Manual for Marine Monitoring in the

COMBINE

Programme of HELCOM

Part C

Programme for monitoring of eutrophication and its effects

Annex C-5
Phytoplankton primary production

ANNEX C-5 PHYTOPLANKTON PRIMARY PRODUCTION

Introduction

Primary production is the only regular rate measurement in the Baltic Monitoring Programme. From these measurements it is possible to calculate the amount of organic material formed from light, carbon-dioxide and nutrients. Primary production has important links to eutrophication and sedimentation and, consequently, to deep water oxygen concentrations.

Purpose

The measurement of primary production in water is carried out for, inter alia, the following purposes:

- to measure the ecophysiological response on different nutrient availability;
- to describe temporal trends in primary production.

Method

Primary production should be measured with the "P/E - method", in an incubator. With this method the uptake rate of carbon is measured at a range of irradiance levels in order to get a relationship between photosynthesis and light. Pmax (maximum photosynthetic rate) and (initial slope of the P-E relationship) and E_k (the light saturation irradiance) can be calculated using this method.

The advantage of the method is that ecophysiological information of the phytoplankton assemblage can be derived from the P/E - relationship. It is also possible to calculate the daily production per m^3 from these measurements after calculation and incorporation of the vertical attenuation coefficient and solar irradiance, and with some assumptions the annual primary production.

Sampling and analytical procedure

WORKING MANUAL AND SUPPORTING PAPERS ON THE USE OF A STANDARDIZED INCUBATOR-TECHNIQUE IN PRIMARY PRODUCTION MEASUREMENTS

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Annex 2 Light measurements and intercalibration of standard ICES incubators
Annex 3 Irradiance Differentiation and Control in the ICES incubatorPreface

The ^{14}C method for the measurement of primary production in the sea has been used for more than 45 years. The database is considerable and it seems, as the method will continue to be an important tool in the monitoring of the status of the marine pelagic ecosystem. A major problem in the comparison of productivity data is, however, the use of different measuring methods. The differences stem from both conceptual and practical reasons. Within ICES long discussions have been held to create a database on primary production. However, the fear that the data were not comparable resulted in a workshop, where the methods applied by different laboratories were intercompared. Very significant differences in results were found between laboratories (Richardson, 1991). During its meeting in 1988 the ICES Working Group on Primary Production found that there was a need for a standardized method for primary production measurements to be used in monitoring studies of which the data were to be stored in the ICES data bank. It was decided to make a strict protocol for primary production measurements performed in an incubator. The intention was to make the incubator inexpensive and the method with as few steps as possible. Over the years that have passed since this decision, there have been long detailed discussions, but also fruitful tests of the incubator developed by Colijn et al (Annex 1, Annex 2). This manual with supporting papers is meant to serve as the protocol for future monitoring of primary production in the ICES area, and hopefully far beyond.

Although the initially intended simplicity has been left due to the wish to be able to measure full P-E relations, we still have given emphasis to obtain a concise and strict protocol, which does not leave much room for alternatives. Sometimes we have given alternatives where these do not affect standardization. However, in order to produce comparable data for a data bank we were obliged to keep the alternatives to a minimum and enable a rigorous quality assurance.

In summary, the purpose of this manual is to provide a strict protocol of the monitoring of Primary Production. Following this manual will ensure comparable data in the ICES database.

INTRODUCTION

The P-E curve method should be used (for terminology we refer to Sakshaug et al., 1997). With this method the ^{14}C uptake is measured at a range of irradiance levels in the incubator, in order to get an estimate of the photosynthesis rate versus irradiance. This can then be parameterized and give values of P_{max} (maximum photosynthesis), a (maximum light utilization coefficient measured as the slope of the linear increase of photosynthesis against irradiance), E_k (the saturating irradiance) and, after calculation and incorporation of vertical attenuation and solar irradiance, the daily primary production per m^2. Together with data on chlorophyll-a, P_{max} can be normalized to obtain assimilation numbers.

