GUIDELINES FOR THE BALTIC MONITORING PROGRAMME FOR THE THIRD STAGE

PART D.
BIOLOGICAL DETERMINANTS
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PART D. BIOLOGICAL DETERMINANDS

BALTIC MARINE ENVIRONMENT PROTECTION COMMISSION
— HELSINKI COMMISSION —
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PREFACE

The Guidelines for the Third Stage of the Baltic Monitoring Programme (BMP) are based on the Guidelines for the Second Stage of the BMP, published by the Commission as Baltic Sea Environment Proceedings No. 12 (BSEP No.12). They have been revised by an expert group nominated by the Commission. The group was chaired by Dr. Gunni Aertebjerg and experts from all the Baltic Sea States participated in the work, with assistance from the International Council for Exploration of the Sea (ICES) and experts of the Baltic Marine Biologists (BMB).

The ninth meeting of the Helsinki Commission (15-19 February 1988) accepted the Guidelines in general as HELCOM Recommendation 9/7. The Commission recommends that the Governments of the Contracting Parties to the Helsinki Convention should apply the Guidelines for the Third Stage of the BMP, i.e. from 1989 to 1993, and also, whenever possible, to follow the Guidelines in the monitoring of the internal waters as well. The data is to be submitted to the data bases of the Commission, as specified in the Guidelines.

The Guidelines for the Third Stage of the BMP are published in the BSEP series as four separate volumes (27 A, 27 B, 27 C, 27 D) and also as one combined volume of loose sheets.

The contents of the Guidelines for the Third Stage of the BMP is as follows:

BSEP 27 A; Part A; Introductory Chapters
27 B; Part B; Physical and Chemical Determinands in Sea Water
27 C; Part C; Harmful Substances in Biota and Sediments
27 D; Part D; Biological Determinands

Volumes B, C and D are intended to be used together with Part A which contains general information on e.g. station networks, sampling requirements and data submission.
GUIDELINES FOR THE BALTIC MONITORING PROGRAMME FOR THE THIRD STAGE

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Biological determinands to be monitored

- Phytoplankton primary production
- Phytoplankton chlorophyll-a and phaeopigments *)
- Phytoplankton (species composition, number of counting units, biomass)
- Zooplankton (species composition, abundance and biomass of mesozooplankton, protozooplankton *)
- Soft bottom macrozoobenthos (species composition, abundance, biomass)
- Micro-organisms *) (total number and biomass of bacteria, production of bacteria, number of colony-forming bacteria)

New sampling methods: To improve the significance and reliability of pelagic sampling, the use of additional new sampling methods is recommended, such as automatic sampling and sediment trapping.

Remark:
It is essential that sampling of macrozoobenthos is accompanied by some hydrographic measurements to provide information about the hydrographic situation. Therefore, as a minimum requirement, water should be sampled as close to the sea bottom as possible for determination of salinity, temperature and oxygen/H2S concentration. Preferably a complete hydrographic series should be taken.

Sampling depths

The sampling depths are described under the sections for each determinand.

*) tentative determinands
1. Phytoplankton primary production

Introduction

In order to make it possible to calculate the daily phytoplankton primary production per m\(^2\) water surface by the data consultant the following measurements are obligatory and shall be reported:
- Temperature corrected potential production at the sampling depths, measured in incubator
- Irradiance in the incubator used
- Vertical irradiance attenuation in the water at the sampling station, measured either by Secchi-disc or an irradiance meter.

a) Production measurements

Sampling depths

Sampling depths are selected to give an adequate vertical production curve. The standard sampling depths are 1 m, 2.5 m, 5 m, (7.5 m), 10 m, 15 m and (20 m) (non-obligatory depths in brackets).

In water bodies with a thermocline and/or a halocline, a higher concentration of phytoplankton is often observed in the discontinuity layer than above or below this layer. If a pycnocline is found within the euphotic zone and does not correspond to one of the standard sampling depths, it is recommended to collect an additional sample in the discontinuity layer.

Sampling time

Water samples for production measurements should preferably be sampled between 8 a.m. and 4 p.m. Central European Time.

Samples

Non-transparent and non-toxic sampling devices must be used.

Experimental bottles

25 cm\(^3\) bottles made of high quality laboratory glass and with standard grinding and glass stoppers are recommended.

The experimental bottles must be thoroughly cleaned before every experiment in order to avoid bacterial film or adsorption of toxic substances to the inside of the bottles. The bottles must be cleaned with a 10 % HCl-solution, then rinsed in tapwater and in distilled water. If possible the bottles should be dried at 170°C. Before use the bottles must be washed with water from the respective samples.

All handling of samples before and after the incubation experiment must take place in dimmed light.

The incubator experiment must be carried out as soon as possible after sampling.

From each sampling depth, two clear experimental bottles are filled with water. Additionally, two dark experimental bottles are filled with water from 1 m and 15 m depths, respectively.

For determination of the irradiance production relation (the PI-curve) seven clear experimental bottles are filled with water from 1 m depth or, with water from the integrated phytoplankton sample (see Section D.3.a)). If a pycnocline is found within the euphotic zone, additionally seven clear experimental bottles are filled with water from 15 m depth.
The experimental bottles must not be filled totally, but space for the $^{14}$C-solution and a little air bubble should be left.

$^{14}$C-solution

The $^{14}$C-ampoules for use in production studies can be purchased from different manufacturers. These ampoules must fulfill the following specifications:

- alkalinity 1.5 mM/dm$^3$
- specific activity $4 - 20 \mu$Ci/cm$^3$

Standardization of $^{14}$C-solution

Liquid scintillation counting shall be used as the basis for determination of the absolute activity.

Concentration of $^{14}$C-solution

The $^{14}$C-solution should be added to the experimental bottles in such concentrations that statistically sufficient estimations of the radioactivity fixed by photosynthesis in the sample can be obtained. However, it is also important not to disturb the CO$_2$ equilibrium in the water sample by adding too much NaH$^{14}$CO$_3$ solution.

Concentrations corresponding to a 1 cm$^3$ $^{14}$C-solution with a radioactivity of 1 - 4 $\mu$Ci/cm$^3$ per 25 cm$^3$ sample have been shown to be applicable for primary production studies in the Baltic.

Dark fixation of carbon

As the dark fixation of carbon is not directly related to photosynthetic production, it has to be reported separately.

Incubator

The incubator used must have the following specifications:

- thermostatically controllable
- irradiance conditions ensuring photosynthetic saturation: at least $250 \times 10^{18}$ quanta m$^{-2}$ s$^{-1}$ (400-700 nm) or 100 joules m$^{-2}$ s$^{-1}$ (400-700 nm). (Philips TLD 18 W/33 meets these demands).

Measurements of irradiance in the incubator

A calibrated irradiance meter (quanta meter, 400-700 nm) shall be used.

The irradiance meter is placed in the water-filled incubator facing the light source of the incubator and at the same distance from the light source as the experimental bottles during experiments.

The irradiances are measured in five different positions: At the center of the bottle-wheel of the incubator and at the outermost positions of the experimental bottles on each side and above and below the center of the bottle-wheel.

The measured irradiances are corrected for the immersion effect by multiplying by the immersion factor of the irradiance meter used.

Immersion factor: The ratio between the sensitivity of the irradiance meter in air and in water.

The mean of the four outermost measurements are calculated, and the irradiance in the incubator is expressed as the mean of the center measurement and the calculated mean of the four outermost measurements.
Incubation temperature

The temperature in the incubator during the experiment has to be adjusted to the mean temperature of the euphotic zone, or to the mean temperature found above an eventual pycnocline if a pycnocline is present within the euphotic zone.

Incubation

The experimental bottles are placed at the bottle-wheel of the incubator in such a manner that only clear experimental bottles face the irradiance source.

Five of the experimental bottles in each of the series for determination of the PI-curves are covered with neutral filters of different transmissions, for example 5%, 10%, 15%, 25% and 50%. The exact transmission should be known. Further, the production is determined at 100% incubator irradiance by the 6th bottle of the series and at 175% incubator irradiance by the 7th bottle of the series. The 175% incubator irradiance is obtained by applying a tinfoil coating as reflector behind the experimental bottles.

Incubation period

The incubation period is 120 minutes.

Filtration of samples

The samples should be filtered immediately after the production experiment is stopped, in order to avoid loss of $^{14}$C due to respiration.

Filters with even distribution of pore size, and good solubility with respect to scintillation liquids are preferred. Pore size should not exceed 0.45 μm. The filters should be wetted before the filtration starts.

The suction pressure should not exceed 0.3 $10^5$ Nm$^{-2}$ (0.3 atm.).

The whole filtration procedure should not exceed 0.5 hour for the entire series of bottles. It is possible to comply with this requirement by arranging a series of filtration units. In case it is impossible to filter the whole contents of a single sample, a subsample may be filtered. The subsample shall be of at least 15 cm$^3$.

If filtering a subsample, both the volume of the subsample and the whole volume of the experimental bottle has to be measured. In the case of filtering the whole volume of an experimental bottle, such measurements are not necessary.

The filters should not be washed but, whenever bottles and filtration funnels need to be rinsed, this should occur at the end of the filtration procedure, but before the last cm$^3$ has passed through the filter.

Preparation of filters

In order to stop all biodegradation and thus losses of radioactivity, and to remove possible $^{14}$C precipitates extracellulary, immediately after filtration the wet membrane filters are placed in a desiccator and exposed to vapours of fuming HCl for 3 minutes.

When scintillation counting is used, the filters can now be placed at the bottom of the empty scintillation vials.
When Geiger counting is used, the filters must be dried in a desiccator with freshly dried silicagel and with soda lime, the latter for removal of excess HCl-fumes.

Radioactivity measurements

Various counting techniques are now available, ranging from Geiger counting and proportional counting to liquid scintillation counting and combustion. There are numerous and specific problems with all methods. It is therefore strongly recommended to consult specialized publications for details.

As liquid scintillation counting is one of the most efficient and has the highest versatility, the radioactivity measurements shall be carried out according to the liquid scintillation technique. When this is not possible, the Geiger counting technique shall be used.

In both cases, the efficiency of the counting technique has to be known, including the relation of efficiency to the amount and nature of filtered material (quenching and self-absorption problems).

The counting results should be given as disintegrations per minute (dpm).

Total CO₂ concentration

For estimating the total carbon dioxide concentration, the formulae given by Buch (1) and reproduced by Gargas (2) are recommended:

\[
TA = 1.26 + 0.031 S \quad \text{for } 18 < S < 25 \\
TA = 0.90 + 0.080 S \quad \text{for } 11 < S < 18 \\
TA = 0.90 + 0.083 S \quad \text{for } 8 < S < 11 \\
TA = 0.43 + 0.156 S \quad \text{for } 5 < S < 8 \\
TA = 0.48 + 0.157 S \quad \text{for } 3 < S < 5 \\
TA = 0.16 + 0.198 S \quad \text{for } 1 < S < 3
\]

where: \( TA \) = titration alkalinity in mM/dm³
\( S \) = salinity in °/oo

Titration alkalinity is not equal to carbonate alkalinity due to other weak acids present in the water. Titration alkalinity can be expressed:

\[
TA = \text{Carbonate alk.} + (H₂BO₃) + \text{OH}⁻ - \text{H}⁺.
\]

The concentration of \( H₂BO₃ \) is about 1% of the titration alkalinity in the Baltic Sea. Carbonate alkalinity is then: \( 0.99 \times TA \). To get a value for total \( CO₂ \), temperature, pH and salinity must be determined. Knowing these parameters a factor \( F \) can be found in Table D.1. The total \( CO₂ \) can then be expressed:

\[
CO₂ = \text{Carbonate alkalinity} \times F.
\]

If more accurate \( CO₂ \) values are requested, which is recommended at higher salinities (the Kattegat and Belt Sea) and also in coastal areas, where the relations given by Buch (1) are less suitable, they can be estimated according to Strickland & Parsons (11), Glowińska et al. (4) or Mackereth et al. (9).
## Table D.1

The factor, $F$, for calculating total CO$_2$ from carbonate alkalinity, temperature, pH, and salinity.

<table>
<thead>
<tr>
<th>pH</th>
<th>7.0</th>
<th>7.1</th>
<th>7.2</th>
<th>7.3</th>
<th>7.4</th>
<th>7.5</th>
<th>7.6</th>
<th>7.7</th>
<th>7.8</th>
<th>7.9</th>
<th>8.0</th>
<th>8.1</th>
<th>8.2</th>
<th>8.3</th>
<th>8.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F$</td>
<td>1.084</td>
<td>1.34</td>
<td>1.189</td>
<td>1.150</td>
<td>1.147</td>
<td>1.093</td>
<td>1.074</td>
<td>1.057</td>
<td>1.040</td>
<td>1.023</td>
<td>1.014</td>
<td>1.008</td>
<td>1.002</td>
<td>0.996</td>
<td></td>
</tr>
</tbody>
</table>

### b) Production calculations

**Calculation of carbon uptake**

The total carbon uptake, $P_t$, during the time $t$, is calculated for all of the experimental bottles from the following equation:

$$P_t = \frac{dpm(a).total\;CO_2(c).12(d).1.05(e).1.06(f).k_1.k_2.k_3}{dpm(b)}$$

where:

- $P_t = \text{total carbon uptake, mg C m}^{-3}\;\text{h}^{-1}$

- $a = \text{filter dpm - background dpm = net dpm/}\text{filter}$

- $b = \text{the activity of the added }^{14}\text{C-solution, dpm}$

- $c = \text{concentration of total CO}_2\text{ in the experimental water, mM/dm}^3$

- $d = 12$: the atomic weight of carbon, converts mM/dm$^3$ to mg/dm$^3$

- $e = \text{a correction for the effect of }^{14}\text{C discrimination, the uptake of the }^{14}\text{C-isotope is 5 \% slower than that of the }^{12}\text{C-isotope}$

- $f = \text{a correction for the respiration of organic matter produced during the experiment. This has empirically been found to represent 6 \% at optimal photosynthesis. The production rate is thus corrected to represent the rate of gross production}$

- $k_1 = \text{a correction factor for subsampling, e.g. 15 cm}^3$ were filtered from a sample of 27 cm$^3$ and 1 cm$^3$ NaH$^{14}\text{CO}_3$ was added, then $k_1 = 28/15 = 1.87$

- $k_2 = \text{a time correction factor, e.g. when converting production per 2 hours to production per hour, then } k_2 = 0.5$

- $k_3 = \text{a unit conversion factor, e.g. when converting mg C dm}^{-3}$ to mg C m$^{-3}$, then $k_3 = 10^3$.  

---

**Note:**

- The table above provides factors for calculating total CO$_2$ from carbonate alkalinity, temperature, pH, and salinity. Each factor is labeled with a letter from a to f, which are used in the production calculation equation.

- The production calculation equation accounts for corrections for subsampling, time, and unit conversion factors.

- The factor $k_1$ adjusts for subsampling, $k_2$ for time conversion, and $k_3$ for unit conversion.

- The total carbon uptake, $P_t$, is calculated by multiplying the net dpm by the concentration of total CO$_2$, adjusting for the added $^{14}$C-solution activity, and correcting for time and unit factors.
Subtraction of dark fixation

At stations with homogeneous water within the euphotic zone, the mean carbon uptake in the two dark experimental bottles is subtracted from the total carbon uptake in all the clear experimental bottles. At stations with a pycnocline within the euphotic zone, the dark-uptake at the 1 m depth is subtracted from all the clear experimental bottles from the pycnocline and above, and the dark-uptake at 15 m depth is subtracted from all the clear experimental bottles from below the pycnocline.

Calculation of potential production

The potential production at a sampling depth is expressed in mg C m$^{-3}$ h$^{-1}$, and calculated as the mean of the production rates measured in the 2 clear experimental bottles from that depth and exposed to 100% incubator irradiance, with the dark fixation subtracted.

Temperature correction

The potential production rates obtained must be corrected for eventual differences between the mean temperature in the incubator during the experiment and the in situ temperatures at the sampling depths. The temperature correction factors are determined from the equation:

$$\text{Temp. correction factor} = \exp \left( \frac{\ln 2 \times (t_2 - t_1)}{10} \right)$$

where $t_1$ = the mean temperature in the incubator during the experiment

$t_2$ = the temperature at the sampling depth

Measurements of vertical irradiance attenuation

Introduction

In order to make it possible to calculate the daily phytoplankton primary production per m$^2$ water surface by the data consultant, the following measurements are obligatory and shall be reported:

- temperature-corrected potential production at the sampling depths, measured in incubator,
- irradiance in the incubator used,
- vertical irradiance attenuation in the water at the sampling station, measured either by Secchi disc or a quanta meter.

Secchi disc measurements

In cases when irradiance meters are not available, a Secchi disc must be used to estimate the vertical attenuation of irradiance in the water column.

When a Secchi disc is used, the Secchi depth measured must be corrected for the wave height:

$$D_0 = D_H (1 + 0.4.H) \text{ m},$$

where

$D_0$ = Corrected Secchi depth, m

$D_H$ = Measured Secchi depth, m

$H$ = Wave height, m

The corrected Secchi depth, $D_0$, equals approximately the 10% quanta depth.

