OSPAR COMMISSION

Protecting and conserving the North-East Atlantic and its resources

Revised JAMP Eutrophication Monitoring Guideline: Nutrients[1]

(Agreement 2013-04)

1. Introduction

Nutrient enrichment of seawater may give rise to eutrophication if other essential conditions are favourable. Knowledge of nutrient concentrations may be used to assess the trophic status of marine waters and to determine the cause of eutrophication. These guidelines provide direction for sampling and analysis of nutrients as part of the monitoring requirements of the OSPAR Eutrophication Monitoring Programme. In addition they will support monitoring and assessment requirements for the Water Framework Directive (WFD) and Descriptor 5 of the European Marine Strategy Framework Directive (MSFD).

2. Purposes

The measurement of nutrients in seawater is carried out for, *inter alia*, the following purposes:

a) to monitor the spatial distribution of nutrient concentrations within the maritime area where nutrient levels are potentially influenced by anthropogenic inputs;

b) to monitor temporal trends in nutrient concentrations over periods of several years (in areas identified under purpose a) in order to assess whether there are increasing or decreasing trends in concentrations as a result of changes in inputs;

c) to support an assessment of the degree of nutrient enrichment within the maritime area, within the context of the work on the development and implementation of a Common Procedure for the Identification of the Eutrophication Status of the Maritime Area, and to contribute to EU Member States' monitoring under the Marine Strategy Framework Directive; and

d) to further the work on understanding the relationship between nutrient concentrations and/or fluxes and the eutrophication effect parameters specified in the monitoring requirements of the Eutrophication Monitoring Programme.

3. Quantitative objectives

The quantitative objectives must take into account the characteristics (e.g. spatial and temporal variability of various scales) of the marine areas concerned.

It is intended that the region-specific temporal trend monitoring programme should have the power (e.g. 90%) to detect a change in concentration (e.g. 50%) over a selected period (e.g. 6 years – the reporting cycle of the MSFD). If necessary, to clarify the situation and to help define objectives, contracting parties should undertake statistical analyses of their existing data sets. This helps to determine the representativeness of the monitoring stations and also helps to determine the selection of suitable sampling stations and sampling frequencies as per the OSPAR Agreement on the Eutrophication Monitoring Programme.

The spatial distribution component of the monitoring programme should enable contracting parties to determine the representativeness of their monitoring stations with regard to spatial variability in nutrient concentrations. This would include a definition of the extent of the monitoring area.

4. Sampling strategy

[1] Replaces Agreement 1997-02

Monitoring should include the analyses of the following nutrient species[2]:

- ammonia, nitrate, nitrite,
- phosphate, and
- silicate.

The most important inorganic nutrients with respect to eutrophication problems are phosphate and the sum of nitrite plus nitrate (TOxN). Silicate and ammonia are important mainly in relation to particular events and situations. Ammonia is often present in high concentrations in low oxygen waters, e.g. anoxic stagnant bottom waters. Total and particulate phosphorus and nitrogen may also be measured and are useful for the identification of temporal trends, ecosystem analysis, and nutrient budgets.

The dissolved organic fractions should also be recognized as a significant source of matter for the recycling of inorganic nutrient species within the system. The dissolved organic fractions of nitrogen (DON) and phosphorus (DOP) can be calculated from the inorganic and total nutrient concentrations. Dissolved organic carbon concentration is also necessary for the interpretation of organic nutrient concentrations.

The OSPAR Agreement on the Eutrophication Monitoring Programme classifies waters as non-problem areas, potential problem areas, or problem areas. The latter two classes require the collection of additional information that relates to the effects of eutrophication. In all areas, it is also important to collect ancillary information to characterize the samples. In addition to basic sampling station information, temperature and salinity are essential supporting parameters. Additional parameters such as chlorophyll pigments, Secchi disk depth, turbidity, suspended particulate matter, current speed, or information about tides, may be needed depending on the site and purpose of the investigation.

4.1 Monitoring

Generally, monitoring for the spatial distribution of nutrients should take place at the time of lowest algal activity, usually winter. This is because surface waters become depleted in inorganic nutrients during spring, summer, and autumn due to the uptake of nutrients by phytoplankton. However, it is also possible that the input of nutrients into coastal waters, in particular, could have a strong temporal variation. Therefore, for the maritime area as a whole, the sampling period and the sampling frequency cannot be specified in terms of months or dates; the period is dependent on regional and inter-annual differences in input and uptake of nutrients.

Monitoring for nutrients should take account of inputs, including terrestrial and atmospheric inputs, as well as the oceanographic characteristics of each region. For example, monitoring for nutrients should take place along salinity gradients in order to account for freshwater run-off from land to sea.