The method for estimating primary production by the "ICES Incubator" given in this manual (cf. Annex 1) is intended for monitoring purposes. Measurements should be possible from small, as well as from large vessels. Because of this, simplifications from what could be considered to be the ideal method have been introduced. It should be pointed out that the "ICES Incubator" method is not meant as a replacement of other "P-E techniques". It has been designed to provide a reliable measurement of primary production, using a simple incubator and a standard protocol.

The incubator is a rectangular perspex tank (33 x 33 x 9 cm) with a turning wheel on which a maximum of 12 experimental bottles can be clamped. 10 fluorescent tubes (TLD 8W J8, no 33) illuminate it. The full description of the incubator is given in Annex 1. The standard protocol is presented here. The incubator is manufactured by HYDROBIOS, Kiel, Germany (see List of Manufacturers).

Standardization of the method involves strong reduction of the number of alternatives. However, a few are indicated (see text in*italics*) but the standard method is to be used to obtain quality assured data for the ICES data bank.

SAMPLING STRATEGY

MIXED WATER COLUMNS

In areas where the euphotic zone is mixed and the phytoplankton community is uniformly distributed, **one** representative sample, obtained at 5 m depth is sufficient. It is important, however, to make sure that the water layer is mixed. This is easiest done with a CTD and fluorescens profile.

As an alternative an integrated sample can be taken with a hose (0-10 m) (Lindahl, 1986 and Annex 3). Mixed discrete samples from 0 to 10 m depth can also be used.

STRATIFIED WATER COLUMNS

In stratified waters, where the phytoplankton community is not homogeneously distributed, a water sample should be obtained with a hose (see Appendix), covering the water column of interest. This single sample is treated as a mixed water sample.

If preferred, samples from different depths can be taken and incubated separately at temperatures similar to temperatures from the sampling depths. In that case more incubators may be needed, or subsequent incubations are to be made.

The hose sampling method can also be used as an alternative to sampling with water bottles, as the complete sample can easily be divided by depth for individual incubations by using clamps (Lindahl, 1986).

In conclusion, measurements of primary production in stratified water bodies are more complicated and will normally fall beyond "simple" monitoring strategies.

MEASURING PROTOCOL (SEE FIG. 1)

Experts to add figures

General Preparation

1. PLACEMENT OF THE INCUBATOR.

The incubator must be placed so that light conditions outside the incubator do not disturb the light climate inside the incubator. The incubator needs to be thermostatically controlled, to give the same temperature as the water sample. For samples from stratified waters differing in temperature two separate incubators should be used, or two consecutive incubations should be performed. The second water sample(s) should be kept dark and at the original temperature during the first incubation.

2. INCUBATION FLASKS.

Tissue culture flasks (see also 3.) of about 50 ml should be used. These flasks also work as paddles for the water-jet driven rotation of the flask-wheel. After each incubation, the flasks and the caps should be rinsed with diluted HCl (10%) and then several times with distilled water to avoid contamination. The flasks should be dried at 70 °C.

3. IRRADIANCE LEVELS IN THE INCUBATOR (FOR DETAILS SEE ANNEX 3)

A set of incubation flasks with different transmission levels, from 0 to 100% should be used (for manufacturer of special prepared bottles see Appendix). It is important that there should be enough measuring points to obtain a good measurement of P_{max} and a. With the special prepared 12 bottles this is

not a problem and after some experimentation with the incubator normally a series of 6 bottles will suffice to measure a reliable P-E relationship. The exact irradiance in each bottle should be measured, despite the transmission the manufacturer gives percentages. This can be done with a small sensor, which can be introduced into the bottles (a manufacturer of this calibrated sensor can be found in the Appendix). To obtain irradiance saturated photosynthetic rates (P_{max}) a minimal irradiance of 500 $\mu E \cdot m^{-2} \cdot s^{-1}$ should be available. This is achieved by using 10 fluorescent lamps (TLD 8W J8, no 33). In case 500 $\mu E \cdot m^{-2} \cdot s^{-1}$ is not reached, a mirror behind the lamps and possibly on the other side of the tank will increase the irradiance flux.