The relative irradiance at a given depth, or the depth for a given relative irradiance, is determined by graphical interpolation. On semi-logarithmic paper (irradiance: log scale, two decades; depth: linear scale), the curve becomes a straight line through 100% irradiance at 0 m depth and 10% irradiance at $D_0$ m depth.
Irradiance meter measurements

If possible, instead of Secchi disc measurements, the vertical attenuation of irradiance in the water at the sampling stations should be measured using a quanta meter (400-700 nm).

The underwater detector is lowered from the side of the ship facing the sun and so far from the side of the ship that the disturbance of the normal distribution of light is minimized.

The irradiance above the water surface, $E_d$ (air), is measured with the dry underwater detector. The irradiance immediately below the water surface, $E_d (z=0)$, is then calculated as 93% of $E_d$ (air). (The mean reflection of irradiance by the water surface approximates 7%.)

The underwater detector is lowered into the water and the depths and irradiances are measured at the sampling depths of primary production.

All underwater irradiance readings are corrected for the immersion effect by multiplying the reading by the immersion factor of the underwater detector used. Immersion factor: The ratio between the sensitivity of the detector in air and in water.

In immediate connection with every measurement with the underwater detector, the irradiance in air is measured using a deck detector. From the deck detector measurements, the underwater measurements are corrected for eventual changes in the irradiance above the water surface after the measurement in air with the underwater detector.

The relative irradiances in the different water depths are calculated, setting $E_d (z=0) = 100\%$.

The relative irradiance at a given depth, or the depth for a given relative irradiance, is determined by graphical interpolation: The measured relative irradiances are depicted (log scale) as a function of the water depth (linear scale) on semi-logarithmic paper (2 decades), and a smooth curve is drawn through the points.

d) Data reporting

All data should be reported according to the details given in Sections D.10 and D.11.

e) References in Chapter D.1.

5. Guidelines for the measurement of phytoplankton primary production. BMB Publ. 1, 2nd ed. 1984.
2. Phytoplankton chlorophyll-a and phaeopigments

Measurements of phytoplankton chlorophyll-a are obligatory, while measurements of phaeopigments are only tentative.

a) Sampling

Water for chlorophyll-a analyses should be taken from the same samples as for primary production and phytoplankton and, if possible, for chemical analyses.

The standard sampling depths for chlorophyll-a are the same as for primary production: 1 m, 2.5 m, 5 m, (7.5 m), 10 m, 15 m and (20 m), non-obligatory depths being given in brackets.

Chlorophyll should also be analysed from the integrated phytoplankton sample (see Section D.3.a)). Additional chlorophyll sampling depths are recommended, e.g., one just above and one below the halocline.

Non-transparent and non-toxic sampling devices must be used.

b) Storage of water samples

It is important that the water is filtered as soon as possible. If the filtration cannot be carried out immediately, the sample must be stored cool and dark, preferably in a refrigerator, and no longer than 8 hours.

c) Volume to be filtered

In the Baltic Sea Area the chlorophyll a concentration varies from about 0.1 mg m⁻³ during winter and in the deep water to 15 mg m⁻³ or more in the surface water during the spring bloom. During summer the concentration in the surface water is most often between 1 mg m⁻³ and 5 mg m⁻³.

At a concentration of 0.1 mg m⁻³ and the use of 10 cm³ extraction solvent and a 5 cm cuvette, at least 12 dm³ have to be filtered to get a spectrophotometric absorbance of at least 0.05.

When using 10 cm³ extraction solvent and a 1 cm cuvette at chlorophyll-a concentrations of 1 mg m⁻³ and 10 mg m⁻³ at least 6 dm³ and 0.6 dm³ water, respectively, have to be filtered to get absorbances of at least 0.05.

d) Filtration

The samples shall be filtered in subdued light through Whatman GF/C filters.

The suction pressure should not exceed 0.5 • 10⁵ Nm⁻² (0.5 atm). After filtration the filter shall be folded together.
The filtration time should not exceed 1/2 hour per sample. If this is not possible, a smaller water volume has to be used.

e) Drying of filters

As drying of filters has been shown in several experiments to increase the amount of extractable chlorophyll pigments, it is recommended to dry the folded filter wrapped in a piece of clean filter paper in a room temperature airstream in darkness.

f) Storage of filters

The filters ought to be analysed immediately after drying.

If storage is necessary, the filters shall be stored deepfrozen (-20°C) in a desiccator with silica gel, for no longer than three months.

g) Extraction

96% ethanol shall be used as extraction solvent.

All work with the chlorophyll extract shall be carried out in subdued or green light.

Storage of the extract shall be in total darkness.

The folded filter is transferred to a graduated centrifuge tube, 10 cm³. 96% ethanol is added, and the tube is stoppered to avoid evaporation.

Extraction shall last 24 hours at room temperature in total darkness. The samples are shaken a few times during the extraction time.

h) Centrifugation

If necessary, the extract volume is adjusted to 10 cm³ before centrifugation. The stoppered centrifuge tube is shaken vigorously to get a homogeneous distribution of the chlorophyll in the extraction solvent.

The sample is centrifuged for 10-20 minutes at about 10,000 m s⁻², in order to reduce the spectrophotometric blank reading (750 nm) which should not exceed 0.005 for a 1 cm cuvette.

i) Storage of extract

The measurements shall be made immediately after centrifugation. If this is not possible the extract may be stored in a deep freezer (-20°C) for no more than 24 hours.

j) Chlorophyll-a measurement procedure

The measurements of chlorophyll-a may either be made by a spectrophotometer or a fluorometer, depending on the equipment available. The measurements of phaeopigments shall only be made by a fluorometer.

1) Spectrophotometric readings

A spectrophotometer with a bandwidth of 2 nm should be used. 96% ethanol should be used as a reference.

Cell-to-cell blanks should be measured at all wavelengths used. The absorbence should be measured at 750 nm and 663-665 nm (at the peak).

For the measurement of phaeopigment, which is non-obligatory, the extract shall be acidified with 1 M
HCl (0.06 cm³ to 5 cm³ of extract) after all other readings have been taken. 0.5-3 min. after acidification, the absorbence at 750 and 663-665 nm should be measured.

Calculations

To calculate the chlorophyll-α content using the spectrophotometric technique, the following equation should be used:

\[ C_V = \frac{10^3 \cdot e \cdot A(665 \text{ k})}{83 \cdot V \cdot l} \]

where:
- \( C_V \) = Chlorophyll-α concentration, mg/m³
- \( e \) = volume of ethanol, cm³
- \( A(665 \text{ k}) \) = absorbence at 665 nm (the peak) minus the absorbence at 750 nm after correction by the cell-to-cell blanks
- \( l \) = length of cuvette, cm
- \( V \) = water volume filtered, dm³
- \( 83 \) = absorption coefficient in 96% ethanol

The volume of chlorophyll sample, volume of ethanol and the length of cell (cuvette) must be chosen to give absorbence values at 663-665 nm of 0.05-0.8, i.e. the optimum range of the spectrophotometer.

2) Fluorometric reading

The sample volume and the quantity of ethanol have to be chosen so that the fluorescence reading is in the optimum range of the equipment used (normally 50-200 cm³ water and 5-10 cm³ ethanol).

A fluorometer with excitation setting of 425-430 nm and emission setting of 663-665 nm, or a filter fluorometer equipped with a blue lamp corresponding to GE F4T5B, red-sensitive photosensitive multiplier and primary filter corresponding to Corning 5-60 and secondary filter corresponding to Corning 2-64 should be used.

The fluorescence shall be measured with the appropriate slit.

For the measurement of phaeopigment the extract shall be acidified with 1 M HCl (0.06 cm³ to 5 cm³ of extract) after the first reading. 0.5-3 minute after acidification, a new reading should be made.

Calculations

Use the equation:

\[ \text{Chl.-α (mg.m}^{-3}\) = R.f.s.e.V^{-1} \]

where:
- \( R \) = fluorescence reading
- \( f \) = calibration factor
- \( s \) = slit correction
- \( e \) = volume of ethanol (cm³)
- \( V \) = volume of filtered water (dm³)

The calibration factor is determined as follows:

\[ f = K.R^{-1}.V.e^{-1} \]

where:
- \( K \) = concentration of chlorophyll-α (mg.m⁻³) determined spectrophotometrically as described in Section D.2.k) and (l).
When phaeopigment is to be calculated use the equation:

\[
\text{Phaeopigment (mg.m}^{-3} = f_a \cdot ((r \cdot R_a) - R) \cdot \frac{s}{e} \cdot V^{-1}
\]

- \( R_a \) = fluorescence reading after acidification
- \( r \) = ratio \( R/R_a \) obtained from an extract free from phaeopigment
- \( f_a = f \cdot r \cdot (r-1)^{-1} \)
- \( f, R, s, e, V \) = see above

k) Reproducibility

Every worker should check the reproducibility of the method at least once a year by doing 10 replicate samples. The value of the coefficient of variation (CV %) should be stated for each pigment.

l) Data reporting

For the data reporting see Sections D.10 and D.11.

Any deviation from the procedure recommended here should be stated on the Plain Language Record when reporting the data.

m) References in Chapter D.2.


3. Phytoplankton

a) Sampling design

For the purpose of quantitative studies, an integrated sample from the uppermost 10 m shall be obtained by pooling equal volumes of discrete water samples taken with a water sampler from 0-1 m, 2.5 m, 5 m, 7.5 m and 10 m. If possible, a true integrated sample can be made instead using a tube sampler (Willén 1986, Ramberg 1976).

*) Blue-green algae may concentrate close to the surface. Care should be taken to ensure that these blue-green algae become represented in the integrated sample.
The integrated sample (independent of the sampling design) should be thoroughly mixed in a large bucket. A first-level subsample of 200 ml is drawn from the well-mixed sample for quantitative phytoplankton counts. A second-level subsample is drawn for chlorophyll-α analysis (see Section D.2).

A third-level subsample might be drawn for measurements of the irradiance-primary production relation (see Section D.1.b)).

Other determinands (e.g. nutrients) might also be determined from the integrated sample.

Additionally, two quantitative samples are recommended: one just above and one below the halocline.

It is recommended that a net sample be taken from the 0-10 m water column for qualitative study of the microplankton fraction. A plankton net made of gauge with 10-20 (-25) μm meshes, with a mouth diameter of 15-20 cm, a cylindrical part 25-30 cm long, and a tapering tail end 15-20 cm long will work quite satisfactorily.

If net samples are used for biometric measurements, it should be noted that for species with the smallest dimension around or below the size of the meshes, cells towards the lower size range tend to be under-represented. Therefore, the quantitative samples have to be used for biometric measurements of these and smaller species.

b) Preservation and storage of samples

Qualitative samples

Net samples should be preserved immediately with about 5 ml conc. formalin per 100 ml. If coccolithophorids are present, 5-10 ml hexamine-neutralized formalin should be used if the coccoliths need to be preserved. Any glass container with a tight screw cap will do, preferably a rather wide-necked one. Plastic containers are not recommended, since some plastics may be permeable to formaldehyde vapour. The samples should be stored in the dark at room temperature, since formaldehyde may polymerize to a white precipitate of paraformaldehyde at low temperatures. Properly stored samples will deteriorate only slowly over a period of several years. Weakly silicified diatom frustules are dissolved first.

Subsamples to be studied alive can be kept fresh for a few hours in an open container in a refrigerator. If water samples are to be processed alive according to the relevant parts of Annex I of this Chapter, it should be done as soon as possible. They can be kept unpreserved for up to 24 h in 0.5-1.0 l polypropylene or polyethylene bottles at in situ temperature if it is below +10 °C or 1 month in a deep freeze. Warmer samples should be refrigerated.

Quantitative samples

Samples for quantitative phytoplankton counts should be preserved immediately with 0.5-1.0 ml acid Lugol's solution per 200 ml subsample. If coccolithophorids need to be preserved with the coccoliths intact, a parallel subsample should be fixed with 0.5-1.0 ml alkaline Lugol's solution or 4 ml neutralized formalin. Clear, colourless 200 ml glass bottles with tightly
fitting screw caps should be used for iodine-preserved material. With such bottles it is easy to see when the iodine becomes depleted and more preservative needs to be added.

Plastic containers should not be used. Formalin should not be added to samples preserved with Lugol's solution unless the storage period is going to exceed one year.

The samples should be counted as soon as possible, preferably within half a year. They should be stored dark and cool (ca. +4°C). Samples stored for more than one year are frequently of little use.

Preservatives

Acid Lugol's solution (Willén 1962):

- 1000 ml distilled or deionized water
- 100 g potassium iodide (KI)
- 50 g resublimated iodine (I₂)
- 100 ml glacial acetic acid (conc. CH₃COOH)

Mix in the order listed. Make sure the previous ingredient has dissolved completely before adding the next one. Store in a tightly stoppered glass bottle at +4°C. Add 0.5-1.0 ml per 200 ml sample.

Alkaline Lugol's solution (modified after Utermöhl 1958):

Replace the acetic acid of the acid solution by 50 g sodium acetate (CH₃COONa). Use a small part of the water to dissolve the acetate. Other comments as above.

Neutralized formalin (Thröndsen 1978):

- 500 ml conc. formalin (37-40 % aqueous HCHO solution)
- 500 ml distilled or deionized water
- 100 g hexamethylenetetramine (CH₂)₆N₄ (hexamine)

Analytic grade formalin is to be preferred. Still better is a solution freshly made from paraformaldehyde. Commercial formalin may contain up to 15 % methanol as a stabilizer, and will not give as good fixation. Filter after one week if the solution forms precipitate or becomes turbid. Add 4 ml per 200 ml water sample. Net samples require 5-10 ml per 100 ml.

c) Qualitative determinations

General

While quantitative investigations have a long standing, and are performed routinely by most laboratories, the greatest weakness has proved to be the identification of the organisms.

It is becoming more and more evident that the taxonomic composition of phytoplankton and the species succession have considerable value as indicators of the trophic status of a water mass. Especially the nano-plankton (2-20 μm) and picoplankton (<2 μm) fractions need much more attention than they have hitherto received.

Quantitative phytoplankton counting is a labour-intensive procedure, and the cost-benefit of the resulting data is questionable. Investing less effort in the actual counting process, and more in cheap chlorophyll-a determinations and skill-requiring survey of the floristic content of samples would apparently give more useful results, provided the taxonomic knowledge of the analyst is good enough.
Determination

Because of the limitations of the Utermöhl technique and the inverted microscope, accessory techniques are needed for the determination of a number of phytoplankton species and groups, especially when as complete as possible species lists are required. Some of these non-obligatory methods are reviewed in Annex I of this Chapter.

Net samples are recommended to be taken routinely for the investigation of sparsely occurring microplankton species with a standard research microscope. The advantages include potentially higher resolution, thinner preparations and the possibility to turn the cells around by tapping the cover glass. This is especially helpful when examining the plate structure of dinoflagellates.

d) Quantitative determinations (phytoplankton counting)

Introduction

The recommendation is based on the counting technique with an inverted microscope as described by Utermöhl (7). In order to elucidate sources of error caused by subsampling and the settling and counting of subsamples, the practical application of the method is emphasized. To make phytoplankton counting less time-consuming and the results more comparable, a simplified procedure along the lines proposed by Willén (11) has been adopted.

Statistically settling and counting represent two more subsampling stages (unless the preserved subsample is counted in toto, which is unrealistic), which reduce the precision of the population estimates (cf. 8, 9).

Settling procedure

Before sedimentation the sample should be adapted to room temperature for about 24 hours to avoid excessive formation of gas bubbles in the sedimentation chambers. Gas bubbles will adversely affect sedimentation, the distribution of cells in the bottom-plate chamber, and microscopy.

Immediately before the sample is poured into the combined sedimentation chamber, the bottles should be shaken firmly but gently in irregular jerks to homogenize the contents. Too violent shaking will produce a lot of small bubbles which may be difficult to eliminate. If the sample must be shaken vigorously in order to disperse tenacious clumps, this should not be done later than one hour before starting sedimentation.

The chambers should be placed on a horizontal surface and should not be exposed to temperature changes or direct sunlight. Covering the settling chamber(s) with an overturned plastic box will provide a fairly safe and uniform environment for sedimentation. If moistened tissue paper is included under the hood, problems caused by evaporation will be reduced considerably. It is essential that the supporting surface is vibration-free, since vibrations will cause the cells to collect in ridges.

Settling time is dependent on the height of the chamber and the preservative (e.g. 1, 4). The times given below are recommended as minimum. If vibration caused e.g. by traffic is a problem, the minimum time should not be significantly exceeded. Otherwise it is suggested that counting be performed within four days. Sedimented samples not counted within a week should be discarded. Separated bottom chambers not counted immediately should be kept in an atmosphere saturated with humidity.
Settling time (h) | Volume of chamber (ml) | Height of chamber (cm) | Lugol's solution | Neutr. formalin
---|---|---|---|---
2 | 1 | 3 | 12
10 | 2 | 8 | 24
50 | 10 | 24 | 48
100 | 20 | 48 | 48

One hundred ml chambers should be used with caution since convection currents are reported to interfere with the settling of plankton in chambers taller than five times their diameter (2, 1). Such chambers can be used only when phytoplankton is very sparse, as in late autumn and winter.

If the cells are too strongly stained by iodine for comfortable identification, surplus iodine can be chemically reduced to iodide by dissolving a small amount of sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$) in the aliquot to be sedimented.

