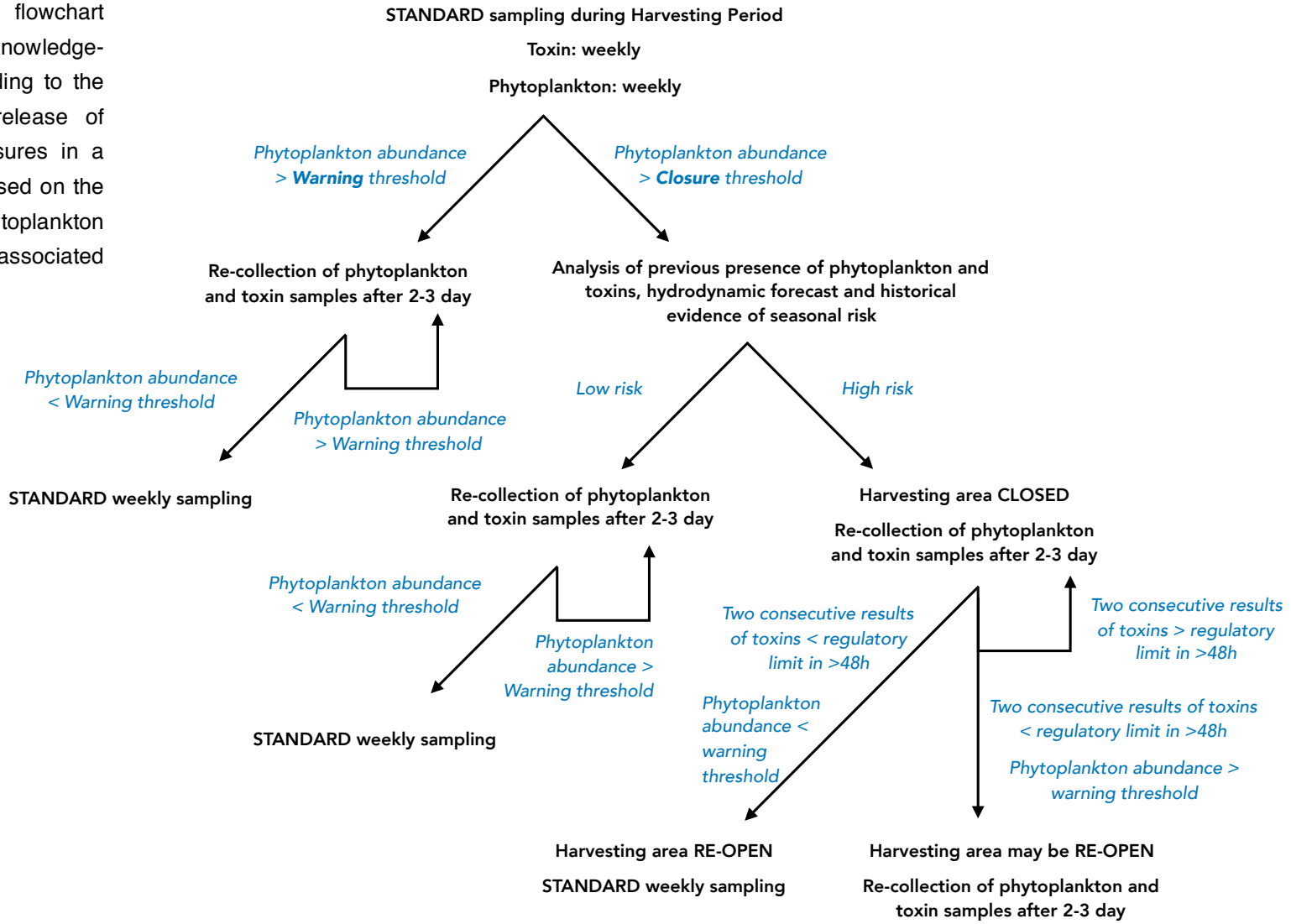

Table 5.1. Concentration of toxin-producing species of phytoplankton used as warning and closure threshold in several EU countries. The species reported are indicative and the list must be periodically updated (in accord to IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae: http://www.marinespecies.org/hab/). Note that not all the listed species have already been found in European waters, however they should be included, in view of the increasingly frequent spread of non-indigenous species

