Guidelines for sampling and determination of dissolved oxygen in seawater

1 Background

1.1 Introduction

The dissolved oxygen (DO) content in seawater is controlled by several unrelated processes including exchange with air, metabolism of plants and animals, microbial and chemical decomposition of organic matter, hydrodynamic features such as mixing, advection, convection, and up- or down-welling. The DO content is always the result of multifactorial influences and the reasons for changes may be difficult to assess. In stratified Baltic waters, DO depletion occurs regularly below the halocline.

When only physical processes are involved, the DO concentration in water is governed by the laws of solubility, i.e., it is a function of atmospheric pressure, water temperature, and salinity. The corresponding equilibrium concentration is generally called solubility. It is an essential reference for the interpretation of DO data. Precise solubility data, tables, and mathematical functions have been established (Carpenter, 1966; Murray and Riley, 1969; Weiss, 1970) and adopted by the international community (UNESCO, 1973). However, Weiss (1981) drew attention to an error in the international tables in which the values are low by 0.10 % since they are based on ideal gas molar volume instead of actual dioxygen molar volume. Later, the Joint Panel on Oceanographic Tables and Standards (JPOTS) recommended that the oxygen solubility equation of Benson and Krause (1984), which incorporated improved solubility measurements, be adopted and the tables updated (UNESCO, 1986). However, the UNESCO paper only referred to the equation that gives concentrations in the unit µmol kg^{-1}.

1.2 Purpose and aims

Monitoring of dissolved oxygen and hydrogen sulphide can provide information of an indirect effect of eutrophication, but hydrogen sulphide and oxygen depletion is also a naturally occurring condition in some areas. The purpose of the monitoring is to map the spatial distribution of concentrations of dissolved oxygen and hydrogen sulphide, with the aim to be able to assess the status of the seafloor and the waters above and to ensure that the data is comparable for the HELCOM pre-core indicator 'Shallow water oxygen' and core indicator 'Oxygen debt'. The indicator descriptions, including their monitoring requirements, are given in the HELCOM core indicator web site: http://www.helcom.fi/baltic-sea-trends/indicators/oxygen-debt

2 Monitoring methods

2.1 Monitoring features

The monitoring of DO is preferably done in combination with hydrogen sulphide measurements to assess the level of oxygen depletion (see *HELCOM Guidelines for sampling and determination of hydrogen sulphide*). If Hydrogen sulphide cannot be sampled and measured, its presence should be noted in sampling protocol.

2.2   Time and area

The DO/hydrogen sulphide should be monitored regularly throughout the year, in particularly in all sub-basins for which the Oxygen debt indicator applies.

2.3   Monitoring procedure

2.3.1   Monitoring strategy

If possible, a DO profile should be recorded for each CTD cast.

Water samples are collected at depths of 1, 2.5 (Polish marine areas), 5, 10, 15, 20, 25 (Kattegat and the Belt Sea only), 30, 40, 50, 60, 70, 80, 90, 100, 125, 150, 175, 200, 225, 250, 300 and 400 meters; and as close to the bottom as possible.

2.3.1.1   Winkler method

The standard procedure for the determination of DO in water is the Winkler method in several modifications (e.g., Carpenter, 1965; Hansen, 1999; ICES, 1997). It is based on the reaction of DO with manganese(II) ions in alkaline solution and subsequent oxidation of iodide to iodine after acidification. Iodine is back titrated with standardized thiosulphate in acid solution. The endpoint can either be detected visually (see EN 25813:1993 and ISO 5813:1983) or in automated methods, by spectrometric or electrochemical means.

2.3.1.2   Galvanic sensors

Electrochemical probes for DO exploit the reduction of oxygen to produce a current that is expressed in DO equivalents.

Sensors of galvanic type using a silver cathode are poisoned by hydrogen sulphide, and hence not suited for use in anoxic water. Sensors of this type are therefore not recommended for monitoring in the Baltic area.

2.3.1.3   Polarographic sensors

Polarographic sensors use electrodes of noble metals, typically a gold cathode and a silver anode. The electrodes are mounted behind a membrane permeable to DO, and when diffusing oxygen molecules are reduced, the electrons delivered are recorded.

For polarographic based sensors, hydrogen sulphide is not considered being a problem. The sensors can be used both for profiling and moored instruments.

2.3.1.4   Optical sensors

Optical or luminescent sensors for DO are also available. They operate by utilizing the interaction between DO and light emitted from a fluorescent dye. Light emitted from a LED light source causes fluorescence in the dye, and the fluorescence is recorded. Presence of DO quenches the fluorescence.