Counting procedure

In order to save time and to achieve a reasonable accuracy in counting, the sedimented sample should first be examined for general distribution of cells on the chamber bottom, and the abundance and size distribution of the organisms. All species found should be listed. If the distribution is visually uneven, one-sided or in ridges, indicating convection or vibration, respectively, the settled sample should be discarded. If this occurs consistently, measures should be taken to eliminate the sources of disturbance.

How much of the chamber area should be counted and the magnification to be used is dependent on the size of the organisms and their abundance, and on the kind of counting units used. The following counting units are recommended:

**CELL:**
- All non-colonial unicellular species
- Dinobryon
- Uroglena (disintegrated colonies)
- Aulacosira
- Chaetoceros
- Detonula
- Leptocylindrus
- Melosira
- Skeletonema (and other chain-forming diatoms)
- Planktonema (and other filamentous green algae)

**COLONY:**
- Aphanothece
- Coelosphaerium
- Gomphosphaeria
- Microcystis (incl. Aphanocapsa)
- Gloeotrichia
- Uroglena (when colonies well preserved)
- Halosphaera
- Sphaerocystis (and similar genera)

**COENOBIIUM, with a ± fixed number of cells (n):**
- Eudorina (32)
- Pandorina (16)
- Coelastrum (8, 16)
- Crucigenia (4)
- Microcystis (4)
- Pediastrum (4, 8, 16, 32, etc.)
- Scenedesmus (2, 4, 8)

**SOME COLONIAL ALGAE** are most conveniently counted as groups of four cells, e.g.:
- Chroococcus
- Merismopedia
- Crucigeniella
- Dictyosphaerium

**FILAMENTS** to be counted in lengths of 100 μm:
- Achroonema
- Anabaena
- Anabaenopsis
- Aphanizomenon
- Beggiatoa
- Lyngbya
- Nodularia
- Oscillatoria
- (etc. filamentous blue-green algae)

The following combinations of objectives and oculars are recommended for quantitative microscopy with the inverted microscope:
<table>
<thead>
<tr>
<th>Count</th>
<th>95 % C.L. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>89</td>
</tr>
<tr>
<td>7</td>
<td>76</td>
</tr>
<tr>
<td>10</td>
<td>63</td>
</tr>
<tr>
<td>15</td>
<td>52</td>
</tr>
<tr>
<td>20</td>
<td>45</td>
</tr>
<tr>
<td>25</td>
<td>40</td>
</tr>
<tr>
<td>40</td>
<td>32</td>
</tr>
<tr>
<td>50</td>
<td>28</td>
</tr>
<tr>
<td>75</td>
<td>23</td>
</tr>
<tr>
<td>100</td>
<td>20</td>
</tr>
<tr>
<td>200</td>
<td>14</td>
</tr>
<tr>
<td>400</td>
<td>10</td>
</tr>
<tr>
<td>500</td>
<td>8.9</td>
</tr>
<tr>
<td>700</td>
<td>7.6</td>
</tr>
<tr>
<td>1000</td>
<td>6.3</td>
</tr>
<tr>
<td>2000</td>
<td>4.5</td>
</tr>
<tr>
<td>5000</td>
<td>2.8</td>
</tr>
<tr>
<td>10000</td>
<td>2.0</td>
</tr>
</tbody>
</table>

It should be recognized that these are not maximum errors. The statistics assume perfectly random distribution of cells on the bottom of the sedimentation chamber, a condition which is probably never realized. The several subsampling steps involved also tend to increase the variance (cf. 9, 10).

With species for which the counting unit is smaller than the individual, e.g. some colonial forms, chain-forming diatoms, and filamentous species with average filament length in excess of 100 μm, the distribution of the counting units will be aggregated even in perfectly sedimented samples. The variance will be higher, and the precision accordingly lower. If it is necessary to keep the error within the same limits as for "randomly" distributed units, the number of counted units should be increased in the ratio average size of individual/size of counting unit.

At low magnification, a sufficient number of crossed transects should be counted to reach the required number of counting units. At high magnification, fields
of view evenly spaced along crossed transects should be used. At intermediate magnifications either method can be employed, depending on the density of the units to be counted. Considering the many other sources of error involved in the method, it is unnecessary to spend time on randomization of the fields to be counted. Only exceptionally will it be necessary to count the whole chamber bottom for very large cells, such as Ceratium and Coscinodiscus.

The number of counting units per dm$^3$ sea water is calculated by multiplying the number of units counted with the coefficient $C$, which is obtained from the following formulas:

$$C = \frac{A \cdot 1000}{N \cdot a_1 \cdot V} \quad \text{or} \quad C = \frac{A \cdot 1000}{a_2 \cdot V}$$

where

- $A$ = cross-section area of the top cylinder of the combined sedimentation chamber; the usual inner diameter is 25.0 mm, giving $A = 491 \ 000 \ 000$ $\mu$m$^2$ (the inner diameter of the bottom-plate being irrelevant)
- $N$ = number of counted fields or transects
- $a_1$ = area of single field or transect
- $a_2$ = total counted area
- $V$ = volume (cm$^3$) of sedimented aliquot

Reliable quantitative counting of the picoplankton fraction requires fluorescence microscopy (cf. Annex I of this Chapter).

When counting phytoplankton in a sedimentation chamber, it is suitable to count protozooplankton (e.g. ciliates and colourless flagellates). This recommendation is also valid for these forms (cf. Annex I of this Chapter). However, it must be stressed that the protozooplankton are a separate group and must not be mixed with the phytoplankton. Thus, they must not be included in abundance or biomass values of phytoplankton.

Cleaning of the sedimentation chambers

After use no part of the combined sedimentation chamber should be allowed to dry out before it is carefully cleaned. Dried phytoplankton or formalin preservative may be quite difficult to remove. The separate parts are first rinsed under running tapwater, then soaked for a few minutes in luke-warm water with some non-abrasive detergent added, then cleaned with a soft brush or soft tissue paper, and rinsed with tapwater. Finally they are given a rinse with deionized or distilled water, and are put away to dry. Special care should be taken not to scratch either end of the top cylinder and the entire upper surface of the bottom plate.

c) References in Chapter D.3.


f) Identification literature

The following list is not complete. Articles concerning single or only a few species have not been included.

Phytoplankton identification literature


Lebour, M., 1925. The dinoflagellates of northern seas. Plymouth.


Phytoplankton checklists

In the BMP the Preliminary Check-list of the Phytoplankton of the Baltic Sea (Section D.4.) shall be used. Additional information can be found in the following check-lists:


Protozooplankton identification literature


**g) Biomass transformations**

**Introduction**

Depending on the purpose of the investigation, phytoplankton biomass can for example be expressed as cell volume (weight), plasma volume or carbon. The transformations to cell volume rely on measurements of the size of the species, and a large number of shapes have to be used for the different organisms. The transformation of cell volume to plasma volume includes an estimate of the vacuole volume, and the calculation of cell carbon is in turn based on the plasma volume. The formulas recommended below are a step towards a more uniform treatment of counted organisms, in order to get comparable results from different parts of the Baltic.

**Stereometric shapes and formulas for common phytoplankton and protozooplankton in the Baltic**

Volumes of species with a small size variation can be calculated as annual median values. For species present only seasonally, median values from several years can be used. Species with a large size variation are suitably divided into size groups during counting (e.g. diatoms and monads).

Methods for calculating volumes of different plankton species are given in Table D.2.

For comparison of the volume of different species in some parts of the Baltic Sea reference is made to (1, 2, 3, 4, and 12).


**TABLE D.2. Stereometrical formulas for common phytoplankton taxa in the Baltic**

<table>
<thead>
<tr>
<th>Stereometric Formulas Used</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cylinder</strong></td>
<td>$\frac{\pi d^2 h}{4}$</td>
</tr>
<tr>
<td><strong>Sphere</strong></td>
<td>$\frac{\pi d^3}{6}$</td>
</tr>
<tr>
<td><strong>Rotational Ellipsoid with ellipse-shaped cross-section</strong></td>
<td>$\frac{\pi l b d}{6}$</td>
</tr>
<tr>
<td><strong>Rotational Ellipsoid with circular cross-section</strong></td>
<td>$\frac{4\pi a b h}{3}$, $\frac{\pi l d h}{6}$</td>
</tr>
<tr>
<td><strong>Ellipsoid</strong></td>
<td>$\pi a b h$</td>
</tr>
<tr>
<td><strong>Parallelepiped</strong></td>
<td>$l b h$</td>
</tr>
</tbody>
</table>

**Species**

- **Nostocophyceae (Cyanophyceae)**
  - *Achenopoma*: cylinder
  - *Anabaena*: cylinder
  - *Aphanizomenon*: cylinder
  - *Chroococcus*: cells as spheres, dividing cells as spheres
  - *Gomphosphaeria*: outer sphere - inner sphere
  - *Nostoc*: obligate heterotrophic
  - *Aphanizomenon*: facultative heterotrophic
  - *Achnanthes*: autotrophic/heterotrophic unknown

---

**Cone**

$$\frac{\pi r^2 h}{3} = \frac{\pi d^3 h}{12}$$

**Truncated Cone**

$$t \cdot h \cdot (d_1 d_0) = \frac{\pi t \cdot h \cdot (d_1^2 d_0 + d_0^2)}{12}$$

**Trapezoid**

$$h \cdot d \cdot (a + b)$$

---
Sympysis lomata: cylinder
Nannophyceae: cells as spheres, dividing cells as spheres/2
Microcystis: cells as spheres (cf. Reynolds & Jaworski, 1978)
Rhopodium: cylinder
Gloeocystis: cylinder
Phaeodactylum: cylinder
Pseudonannochloris: cylinder

CRYPTOPHYCEAE

- Chlamydomonas: cone + sphere/2, rotational ellipsoid (ellipse-shaped cross-section)
- Chroomonas: cone + sphere/2, rotational ellipsoid (ellipse-shaped cross-section)
- Cryptomonas: rotational ellipsoid (ellipse-shaped cross-section)
- Rhoodymonas: cone + sphere/2

DINOPHYCEAE

- Amphidinium: rotational ellipsoid $\frac{8}{9}$ (ellipse-shaped cross-section)
- Ceratium furca: cone + parallelepiped + trapezoid + cylinder
  - Lineatum:
  
Ceratium fusus: cylinder + truncated cone

Ceratium longipes:
- Macroceras: cone + cylinder + sphere/2
- Stripa:

- Dinophysis acutata: ellipsoid
  - Dinophysis rotundata: cylinder
  - Glenodinium: sphere/2 - 30% to 40%
  - Gonyaulax catenata: sphere/2
  - Gonyaulac sacculata: trapezoid + parallelepiped/2
  - Gonyaulac disaenathis: trapezoid + parallelepiped/2
  - Scrippsia:
Gorysta: grindsleyi:
 polyedra:
 tomaropsis:

 Goniodoma: sphere

 Gymnodinium spp.:
 2 cones or 2 spheres/2 - 10 to 40%

 Gymnodinium simplex: 2 spheres/2

 Gymnodinium splendens: (sphere/1 + cone)/2

 Heterocapsa triqueta: 2 cones - 20%

 Minamia bipes: cone - 50%

 Noctiluca millarisis: sphere

 Oxytoxum: 2 cones

 Polykrikos: cylinder

 Prorocentrum balthicum: sphere - 10%, measurement should be made at maximum diameter

 Prorocentrum micans: parallelepiped + parallelepiped/2

 Protoperidinium: General formulas for volume calculations:
 cone + sphere/2, 2 cones, sphere, 2 cones + cone. Generally the volumes have to be reduced by 10 to 30% because of the concavity of the ventral side.

 X. P. breve:

 X. brevispinus: cone + sphere/2

 X. contiosoides:

 X. foetidus:

 X. clausius: (cone + 2 cones) - 30%

 X. contius: (cone + 2 cones) - 25%

 X. depressus: (cone + 2 cones) - 30%

 X. divergens: (cone + 2 cones) - 30%

 X. graniti: (cone + sphere/2) - 20%

 X. pulicarius: (cone + sphere/2) - 30%

 X. pellucidae: (cone + sphere/2) - 10%

 Semispirella faurcensis: sphere

 ? Harmocysta: rotational ellipsoid

 CHRYSOPHYCEAE

 X. Biocoecae:

 Dinobryon: rotational ellipsoid or sphere

 Kephyrion: cylinder

 Miliammina: cone + sphere/2

 Synura: cone + sphere/2

 Uroglena: sphere or rotational ellipsoid (ellipse-shaped)

 Urgionopsis: cross-section

 BACILLARIOPHYCEAE

 Achnanthas: parallelepiped

 Achnanthes: parallelepiped

 Amphiprora: ellipsoid

 Amphora: ellipsoid

 Asterionelloa: parallelepiped

 Attheya: ellipsoid

 Bacillaria: parallelepiped

 Biddulphia: ellipsoid

 Ceratiosoma: cylinder

 Chaetoceros: ellipsoid

 Cocconeis: ellipsoid

 Coccosphaera: cylinder + two caps

 Cyclotella: ellipsoid

 Detoniella: cylinder

 Dictyoa: parallelepiped

 Diploneis: ellipsoid

 Ditylum: cylinder
Spithemia: ellipsoid
Eunotia: ellipsoid
Fragilariopsis: parallelepiped
Gomphonema: ellipsoid
Gomphonemoida: parallelepiped
Gomphonemella: cylinder
Gyrospira: ellipsoid
Kontzeskia: cylinder
Lauderia: cylinder
Leptocylindrus: cylinder
Limnionema: parallelepiped/2
Mediastrom: cylinder
Mediastromella: parallelepiped, parallelepiped/2
Pezoscleria: parallelepiped
Rhodomonas: parallelepiped
Rhizosolenia: parallelepiped
Rhizosolenella: parallelepiped
Rhoicosphenia: parallelepiped/2
Rhopalodia: ellipsoid
Skeletonema: cylinder
Stephanodiscus: parallelepiped
Surirella: ellipsoid
Synedra: parallelepiped, parallelepiped/2
Tisellaria: parallelepiped
Tisellaria: parallelepiped
Thalassiothrix: cylinder
XANTHOPHYCEAE

Centritractus: rotational ellipsoid
Gloeochloris: cells as spheres or rotational ellipsoids
Ophiocytium: cylinder

EUGLENOPHYCEAE

Colacium: rotational ellipsoid
Rhizostoma: cylinder
Rhizostoma: rotational ellipsoid
Skeletonema: parallelepiped
Scenedesmus: cells as rotational ellipsoid or cone + cone

XX Masses size groups 2 -< 3 µm
3 -< 5 µm
5 -< 7 µm
7 -< 10 µm
10 -< 15 µm
15 -< 20 µm, etc.

PICOPLANKTON < 2 µm

CHOANOFLAGELLIDA

Anomalota: rotational ellipsoids or spheres
Ciliates with Endosymbiotic Algae

Mesodinium
- < 30 μm: 2 - sphere \( \frac{5}{3} \)
- ≥ 30 μm: sphere

Ciliates

- ciliate: sphere \( \frac{4}{3} \), cone or ellipsoid
- Didinium: truncated cone
- Lohmanniella: sphere
- Strombidium concavum: cone
- Strombidium lagerula: cone
- Strombidium stylifer: cone
- Tintinnopsis: cylinder + cone
- Tontonia graniformis: sphere
- Tontonia appendiculariformis: 2 truncated cones

Tintinnids

- Coelocella helia: cylinder + cone
- Favella denticulata: cylinder + cone
- Helicotricha subulata: sphere - 30%
- Stenosomella monella: sphere - 30%
- Stenosomella ventricosa: cone
- Tintinnopsis berorides: cone
- Tintinnopsis tubulosa: cone

Volume calculations for Tintinnoids may be difficult due to the shells of the organisms. The measurements must be done when the ciliate is outside the shell. In preserved samples, however, the ciliate usually stays inside the shell. In that case a certain amount must be subtracted from the shell volume. This amount must be found from measurements of the shell and the ciliate. It is sufficient to measure this in one sample.

Plasma volume calculations

All phytoplankton, except diatoms.

In this recommendation it is assumed that plasma volume (PV) equals cell volume (CV). See, however, Sicko-Goad et al. (5).

\[ PV = CV \]

unit: μm³

Diatoms

Different methods for calculation of the plasma volume have been suggested by (6 and 7). The method recommended here is a modification of that given by Strathmann to include pennate diatoms. Plasma volume (PV) equals cell volume (CV) minus vacuole volume (VV).

The vacuole volume is calculated by subtracting plasma thickness (c) from the measurements of length, breadth etc. (see Figure D.1. below). If a measurement extends from cell wall to cell wall, 2 c should be subtracted from the measured value. When using a measurement such as radius that is not bounded by the cell wall and thus contains only one layer of plasma, only c should be subtracted. Values of c have been given by Lohmann (8) and Smayda (6) and are in the range of 1 μm.

Due to the high content of organic matter in the sole, only 90% of the vacuole volume should be subtracted from the cell volume to give the plasma volume.

\[ PV = CV - (0.9 \cdot VV) \]

unit: μm³
For comparison of the carbon content of different species in some parts of the Baltic Sea, see Smetacek (2) and Edler (4).

h) Presentation of results and data reporting

For the data reporting see Sections D.10 and D.11.

i) References


Annex I to Section D.3. Phytoplankton

Notes on accessory methods for species determination, and on the determination of protozooplankton vs. non-photosynthetic phytoplankton.