	toxin-producing plankton (Reg. EC 853/2004)	**Warning threshold (cells/L)**						
		POR	**GRE**	**FRA**	**UK**	**CRO**	**NL**	**DK**
PSP	*Gymnodinium catenatum* *Pyrodinium bahamense*	500	500	None	Not monitored		500	Not monitored
	Alexandrium spp. (*A.minutum, A.ostenfeldii, A.andersoni, A.australiense, A.catenella, A.minutum, A.pacificum, A.tamiyavanichii, A.taylorii*)	500	500	10000 (all species together) 1000-5000 for lagoons	40		500	500
	Marine cyanobacteria (e.g *Anabaena, Aphanizomenon, Plankthotrix, Lyngbya, Cilindrospermopsis*)	10000000		None			10000000	Not monitored
ASP	*Pseudo- nitzschia* spp. Seriata group (> 3 µm wide)	80000	50000	100000	Scotland: 50000 England, Wales, N Ireland: 150000		80000	200000
	Pseudo- nitzschia spp. Delicatissima group (< 3 µm wide)	200000		300000			200000	200000
	Nitzschia bizertensis *Nitzschia navis-varingica*							Not monitored

(contd.)

(Table 1 contd.)

	toxin-producing plankton (Reg. EC 853/2004)	Warning threshold (cells/L)						
		POR	**GRE**	**FRA**	**UK**	**CRO**	**NL**	**DK**
DSP	*Dinophysis* spp. (*D.acuta, D.acuminata, D.fortii, D.ovum, D.caudata*, *D.infundibulum*, *D.miles, D.norvegica, D.sacculus, D.tripos*) *Phalacroma* spp. (*P.mitra, P.rotundatum*)	200	200	100	100	9900 cells m^{-2}	200	1000
	Prorocentrum spp. except *P. cordatum* (*P.lima, P.belizeanum*, *P.concavum, P. caipirignum, P.foraminosum, P.faustiae, P.hoffmannianum, P. mexicanum/rhathymum)*	500	200	10000	100		500	500
AZP	*Azadinium* spp. *(A.poporum, A.spinosum, A.dexteroporum, A.luciferelloides)* *Amphidoma languida*	50000	1000	None			50000	Not monitored
YTXs	*Gonyaulax spinifera* *Gonyaulax taylorii* *Lingulodinium polyedra* *Protoceratium reticulatum*	1000	200	10000	None set. Monitored		1000	Not monitored

(contd.)

(Table 1 contd.)

	toxin-producing plankton (Reg. EC 853/2004)	Closure threshold (cells/L)		
		POR	**GRE**	**NOR**
PSP	*Gymnodinium catenatum* *Pyrodinium bahamense*	1000-2000	>1000	Not monitored
	Alexandrium spp. (*A.minutum, A.ostenfeldii, A.andersoni, A.australiense, A.catenella, A.minutum, A.pacificum, A.tamiyavanichii, A.taylorii*)	1000-2000	>1000	200
	Marine cyanobacteria (e.g *Anabaena, Aphanizomenon, Plankthotrix, Lyngbya, Cilindrospermopsis*)	>20000000		Not monitored
ASP	*Pseudo- nitzschia* spp. Seriata group (> 3 µm wide)	100000 – 200000	>200000	50000
	Pseudo- nitzschia spp. Delicatissima group (< 3 µm wide)	300000 – 500000		1000000
	Nitzschia bizertensis *Nitzschia navis-varingica*			Not monitored
DSP	*Dinophysis* spp. (*D.acuta, D.acuminata, D.fortii, D.ovum, D.caudata*, *D.infundibulum*, *D.miles, D.norvegica, D.sacculus, D.tripos*) *Phalacroma* spp. (*P.mitra, P.rotundatum*)	500 - 1000	>1000	100-200 (D. acuta) 1000 (D. acuminata) 4000 (D. norvegica)
	Prorocentrum spp. except *P. cordatum* (*P.lima, P.belizeanum*, *P.concavum, P. caipirignum, P.foraminosum, P.faustiae, P.hoffmannianum, P. mexicanum/rhathymum)*	2000-3000 P. minimum $1x10^6$	>1000	Not monitored
AZP	*Azadinium* spp. *(A.poporum, A.spinosum, A.dexteroporum, A.luciferelloides)* *Amphidoma languida*	>100000	5000	Not established
YTXs	*Gonyaulax spinifera* *Gonyaulax taylorii* *Lingulodinium polyedra* *Protoceratium reticulatum*	100000	>1000	1000

6. Quality Control

6.1 Introduction

Quality control includes all important tests which support and assure the quality of results and the competence of the operator. It is important that these results are supported by a good quality system to allow for traceability and give confidence in the results. It is good working practice that these results contain some indication of their quality, that is, how much they can be relied on (Ellison and Williams 2012). It is a requirement for laboratories taking part in official control programmes to be accredited and thus to have quality assurance measures in place in order to provide data and measurements to a set standard.