4. ^{14}C SOLUTION.

Dilution of commercially available ^{14}C solution should be avoided due to the risk of contamination. The standard activity of every batch of ^{14}C solution should be controlled by the liquid scintillation technique (see point 11). It is recommended to use ampoules which contain the whole amount of ^{14}C needed for one incubation series. This reduces the number of measurements of the added ^{14}C activity.

In case ^{14}C solutions are prepared 'home-made' high grade chemicals and UHQ water must be used.

The final carbonate concentration of the solution should agree with the average carbonate concentration of the sea area being studied and the pH of the solution should be in the range of 9.5 - 10.0.

5. ACCOMPANYING FIELD MEASUREMENTS.

In order to obtain a representative sample of phytoplankton it is important to have knowledge of the vertical distribution of the algae. This is easiest accomplished by a CTD-cast combined with an *in situ* chlorophyll-fluorescence cast. Measurements of the under-water irradiance in at least 5 different depths, in order to calculate the vertical irradiance attenuation coefficient are also necessary. If the daily production is going to be calculated, the daily surface irradiance must also be measured in hourly intervals.

6. SAMPLING.

Non-transparent and non-toxic sampling devices must be used. Sampling should take place in daylight, to avoid strong interference of inequality due to diel rhythms of the phytoplankton (Annex 1, Gargas et al., 1979).

After sampling but before incubation subsamples are taken for chlorophyll (Fig.1, Step 1) and TCO_2 analysis (Fig. 1, Step 2).

The incubation should start as soon as possible, preferably within half an hour after sampling. All transfers of water samples should take place in subdued light, in order to avoid light-shock of the phytoplankton.

7. TOTAL CO_2 CONCENTRATION. (FIG. 1, STEP 2)

Total CO_2 concentration should be calculated according to other standard methods, using titration of carbonate (Strickland and Parsons, 1972). In brackish waters, such as the Baltic Sea, the CO_2 concentration

can be calculated by the formulas of Buch (1945). In both cases temperature, salinity and pH must be measured.

8. ADDITION OF ^{14}C (FIG. 1, STEP 3).

The ^{14}C solution is added to the whole volume of sample needed to fill all the flasks. After thorough mixing, the flasks are filled. This procedure minimizes errors compared to pipetting the radioactive tracer to every individual incubation bottle.

Incubation bottles are filled with a measuring cylinder. The flasks should be filled up to the neck, leaving an air bubble in the flask. One dark flask from each original sample should be incubated.

The ^{14}C solution should be added to the sample in such concentrations that statistically sufficient counts of the radioactivity in the phytoplankton can be obtained. A triplicate measurement of the added activity is needed (Fig.1, Step 4). These samples should be counted immediately to avoid loss of activity. Therefore in case direct counting is impossible the inorganic ^{14}C should be mixed with ethanol-amine by pipetting 0.25 ml of sample with added activity together with 0.25 ml of ethanolamine. Scintillation cocktail can be added later and radioactivity determined.

As an alternative incubation flasks are first filled and then the ^{14}C solution is added to every flask. It is important that the added volume is small and that a precise, calibrated micropipette is used.

9. INCUBATION. (FIG.1, STEP 5)

The incubation time should be about 2 hours and the rotation speed should be approximately 10 rpm. Start and end of the incubation should be given in the protocol so that the precise incubation period (in minutes) can be used for the calculation. To achieve an unhampered rotation of the samples all positions on the wheel need to be filled (e.g. by using flasks filled with water).

10. END OF INCUBATION. (FIG. 1, STEP 6)

After incubation the flask contents are filtered immediately. In case of high algal biomass or high sedimentation load it might be needed to filter a subsample. A defined portion should be taken and filtered.

Glass-fibre filters (GF/F, Ø 25 mm) should be used, since these filters are cheap, become opaque and are known not to disturb the counting procedure of the radiotracer. To avoid any contamination of the filter edges, prewetted filters should be used. The suction pressure should not exceed 30 kPa during filtration. The filters should be rinsed once with a small volume (5 ml) of filtered seawater from the original sample (use filtrate of the chlorophyll-a measurement!).