This type of sensors generally has longer response times compared to electrochemical probes, with sampling frequencies of 1-2 Hz. The main advantages of optical sensors over polarographic sensors are a higher sensitivity to low DO concentrations, and lower sensitivity to membrane fouling. While many of these sensors are unsuitable for profiling due to their long response times, they are very well suited for moored instruments.

2.3.2   Sampling methods and equipment

2.3.2.1   Winkler method

The basic method for the determination of oxygen concentration is the Winkler method (Grasshoff et al., 1983). Oxygen sensors may be used, though it is highly recommended to take water samples in areas with low oxygen concentration (below 2 ml l^{-1}).

It should first of all be noted that the subsampling of oxygen samples is the most critical step of the total analysis. It is of utmost importance that this step is carried out by trained and experienced staff. Samplers suitable for other water sampling can be used for oxygen. A special bottom water sampler could be useful for studying the oxygen conditions in the near-bottom water layer.

DO samples should be the first to be drawn from the water samplers. For subsampling and titration, only glass bottles with conical-shaped tops and with glass ground stoppers meet the requirements of the Winkler method. Subsample bottles must be calibrated and identified with their stoppers since they must not be interchanged. Subsamples are drawn with a flexible plastic tube attached to the water samplers reaching to the bottom of the glass bottle. Fill and overflow each bottle with at least three volumes. Make sure not to draw any air bubbles into the sample. Reagents are added with the dispenser tip submerged at least 1 cm below the neck of the vial. The inserted stopper displaces the excess of water. Carefully avoid contact with reagent and trapping bubbles. The sample is mixed by thoroughly shaking, as this is a very critical step in the fixation of the oxygen. Some laboratories prefer to mix a second time after a few minutes to maximize the contact between the sample and the reagents.

2.3.2.2   Sample handling and analysis

Oxygen samples may be stored for up to 12 hours after adding the reagents and after complete fixation. The bottles should be kept in the dark and any change in temperature should be avoided. The bottles can be stored under a waterlock for up to one month.

When sensors for DO are used (whether on moored or profiling instruments), regular checks and calibrations have to be made. Sensor membranes are susceptible to biological fouling and contamination by oil and grease. It is recommended to observe manufacturer's instructions for calibration and maintenance of sensors. Water samples analysed with the Winkler method are useful as reference samples for *in situ* measurements.

2.4   Data analysis

The 100% saturation of DO can be calculated for the specific water when salinity and temperature are known. Below, equations are given for the calculation of solubility values of DO, in various units, according to the UNESCO recommendation. These equations are directly taken from the paper of Benson and Krause (1984), where two equations are provided for calculation in either µmol kg^{-1} or in µmol l^{-1}. The calculation of saturation requires the in situ temperature known to ±0.1 °C and salinity within 0.2 (PSS 78). To allow conversion between different units, the sample temperature at the addition of the reagents should be reported, if significantly different from the *in situ* sample temperature.

The following symbols are used:

T : temperature in Kelvin (K) = temperature in (t °C) + 273.15
S : salinity on the Practical Salinity Scale 1978 (PSS78)
Cs : DO solubility concentration (the unit is mentioned using subscripts)

The equations can be expressed as follows:

$$\ln Cs(\text{µmol kg}^{-1}) = A + B/T + C/T^2 + D/T^3 + E/T^4 - S \times (F + G/T + H/T^2),$$

and

$$\ln Cs(\text{µmol l}^{-1}) = I + J/T + K/T^2 + L/T^3 + M/T^4 - S \times (N + P/T + Q/T^2).$$

The constants A to Q are the following:

micromole per kilogram
micromole per litre
A = −135.29996
B = +1.572288 × 10^5
C = −6.637149 × 10^7
D = +1.243678 × 10^{10}
E = −8.621061 × 10^{11}
F = +0.020573
G = −12.142
H = +2363.1
I = −135.90205
J = +1.575701 × 10^5
K = −6.642308 × 10^7
L = +1.243800 × 10^{10}
M = −8.621949 × 10^{11}
N = +0.017674
P = −10.754
Q = +2140.7

Application domain: t = 0–40 °C; S = 0–40.

Cs is obtained as:

Cs = exp (ln Cs),

Solubility in milligram per litre is obtained from the value in micromole per litre by multiplying by the molar mass of O_2 and 10^{-3} for unit consistency:

$Cs(mg\ l^{-1}) = Cs(\mu mol\ l^{-1}) \times 31.9988\ g\ mol^{-1} \times 10^{-3}$

Solubility in millilitre per litre is obtained from the value in micromole per litre by multiplying by the molar volume of the gas at standard temperature and pressure (STP; 0 °C, 1 atmosphere). For that conversion, some data previously published refer to the molar volume (STP) of dioxygen (O_2; 0.0223916 ml μmol^{-1}), like those of Weiss (1970), while others refer to that of an ideal gas (0.022414 ml μmol^{-1}), like those of the UNESCO tables and Benson and Krause (1984).