These measures include having a validated method in place, well-trained personnel, a series of internal quality controls, traceability of results and participation in proficiency testing schemes (ISO/IEC 17025).

Since accreditation is mandatory for laboratories taking part in official controls for phytoplankton analysis, it is necessary for the test method being used for a particular measurement to be validated and fit for purpose. A validation plan and report for the test method is fundamental to provide a measure of the 'uncertainty of measurement' for the test method (Ellison and Williams 2003) amongst other requirements. "*There should be a regular independent assessment of the technical performance of a laboratory*" and "*Analytical measurements made in one location should be consistent with those made elsewhere*" (Magnusson and Örnemark 2004).

This means that laboratories must:

i) have internal quality controls in place in the form of intercalibration and intercomparative studies within the laboratory and

ii) have external quality controls by participating in proficiency testing (PT) schemes and in intercalibration/intercomparative studies between laboratories.

PT participation provides an independent assessment of the quality of results in routine analysis and also anticipates comparative information about method and instrument performance.

6.2 Internal quality assessments

Quality control samples should be set up on a regular basis in the laboratory to ensure that analysts are competent at carrying out phytoplankton analysis within the laboratory. Ongoing competency is an important aspect of these internal quality controls, where analysts must show consistent performance over time, in terms of both taxonomical identification and also on the determination of the abundance of target organisms in the samples.

Internal quality controls should check the ability of the analysts to identify phytoplankton taxa correctly and also to enumerate the species under repeatability conditions within the precision set for the test:

- The quality control for species composition could be an **ongoing audit trail for species identification** of the most important toxic/harmful species found in the samples. Analysts carry this out to provide an audit trail of their work, and thereby accountability for correct identification.
- Quality control for species abundance should also be carried out regularly as **ongoing cell estimate verifications** between analysts within a laboratory to provide a measure of precision within set limits and thereby accountability for correct cell estimation.

6.2.1 Ongoing taxonomic identification quality control.

It is recommended that all operators regularly attend a phytoplankton taxonomic course (e.g. IOC/Copenhagen course) to maintain and enhance their knowledge.

Analysts should receive ongoing training to improve identification skills. This internal quality control is designed to provide an audit trail of correct identification of phytoplankton organisms to the highest taxonomic level possible. This can be done by each analyst capturing regular images of toxic/harmful species in their samples using microscope cameras. It is recommended that phytoplankton analysts carry out these quality controls as toxic/problematic phytoplankton species appear in samples.

A list of target phytoplankton species specific to the particular geographical area should be kept in the laboratory. This list should be based on knowledge of the area and the species likely to occur there. It is recommended that the species in this list, a subset of the IOC-UNESCO taxonomic reference list mentioned in section 4.2.3 of this guide, are reviewed at regular intervals. This can be done through mutual cross check of species identification by direct examination of samples by analysts in the lab. Recording high quality images (ideally, a minimum of one image of each taxonomic species per month/period/event) is a means to facilitate such cross checking. The photos can then form part of a taxonomic library of images for the laboratory. If possible, the measurements of length and width of the cells photographed should be included as it can aid in the review of the images.

6.2.2 Ongoing cell counting quality control

The scope of the verification of cell estimates should be carried out at regular intervals (e.g., quarterly) by the laboratory and should include species from samples at different cell density ranges. This can be done by comparison of cell counts between analysts on same samples, within a specified time period under repeatability conditions.

This means carrying out the analysis under standardised conditions and, whenever possible, using the same counting technique (e.g., whole chamber, transects, random fields), microscope, chamber, cell counter, objective magnification and analyzing the sample in the same day. If counting a full sedimentation chamber, the direction of the analysis

should also be the same and intermediate cell counts, for example: transect to transect counts should be used to identify possible deviations during the analysis.

Quality control of cells quantification could also be accomplished by Q charts (Shewhart charts) (Bartram and Balance 1996). It is the same rationale as the intercalibration but on an internal laboratory routine. A large sample is produced usually from a culture collection or from selected adequate natural/field samples. All analysts in the lab count 4-5 chambers which they have prepared themselves and use the microscope and all the conditions used for routine sample analyses. In the same sample there should be abundant and less abundant taxa, so that all the possible couning techniques are checked at once. Then the results are analysed expressing a factor like z-score.

Another quality control procedure for cell counts is the double sample (duplicate analysis): each analyst performs a double analysis of a sample (preparing 2 subsamples, counting them under exactly the same conditions) either every 30th sample that they examine or once per month (Bartram and Balance 1996).