Fluorescence microscopy is needed in order to obtain reliable counts of unicellular autotrophic picoplankton species. Experienced workers may reach order-of-magnitude accuracy with relatively large cells such as Micromonas pusilla and Nannochloropsis sp. by the Utermöhl technique, but if mineral particles are abundant, the results may be questionable. Coccoid blue-green algal cells about 1 μm in diameter or smaller can be distinguished from true bacteria, and thus quantified, only by fluorescence microscopy. Methodological details are given by Hoppe et al. (3), Vargo (8) and Haas (2).

Centrifugation of living water samples is recommended for the study of delicate nanoplanckton flagellates whose morphology usually deteriorates upon fixation to the extent that they become virtually unrecognizable (e.g. small naked dinoflagellates, many chrysophytes and prymnesiophytes, some prasinophytes, and most zooflagellates). Besides, several genera or even some species are well characterized by their mode of swimming (e.g. Pseudopedinella, Prymnesium, Chrysochromulina, Pavlova, Micromonas pusilla, Nephroselmis).

A simple bench centrifuge with a swing-out head taking 4-6 25 ml pointed centrifuge tubes is adequate. At 1500-2000 rpm a centrifugation time of 10 min is sufficient.
To optimize the viability of the cells under the microscope, the glassware should be as clean as possible (cf. 9).

**Dry preparations** observed with a good oil immersion phase-contrast objective make possible the determination of a limited number of species in the genera *Paraphysomonas* and *Chrysochromulina* on the basis of their scale morphology, some coccolithophorids, and most choanoflagellates.

Suitably concentrated material obtained by centrifugation as described above is pipetted onto cleaned cover glasses, fixed by exposure to the vapour of a few small drops of 1-2 % OsO₄ solution inside a Petri dish (under a fume hood), and allowed to dry. The salt is washed away by immersing the cover glasses into distilled or deionized water for 10 min, after which they are dried again. Finally the cover glasses are turned specimen side downwards, and are glued to glass slides.

**Electron microscopy** is required for a positive determination to species level of most scale-covered organisms. These include most prymnesiophytes and prasinophytes, some chrysophytes (*Mallomonadaceae* and *Paraphysomonadaceae*), and certain protozoan groups.

The method of making direct preparations onto specimen grids is very similar to that described for dry preparations. A detailed account of the technique has been given by Moestrup & Thomsen (5). Species with thin organic scales require shadowing for TEM. Most silicified scales can also be determined without shadowing. Strongly calcified coccolithophorids usually require SEM, as do a number of dinoflagellates with thin thecal plates.

**Diatom preparations** are necessary for the determination of most nanoplanktonic disc-shaped centric diatoms (*e.g.* *Cyclotella*, *Stephanodiscus* and *Thalassiosira*) even at the generic level. The majority of littoral pennate diatoms also require diatom preparations for determination at the species level; for some naviculoids, even at the generic level.

A rapid and satisfactory method of making diatom preparations is as follows: Put less than 0.5 ml of concentrated sample into a test tube. Add ca. 1 ml conc. HNO₃. Hold the test tube with a clothes-peg or similar, and heat on a small flame inclining the test tube 45-60° from the vertical and continuously shaking with caution in order to avoid overheating and spitting. Keep the opening of the test tube away from yourself in a safe direction. Boil for 1-5 minutes until the solution is clear and white vapours start to form. If the material is not completely oxidized after 5 min., add a few drops of conc. H₂SO₄ (beware of overheating) and repeat.

The oxidation with acids should be performed under a fume hood. If a fume hood is not available, the acids can be replaced by enough K₂S₂O₈ to make a nearly saturated solution at +100°C. The sample volume can be increased to ca. 2 ml. Heat for 1-2 h in a boiling water bath occasionally replacing the evaporated water.

Cool and dilute the contents of the test tube by adding at least 10 ml water and transfer to a centrifuge tube. Spin the material down at 1500-2000 rpm for 20 min. Remove the supernatant preferably using a Pasteur pipette attached to a water suction pump. Resuspend the sediment with a jet of water from a wash bottle, and spin it down again for 10 min. Repeat the rinsing procedure at least five times. Use distilled or deionized water in no less than the two last rinses. Dilute with
water to a suitable (by experience) turbidity and transfer a few drops to a clean cover glass. Evaporate the water, preferably slowly under cover to achieve an even distribution. Add a suitable amount of embedding medium with a high refraction index (e.g. Clophenharpiks, Hyrax, Styrax) onto a clean slide, place the cover glass on the top (or pick it up with the embedding medium on the slide), and heat gently on a hot plate to expel air bubbles and evaporate the solvent.

It should be recognized, however, that some of the smallest centric species, and small species of the genera Fragilaria, Achnanthes, and Navicula, at least, and some Nitzschia species, still require electron microscopy (TEM or SEM) for a safe determination.

Protozoa and non-photosynthetic phytoplankton

When working with phytoplankton, it is convenient to determine the protozooplankton as well. The distinction between algal and protozoan higher systematic units is vague to the extent that some researchers tend to classify them together as protists. Most genuine algal classes contain species or even whole genera which are non-photosynthetic, and which thus functionally should be regarded as zooplankton, for example, Cryptophyceae: Cryptaulax, Cyathomonas, Katablepharia, Leucopeys; Dinophyceae: Amphidinium P.-P., Gymnodinium P.-P., Gyrodinium P.-P.; Oxyrrhis; Nootilus, Oblea, Protoperidinium, Ebria; Prymnesiophyceae: Balaniger; Chrysophyceae: Paraphysomonas; Euglenophyceae: Astasia; Peranematales; Chlorophyceae: Polydora.

A major obstacle to a uniform treatment is the distinction between the Botanical and Zoological Nomenclatural Codes and the incompatibility of the systematic superstructures. A number of different phyletic groups generally considered by botanists as algal classes within a number of divisions (e.g. 1) are treated by zoologists as orders of the class Phytomastigophorea (Phylum Sarcomastigophora, Subphylum Mastigophora: 4). While groups like the choanoflagellates and the bodonoid flagellates, which until recently were claimed by phycologists, now seem to be generally accepted to belong in the animal kingdom, some groups are still disputed or uncertain, or lack a satisfactory systematic superstructure, e.g. the bicosoecoids and many colourless members of the Proto- and Polyblepharidinae and Polymastiginae (6, 7), etc. On the other hand, connections have been found in the Pedinellales (9) between a chrysophyceen lineage and members of the protozoan order Actinophryida (Phylum Sarcomastigophora, Class Heliozoa). There are also a few true animals which function as plants because they have intracellular photosynthetic symbionts (e.g. Mesodinium).

Even with the procaryotes, a uniform conception is lacking regarding the limits of the systematic entities and their names. The blue-green algae seem to be a rather homogeneous group, regardless of whether they are referred to as Cyanophyceae, Nostocophyceae or Cyanobacteria. Problems of delimitation arise when morphologically similar non-photosynthetic types are included (e.g. Beggiatoa, Achroonema), or when the organisms are so small, i.e. usually less than 1-1.5 μm wide, that special methods (e.g. fluorescence microscopy) are required to determine whether the cells contain chlorophyll or not. This concerns for instance narrow Oscillatoria spp. vs. Achroonema spp., Merismopedia warmingiana vs. Lampropedia hyalina, and very small photosynthetic coccoid cells vs. true bacteria.
References to Annex I of D.3.


4. Checklist of phytoplankton


A preliminary check-list of the phytoplankton of the Baltic Sea

LARS EDLER, GUY HÄLLFORS and ÅKE NIEMI

INTRODUCTION

Studies of phytoplankton in the Baltic Sea have a long tradition. They started about one hundred years ago (Hensen 1887) and, since then, numerous investigations have been carried out. During the past 15 years it has become more and more evident that phytoplankton research has been hampered by the lack of a modern determination manual. Usually determination has to be based on extensive taxonomical literature which very often is difficult to obtain.

The objective of compiling the present list was to record available information concerning species composition of Baltic Sea phytoplankton, the nomenclature of the species, and to some degree their spatial and temporal distributions. By this means we hope to facilitate and encourage further research. The list is intended to be used as an easily cited source in matters concerning nomenclature and the distribution of phytoplankton species in the Baltic Sea. It is an extension of the list of Hällfors (1979) and includes the whole Baltic Sea.

The list is not to be considered as complete. It is mainly based on our own material and experience supplemented with literature data. However, the literature coverage is not extensive. We lack material from certain regions (especially AS and RB), and have been unable to secure enough published information. For these subareas, as well as other subareas we shall be grateful to receive records, corrections and improvements in order to make the next edition as useful as possible.

ARRANGEMENT OF THE LIST

For the purpose of the check-list the Baltic Sea has been divided into ten subareas (Fig. 1), the

<table>
<thead>
<tr>
<th>Subarea</th>
<th>Description</th>
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<tr>
<td>Bothnian Bay (BB), the Bothnian Sea (BS), the Archipelago Sea (AS), the Gulf of Finland (GF), the Riga Bay (RB), the Northern Baltic proper (NB), the Central Baltic proper (CB), the Southern Baltic proper (SB), the Arkona Basin</td>
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Key words: Phytoplankton, check-list, the Baltic Sea

Lars Edler, Department of Marine Biology, University of Lund, Box 124, S-221 00 Lund, Sweden. Guy Hällfors and Åke Niemi, University of Helsinki, Zoological Institute, SF-00014 University, Helsinki, Finland
Fig. 1. The Baltic Sea with the subareas indicated for which records of planktonic species are included in the check list. BB = the Bothnian Bay, BS = the Bothnian Sea, AS = the Archipelago Sea, GF = the Gulf of Finland, RB = the Riga Bay, NB = the Northern Baltic proper, CB = the Central Baltic proper, SB = the Southern Baltic proper, AB = the Arkona Basin, and KB = the Kattegat and Belt Sea Area.

(AB), and the Kattegat and Belt Sea Area (KB). To facilitate rapid location of samples, well-defined borders have been drawn which differ somewhat from previously proposed 'natural' borders. When considering division of the area for other purposes the borders suggested by Ekman (1931) and by Wattenberg (1949) should be taken into consideration.

Species are listed alphabetically under each Order. We have tried to consider recent nomenclatural changes and have included the most important synonyms used in the Baltic Sea in brackets below each species. The number(s) in brackets after a name refers to an annotation at the end of the list. For the sake of clarity, author's names are mostly given in full. For abbreviations, see Christensen & Thomsen (1974) and Parke & Dixon (1976). When a species has been recorded from an area, there is a symbol in the proper column.

**Explanation of the symbols**

- + occurrence without ecological characterization
- C cold water species (CIMP C)
- W warm water species (CIMP C)
- L main occurrence in the littoral
- F freshwater species which does not tolerate the full salinity of the area
- ± species belonging to waters of lower salinity than that of the area
- ± species belonging to waters of higher salinity than that of the area
- ± main occurrence in eutrophed waters
- ± questionable record
- ( ) symbol not very strictly applicable, e.g. (C) = generally but not exclusively cold water species
- ± no record

**CYANOPHYTA (CYNOBACTERIA)**

*Nostocaceae* (Cyano)phyceae

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**CYNODONDALES**

Acheterina S. Skuja (3)

*Actions severe* (C.A. Agardh) Skuja

*Draba* (C.A. Agardh) Skuja

Aphanizomenon Flahault & Gigiard (1)

*Aphanizomenon* (C.A. Agardh) Flahault & Gigiard

*Gigio* (C.A. Agardh) Flahault & Gigiard

*Oleosira* (C.A. Agardh) Flahault & Gigiard

*Pleurocapsa* (C.A. Agardh) Flahault & Gigiard

**CRYPTOPHYTA**

Cryptophyceae

Cryptomonadales (5)

*Chlorella* (C.A. Agardh) Flahault & Gigiard

*Chlamydomonas* (C.A. Agardh) Flahault & Gigiard

*Euglenophyceae* (10)

*Euglena* (C.A. Agardh) Flahault & Gigiard

**DINOPHYTA**

Dinophyceae

Procentrales

Protoceratium Ehrenberg

*Protoceratium* (C.A. Agardh) Flahault & Gigiard

*Protoperidinium* (C.A. Agardh) Flahault & Gigiard

*Pseudogonyaulax* (C.A. Agardh) Flahault & Gigiard

*Pseudo-nitzschia* (C.A. Agardh) Flahault & Gigiard

**DINOPHYSEAE**

Dinophyceae

Protoceratium Ehrenberg

Protoceratium Ehrenberg

Protoceratium Ehrenberg

Protoceratium Ehrenberg

Protoceratium Ehrenberg

Protoceratium Ehrenberg

Protoceratium Ehrenberg
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<td>Gonyaulax Light</td>
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**Gymnodiniales**
- *Actinascus* (Ehrenberg) Dujardin
- *Gonyaulax* (Gymnodiniales) Dujardin
- *Peridinium* (Gymnodiniales) Dujardin

**Peridiniales**
- *Pyrocystales*
  - *Gonyaulax* (Ehrenberg) Dujardin
  - *Peridinium* (Ehrenberg) Dujardin

**Peridiniales**
- *Ctenophora* (Ehrenberg) Dujardin
  - *Gonyaulax* (Gymnodiniales) Dujardin
  - *Peridinium* (Gymnodiniales) Dujardin
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**CHRYSOPHYTA**

**CHRYSOPHYCEAE**

**OCHROMONADALES**

- *Asthephyca Berg*
- *Heteroecia (O. F. Müller) Stein*
- *Bacillomonas (Stein) Stein*
- *Dinobryon (Stein) Stein*
- *Dinolykton Ehrnberg*
- *Eutreption Ehrenberg*

**EUSTIGMOPHYCEAE**

- *Eustigmatophycus Ehrenberg*
- *Eustigmatea Ehrenberg*
### Tribophyceae (Xanthophyceae, Heterocontae)

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### MISCHOCOCCALES

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### TRIBONEMATACEAE

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### DIATOMACEAE (Bacillariophyceae)

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### EUPODISCACEAE (Biodulphiales, Centrales)

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### ACTA BOT. FENNICA 128 (1964)

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**Raphidophyceae (Chloromonadophyceae)**

**RAPHIDOMONADALES**

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**EUGLENOPHYTA**

**Euglenophyceae**

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PEDINOMONALES

Micromonas

Puudoscourfa/dia

Mantoniel/a

Pterosperma

Mametella

Trechonomonas

Entheberg

Hugada (Peray Stein em. Delandre)

voluntas (Entheberg)

PTEROSPERMATALES

Micromonas Manton & Parke

puddle (Butcher) Manton & Parke

Pseudomonas (Manton) Manton

marina (Thondsen) Manton

Mesonella Descadary

pseudisetum (Manton em. Parke) Descadary

Nephromenista Stein

minosa (Carter) Butcher

dilectus Stein em. Menonrup & Enti

pyramidum (Carter) Enti

rotundata (Carter) Fott

Parapinnularia Buschet

nematoceros J. Schiller

vandenhoofii (Jorgensen) Ostenfeld

in Ostenfeld et Schmidt

Plectophyta Ostenfeld in Korsikov et Korsikov

pelagios Ostenfeld in Korsikov et Ostenfeld

MAMIELLALES

Mamellia Mennop

gelis (Parke & Rayns) Menonrup (49)

PYRAMIMONDALES

Azorhizoma Aartari

ploriclisia Stein

flaviphora Schnitz

marea Ostenfeld

viridis Schnitz

Pyramimonas Schmidta (5)

goazzl Park

obesalis N. Carter

oralis Butcher

PRASINOCYTOPALES

Tetraselmis Stein (5)

concentra (Parke et Manton) Norris, Horn & Chihara

condiformis (Carter) Stein

(Carter condiformis (Carter Diels)

(Pseudomonas arietiniae G. S. West)

CHLOROPHYTIA

Prasinophyceae (incl. Loxophyceae)

PEDINOMONALES

Micromonas

Pterosperma

Mametella

Trechonomonas

Entheberg

CHLOROCOCCEAE

Prasinophyceae (incl. Tetrastromatales)

Bryopsidomonas

Bryopsidomonas

Chlorophyceae

Chlorococcales

Chlorococcales

Pyramimonadales

Pyramimonadales

Prasinocladales

Tetraselmis Stein (5)

concentra (Parke et Manton) Norris, Horn & Chihara

condiformis (Carter) Stein

(Carter condiformis (Carter Diels)

(Pseudomonas arietiniae G. S. West)
Monoraphidium
Nephrocytium
Oocystis
griffithi
minutum
mtrablt
Polyedriopsis
pelagica
solitario
argulosum
comsctum
duplex
Brun
haler
(\textit{Sirosimus}
\textit{Stiltnassum}
\textit{Arrkistrodesmus}
apiculatus
acumirratus
armatus
communis
derrticulatus
ecornis
\textit{L.}
W.
Eldet.
Snow
Nageli
\textit{Berkeley}
Meyen
\textit{Chodat}
Lagerhe1m
\textit{Schroterl}
Lemmermann
Hegewald
G.
S.
Smith
Printz
Nygaard
\textit{KB AB}
\textit{SB CB}
\textit{NB RB}
\textit{GF AS}
\textit{BS BB}