A nutrient–salinity relationship for a coastal area can provide information about processes affecting nutrient concentrations and eutrophication effects. A linear relationship indicates that physical mixing is the dominant process regulating the nutrient concentration, while non-linearity indicates the additional influence of chemical and/or biological processes. Several sources of freshwater or offshore water may add complexity to nutrient–salinity mixing diagrams, and temporal variability in the nutrient concentrations of the sources may contribute additional scatter and variability to the relationship.

The sampling strategy must also consider possible water column stratification due to the presence of a pronounced halocline and/or thermocline. It should also consider the potential impact of the depth of the euphotic zone on the uptake of nutrients by phytoplankton.

The temporal trend monitoring strategy should ensure that sufficient data are collected in order to confirm that maximum winter nutrient concentrations are included and that a nutrient–salinity curve can be constructed from which “salinity” normalized (e.g. to 30 psu) nutrient concentrations can be calculated.

In most cases, it will be possible to decide that the data are suitable for temporal trend studies only after sampling with suitable temporal and spatial resolution, and with the assistance of supporting measurements of algal activity (e.g. chlorophyll *a*).

4.2 Monitoring for research purposes

For purpose (d), i.e. research, the sampling strategy for nutrients will be influenced by the specific goals of

[2] The nutrient species specified in the minimum monitoring requirements of the Eutrophication Monitoring Programme are: ammonia, nitrite, nitrate, and phosphate. Silicate is a required parameter only in problem and potential problem areas.

the research but should be in accordance with the sampling strategy for the eutrophication effect parameters required for potential problem and problem areas.

5. Sampling equipment

5.1 Equipment

For general requirements for sampling, preservation, handling, transport and storage of water samples see EN ISO 5667-3.

A variety of sampling bottles can be used for the collection of nutrient samples. These are commonly deployed on either a CTD[3]-rosette or are clamped to a hydrographic wire and lowered to the prescribed depth. Reliability of CTD measurements should be regularly checked and quality assurance (QA) procedures documented.

Working in (shallow) estuaries and coastal areas sometimes requires special equipment and sampling, e.g. samples collected by pumping water through a flexible plastic hose deployed over the side of the ship. It is essential to validate that the equipment used is adequate for the desired purpose.

It is important to use suitable bottles to collect and store samples, i.e. glass bottles may leach silicate and phosphate into samples. Polyethylene or polypropylene bottles may be used. The sampling bottles and storage containers should always be rinsed with sample water before filling.

5.2 Contamination

Sampling activities always include the risk of contamination, which may have various sources depending on specific sampling situations. Care should be taken to ensure good laboratory practice during sampling (e.g. avoidance of contamination from ship, cleaning of instrumentation and bottles, etc.). It is recommended that laboratories evaluate contamination risks and document how they minimize and control these risks during sampling. Among the common nutrients, ammonia is usually the most challenging to determine due to airborne contamination, both onboard ship and onshore. Contact with cigarette smoke has to be avoided, both in the air and on workers' fingers, and exposure of samples to the atmosphere should be minimized.

6. Storage and pre-treatment of samples

6.1 Storage

Nutrient determinations should be carried out as soon as possible after sampling. Ammonia should be determined immediately after sampling, while nitrate, phosphate, and silicate should be determined within a few hours after sampling, with samples protected from light and stored in a refrigerator.

"If analysis is not possible within a few hours then samples must be preserved. Commonly used preservation methods are freezing (for silicate preferable at temperatures between –18 °C and –20 °C) or adding a preservative, e.g. $HgCl_2$. If the sample contains amounts of particulate matter, which may compromise the analysis, it should be filtered to remove the particles before freezing (see Section 6.2). Samples for the determination of silicate that have been frozen should be defrosted for sufficient time for de-polymerisation to occur. This is particularly important for water with high silicate concentrations.

Since no preservation method for nutrients can presently be recommended for general use, each laboratory must validate and document its storage methods for each nutrient, taking account of the likely differences in properties of estuarine, coastal, and offshore waters. The validation should be done over the whole seasonal cycle to investigate the potential impact of varying conditions, e.g. during periods of high and low nutrient concentrations and during high and low primary productivity. The QUASH (Quality Assurance of Sampling and Sample Handling) project (1996–2000) carried out an inter-comparison of sampling handling and preservation methods for nutrients in seawater for a number of laboratories. The outcome demonstrated the need for laboratories to validate and document their procedures and it highlighted the particular challenges of preserving samples for subsequent ammonia analysis (QUASH, 2000).

6.2 Pre-treatment

[3] CTD is conductivity (to calculate salinity), temperature, and depth.

Unnecessary manipulation of the samples should be avoided. However, filtration at constant pressure or centrifugation may become necessary in particle-rich waters (e.g. in coastal zones, estuaries, or during phytoplankton blooms). Filtration using glass fibre filters (e.g. Whatman GF/F) or hydrophilic cellulose acetate filters (e.g. Sartorius Minisart, 0.45 µm pore size) should generally be adequate. Each laboratory should validate the filtration methodology on test samples, including the pressure at which filtration is carried out and potential contamination from filters, before using them routinely. If unfiltered samples are analysed, the need for a correction for turbidity should be assessed.