In order to use a statistical result from small exercises of this kind, a measure of precision can be used to test whether analysts are within the tolerance limits of the mean count as a measure of closeness between analysts and their counts. To this end, the quantitative analyses in EN 15204 and in Venrick (1978), Edler (1979) and Andersen and Throndsen (2004) are recommended.

Ongoing cell counting verification should be carried out several times a year to assure cell counting is carried out to a high standard. The laboratory technical manager should choose the right interval for these exercises (development of an internal quality control schedule) and identify any source of potential error or bias in the application of the Utermöhl method.

Table 6.1 - Plan for the scheduling of quality control and validation procedures of the Utermöhl method according to EN 15204: 2006.

Type of control	Frequency	Goal	Scope
Duplicates - a sample tested twice by the same operator	Every 30th sample / Monthly	Determine operator error and reproducibility	Internal control
Inter-operators - The same sample is tested by all operators	Monthly/Quarterly	Error associated with the operators	Internal control
Accuracy - from a sample are assembled three replicates of 25ml and three replicates of 50ml that are tested by all the operators simultaneously	Semester/Annual	Determine error associated with the repetition of identification and quantification of phytoplankton in the sample	Method validation
Randomness – compare the quantification of cells in the horizontal transect and vertical transect	Semester/Annual	Validate the homogeneous and random distribution of cells in the sedimentation chamber	Method validation
Homogenization and sub-sampling - three sub-samples from one sample are simultaneously placed to sediment	Semester/Annual	Determine the error associated with sample preparation for the identification and quantification of phytoplankton and reproducibility	Method validation
Cleansing of sedimentation chambers and whites - a sample of distilled water is placed to sediment in a randomly selected, numbered sediment chamber. An operator analyses the unknown sample.	Monthly - there is sequential rotation between the various operators throughout the year	Ensure the non-contamination of the sedimentation chamber, bottle and false positives.	Validation or internal control

6.3 External quality assessments: Proficiency testing schemes

ISO17025 indicates clearly in its technical requirements, under the section '*assuring the quality of the test and calibration of results*' that QC procedures for monitoring the validity of the results should be in place. These measures include internal quality controls and participation in interlaboratory comparisons and proficiency testing schemes. It also mentions the use of certified reference materials and standards, but at present these certified or standardized biological materials are not available to phytoplankton monitoring programmes.

Laboratories participating in official control programmes should participate in intercomparison exercises (e.g., International Phytoplankton Intercomparison- https://www.iphyi.org, Proftest SYKE- https://www.syke.fi). These should:

1) require the identification and enumeration of marine phytoplankton including potentially toxic species and

2) run annually in order to fulfil the accreditation requirement for participating in an intercalibration scheme every year.

The aim of intercomparison is to compare results with other laboratories and analysts using the same or equivalent test methods. Intercomparison exercises study variability in abundance and/or diversity of marine phytoplankton species found in test samples between and within laboratories and analysts. Test materials can be prepared by either spiking water samples with marine phytoplankton species of interest, by using cultures, field samples or using artificial materials for enumeration purposes.

Analysts involved in phytoplankton monitoring should participate in external quality assessments like interlaboratory comparisons. When feasible, it is recommended that at least half of the lab analysts participate each year, and during a period of 3 or maximum 4 years, all operators involved in monitoring complete at least one international intercomparison test. This continued evaluation is not only important to the laboratories themselves but also to their

customers and regulatory bodies and organizations that specify their requirements.

The data produced from intercomparison tests can be used to address continued staff performance and assessment. Also, it can be used to quantify the uncertainty contribution for the test method to be used for validation purposes (e.g., Magnusson et al. 2012).

The intercomparison schemes should be accredited and/or follow the rules of ISO17043:2010 in relation to the general requirements for proficiency testing and evaluate the performance of analysts using the statistical methods as set out in ISO13528:2015.

The International Phytoplankton Intercomparison (IPI), run annually by the Marine Institute Ireland since 2005 and in collaboration with the IOC UNESCO Science and Communication Centre on Harmful Algae in Copenhagen since 2011, is an independent external proficiency testing scheme, that evaluates and determines the performance of participants from laboratories worldwide and monitors their continued competency (www.iphyi.org). IPI follows the rules of ISO17043:2010 in relation to the general requirements for proficiency testing and evaluates the performance of analysts using the statistical methods as set out in ISO13528:2015. IPI uses ProLab Plus a dedicated software for PT schemes to analyse the data. The exercise is divided into two defined sections. The first is the analysis of homogenized and stable materials including species of interest at different concentration ranges and secondly an online phytoplankton taxonomic assessment setup in a remote platform 'Oceanteacher' https://classroom.oceanteacher.org/ run by the IODE office (IOC project office) in Oostend, Belgium.

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