-- var. disciformis Chodat
effusus Chodat
prevalens W. & G. S. West
gramus Chodat
intermedia Chodat
\textit{levis} Dobbie
\textit{obtusus} Trevis. Kützng
\textit{var. Mereni}
spicatus P. Richter
sivalis Chodat
\textit{var. Hörtlnapns}
spicatus Chodat
Schoderae Lemmermann
\textit{var. (Schodera) Lemmermann}
(\textit{Levanderesmus} argenosus (Schöder)
G. S. West
Sorocarium Kützing
\textit{sporadum Nageli}
Trehardiae Kützing
\textit{circularis} Hangiig
\textit{lanceolatum} Borge
\textit{minimum} (A. Braun) Hangiig
\textit{planctonicum} G. M. Smith
\textit{quadraanum (Reischl) Hangiig}
\textit{regularis Kützing var. regularis (4)}
-- var. inus. Teiling (4)
\textit{membrum} (Nageli) Hangiig
\textit{Trachura} Bernard
\textit{irregularilactules Bernard}
\textit{Trevirata Kützing}
\textit{bruchkou (Mobius) Lemmermann}

ULGTRICHALES
Elsinoeae Wille
zygodeum Wille (11)
\textit{avinum} Br.-Schott (Printz) (11)
\textit{Klebsormidium} Silva, Mattos & Blackwell
\textit{brevipalma} (Kützing) Silva, Mattos & Blackwell (11)
\textit{Kolodrini} Hinde
\textit{lomninae Hinck.} (\textit{var.} \textit{diaphanum} (46)
\textit{Plaktonema Schmid}
\textit{lauterbornii Schmid}
\textit{(Brun}unlz lauternbornii (Schmid)
\textit{Probstus-Lauter})

ODOGONIALES
Oedogoniae Link ez Him (46)

ZYGOMATALES
Archaeodermata Ehrenberg
\textit{nov. (Benthosum) Hangiig
(1) Requires taxonomic re-investigation.
(2) Halme & Møller (1958) further report the species C. aduanum Grev. and C. monomorium Lemmermann from the Porjusa Bay Sea, however, Pankow (1976: 26).
(3) Species in this genus need taxonomic re-investigation.
(4) See Pankow (1976).
(5) In all areas probably several species are present which need to be worked out.
(6) Recorded as an unidentifiable species in Halfors (1979).
(7) This species has mostly been recorded as sternbaltica (Leyss.) Böttger. In our opinion C. flavoviridis and C. lemmermanni should be kept as separate species. All samples with specimens from the Baltic Sea that have been studied have been A. lemmermanni. It has also been confused with A. baltica (Niemi & Halftors 1974).
(9) Given by Pankow (1976) for the Baltic Sea. The determination needs confirmation.
(10) Recorded from the sea only without algae. Alkalinity formation is at times abundant in certain baltic breakwater rock-pools (Hالفس 1984). See also Warn (1952).
(11) Jannfelt (1964) reports what is probably this species under the name Anabaena circinalis var. circinalis.
(12) Systematic position uncertain.
(13) Thinly plate like as a normal component of the flora in the southern part of the Porjusa and Gult Sea areas (KB).
(14) Easily confused with species of Anabaena and Planktonella.
(15) Accidentally planktonic filaments are easily confused with Diatoma spp.
(16) The species have not been worked out.
(17) The specimens B. Carter and C. mariae (Böttger) Bucher recorded from the shore of Kiel (Böttger 1961).
(18) For Niemi et al. (1975) should be referred to O. punctata.
(19) Recorded twice in the south east Kustadis 1983 (Edler & Wallis, unpubl.).
(20) Given by Pankow (1976) from the western Baltic Sea.
(21) Main occurrence in rock-pools (see Droop 1955) in the western Baltic Sea.
(22) See Swift (1973).
(23) See Perké & Dixon 1978.
(24) Commonly named Diploneis leptica (Niemi & Halftors 1974) and Gymnodinium leptica (Niemi 1971, 1972). Diploneis leptica Bergh (= Gymnodinium leptica (Bergh) Schiller), however, is a different species.

(25) The record is based on the observation of single scales only.
(26) Marzesse & Pankow (1972) and Pankow (1976) in addition record Berghia varians Lemmermann, which, however, probably does not belong here.
(27) Especially in older literature the species has been comprehended in a collective sense, including the other species of the genus (= S. acuta Ehrenberg). Such determinations should primarily be rather given as S. varians var. S. varians the species should be used S. acuta sens. Kalckows. Electron microcopy of the scales in usually required for correct determination of most species of the genus.
(29) In addition, Halme & Møller (1958) mention C. varians. M. Valv. var. algarum Grev. and var. levis (Ehrenberg) Böttger from the intertidal part of the Porjusa Bay Sea.
(30) Lergaard (1920) incorrectly used the name C. debilis for this species.
(31) In the northern Baltic Sea, chains of C. venter with very narrow apertures have previously erroneously been determined as C. oestrina according to Hustedt (1927). 1930, however, generic characters are quite different (see Granfelt 1936).
(32) In addition to valvulae, the species also forms clumps which apparently have been referred to C. ochro- Grev. & Wohlfahrt 1935 and C. jactata Bølsiek (uncited papers).
(33) Niemi et al. (1970) report this species erroneously as C. acutangula Ehrenberg. Apparently the same species is also reported as C. acutangula Ehrenberg in several papers. C. acutangula is characterized by the incised, incised curve of the valve, guide bands tapering towards the end and the wedge-shaped girdle view. C. acutangula and C. acutangula probably do not belong to the recent flora of the northern Baltic Sea.
(34) C. acutangula is probably restricted to the southern and western Baltic Sea. Reports from GB are erroneous (Niemi et al. 1970) or dubious (Halme & Møller 1958. Møller & Tynni 1968).
(36) Records of this species from the open sea (Møller 1962) should probably be ascribed to C. propinqua (Halme & Møller 1958).
(37) See Lergaard (1920). The species is very rare in NB. GF and BS. It should not be confused with the green algae Phaeocystis protococcaea.

REFERENCES


5. Rubin codes of phytoplankton

Code List P2, Phytoplankton, version 84334-GUZ, which was distributed as Annex IX of the Guidelines for the Second Stage of the BMP (amendment to the Baltic Sea Environment Proceedings No. 12, 19 June 1985), should be substituted by the new list published in:

Gunilla Zetterberg
Code List P 4, Phytoplankton
version 86165-GUZ
Published 1986, Stockholm
ISSN 0282-8375
176 pp.

Contact address: 86165-GUZ, Kodcentralen,
Riksmuseet
S-104 05 Stockholm

6. Phytoplankton identification sheets

The phytoplankton identification sheets are under preparation by the expert group convened by Dr. Ulrich Horstmann of the Federal Republic of Germany.

The phytoplankton sheets will be printed in the series Annales Botanici Fennici, Acta Botanica Fennica, published by the Finnish Botanical Publishing Board, Helsinki. The reprint copies will be distributed to the Baltic Sea States through the Secretariat of the Helsinki Commission.
7. Zooplankton

For the Baltic Monitoring Programme (BMP), mesozooplankton (0.2-20 mm) is the only obligatory determinand. However, the undisputed significance of nano- (2-20 µm) and microzooplankton (20-200 µm) in the pelagic ecosystem justifies a recommendation to include them into the Monitoring Programme, but only as a tentative parameter (see also Section D.3.d) and D.3. Annex I).

a) Sampling

Mesoplankton shall be sampled by means of vertical hauls with a plankton net (WP-2 net). The WP-2 net should have a mesh size of 100 µm and should be hauled vertically with a speed of about 0.5 m/s. The hauls shall be fractionated and the number of hauls to be made at each station is dependent on local water stratification. In principle, the following depth intervals shall be used:

- bottom - halocline (included)
- halocline - thermocline (included)
- thermocline - surface

The wire angle must be kept as small as possible during sampling to assure that the whole depth fractions are sampled.

The use of sonars and pingers is recommended to get exact knowledge of the depth position of the net.

Flowmeters can be used, but they are non-obligatory.

Pumps and other sampling devices can be used if calibrated against the WP-2 net for the specific areas and for different times, and the proper conversion factors have been determined. Nano- and microzooplankton should be sampled by means of water samplers or a plankton pump, macro- (2-20 cm) and megazooplankton (20-200 cm) by means of horizontal or oblique hauls with larger nets (like the Bongo net), high speed samplers or small trawls (like the IKMWT, Isaacs-Kidd Midwater Trawl).

b) Preservation

The samples shall be preserved in 4% formaldehyde solution (1 part 40% formaldehyde solution and 9 parts water). The formaldehyde has to be buffered to pH 8-8.2 with disodiumtetraborate (borax) (Na₂B₄O₇ • 10 H₂O).

c) Subsampling

For subsampling the whirling apparatus by Kott (2) or the Folsom sample splitter (3) should be used.

d) Species composition and abundance

When subsampling is needed, the volume of the subsample must be chosen so that at least 500 specimens are retained. Copepods shall be analysed to developmental stage and sex of adult (c. I-III, c. IV-V, c. VI, c. VI O).

Since certain copepod nauplii and rotifers will pass the 100 µm meshes, they can be excluded from the obligatory mesozooplankton analysis.

Macrozooplankton (medusae, mysids, chaetognaths, etc.) are not sampled adequately by the WP-2 net either and thus can be excluded from the obligatory analysis.
e) Biomass

The biomass is calculated as the sum of individual volumes. The list of individual volumes elaborated by BMB WG 14 (4) shall be used.

f) Data reporting

Data shall be reported on standardized formats (according to Sections D.10 and D.11). The abundance and the biomass should be expressed both per m' and per m'. If flowmeters have been used, both the original and the corrected values shall be listed.

g) References in Chapter D.7.


8. Macrozoobenthos

a) Soft bottom macrozoobenthos

The main responsibility for zoobenthos sampling and analysis is as follows:

- Denmark: BMP K7, P1, Q1, R3
- Finland: BMP A2, A3, C1, C4, D1, F2, F5
- German Dem. Rep.: BMP K4, K7, R8, R2
- Poland: BMP K1, K2, L1
- Sweden: BMP I1, K2, K4, R6, R7
- Soviet Union: BMP F2, F5, J2, K1

b) Soft bottom macrozoobenthos analysis

For the purpose of the Baltic Monitoring Programme, the macrofauna is defined as that part of the soft bottom fauna which is retained on a sieve with a mesh size of 1.0 x 1.0 mm (see 8).

Sampling

Sampling on shallow stations (70 m or less) should preferably be conducted during the daytime, since some benthic species have semipelagic activity during the night. It is essential that sampling of macrozoobenthos is accompanied by some hydrographic sampling to provide information about the hydrographic situation. Therefore, as a minimum requirement, water should be sampled as close as possible to the seabottom, or a profile with a calibrated CTD and oxygen probe should be taken.

The widely applied 0.1 m³ Van Veen grab (modified version after 2) has to be used as the standard gear for benthic macrofauna sampling in the Baltic Sea, because of its comparative reliability and simplicity of handling at sea. The grab should weigh about 25-35 kg when emptied. In order to reduce the shock wave caused by lowering the grab, the windows on the upper side shall cover an area as large as possible, in practice around 60% of its upper surface. The windows
shall be covered with metal gauze of 0.5 x 0.5 mm mesh size.

There may be cases where the use of other gear with smaller sampling area is advisable, e.g. if the fauna is very dense and uniform. When other gears than the standard grab are employed, intercalibrations have to be done on a regional basis and on specific sediments on which these samplers will be used. When a change of gear is intended, it is recommended to sample parallel with both gears for a period of 3-5 years.

Means are to be provided for attaching 20 kg of lead to the upper edges of the jaws or inside the grab.

Precautions that must be taken when using the grab:

- The settling down and the closing of the grab must be done as gently as possible. This will reduce the shock wave and the risk of sediment loss as a result of lifting the grab before completed closure.

- The wire angle must be kept as small as possible to ensure that the grab is set down and lifted up vertically.

If, as often happens on sandy bottom or erosion sediments, less than 5 l of sediment is collected, the sample should be regarded as not quantitative, and a new sample should be taken after loading the grab with an extra 20 kg of lead as described above. This may as much as double the effective sampling depth of the grab. If less than 5 l of sediment is still collected, the sample may be used, but the low sample volume should be stressed when results are given (cf. 2, p. 64). The evidence of this problem may be different in different parts of the Baltic Sea, depending on, e.g., how deep in the sediment the species live.

The choice of sample size and number of samples is always a compromise between the need for statistical accuracy and the effort which can be put into the study. One way to do this is to calculate an index of precision. The ratio of standard error to arithmetic mean may be used (3), i.e. \( \frac{s}{x} \) (standard deviation, \( x \) = arithmetic mean, \( n \) = number of samples). A reasonable error would probably be \( \pm 0.2 \pm 20\% \).

\[ D = \frac{1}{X} \sqrt{\frac{s^2}{n}} \quad (D \leq 0.2) \]

On the representative stations, at least five samples should be taken to enable the investigator to reach a certain level of precision by sorting as many samples as necessary. The same procedure is strongly recommended for all other benthos stations unless another sampling strategy (area sampling) is employed in national/coastal monitoring programmes (see also Sections A.1 and A.2).

The depth to bottom must be recorded and documented separately for every sample at the time of sampling. Nevertheless, the depth range shall be kept as small as possible.

Each laboratory shall carefully check the exact sampling area of its grab in order to make possible a correct calculation of the number of individuals per square metre.

Sediment description shall be given by visual observation stating sediment type, colour, and the approx. depth of the oxygenated surface layer (Section D.10, last page).
Sievving

The standard sieve for the Baltic Monitoring Programme shall be of metal gauze (stainless steel, brass or bronze) and have a mesh size of 1.0 x 1.0 mm. In order to collect quantitatively developmental stages of the macrofauna and abundant smaller species it is, however, recommended to use an additional sieve with mesh size of 0.5 x 0.5 mm. This sieve must have the same qualities as the 1 mm sieve. The mesh size of the sieves has to be checked from time to time for damage and wear.

Attention must be paid to the following points:
- Each sample must be sieved, stored and documented separately;
- The volume of each sample must be measured. This can be done by grading the container or by using a ruler;
- The grab has to be emptied into a container and should be brought portion by portion onto the sieve as a sediment - water suspension. The use of sprinklers and hand-operated douches to suspend the sample is recommended. Very stiff clay can be gently fragmented by hand. Between the pourings the sieve must be cleaned to avoid clogging and to ensure an equal mesh size during the whole sieving procedure;
- The sieving of the sample has to be done carefully in order to avoid damage of fragile animals. Therefore, a direct jet of water against the sieve should be avoided;
- Visible fragile animals, e.g. some polychaetes, shall be hand-picked during the sieving; stones and big shells should be picked out to avoid the grinding effect;
- All residues retained on the sieves should be carefully flushed off the sieves with water from below and fixed. Spoons and other tools for sample transfer should be avoided;
- When the 0.5 x 0.5 mm sieve is used, the 0.5 and 1.0 mm sieve fractions must be kept separate throughout all further processing.

Other sampling methods

Dredge hauls may be valuable as a complement to grab samples, since mobile as well as large but comparatively rare species are more easily caught by dredging. This is especially true in areas almost devoid of benthic fauna, where grab samples may be too small to collect the remaining specimens. Dredge samples may also be used for describing the relative dominance. Descriptions of suitable dredges can be found in (4).

In areas where the burrowing depth of the fauna are beyond the penetration depth of the grabs (or that type of gear cannot be used), core samplers may be advisable to use, provided that their efficiency has been satisfactorily proven by intercalibrations. (see Section D.8.b)).

In order to survey a large area and interconnect point-like (station) information on epibenthos, large-scale survey methods such as side-scan sonar and underwater-TV may be used.

Fixation

The hand-picked animals and the sieving residue shall be fixed in buffered 4% formaldehyde solution (1 part 40% formaldehyde solution and 3 parts water). It should be noted that formaldehyde is regarded as toxic and
probably carcinogenic. Therefore, it should be handled with great care and means for waste air exhaustion should be provided for all laboratory procedures. For buffering, 100 g of hexamethylenetetramine (Hexamine = Urotropin) shall be used per 1 dm³ of 40% formaldehyde.

Sodiumtetraborate (= Borax) in excess may also be used.

Preservation and storage

The samples shall be stored in dark jars or in the dark. The pH of the samples is to be checked every third month and adjusted to about pH 7 by the addition of buffer if necessary.

Staining

In special cases, i.e. samples from sandy bottoms, it may be advisable to stain the 1 mm sieve samples to facilitate the sorting process.

When the 0.5 x 0.5 mm sieve is used, this size fraction shall always be stained with Rose Bengal for more effective sorting. The staining shall be done before sorting by:

- washing the sample free from the preservation fluid by using a sieve with a mesh size smaller than 0.5 x 0.5 mm;
- allowing the sieve to stand in Rose Bengal stain (1 g/dm³ of tap water + 5 g of phenol for adjustments to pH 4-5) for 20 minutes with the sample well covered.

However, Rose Bengal (4 g/dm³ of 40% formaldehyde) may be added already to the fixation fluid.

Analyses

Species composition, abundance and biomass should be determined.