7. Analytical procedures

The determination of nutrients is mostly based on colorimetric methods (e.g. Grasshoff *et al*., 1999). There are also fluorometric methods available, e.g. for the analysis of ammonia in seawater (Holmes *et al.*, 1999; Aminot *et al.*, 2001), and UV spectrophotometric methods for the direct determination of nitrate (Johnson and Coletti, 2002).

Most methods commonly used are manual methods adapted to automated analytical equipment (continuous flow analysis or flow injection analysis) (Kirkwood, 1996). In addition to the validation of the chemical method itself, the validation of the handling procedures and maintenance of the automated equipment is important.

Publications and manuals are available that provide detailed guidance for working at sea with continuous flow analysis of nutrients (Aminot and Kerouel, 2007; Hydes *et al*., 2010).

8. Analytical quality assurance

Laboratories carrying out analyses of nutrients have to establish a quality management system according to EN ISO/IEC 17025. An accreditation by a recognized accreditation authority is recommended. The quality assurance programme should ensure that the data are fit for the purpose for which they have been collected, i.e. that detection limits are adequate and accuracy is compatible with the objectives of the monitoring programme. The quality assurance procedures must cover all steps of the nutrient determinations, including sampling, storage of samples, analytical procedures, maintenance and handling of the equipment, training of the personnel, as well as an audit trail. The laboratory should also take part in interlaboratory comparisons and proficiency testing, e.g. QUASIMEME, to provide external verification of laboratory performance.

Specific technical information on quality assurance may be found in Kirkwood (1996), Vijverberg and Cofino (1987), and in the Nordtest report (Nordtest, 2006).

Currently certified reference materials (CRMs) for nutrients in seawater are commercially available from:

- KANSO Technos in Japan (http://www.kanso.co.jp/eng/production/index.html), currently for nitrate plus nitrite, nitrite, phosphate, and silicate.
- National Research Council of Canada

  (http://www.nrc-cnrc.gc.ca/eng/solutions/advisory/crm/certificates/moos_2.html)[4] for nitrate plus nitrite, nitrite, phosphate, and silicate.
- Eurofins, Denmark (http://www.eurofins.dk/dk/milj0/reference-materialer/certified-reference-materials.aspx)[5]for ammonia, total nitrogen, total phosphorous, nitrate plus nitrite, nitrite, phosphate, and silicate.

Use of these materials should enable comparability of data to be achieved. For samples with concentrations greater than 100 times the detection limit of the method, deviations from the certified values should be within a few percent for silicate and approaching 1% for nitrate and phosphate.

Performance requirements for individual laboratories will depend on the concentrations encountered in the specific monitoring programme. Laboratories must determine limits of detection and also limits of quantification for their analyses and confirm that these are appropriate for the specific monitoring programme. For example, for determining temporal trends of winter nutrients in European Atlantic shelf waters (waters of salinity ~35 psu), laboratories should aim for limits of quantification of approximately 0.2 µM for TOxN, 0.03 µM for nitrite, 0.06 µM for phosphate, 0.2 µM for silicate, and 0.3 µM for ammonia.

[4] Mailing address: NRC Institute for National Measurement Standards (NRC–INMS), 1200 Montreal Road, M-36, Ottawa, Ontario K1A 0R6, Canada.

[5] Mailing address: Eurofins Miljø A/S, Ladelundvej 85, DK-6600 Vejen, Denmark.

9. The use of *in situ* nutrient analysers

9.1 Platform types

Autonomous nutrient analysers are increasingly being used for providing *in situ* semi-continuous measurements of nutrient concentrations. Where a static platform is used (such as on a mooring), high frequency measurements of nutrient concentrations at a single point may be obtained. Such a mooring may also allow deployment of analysers at multiple depths or the use of new technologies such as the SeaHorse®[6], which can generate time-series of nutrient concentrations as a function of depth at the mooring site. When nutrient sensors are used with a ship's pumped seawater supply (such as a Ferrybox system), a map of nutrient concentrations over a wide area may be obtained. A Ferrybox system allows samples from a fixed depth to be obtained while deployment of nutrient sensors on autonomous underwater vehicles (AUVs) can generate data series for variable or fixed depths over a prescribed track. In summary, there is a wide range of options for collecting nutrient data over time, space, and depth that can be selected to provide the most useful data for specific monitoring programmes.

[6] The SeaHorse is a product of Brooke Ocean, http://www.brooke-ocean.com/seahorse.html.