After filtration the filters should be placed in scintillation vials and dried at room temperature for 24 hours. Following addition of scintillation liquid, the samples should be kept in dark for at least 3 hours to reduce chemiluminescence.

As an alternative to filtration many scientists use the bubbling method to obtain the total (dissolved and particulate) primary production.

From each incubated sample a sub-sample of 10 ml (exactly) is pipetted into a scintillation vial and 0.2 ml of 80 % HCl is immediately added. In a ventilated cupboard, the vials are then bubbled with a fine jet of air bubbles for 20 minutes, or are left open for 24 hours. 10 ml of scintillation cocktail is added and the vials are shaken by hand for some seconds before scintillation counting.

11. COUNTING OF THE RADIOACTIVITY. (FIG.1, STEP 8)

The liquid scintillation technique should be used when counting the uptake of ^{14}C. In order to get a statistically accurate measurement, 40 000 DPM, or counting for 10 minutes is needed to get a result with 1-% accuracy. Quench curves for different amounts of chlorophyll should be established and adding an internal standard, e.g. ^{14}C -hexadecane or toluol, should check the measuring efficiency of the liquid scintillation counter. Normal counting efficiency calculation is done by using the channel ratio method. Modern scintillation counters are equipped with programs to facilitate efficiency calculations. The user is referred to the instructions of the manufacturer.

12. CALCULATION OF CARBON UPTAKE (FIG. 1, STEP 9).

The total carbon uptake is calculated from the equation:

$$dP/dt\ (\mu g C \cdot L^{-1} \cdot hr^{-1}) = \frac{dpm\ (a) \cdot total\ ^{12}CO_2\ (c) \cdot 12\ (d) \cdot 1.05\ (e) \cdot k1 \cdot k2}{dpm\ (b)}$$

Where

(a) = Sample activity (minus back-ground), dpm
(b) = Total activity added to the sample (minus back-ground), dpm
(c) = Total concentration of $^{12}CO_2$ in the sample water, µmol/L (or µM)
(d) = The atomic weight of carbon
(e) = A correction for the effect of ^{14}C discrimination
k1 = subsampling factor (e.g. sample 50 ml, subsample 10 ml: k1=subsample factor 50/10= 5)
k2 = time factor (e.g. incubation time 125 minutes: k2= 60/125= 0.48)

The results will be given as µg $C \cdot L^{-1} \cdot h^{-1}$ per irradiance level and as well as the photosynthesis at light saturation (P_{max}), the maximum light utilization coefficient (a), and light saturation parameter E_k , from the P-E curve (see below).

13. CALCULATION OF DAILY PRIMARY PRODUCTION

In order to calculate the daily primary production a number of parameters are needed. These include:

1. Vertical attenuation (extinction) coefficient, in m^{-1}, measured with a calibrated irradiance meter.

In case no attenuation can be measured, Secchi disc values can be used by conversion. The attenuation coefficient is calculated as

Att. Coef. = x / Secchi depth (m)

where x is 1.7 - 2.3 (1.7 (Raymont, 1967), 2.3 (Aertebjerg and Bresta, 1984), 1.84 (Edler, 1997)). This factor changes with sea area. In principle, it increases with decreasing salinity.

2. Insolation (Hourly measurements of incoming radiation between 400 and 700 nm (PAR), in $E \cdot m^{-2} \cdot s^{-1}$

3. P_{max}, E_k, and a.

The transformation of the hourly production corrected for dark uptake into daily production, which is the ultimate ecological goal, should follow the protocol outlined in Appendix 2.

A simple computer program for the calculation of the daily production is available (see list of manufacturer). After giving the raw data to the protocol, the software will calculate the daily production and combine the data in a database for the ICES data bank.

14. QUALITY ASSURANCE

GENERAL

In order to produce specified and confident data on primary production the performance of the measurement and the analytical procedures must follow a high quality system and operate in a state of statistical control. The method used shall be validated to meet the required specifications related to the use of the results. The validation includes selectivity, sensitivity, range, limit of detection and accuracy.