Sorting

Sorting should always be done using magnification aid (magnification lamp, Stereo-Microscope). Small portions of the unsorted material shall be put on a 0.5 mm mesh size sieve and washed with tap water. The portions must be flushed into a transparent glass dish of about 20 cm diameter covering the bottom of the dish as only a loose and semi-transparent layer. The material shall be looked through twice by regular scanning. The dish must be shaken before the repeated search.

During the first examination, a piece of dark foil shall be placed under the dish while a piece of light-coloured foil shall be used for the second examination. This ensures equal visibility of light- and dark-coloured animals.

After having sorted each sample (except the first!) the index of precision (3, p. 129; see also the above paragraph on sampling) has to be calculated. When reaching the proposed level of precision (\( D \leq 0.2 \leq 20\% \)) no further sample from this station needs to be sorted. This applies only for the dominant species, each of which takes up more than 10% of the total abundance.

When the 0.5 x 0.5 mm sieve is used, this size fraction shall be sorted by scanning the sample twice under a stereomicroscope. Before sorting, the size fraction could be subsampled using a subsampler described by Dybern et al. (2, page 61).
Broken animals shall only be counted as individuals by their heads (e.g. polychaetes) or hinges of bivalves with adhering pieces of tissue.

**Biomass determination**

The biomass shall be determined as formalin wet weight, formalin dry weight and ash-free dry weight.

The biomass determination shall be carried out for each taxon separately.

During the initial period after fixation the biomass of organisms changes, and therefore formalin wet weight shall be measured not earlier than three months after preservation (1).

The formalin wet weight is obtained by weighing after external preservation fluid is removed on filter paper. While removing the water, the larger animals shall be carefully and delicately turned over on the filter paper. The animals are left on the filter paper until no more distinct, wet traces can be seen. In practice, tiny animals dry within a few seconds, while larger ones take 20 seconds or longer to dry. Shelled animals are weighed together with their shells. In the case of large bivalves (length over 15 mm), the two shells are opened with a scalpel and laid on filter paper. The opened shells must be squeezed so that all the water flows out of the mantle cavity. As soon as the non-tissue water has been removed, the organisms are weighed with an accuracy of 0.1 mg.

The formalin dry weight shall be estimated after drying the formalin material at 60-80°C to constant weight (for 12-24 hours, or an even longer time, as may be necessary for this process). Ash-free dry weight should be estimated after measuring dry weight. It is determined after incineration at 500-520°C in an oven until weight constancy (about 12 hours, depending on sample and object size). The temperature in the oven should be checked with a calibrated thermometer, because there may be considerable temperature gradients (up to 50°C) in a muffle furnace. Caution is advised not to pass a certain temperature (550°C) since then a sudden loss of weight may happen due to the formation of CaO out of the skeletal material of many invertebrates (CaCO₃). This can reduce the weight of the mineral fraction by 44%. This decomposition occurs very abruptly and within a small temperature interval (6).

Before weighing, the samples must be kept in a desiccator, while cooling down to room temperature after drying as well as burning. Ash-free dry weight may also be calculated using the conversion factors published by BMB (5).

**c) Data reporting**

All data should be reported according to details given in Sections D.10. and D.11.

Whenever some taxon found on the sieves is excluded from the reported results, this should be explicitly stated and the reason explained (e.g. Piscicola geometra not included because it is a parasite) on the Plain Language Record. Also results from dredge hauls and any other survey methods should be reported in the Plain Language Record.

A sediment description can be given in the Plain Language Record or, if a more comprehensive sediment analysis has been made, in the reporting format for contaminants in sediments (see Section C.III).
9. Micro-organisms

Methods to be used for microbiological monitoring

- Total number and biomass of bacteria
- Production of bacteria
- Colony-forming bacteria (colony count)

Sampling sites and frequency

On monitoring cruises, including standard stations, microbiological measurements should be performed on the same samples as 14C-primary productivity measurements are made.

For baseline studies near-shore stations (at least one, but preferably three) should be monitored with higher frequency, at least once a month.

Sampling

Water for microbiological analyses should be taken from the same samples as for primary production and chlorophyll-\(a\).

The standard sampling depths for microbiological measurements are: 1 m (2 m), (3 m), 5 m, 10 m, (15 m) and 20 m, non-obligatory depths being given in brackets.

Sampling should preferably be performed in the morning.

Data reporting

All data reporting should follow the biological data reporting format and should be given along with the phytoplankton determinands (primary productivity, chlorophyll-\(a\) and phytoplankton):
for bacteria counts: total number, mean cell volume and the factor for converting cell volume to carbon should be given,

- for production of bacteria: thymidine incorporation and factors for calculating net production should be given,

- for colony-forming bacteria: the number of colony forming bacteria per ml of water sample should be given.

a) Method for measuring total number and biomass of bacteria

The method for measuring total number and biomass of bacteria with Acridine Orange (AO) staining follows essentially the method presented by Hobbie et al. (2). However, one should note that other stains, DAPI (3), ACRIFLAVIN (1) etc. may work better than AO in different types of waters.

Acridine orange direct count (AODC)

Materials

- Scintillation or other glass vials (particle free, heated in 500 °C for 4 hours) for collecting and storing the samples
- Formalin (35-39%), unbuffered
- Sterile filtration units for making particle-free liquids
- Syringes
- Membrane filters
- Nuclepore filters (pore size 0.2 μm)

AO-solution

1. Acridine orange (AO) solution: 0.5 mmol

15.1 mg of AO + 98 ml of deionized or distilled water + 2 ml of unbuffered formalin (35-39%)

- make the solution particle-free by filtration with a disposable filtration unit (pore size 0.2 μm) just prior to staining as described below, or

- make the solution particle-free by filtration, cover it with aluminium foil (solution is light sensitive) and store it in the refrigerator (may be stored up to several weeks).

2. Irgalan black solution

2 g of Irgalan black/one liter of deionized water. Add 20 ml of acetic acid to make the solution 2% acid.

Procedure

Shake the sample well and draw a 20 ml subsample from the sample bottle.

Add 0.5 ml of formalin into the 20 ml sample through the sterile filtration unit. Let a few drops out of the unit before starting to add it into the sample vials.

Store the samples in the refrigerator for further processing. Good preparations are obtained if staining is done within 24 hours after formalin fixation. However, good preparations have also been obtained after storage times of several months.
Irgalan black staining of Nuclepore filters

Stain the Nuclepore filters (25 mm, pore size 0.2 μm) 30 min. in 0.2 μm filtered Irgalan black. Rinse carefully 3 times on 0.2 μm filtered deionized water. Dry the filters (5-10 minutes) and place them back in their case. Use clean, particle-free petri dishes for staining and rinsing.

Sample preparation

Rinse the filtration system thoroughly with sterile water to start the procedure.

Place the Sartorius, Millipore etc. membrane filter (pore size 0.2 μm) on the sinter as the supporter.

Place the Irgalan black stained filter on the wet Sartorius: avoid air bubbles.

Staining procedure

Add 5 ml of particle-free deionized or distilled water into the funnel.

Pipette 1-2 ml of sample water into the funnel (mark the date and filtered volume on the sheet) and filter to dry. Add 2-5 ml of particle-free deionized or distilled water into the funnel and continue filtering to dry.

Stain the sample with 300-500 μl of particle-free AO solution (0.5 mmol). Use the sterile filtration unit. Keep the filtration unit covered with aluminium foil.

Allow two to five minutes for staining with AO.

Allow the filter to dry.

Take off the Nuclepore filter when vacuum is still on and write with a pen at the edge of it: sample label and volume of filtration.

Allow the filter to dry for a few minutes (5-10 min) and place it back into the box.

Store the filters in a dry, dark place (may be stored for several months).

Microscopy

Place a drop of immersion oil (nd.1.515) on the objective glass.

Place the filter (or a part of it if you want to look at it again later) on the oil so that air bubbles are not caught under it.

Place a drop of immersion oil on the filter.

Place a cover slip on the filter without creating air bubbles.

Place a drop of immersion oil on the cover slip. The sample is ready for counting.

Count at least 20 fields (10-20 cells/field). For calculations, you need the filtration surface area and the area of the counting (equation 1).

\[
N \text{ (cell/ml)} = X \cdot A \cdot d \cdot 10^6 \cdot a^{-1} \cdot n^{-1} \cdot v^{-1}
\]

- \(A\) = area filtered (e.g. 193.59 mm²)
- \(d\) = dilution due to formalin (20.5/20) = 1.025
- \(a\) = area counted (e.g. 640 μm²)
- \(n\) = fields counted (e.g. 20)
- \(v\) = volume filtered (1-2 ml)
- \(X\) = total count of cells
Cell volume determination

Bacterial cell size distribution is determined by calibrated ocular graticules e.g. Patterson-Cawood or New Porton Grids (Graticules, Ltd., England). At least 20 fields and the total of at least 50 cells are measured (classified). The cells measured must be selected randomly, e.g. count the cell closest to a predetermined spot of the graticule from each field. Do not hesitate to count 50 fields altogether.

The actual measurement is done with the aid of different size circles on the graticule. For cocci select the circle closest to the size of the organism: With Patterson-Cawood grid, and with about 1200 times magnification the smallest circle is close to 0.2 µm. For rods pick the circle that is closest to the width (W) of the rod and another circle that is closest to the length (L) of the rod.

Cell volumes are counted according to equation 2.

\[ \text{Biovolume } \mu m^3 = \frac{\pi}{4}W^2(L-W/3), \text{ where} \]

- L = cell length
- W = cell width
- For cocci L = W.

Results

Results must be expressed as:

A. Total number of bacteria, cells ml⁻¹ *10⁶ (1.00E6)
B. Mean cell volume, µm³ cell⁻¹, and coefficient of variation (1.00E-3), CV%
C. Factor for converting cell volumes to carbon = 0.35 pgC µm⁻³

Biomass of bacteria = A * B * C

References:


b) Production of bacteria

Method for measuring bacterial net production using Tritiated Thymidine Incorporation

Bacterial production is measured by a modified TTI-method (Tritiated Thymidine Incorporation), Fuhrman and Azam (1) (see Figure D.2.). This procedure was adopted for routine use by Larsen et al. (2).

Materials

\[ ^3 \text{H-methyl-thymidine (20-100 Ci/mmol) } \]
Formalin (35-39%)
Trichloroacetic acid 5% (ice-cold)
Filtration unit (e.g. Sartorius SM 16547 or Millipore 1225 Sampling manifold)
Membrane filter (0.2 µm)
Liquid scintillation equipment
FIGURE D.2. Flow chart of TTI-measurement technique, sample volumes, incubation times and liquid scintillation procedure may vary between sites.

- 25 ml seawater
- 10 nM ³H-thymidine
- 30 min incubation
- 0.8 ml 37% formalin
- 10 ml icecold
- 5% TCA
- 500 μl ethylacetate 30 min
- 10 ml Aqualyte
- 0.2 μm

Procedure

Twenty to twenty-five ml water samples are incubated with 10 nM tritiated thymidine (³H-methyl-thymidine, 20-100 Ci mmol⁻¹) for 30 minutes at in situ temperature. When temperature is below 5°C incubation up to 2 hours may be used.

Incubations should be started immediately after sampling. If storage cannot be avoided, larger amounts of water (e.g. 1 l sample bottles) must be kept at in situ temperature, in a water bath, in the darkness and incubations should be started within an hour.

Three formalin-killed (1% final conc.) blanks are incubated along with three subsamples. Allow 10 min. before adding label to blanks. Incubations are stopped by adding formalin to a final concentration of 1%. Formalin-killed samples are stored in the refrigerator until filtration. A known volume of the samples is filtered through 0.2 μm membrane 25 mm diameter filters (Sartorius SM 111, 113, Millipore, Asypore). The filters are rinsed five times with 1 ml ice-cold TCA (trichloroacetic acid) and again five times with 1 ml TCA with the chimneys of the filtration device removed.

The filters are stored in glass (preferably) or plastic vials and, prior to liquid scintillation counting, solubilized by 30 minutes' treatment with 0.5 ml ethyl acetate. Certain scintillants (e.g. PCS, New England) may make use of a solubilizer unnecessary (with Millipore BS, Sartorius 113 and Asypore). This alternative is to be preferred since ethyl acetate is a strong quenching agent and therefore inaccuracies in pipetting will introduce large errors in counting efficiency.
After addition of the scintillation cocktail, the \(^{3}H\)-activity is assayed by liquid scintillation counting and corrected for quench by external standard channels ratios. Formalin pre-fixed samples are used as blanks. Thymidine incorporation (TI, mol l\(^{-1}\) h\(^{-1}\)) is calculated as:

\[
TI = \frac{dpm_s - dpm_B}{SV \times T \times SA}
\]

where \(dpm_s\) and \(dpm_B\) are the \(^{3}H\)-activities of the sample and blank, respectively, \(SV\) is sample volume (l), \(T\) is incubation time (h), and \(SA\) is specific activity of the \(^{3}H\)-thymidine (dpm mol\(^{-1}\)).

Batch cultures to evaluate the conversion of thymidine into bacterial cell production should be performed for each new sampling site during each of the annual succession stages (e.g. spring, summer and winter). For a discussion on this subject, see (3).

For batch cultures, seawater (250 ml) is filtered aseptically through a 0.22 \(\mu\)m Millipore filter. As an inoculum 25 ml seawater, filtered through 0.6 \(\mu\)m to remove predators, is added. The batches are incubated at in situ temperature for 48 h. Every 12 h subsamples are taken and preserved with formalin for bacterial enumeration by AODC. TI-measurements are carried out at 4-8 h intervals following the procedure described above. Finally, for each 12 h period the integrated thymidine incorporation is compared with the observed increase in cell numbers to obtain an empirical conversion factor from thymidine incorporation to cell production. As a guidance, a conversion factor of \(1.1 \times 10^{18}\) cells mol\(^{-1}\) thymidine incorporated may be applied to coastal marine environments (3). The measured average cell volume and a factor of 0.35 pgC \(\mu\)m\(^{-3}\) should be used to convert production data into carbon terms.

Net production is thus calculated according to:

\[
\text{Net production, mgC m}^{-3}\text{h}^{-1} = TI \times CF \times CV \times CC
\]

where

- \(TI\) = thymidine incorporation, mol l\(^{-1}\) h\(^{-1}\)
- \(CF = 1.1 \times 10^{18}\) cell mol\(^{-1}\)
- \(CV = \mu\)m\(^{3}\) cell\(^{-1}\)
- \(CC = 3.5 \times 10^{-7}\) pgC \(\mu\)m\(^{-3}\)

References

C) Colony-forming bacteria

Determination of the number of colony-forming bacteria

Introduction

The determination of the number of colony-forming bacteria is routinely performed by most of the microbiological laboratories. These measurements do not require special apparatus other than basic equipment. The number of colony-forming bacteria is an easily obtainable parameter which gives some information on the degree of pollution of the water bodies under study. It has been found that the colony-forming
bacteria often react rapidly to environmental stress (2, 3). Thus, in eutrophied and polluted areas, the number of colony-forming bacteria and also its percentage of the total bacterial number are substantially higher than in clean waters.

Since the number of colony-forming bacteria is affected by environmental changes, this implies that also the type of nutrient media, the incubation temperature, etc. influence to a great extent the results. The method, therefore, necessarily requires a rigid standardization.

For the determination of the colony-forming bacteria the "pour plate" and the "spread plate" methods exist. In most cases the results obtained by the two methods are quite similar (4). Since the "pour plate" method is more frequently employed in routine measurements, it is suggested that it be used for microbiological monitoring (1).

Equipment

Microbiological water sampler
- ZoBell-samplers are very convenient.

Dilution vials
- Screw cap test tubes with 9 ml of sterile water having a salinity close to that of the sample may be used.

Sterilized 1.0 ml pipettes

Petri plates of 9-10 cm Ø

Thermostatically controlled water bath
- The flasks containing the nutrient medium should be maintained at 42°C.

Nutrient medium
- ZoBell's medium 2216 E is suggested. This medium contains 5 g of peptone, 1 g of yeast extract and 15 g of agar per liter. The pH of the medium should be 7.6.
- Since the composition of peptone, yeast extract and agar may vary according to the supplier, the products from DIFCO (Detroit) should be used.
- The water used for preparing the medium should be aged sea water with a salinity close to that of the water samples to be studied.
- It is convenient to put 10 ml portions in test tubes, since the test tubes allow rapid liquefying of the medium and afterwards fast maintenance at 42°C. The medium should be sterilized for 20 minutes at 121°C in an autoclave.

Incubator
- The incubator should maintain a temperature of 20°C.

Colony counting device

Sampling depths

The water samples should be taken with the microbial water sampler under sterile conditions from 3 m below the water surface, 3 m above the sediment, and at the depth of the halocline. The depths should coincide with those for phytoplankton and chemical analyses.

Storage

Storage of water samples should be avoided. A storage time of 3 hours, however, in a refrigerator may be tolerable.
**Procedure**

The whole procedure has to be done under aseptic conditions.

1) Samples from the chosen depths are taken and thoroughly shaken.

2) The number of colonies growing on the plates should be between 30 and 300. It may, therefore, be necessary to dilute the water samples with sterile water, with the salinity close to that of the sample water.

The appropriate dilutions are prepared by pipetting 1 ml of the sample to the first dilution vial and 1 ml from there to the second vial and, if necessary, continuing to the third vial. Mix well between the dilution steps. Thus, dilutions up to $10^{-3}$ are obtained.