9.2 Instrument selection

Sensors for *in-situ* applications are based on wet chemistry colorimetric methods and, for nitrate, a direct optical UV spectrophotometric measurement. Reviews by Moore *et al*. (2009) and Johnson *et al*. (2007) discuss different types of sensors. Potential problems with *in situ* sensors are biofouling and power constraints. Biofouling may be more readily overcome on a Ferrybox system where cleaning of the measurement system may be programmed into the duty cycle. Controls implemented on some *in situ* optical sensors include wiped sensors, guarding with copper mesh, and chlorination. Power constraints on a Ferrybox system will not usually be a problem but may be a consideration for a moored sensor. The extent of biofouling and power considerations will contribute to determining the length of time sensors can be left *in situ*. Coloured dissolved organic matter (CDOM) has a spectral component to its absorption curve that may interfere with nitrate measurement when using optical sensors, although this is unlikely to be an issue for most marine applications.

9.3 Quality assurance

Appropriate calibration and ongoing quality control must be implemented to ensure that the data collected suit the purpose. Routine laboratory testing and validation of results against discrete samples analysed in the laboratory must be undertaken to ensure that comparable results of known and acceptable quality are obtained.

10 Reporting requirements

Nutrients must be reported in µmol l^{-1}. Data collected as part of the Eutrophication Monitoring Programme should be reported to the ICES database in accordance with the requirements of the latest ICES reporting formats, together with QA information on methods used, detection limits, reference values, and any other comments or information relevant to an assessment of the data.

It is recommended that uncertainty of measurement (UCM) and determination method of the measurement uncertainty is reported. Guidance on calculating UCM is available from OSPAR (2011), Nordtest (2006) and ISO 11352.

11 References

Aminot, A., Kérouel, R., and Birot, D. 2001. A flow injection-fluorometric method for the determination of ammonium in fresh and saline waters with a view to *in situ* analyses. Water Research, 35(7): 1777–1785.

Aminot, A., and Kérouel, R. 2007. Dosage automatique des nutriments dans les eaux marines. Editions Quae, Versailles, France, 188 pp. ISBN 978-2-7592-0023-8.

EN ISO 5667-3*: Water quality - Sampling - Part 3: Preservation and handling of water samples

EN ISO/IEC 17025*: General requirements for the competence of testing and calibration laboratories

Grasshoff, K., Kremling, K., and Ehrhardt, M. (Eds.) 1999. Methods of Seawater Analysis. 3rd ed. Wiley–VCH.

Holmes, R. M., Aminot, A., Kérouel, R., Hooker, B. A., and Peterson, B. J. 1999. A simple and precise method for measuring ammonium in marine and freshwater ecosystems. Canadian Journal of Fisheries and Aquatic Sciences, 56(10): 1801–1808.

Hydes, D. J., Aoyama, M., Aminot, A., Bakker, K., Becker, S., Coverly, S., Daniel, A., *et al.* 2010. Determination of Dissolved Nutrients (N, P, Si) in Seawater with High Precision and Inter-Comparability Using Gas-Segmented Continuous Flow Analysers. The GO-SHIP Repeat Hydrography Manual: A Collection of Expert Reports and Guidelines. IOCCP report N.14, ICPO Publication Series N. No. 134, Version 1, 2010. (www.go-ship.org/HydroMan.html.)

ISO 11352*: Water quality - Estimation of measurement uncertainty based on validation and quality control data

Johnson, K. S., and Coletti, L. J. 2002. *In situ* ultraviolet spectrophotometry for high resolution and long-term monitoring of nitrate, bromide and bisulfide in the ocean, Deep Sea Research I, 49: 1291–1305.

Johnson, K. S., Needoba, J., Riser, S. C., and Showers, W. J. 2007. Chemical Sensor Networks for the Aquatic Environment. Chemical Reviews, 107: 623–640.

Kirkwood, D. S. 1996. Nutrients: Practical notes on their determination in sea water. ICES Techniques in Marine Environmental Sciences, No. 17.

Moore, T. S., Mullaugh, K. M. Holyoke, R. R. Madison, A. S., Yucel, M., and Luther, G. W. 2009. III. Marine Chemical Technology and Sensors for Marine Waters: Potentials and Limits. Annual Review of

Marine Science, 1: 91–115.

Nordtest. 2006. Internal Quality Control, Handbook for Chemical Laboratories. Nordtest report TR 569, www.nordtest.info.

OSPAR. 2011. JAMP guidelines for estimation of a measure for uncertainty in OSPAR monitoring. OSPAR Agreement 2011-3.

QUASH. 2000. Quality Assurance of Sampling and Sample Handling. Final Report. EC contract STM4-CT96-2100. Vijverberg, F. A. J. M., and Cofino, W. P. 1987. Control procedures: Good laboratory practice and quality assurance.

ICES Techniques in Marine Environmental Sciences, No. 6.

* For undated references, the latest edition of the referenced document (including any amendments) applies.