A high quality is maintained by using experienced and well trained personnel. Ring tests and intercomparisons ought to be conducted regularly.

SELECTIVITY

The ^{14}C tracer method is used to measure the incorporation of the added isotope in the form of $NaH^{14}CO_3$ as an estimate of the photoautotrophic growth, measured as photosynthesis of phytoplankton. The method is highly selective but nonphotosynthetic incorporation of ^{14}C and non-biological fixation takes place simultaneously. This is measured as the dark uptake. It can not, however be used as respiration value as in the oxygen method. In the scintillation counting procedure interference may occur from the background values. They are, however, always subtracted from the uptake values.

SENSITIVITY

Apart from the high selectivity the method is also very sensitive. Uptake rates of 0.05 µg $C \cdot L^{-1} \cdot hr^{-1}$ can easily be measured. There is no actual upper and lower limit of the method. The sensitivity can be improved by

adding more ^{14}C to the samples and/or by counting the incorporated radioactivity of the phytoplankton over a longer time which will improve the counting statistics.

Detection limit

The detection limit is set by the background radiation, the use of a zero time blank and dark incubations. The lower limit of detection is a sample should be defined as having activities at least three times the background values.

RANGE

As mentioned above a range virtually does not exist. Uptake rates between 0.05 and 250 µg $C \cdot L^{-1} \cdot hr^{-1}$ can easily be measured. At very high uptake rates an increase in pH may occur. This would affect the distribution and availability of the bicarbonate ion. Under such conditions the incubation period should be reduced. Under natural marine and brackish water conditions this does not happen.

ACCURACY

Random as well as systematic errors occur in this method. Random errors should be kept to a minimum by adopting the standard procedure with only few experimental steps in the whole process from sampling to scintillation analysis. Systematic errors may occur with the light source, irradiance levels, filtration technique and the scintillation counting. These errors should be kept to a minimum. Regular control of handling and function of the instruments used, as well as calibration of the instruments are necessary tools to control the errors. The use of an independent analytical method to measure the systematic error is not possible, since there is no better independent analytical method available as an alternative for the 14C tracer method. Certified reference material (CRM) exists for the calibration of the scintillation counter, or for the calibration of the counting procedure with the original samples by using the internal standard method (adding standard to a sample). Participation in intercomparison exercises is one of the possibilities to test the comparability and therefore the precision and error propagation in this method. In general, however, most variability of this method will be caused by the biological nature of the material. Therefore strict procedures for the measuring protocol are needed to obtain the best possible results.

The quality assurance should ensure that the data are fit for the purpose for which they have been collected, i.e. that they satisfy the detection limits and levels of accuracy compatible with the objectives of the monitoring program.

15. DATA DELIVERY:

In order to have the possibility to check and recalculate daily productivity data it is important that all laboratories deliver their data in the same format and that this includes the fixation rates for all irradiances. Data should be delivered in the ICES, Biological Reporting Format.

ACKNOWLEDGEMENT

To be included

LITERATURE AND FURTHER READING:

Aertebjerg, G. and Bresta, A.M. (ed), 1984. Guidelines for the Measurement of Phytoplankton Primary Production. Baltic Marine Biologists Publication No. 1. 2nd edition.

Buch, K., 1945. Kolsyrejämvikten i Baltiska Havet. Fennia, 68(5):.

Colijn, F., Kraay, G.W., Duin, R.N.M., Tillmann, U. and Veldhuis, M.J.W., 1996. Design and test of a novel Pmax incubator to be used for measuring the primary production in ICES monitoring studies. ICES CM 1996/L3. (Annex 1 is a modified version of the original paper)

de Keyzer, J.. 1994. Irradiance Differentiation and Control in the ICES incubator. Zemoko, Maritiem (unpubl. report)

Edler, L., 1997. In: Report of the ICES/HELCOM Workshop on Quality Assurance of pelagic biological measurements in the Baltic Sea. ICES CM 1997/E:5

Gargas, E. and Hare, I., 1976. User´s Manual for estimating the Daily Phytoplankton Production measured in Incubator. Contribution from the Water Quality Institute. Danish Academy of Sciences. No. 2.