In near-shore regions, dilutions of the samples of $10^{-2}$ and $10^{-3}$ may be necessary, whereas in the open Baltic Sea the original sample or the $10^{-1}$ dilution may be used.

3) 1.0 ml portions of the water sample or of a suitable dilution is pipetted into 3 sterile Petri dishes.

4) A 10 ml portion of the 42°C warm molten nutrient medium is poured into the Petri dish and mixed with the water sample. This is best done by moving the dish several times in a clockwise and anticlockwise direction. The agar medium will solidify within some minutes.

5) When the agar medium has cooled down to room temperature, the dishes are placed upside down and put together into stacks. These are covered with plastic bags and then incubated at 20°C in the incubator.

6) The colonies are counted after 7 and 14 days of incubation. The first counting is considered only in the case of fungal growth, which usually does not appear before the second week of incubation.

7) The results are reported as number of colony-forming bacteria per ml of water sample.

**References**


10. Biological data reporting format

International Council for the Exploration of the Sea
Revised August 1983

REVISIONS TO THE BIOLOGICAL DATA REPORTING FORMAT

In relation to the July 1981 version of the Biological Data Reporting Format, the following changes have been made:

Section 3.2 Phytoplankton Primary Production

3.2.1 Type Master Record (pp. 6 and 23 in July 1981 version and in this version).

Column 53 has been assigned to "Attenuation determination method".

Columns 54 to 72 are unassigned.

The assignment of columns 70-73 to "Total irradiance (in situ)" and columns 74-77 to "Total irradiance (experimental)" has been deleted, and

Column 73 has been assigned to "Irradiance unit".

Columns 74-77 have been assigned to "Incubator irradiance".

3.2.2 Data Cycle Record (pp. 7 and 24 in July 1981 version and in this version).

Columns 24 to 27 have been assigned to "Relative irradiance".

Columns 45-49 are now assigned to "Potential primary production" (addition underlined).

Columns 50-54 are now assigned to "Daily production per m²" (addition underlined).

Columns 60-62 are now assigned to "Carbon dioxide".

Columns 70-74 have been assigned to "Daily production per m²".

Section 3.3 Chlorophyll a

3.3.1 Type Master Record (pp. 9 and 25 in July 1981 version and in this version).

Column 53 has been assigned to "Calculation method".

Section 3.4 Phytoplankton

3.4.2 Data Cycle Record (p. 11 in both versions).

Column 24: a new code number has been added for the RUBIN Code List PL, Phytoplankton, limnic.

Section 3.5 Mesozooplankton

3.5.2 Data Cycle Record (pp. 14 and 30 in July 1981 version and in this version).

Column 24: a new code number has been added for the RUBIN Code List 01 Baltic invertebrates.

Column 37 should be changed to unassigned from identifier.

Column 38 should be changed to "Developmental stage and sex".

Section 3.6 Zoobenthos

3.6.2 Data Cycle Record (p. 18 in both versions).

Column 24: a new code number has been added for the RUBIN Code List 01 Baltic invertebrates.
INTRODUCTION

This format has been developed in response to a request to the International Council for the Exploration of the Sea by the Interim Baltic Marine Environment Protection Commission for a data reporting format which could be used for biological measurements obtained in the Baltic Monitoring Programme. The format has been designed to interface with the ICES Oceanographic Data Reporting System (see Manual on ICES Oceanographic Punch Cards, 4th Ed. 1979), which is used to report hydrographic and hydrochemical data relevant to an interpretation of the biological results, and has been based on the IOC GP-3 format for the international exchange of oceanographic data.

The Biological Data Reporting Format is intended to be flexible and is amenable for recording and exchange of data via standard forms, punch cards or magnetic tapes.

OVERVIEW OF THE SYSTEM

Four types of records are included in this format: a File Header Record (the BioMaster), a Series Header Record (the Type Master), a Data Cycle Record, and a Plain Language Record (see Figure 1).

FIGURE 1: OVERVIEW OF FORMAT
Note:

For the reporting of data on mesozooplankton, the form labelled "zooplankton" should be used. When data on protozooplankton (tentative parameter) are to be reported, the "phytoplankton" reporting form should be used and the Phytoplankton Type Master Record should be marked with the code Z in column 77.

<table>
<thead>
<tr>
<th></th>
<th>Country</th>
<th>Ship</th>
<th>Station No.</th>
<th>Year</th>
<th>Parameter</th>
<th>Method</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

![Diagram of data entry record format]
The BioMaster Record serves as the file header for the full series of biological data obtained at one station. The first 27 columns of the BioMaster Record are identical to the first 27 columns of the cards used in the ICES Oceanographic Data System so that the relevant hydrographic, hydrochemical and meteorological data may be easily correlated with the biological data obtained at the same station. The remainder of the BioMaster Record is used to record the types of observations made at that station and the number of Type Master Header Records which have been filled in for each observation type.

The Type Master Record serves as the header for the information relevant to the individual types of observations made. At present, five parameters are included in this format, with code numbers as follows:

<table>
<thead>
<tr>
<th>Code No.</th>
<th>Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Phytoplankton Primary Production</td>
</tr>
<tr>
<td>02</td>
<td>Chlorophyll a/Pigments</td>
</tr>
<tr>
<td>03</td>
<td>Phytoplankton</td>
</tr>
<tr>
<td>04</td>
<td>Zooplankton</td>
</tr>
<tr>
<td>05</td>
<td>Zoobenthos</td>
</tr>
</tbody>
</table>

For each parameter measured at a station, one or more Type Master Records will be filled in, according to, e.g., whether one or more than one method of measurement has been used. The first eleven columns are identical to Columns 1-8 and 19-21 of the BioMaster Record to provide for proper identification of the observation series (see Figure 2).

The Data Cycle Record provides the record for the individual data obtained with respect to the relevant Type Master Record. For example, the Data Cycle Record may contain information on the number of individual organisms and their biomass according to species for phytoplankton, zooplankton, and zoobenthos. The first 11 columns are identical to Columns 1-8 and 19-21 of the BioMaster Record, while the next 4 columns serve to correlate the Data Cycle Record with the appropriate Type Master Record (see Figure 2).

The Plain Language Record may be used at any level in the format to insert comments which are, e.g., relevant to the interpretation of the data. The first 11 columns of this record are identical to Columns 1-8 and 19-21 on the BioMaster Record. The next 4 columns are used to identify the type of observation to which a particular Plain Language Record is relevant.

### 3. Detailed Description

#### 3.1. BioMaster Record

The BioMaster Record is the file header for the full series of biological data obtained at one station. The BioMaster identifies what type of information is contained in the file, i.e., which parameters have been measured and how many Type Master Records have been prepared for each parameter.

One BioMaster Record should be filled out for each sampling station according to the scheme given below. The first 27 columns should be filled in with information identical to that given in the corresponding Hydro Master Card for that station. For convenience, part of the scheme concerning these first 27 columns is given here (see page 4); full descriptions can be found in the Manual on ICES Oceanographic Punch Cards (Fourth Edition, 1979).

### Table 1

<table>
<thead>
<tr>
<th>Column</th>
<th>Item</th>
<th>BioMaster Record</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2</td>
<td>Country</td>
<td>Code Description</td>
</tr>
<tr>
<td>3-4</td>
<td>Ship</td>
<td>100 Country Code (see Appendix 1).</td>
</tr>
<tr>
<td>5-8</td>
<td>Station No.</td>
<td>Number of the vessel, according to national number code.</td>
</tr>
<tr>
<td>9-12</td>
<td>Latitude</td>
<td>Consecutive numbers given for one year and one ship (starting anew each year).</td>
</tr>
<tr>
<td>13-17</td>
<td>Longitude</td>
<td>Given to nearest minute.</td>
</tr>
<tr>
<td>18</td>
<td>MESSW</td>
<td>Given to nearest minute.</td>
</tr>
</tbody>
</table>

In this connection, the following rules are observed:

- For latitude, take N when N and E are given, S when S and W are given.
- For longitude, take E when E and W are given.
- Insert last three digits of the year.
- Number of the month. January = 01, etc.
- Day of the month.
- Starting time (to the nearest hour) of the hydrographic station in GMT.

#### Table 2

<table>
<thead>
<tr>
<th>Column</th>
<th>Item</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>19-21</td>
<td>Year</td>
<td>By any name.</td>
</tr>
<tr>
<td>22-23</td>
<td>Month</td>
<td>By any name.</td>
</tr>
<tr>
<td>24-25</td>
<td>Day</td>
<td>By any name.</td>
</tr>
<tr>
<td>26-27</td>
<td>Station time</td>
<td>By any name.</td>
</tr>
<tr>
<td>28-29</td>
<td>Parameter</td>
<td>Information on which parameters have been measured at the station is inserted according to the following code:</td>
</tr>
<tr>
<td>30-31</td>
<td>No of Type Masters</td>
<td></td>
</tr>
<tr>
<td>32-33</td>
<td>Parameter</td>
<td>G1 = Phytoplankton Primary Production</td>
</tr>
<tr>
<td>34-35</td>
<td>No of Type Masters</td>
<td>G2 = Chlorophyll a/Pigments</td>
</tr>
<tr>
<td>36-37</td>
<td>Parameter</td>
<td>G3 = Phytoplankton</td>
</tr>
<tr>
<td>38-39</td>
<td>No of Type Masters</td>
<td>G4 = Zooplankton</td>
</tr>
<tr>
<td>40-41</td>
<td>Parameter</td>
<td>G5 = Zoobenthos</td>
</tr>
<tr>
<td>42-43</td>
<td>No of Type Masters</td>
<td></td>
</tr>
<tr>
<td>44-45</td>
<td>Parameter</td>
<td></td>
</tr>
<tr>
<td>46-47</td>
<td>No of Type Masters</td>
<td></td>
</tr>
<tr>
<td>48-72</td>
<td>Unassigned</td>
<td>Reserved for definition later, as needed. Insert blanks.</td>
</tr>
<tr>
<td>73-77</td>
<td>ICES Station No.</td>
<td>Insert station number according to the International Catalogue of Ocean Data Systems.</td>
</tr>
<tr>
<td>78</td>
<td>Unassigned</td>
<td>Reserved for possible future use for coding purposes. Insert blanks.</td>
</tr>
<tr>
<td>79-80</td>
<td>BioMaster Record</td>
<td>Code</td>
</tr>
</tbody>
</table>

Insert the figure 51.
3.2. Phytoplankton Primary Production

3.2.1. Type Master Record

The number of Type Master Records to be filled out will depend on the number of different methods or conditions used to measure primary production at the station. If only one experimental method (incubator or in situ) is used and if the same \(^{14}C\) counting method and incubation times are used for all samples, then only one Type Master Record should be completed for this parameter. However, if different experimental or counting methods are used or different incubation times or durations are employed, separate Type Master Records should be filled out for each different condition. The means of distinguishing among these variations are contained in Columns 14 and 15, with further specification in Columns 41-48, 49-51 and 52, as applicable.

This Record should be filled out as follows:

<table>
<thead>
<tr>
<th>Column</th>
<th>Item</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2</td>
<td>Country</td>
<td>Fill in same information as in Columns 1-8 on BioMaster Record.</td>
</tr>
<tr>
<td>3-4</td>
<td>Ship</td>
<td></td>
</tr>
<tr>
<td>5-8</td>
<td>Station No.</td>
<td></td>
</tr>
<tr>
<td>9-11</td>
<td>Year</td>
<td>Insert last three digits of the year.</td>
</tr>
<tr>
<td>12-13</td>
<td>Parameter</td>
<td>Insert 01 (code number for phytoplankton primary production).</td>
</tr>
<tr>
<td>14</td>
<td>Method</td>
<td>Insert code number of experimental method used, as follows:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 - incubator</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 - in situ</td>
</tr>
<tr>
<td>15</td>
<td>Specification</td>
<td>If measurements have been carried out using two or more incubation times or temperatures, or if different (^{14}C) counting methods have been used, assign a number for each variation, beginning with 1. If no different conditions have been used, insert a blank.</td>
</tr>
<tr>
<td>16-17</td>
<td>No. of depths</td>
<td>Right justified. Insert the number of depths at which samples have been taken.</td>
</tr>
<tr>
<td>18-23</td>
<td>Depth to bottom</td>
<td>Right justified. Insert depth to bottom in meters, to the first decimal if possible or appropriate. If not, insert a blank in Column 23.</td>
</tr>
<tr>
<td>24-30</td>
<td>Detailed Coordinates (optional)</td>
<td>These columns are available to specify the coordinates of the sampling site in greater detail, if desired. Otherwise, the columns should be filled with blanks.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>If used, the columns should be filled as follows: (see next page)</td>
</tr>
</tbody>
</table>

-125-

<table>
<thead>
<tr>
<th>Column</th>
<th>Item</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>39-40</td>
<td>Sampling time</td>
<td>Insert the time of sampling in GMT to the nearest hour.</td>
</tr>
<tr>
<td>41-46</td>
<td>Incubation time</td>
<td>Insert incubation time to the nearest minute in GMT.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cols. 41-46 beginning of incubation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cols. 45-48 end of incubation</td>
</tr>
<tr>
<td>49-51</td>
<td>Incubation temperature</td>
<td>Insert temperature of incubation in °C to the first decimal place.</td>
</tr>
<tr>
<td>52</td>
<td>Counting method</td>
<td>Insert the method used to count (^{14}C) activity:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 - Liquid scintillation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 - Geiger-Müller</td>
</tr>
<tr>
<td>53</td>
<td>Attenuation determination method</td>
<td>Insert the method used to determine the relative irradiance at the sampling depths:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 - quanta meter</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 - photometer with green filter</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 - Secchi disc</td>
</tr>
<tr>
<td>54-72</td>
<td>Unassigned</td>
<td>Fill with blanks.</td>
</tr>
<tr>
<td>73</td>
<td>Irradiance unit</td>
<td>Insert the unit in which the irradiance in the incubator is given:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 - (10^{18}) quanta m(^{-2}) s(^{-1}) (400 - 700 nm)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 - (10^{18}) quanta m(^{-2}) s(^{-1}) (350 - 3000 nm)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 - Joules m(^{-2}) s(^{-1}) (400 - 700 nm)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 - Joules m(^{-2}) s(^{-1}) (350 - 3000 nm)</td>
</tr>
<tr>
<td>74-77</td>
<td>Incubator irradiance</td>
<td>Right justified. Insert the mean irradiance in the incubator during the experiment (or the mean irradiance during the in situ experiment).</td>
</tr>
<tr>
<td>78</td>
<td>Unassigned</td>
<td>Reserved for possible future use for coding purposes. Insert blank.</td>
</tr>
<tr>
<td>79-80</td>
<td>Type Master Record Code</td>
<td>Insert the figure 32.</td>
</tr>
</tbody>
</table>
3.2.2 Data Cycle Record

One Data Cycle Record should be filled in for each sub-sample studied, as follows:

<table>
<thead>
<tr>
<th>Column</th>
<th>Item</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-15</td>
<td>Unassigned</td>
<td>Fill in same information as in Columns 1-15 of the associated Primary Production Type Master Record.</td>
</tr>
<tr>
<td>16-17</td>
<td>Unassigned</td>
<td>Fill with blanks.</td>
</tr>
<tr>
<td>18-23</td>
<td>Observation depth</td>
<td>Right justified. Insert depth at which sample was obtained in meters, to one decimal place if applicable. Insert a blank if no decimal place used.</td>
</tr>
<tr>
<td>24-27</td>
<td>Relative irradiance</td>
<td>Right justified. Insert the relative irradiance in percentage of the irradiance immediately below the water surface, to one decimal place if applicable. Insert a blank if no decimal place is used.</td>
</tr>
<tr>
<td>28-38</td>
<td>Unassigned</td>
<td>Fill with blanks.</td>
</tr>
<tr>
<td>39-40</td>
<td>No. of parallel samples</td>
<td>Right justified. Insert total number of parallel samples taken at the depth given in Columns 18-23.</td>
</tr>
<tr>
<td>41-42</td>
<td>Sample No.</td>
<td>Insert the number of the particular sample being reported on this record, beginning with 01.</td>
</tr>
<tr>
<td>43</td>
<td>No. of sub-samples</td>
<td>Insert the total number of sub-samples obtained from the sample.</td>
</tr>
<tr>
<td>44</td>
<td>Sub-sample No.</td>
<td>Insert the number of the particular sub-sample being reported on this record, beginning with 1.</td>
</tr>
<tr>
<td>45-49</td>
<td>Potential primary production</td>
<td>Right justified. Insert the corrected maximum potential production in µg C m⁻³ hr⁻¹ to the second decimal place.</td>
</tr>
<tr>
<td>50-54</td>
<td>Daily production per m²</td>
<td>Right justified. Insert the day-integrated actual primary production in µg C m⁻³ day⁻¹ to the second decimal place.</td>
</tr>
<tr>
<td>55-59</td>
<td>Dark fixation</td>
<td>Right justified. Insert in µg C m⁻³ hr⁻¹ to the second decimal place.</td>
</tr>
<tr>
<td>60-62</td>
<td>Carbon dioxide</td>
<td>Insert concentration in µM dm⁻³.</td>
</tr>
<tr>
<td>63-69</td>
<td>Unassigned</td>
<td>Reserved for future definition as necessary. Fill with blanks.</td>
</tr>
<tr>
<td>70-74</td>
<td>Daily production per m²</td>
<td>Right justified. Insert the depth-integrated actual primary production in µg C m⁻² day⁻¹ to the first decimal place.</td>
</tr>
<tr>
<td>75-77</td>
<td>Part counted (optional)</td>
<td>Right justified. Insert as a per cent, with no decimal place.</td>
</tr>
<tr>
<td>78</td>
<td>Unassigned</td>
<td>Reserved for possible future use for coding purposes. Insert blanks.</td>
</tr>
<tr>
<td>79-80</td>
<td>Data Cycle Record Code</td>
<td>Insert the figure 33.</td>
</tr>
</tbody>
</table>

3.3 Chlorophyll a

3.3.1 Type Master Record

The number of Type Master Records to be filled out will depend on the number of different methods or conditions used to determine chlorophyll a at the station. If only one method is used for all the samples, then only one Type Master Record should be completed for this parameter. However, if different solvents, filter types, or measurement techniques (spectrophotometric or fluorometric) are used, then separate Type Master Records should be filled out for each different condition. The means of distinguishing among these variations are contained in Columns 14 and 15, with further specification in Columns 44-52, as applicable.