Referring to exact O_2 molar volume:

$Cs(ml\ l^{-1}) = Cs(\mu mol\ l^{-1}) \times 0.0223916\ ml\ \mu mol^{-1}$

The saturation is then expressed as the % O_2 measured compared to the O_2 calculated.

The calculations can be checked against the oceanographic calculator at the ICES webpage, where the same equations are used. They are available on the ICES website at http://ocean.ices.dk/Tools/Calculator.aspx.

CTD data, including DO profiles, should be quality controlled according to ICES Guidelines for CTD Data.

3   Data reporting and storage

DO concentrations should be reported in ml/l O_2 at STP and/or in % of saturation (Weiss, 1970).

Data is reported annually to the HELCOM COMBINE database, hosted by ICES.

The national data centre or designated oceanographic data centre is responsible for storage of master data collected within the national monitoring programs to contribute to the HELCOM COMBINE monitoring programme. The data centres obligations are to store all raw data, metadata and processed CTD data, including DO profiles, in a long term and secure way and to report data and/or changes in data to ICES. The data centre should also make sure that sufficient, confident, and traceable documentation of the samples and measurements is available for further data handling.

The CTD data format, preferably a simple ASCII-format should include all metadata, all processed data and quality flags for each scan and parameter. However, the data centre should follow the direction given by ICES when reporting data yearly; hence data could be submitted in any format as long as it is well described and structured consistently. In order to ensure that all data has been converted correctly a statistical summary should be added to the submission, including range of each parameter. All other relevant information and documentation of methods and special circumstances should be submitted and if available a cruise summary report for each cruise.

The data centre should also make preliminary data available as soon as possible after the cruise, preferably, at a searchable website. Since the data centres have the master data storage and all changes and new data are available here first, access to data could be made via a web service. Then, any data user can access data as soon as the national data centre publishes the data by harvesting of new or changed data from the web service.

4 Quality control

4.1 Quality control of methods

4.1.1 Winkler method

Minimum analytical requirements are;

limit of detection (LOD) 0.02 ml l^{-1},

accuracy 0.03 ml l^{-1},

limit of quantification (LOQ) 0.1 ml l^{-1}.

With the Winkler method, a repeatability of 0.1 % can be achieved in the upper concentration range.

There is no certified reference material for DO readily available. The reference method is the properly performed Winkler method (Hansen, 1999). The quality assurance relies to a very high degree on good practice applied by experienced staff. Water stored with air contact for several weeks at a stable temperature can be used as a laboratory reference material for control charts.

Essential procedures include:

□ calibration and identification of sample bottles and their respective stoppers
□ calibration of volumetric flasks and dispensers
□ control charts for reagent and titration blanks
□ control charts of precision by replicate samples
□ in case automated titration is used, check the accuracy of the addition of the titrand

Replicate samples can be taken from the same sampler, but ideally from different samplers triggered at the same depth in deep water.

Blanks can be checked by adding double or triple amounts of reagents to identical samples.

4.1.2 *In situ* measurements

For sensors used in moored and profiling instruments, a schedule for maintenance and calibration must be established. Follow manufacturer's recommendation for cleaning and maintenance.

It is recommended to use a CTD equipped with dual independent DO sensors, for an immediate evaluation of sensor performance.

Laboratory calibration of the DO sensors needs to be performed with regular intervals. However, it should be noted that not all manufacturers can provide a traceable calibration.

4.1.3 General remarks on quality control of methods

Laboratories should have established a quality management system according to EN ISO/IEC 17025.

Measurement uncertainty should be estimated using ISO 11352. Estimation should be based on within-laboratory reproducibility, and, when available, data from proficiency testings and intercalibrations.

Contracting parties should follow the HELCOM monitoring guideline but minor deviations from this are acceptable if the method achieves comparable results. Validation of the adopted method needs to be performed on the relevant matrix and concentration range e.g. by taking part in intercomparison studies or proficiency testing schemes.

4.2 Quality control of data and reporting

If hydrogen sulphide is present, discard the oxygen results from corresponding depth.

Data must be flagged if normal QA routines or recommended storage conditions cannot be followed.

5 Contacts and references

5.1 Contact persons

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5.2 References

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

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