Gargas, E., Hare, I., Martens, P. and Edler, L., 1979. Diel changes in phytoplankton photosynthetic efficiency in brackish waters. Marine Biology 52: 113-122

HELCOM, 1988. Guidelines for the Baltic Monitoring Programme for the third Stage; Part D. Biological Determinands. Baltic Sea Environment Proceedings No 27 D. Baltic Marine Environment Protection Commission. Helsinki Commission.

Li, W.K.W and Maestrini, S.Y. (ed.). 1993. Measurement of Primary Production from the Molecular to the Global Scale. ICES Marine Science Symposia. Volume 197. ISSN 0906-060X.

Lindahl, O. 1986. A dividable hose for phytoplankton sampling. In Report of the ICES Working Group on Exeptional Algal Blooms, Hirtshals, Denmark 17-19 March, 1986. ICES, C.M. 1986/L:26.

O´Reilly, J.E. and Thomas, J.P., 1983. A Manual for the Measurement of Total Daily Primary Productivity. Biomass Handbook No. 10. SCAR, SCOR, IABO, ACMRR.

Raymont, J.E.G., 1967. Plankton and Productivity in the Oceans. Pergamon Press, Oxford.

Richardson, K. (ed), 1987. Primary Production: Guidelines for measurement by 14C incorporation. ICES.Techniques in Marine Environmental Sciences No. 5

Richardson, K. 1991. The ICES 14C Primary Production measurement intercomparison exercise. Mar. Ecol. Prog. Ser. 72: 189-201.

Sakshaug, E., Bricaud,A., Dandonneau, Y., Falkowski, P.G., Kiefer, D.A., Legendre, L., Morel, A., Parslow,J. and M. Takahashi, 1997. Parameters of photosynthesis: definitions, theory and interpretation of results. J. Plankt. Res. 19: 1637-1670.

Strickland J.D.H. and Parsons T.R. 1972. A practical handbook of seawater analysis. Fish. Res. Bd. Canada. Bull. 167. Ottawa. 310 pp.

List of Manufacturers

Incubator:

HYDROBIOS, c/o H. Fischer, Am Jägersberg 5-7, 24161 KIEL, Germany, Tel. +49-431-3696011, Fax: +49-431-3696021, E-mail: hydrobios@t-online.de

Incubation flasks and light sensor:

ZEMOKO, c/o, ing. Jan de Keyzer, Dorpsplein 40, 4371 AC Koudekerke, the Netherlands, Tel/Fax: +31-118-551182
Working Manual ICES Incubator
Irradiance Differentiation and Control in the ICES incubator.

Calculation program:

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APPENDIX: DESCRIPTION OF HOSE SAMPLING METHOD FOR PHYTOPLANKTON MEASUREMENTS

Manual for Marine Monitoring in the COMBINE Programme of HELCOM, Part C, Annex C-6. (Manual version 1.0 - Revised June 1998, HELCOM EC 8/97, updated by EC MON 3/98.

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In order to get a true integrated sample of phytoplankton the mixing of discrete water bottle samples is not adequate. To overcome this the hose method should be used (Lindahl, 1986).

An armoured PVC hose is suitable. The inner diameter of the hose should be c. 20 mm, giving a sampled volume of c. 3 L with a 10 meter hose. The length of the hose should cover the 10 upper meters of the sea to be sampled, as well as the distance from the sea surface to the boat deck, from where it is operated.

In the end of the hose a PVC tube should be placed and secured with a hose clamp. A snap hook should be fastened at the hose clamp. Ten meters up, corresponding to the surface of the sea, a valve should be placed and secured with hose clamps. At the end of the tap a line should be connected. It must be possible to operate the line from the boat deck.

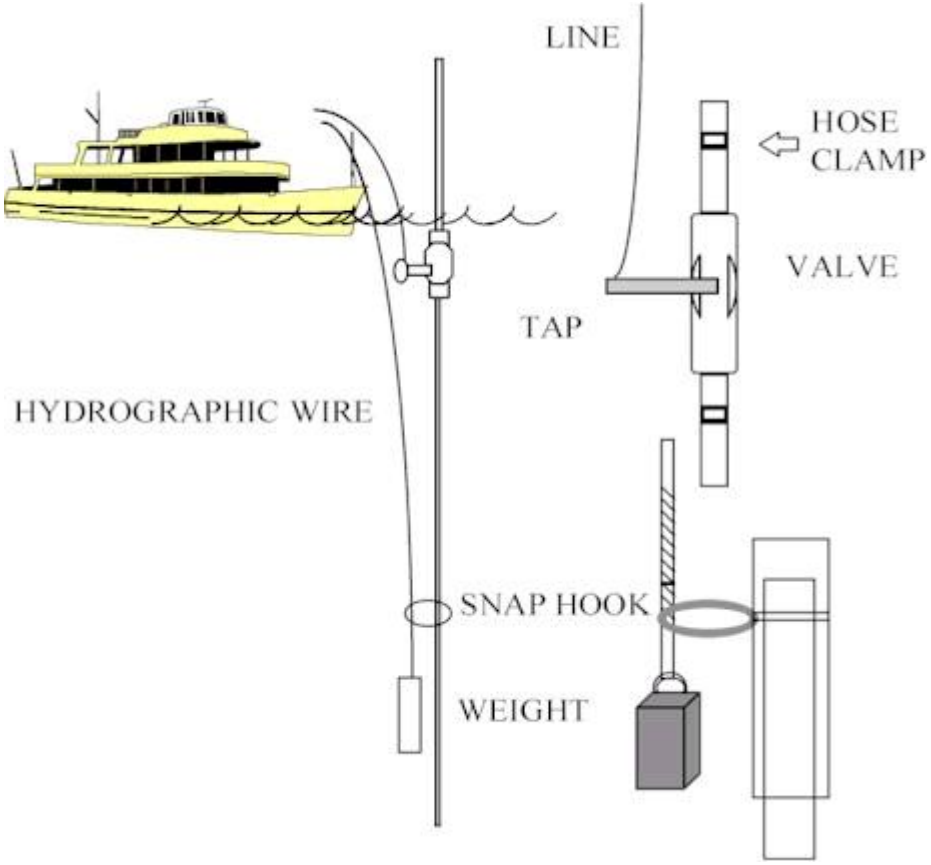
When sampling, the lower end of the hose is connected to the hydrographic wire with the snap hook. The end of the hose should be below the wire weight in order to avoid contamination. The valve must be open when lowering the hose. The hose is lowered slowly until the valve has reached the sea surface. The valve is closed by pulling the string connected to the tap. The hydrographic wire is then elevated. The valve is then

opened and the content of the hose is filled into a bucket and mixed. Subsampling bottles are filled from the bucket.

After sampling the hose is rinsed carefully with fresh water and stoppers are put in both ends in order not to pollute the inside of the hose. A thorough cleaning with diluted HCl or other detergents should be made at the end of a cruise and the hose should be dried.

Reference

Lindahl, O., 1986. A dividable hose for phytoplankton sampling. ICES; C.M. 1986/L:26, Annex 3.



Primary production protocol

Annex 1 Colijn, F., Kraay, G.W., Duin, R.N.M., Tillmann, U. and Veldhuis, M.J.W., 1996. Design and test of a novel Pmax incubator to be used for measuring the primary production in ICES monitoring studies. ICES CM 1996/L3. (Annex 1 is a modified version of the original paper)

Annex 2 L.P.M.J. Wetsteyn[1], L. Edler[2], M.M. Steendijk[1], G.W. Kraay[3], F. Colijn[4] & R.N.M. Duin[5]. Light measurements and intercalibration of standard ICES incubators
(second draft).

Annex 3 de Keyzer, J.. 1994. Irradiance Differentiation and Control in the ICES incubator. Zemoko, Maritiem (unpubl. report)

(revised version 4.December 1997)