This record should be filled out as follows:

<table>
<thead>
<tr>
<th>Column</th>
<th>Item</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-8</td>
<td>Unassigned</td>
<td>Fill in same information as in Columns 1-8 on the Master Record.</td>
</tr>
<tr>
<td>9-11</td>
<td>Year</td>
<td>Insert last three digits of the year.</td>
</tr>
<tr>
<td>12-13</td>
<td>Parameter</td>
<td>Insert 02 (code for chlorophyll a).</td>
</tr>
<tr>
<td>14</td>
<td>Method</td>
<td>Insert code number of measurement method, as follows:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 - spectrophotometric</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 - fluorometric</td>
</tr>
<tr>
<td>15</td>
<td>Specification</td>
<td>If measurements have been carried out using two or more types of filters, extraction solvents, or other condition reported on the Type Master Record, assign a number for each variation beginning with 1. If no different conditions have been used, insert a blank.</td>
</tr>
<tr>
<td>16-17</td>
<td>No. of depths</td>
<td>Follow the instructions given for the identical column numbers in the Phytoplankton Primary Production Type Master Record (Sec. 3.2.1).</td>
</tr>
<tr>
<td>18-23</td>
<td>Depth to bottom</td>
<td>Insert time of sampling in GMT to the nearest hour.</td>
</tr>
<tr>
<td>24-30</td>
<td>Detailed coordinates</td>
<td>Right justified. Insert volume of sample in dm³ to one decimal place.</td>
</tr>
<tr>
<td>39-40</td>
<td>Sampling time</td>
<td>Insert the code for the type of filter used, as follows:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 - Whatman GF/F or GF/C</td>
</tr>
<tr>
<td>45-46</td>
<td>Filter diameter</td>
<td>Insert the active filter diameter in millimeters.</td>
</tr>
<tr>
<td>47</td>
<td>Solvent</td>
<td>Insert the code number for the extraction solvent used, as follows:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 - 90% acetone.</td>
</tr>
</tbody>
</table>
3.3.2. Data Cycle Record

One Data Cycle Record should be filled in for each sub-sample, giving the information as follows:

<table>
<thead>
<tr>
<th>Column</th>
<th>Item</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-15</td>
<td>Unassigned</td>
<td>Insert figure 32.</td>
</tr>
<tr>
<td>16-17</td>
<td>Observation depth</td>
<td>Follow instructions given for the identical column numbers in the Phytoplankton Primary Production Data Cycle Record (Sec. 3.2.2).</td>
</tr>
<tr>
<td>24-38</td>
<td>No. of parallel samples</td>
<td>Follow instructions given for the identical column numbers in the Phytoplankton Primary Production Data Cycle Record (Sec. 3.2.2).</td>
</tr>
<tr>
<td>39-40</td>
<td>Sampling time</td>
<td>Follow instructions given for the identical column numbers in the Phytoplankton Primary Production Data Cycle Record (Sec. 3.2.2).</td>
</tr>
<tr>
<td>41-42</td>
<td>Chlorophyll a</td>
<td>Right justified. Insert in mg m(^{-3}) to one decimal place.</td>
</tr>
<tr>
<td>43</td>
<td>Chlorophyll b</td>
<td>Right justified. Insert in mg m(^{-3}) to one decimal place.</td>
</tr>
<tr>
<td>45-47</td>
<td>Phaeopigment</td>
<td>Right justified. Insert in mg m(^{-3}) to one decimal place.</td>
</tr>
<tr>
<td>51-77</td>
<td>Unassigned</td>
<td>Reserved for future definition as needed. Insert with blanks.</td>
</tr>
<tr>
<td>78</td>
<td>Unassigned</td>
<td>Reserved for possible future use for coding purposes. Insert blanks.</td>
</tr>
<tr>
<td>79-80</td>
<td>Data Cycle Record Code</td>
<td>Insert figure 33.</td>
</tr>
</tbody>
</table>

3.4. Phytoplankton

3.4.1. Type Master Record

Normally only one Type Master Record for phytoplankton will be needed for each station. If, however, different preservatives are used, a separate Type Master Record should be completed for each kind of preservative. A description of the record is as follows:

<table>
<thead>
<tr>
<th>Column</th>
<th>Item</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-8</td>
<td>Unassigned</td>
<td>Insert last three digits of the year.</td>
</tr>
<tr>
<td>12-13</td>
<td>Parameter</td>
<td>Insert 03 (code number for phytoplankton).</td>
</tr>
<tr>
<td>14</td>
<td>Method</td>
<td>If agreed method is used, leave blank. If not, insert 9 and describe differences on Plain Language Record.</td>
</tr>
<tr>
<td>15</td>
<td>Specification</td>
<td>Insert blank, unless two or more preservatives have been used.</td>
</tr>
<tr>
<td>16-17</td>
<td>No. of depths</td>
<td>Follow the instructions given for the identical column numbers in the Phytoplankton Primary Production Type Master Record (Sec. 3.2.1).</td>
</tr>
<tr>
<td>18-23</td>
<td>Depth to bottom</td>
<td>Follow the instructions given for the identical column numbers in the Phytoplankton Primary Production Type Master Record (Sec. 3.2.1).</td>
</tr>
<tr>
<td>24-38</td>
<td>Detailed coordina-</td>
<td>Follow the instructions given for the identical column numbers in the Phytoplankton Primary Production Type Master Record (Sec. 3.2.1).</td>
</tr>
<tr>
<td>39-40</td>
<td>Sampling time</td>
<td>Follow the instructions given for the identical column numbers in the Phytoplankton Primary Production Type Master Record (Sec. 3.2.1).</td>
</tr>
<tr>
<td>41</td>
<td>Preservative</td>
<td>Insert according to the following code:</td>
</tr>
<tr>
<td>42-77</td>
<td>Unassigned</td>
<td>Insert last three digits of the year.</td>
</tr>
<tr>
<td>78</td>
<td>Unassigned</td>
<td>Reserved for possible future use for coding purposes. Insert blanks.</td>
</tr>
<tr>
<td>79-80</td>
<td>Type Master Record Code</td>
<td>Insert figure 33.</td>
</tr>
</tbody>
</table>

3.4.2. Data Cycle Record

One Data Cycle Record should be filled out for each species found in each counting aliquot, which is a known fraction of a sub-sample divided for purposes of counting the organisms. The record should be completed as follows:

<table>
<thead>
<tr>
<th>Column</th>
<th>Item</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-15</td>
<td>Unassigned</td>
<td>Insert figure 33.</td>
</tr>
<tr>
<td>16-17</td>
<td>Observation depth</td>
<td>Follow instructions given for the identical column numbers in the Phytoplankton Primary Production Data Cycle Record (Sec. 3.2.2).</td>
</tr>
<tr>
<td>18-23</td>
<td>Physical variables</td>
<td>Follow instructions given for the identical column numbers in the Phytoplankton Primary Production Data Cycle Record (Sec. 3.2.2).</td>
</tr>
<tr>
<td>39-40</td>
<td>Sampling time</td>
<td>Follow instructions given for the identical column numbers in the Phytoplankton Primary Production Data Cycle Record (Sec. 3.2.2).</td>
</tr>
<tr>
<td>Column</td>
<td>Item</td>
<td>Code Description</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>1-15</td>
<td>Fill in same information as in Columns 1-15 of the associated Phytoplankton Type Master Record.</td>
<td></td>
</tr>
<tr>
<td>16-17</td>
<td>Unassigned</td>
<td></td>
</tr>
<tr>
<td>18-23</td>
<td>Observation depth</td>
<td>Right justified. Insert sampling depth in meters, with a blank in Column 23 (reserved for decimal place). If an integrated sample is taken over a range of depths, give minimum and maximum depths according to the instructions given for the identical column numbers in the Zooplankton Data Cycle Record (sec. 3.5.2).</td>
</tr>
<tr>
<td>24</td>
<td>Species code</td>
<td>Insert identification number for species code used, as follows: 1 - USNMDC Taxonomic Code 2 - Plain language abbreviations of Latin name (6 cols. for genus + 6 cols. for species). 3 - RUBIN Code List PL Phytoplankton, Illinois.</td>
</tr>
<tr>
<td>25-36</td>
<td>Species</td>
<td>Left justified. Insert code number of species or plain language abbreviation. Blanks should be inserted in columns not used.</td>
</tr>
<tr>
<td>37-38</td>
<td>Identifiers</td>
<td>Insert code for appropriate identifiers, e.g., developmental stage, sex, size, etc. (Code to be developed later).</td>
</tr>
<tr>
<td>39-40</td>
<td>No. of parallel samples</td>
<td>Follow the instructions given for the identical column numbers in the Phytoplankton Primary Production Data Cycle Record (Sec. 3.2.2).</td>
</tr>
<tr>
<td>41-42</td>
<td>Sample No.</td>
<td></td>
</tr>
<tr>
<td>43</td>
<td>No. of sub-samples</td>
<td></td>
</tr>
<tr>
<td>44</td>
<td>Sub-sample No.</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>No. of counting aliquots</td>
<td>Insert the number of aliquots into which the sample was divided for counting purposes.</td>
</tr>
<tr>
<td>46</td>
<td>Aliquot No.</td>
<td>Insert the number of the particular counting aliquot for which results are being reported on this record, beginning with 1.</td>
</tr>
<tr>
<td>47-49</td>
<td>Magnification</td>
<td>Insert amount of total magnification used in counting the aliquot.</td>
</tr>
<tr>
<td>50-54</td>
<td>Coefficient</td>
<td>Right justified. Insert the appropriate coefficient for the species reported according to the magnification used and other relevant factors, as is needed to express the number of units of the organisms per dm³.</td>
</tr>
<tr>
<td>55-58</td>
<td>No. of counting units</td>
<td>Right justified. Insert the number of counting units of organisms (as defined for each species - cells, clusters, etc.) observed for the species being reported.</td>
</tr>
<tr>
<td>59-63</td>
<td>Cell volume</td>
<td>Insert appropriate cell volume for the units of organisms counted in µm³ using scientific notation according to the following scheme: Col. 59 integer Col. 60-62 decimal places (insert blanks in columns not otherwise filled) Col. 63 exponent Example: cell volume is 435 µm³ change to scientific notation: 4.35 x 10². Insert in columns: 4 3 5 b 2 59 60 61 62 63</td>
</tr>
<tr>
<td>64-68</td>
<td>Plasma volume</td>
<td>Insert plasma volume in µm³ using scientific notation and the same scheme as for cell volume, above, Col. 64 integer Col. 65-67 decimal places (insert blanks in columns not otherwise filled) Col. 68 exponent See example for cell volume.</td>
</tr>
<tr>
<td>69-73</td>
<td>Carbon content</td>
<td>Right justified. Insert in picograms.</td>
</tr>
<tr>
<td>74-77</td>
<td>Unassigned</td>
<td>Reserved for future definition as necessary. Fill with blanks.</td>
</tr>
<tr>
<td>78</td>
<td>Unassigned</td>
<td>Reserved for possible future use for coding purposes. Insert blank.</td>
</tr>
<tr>
<td>79-80</td>
<td>Data Cycle Record Code</td>
<td>Insert the figure 33.</td>
</tr>
<tr>
<td>3.5.</td>
<td>Mesozooplankton</td>
<td></td>
</tr>
<tr>
<td>3.5.1.</td>
<td>Type Master Record</td>
<td>The number of Type Master Records to be filled in will depend on the number of different methods used to obtain and analyze plankton samples at the station. If only one method is used for all samples, only one Type Master Record should be completed for this parameter. If more than one mesh size sampler is used to obtain the samples, for example, then separate Type Master Records should be filled in for each condition. NOTE: At present, this record is intended for data obtained using vertical hauls only. Revisions must be made before data obtained using oblique hauls can be recorded for this parameter.</td>
</tr>
</tbody>
</table>
### 3.5.2. Data Cycle Record

One Data Cycle Record should be filled out for each species found in each sub-sample counted. The record should be completed as follows:

<table>
<thead>
<tr>
<th>Column</th>
<th>Item</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-15</td>
<td>Fill in same information as inCols. 1-15 of the associated Zooplankton Type Master Record.</td>
<td></td>
</tr>
<tr>
<td>16-17</td>
<td>Haul No.</td>
<td>Right justified. Insert the number of the haul from which the sample being recorded was taken.</td>
</tr>
<tr>
<td>18-20</td>
<td>Maximum depth of haul</td>
<td>Right justified. Insert maximum depth in meters.</td>
</tr>
<tr>
<td>21-23</td>
<td>Minimum depth of haul</td>
<td>Right justified. Insert minimum depth in meters.</td>
</tr>
<tr>
<td>24</td>
<td>Species code identification</td>
<td>Insert identification number for species code used, as follows:</td>
</tr>
<tr>
<td></td>
<td>1 - USNODC Taxonomic Code.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 - Plain language abbreviation of Latin name (6 columns for genus + 6 columns for species).</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 - RUSH Code List.</td>
<td></td>
</tr>
<tr>
<td>25-36</td>
<td>Species</td>
<td>Left justified. Insert code number of species or plain language abbreviation. Blanks should be inserted in columns not used.</td>
</tr>
<tr>
<td>37</td>
<td>Unassigned</td>
<td>Insert a blank.</td>
</tr>
<tr>
<td>38</td>
<td>Developmental stage and sex</td>
<td>Insert code for developmental stage and sex:</td>
</tr>
<tr>
<td></td>
<td>1 - copepodite stage I-III</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 - copepodite stage IV-V</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 - adult female</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 - adult male</td>
<td></td>
</tr>
<tr>
<td>39-40</td>
<td>No. of parallel samples</td>
<td>Follow the instructions given for the identical column numbers in the Phytoplankton Primary Production Data Cycle Record (Sec. 3.2.2).</td>
</tr>
<tr>
<td>41-42</td>
<td>Sample No.</td>
<td>Insert the total number of sub-samples in which zooplankton have been counted.</td>
</tr>
<tr>
<td>43</td>
<td>No. of counted sub-samples</td>
<td>Insert the number of the particular sub-sample being reported on this record, beginning with 1.</td>
</tr>
<tr>
<td>45-47</td>
<td>Portion counted</td>
<td>Insert the denominator of the fraction into which the sample has been divided, e.g., 1/64 is recorded as 064; 1/8 is recorded as 008.</td>
</tr>
<tr>
<td>48-51</td>
<td>No. of individuals counted</td>
<td>Right justified. Insert the total number of individual organisms of the species which were counted in the sub-sample.</td>
</tr>
<tr>
<td>52-56</td>
<td>Biomass - wet weight</td>
<td>Insert the wet weight of the individuals counted in µg. Scientific notation should be used and the number recorded as follows:</td>
</tr>
<tr>
<td></td>
<td>Col. 52 integer</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Col. 53-54 decimal places</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Col. 55-56 exponent</td>
<td></td>
</tr>
</tbody>
</table>
### Carbon content

Insert carbon content for the individuals counted in µg. Scientific notation should be used and the number recorded as follows:
- Col. 67 integer
- Cols. 68-69 decimal places
- Cols. 70-71 exponent

### Unassigned

Insert blank.

### Filtration efficiency

For use only when flow meters are employed on sampling nets. Right justified. Insert filtration efficiency in per cent as the ratio between the amount of water passing through the net and the amount of water flowing outside the net.

### Wire angle

If wire during sampling was vertical, insert 00. If wire was not completely vertical, insert approximate angle by which it deviated from the vertical, e.g., the figure 10 would indicate a 10° deviation from the vertical.

### Data Cycle Record

Insert the figure 33.

### Zoobenthos

#### 3.6. Zoobenthos

The number of Type Master Records to be filled in will depend on the number of different methods used to obtain and analyse the zoobenthos samples. If only one method is used, only one Type Master Record should be completed. However, if different methods are used to obtain the samples different mesh sizes or preservatives are used to treat the sample, separate Type Master Records should be filled in for each different condition.

This record should be completed as follows:

<table>
<thead>
<tr>
<th>Column</th>
<th>Item</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-8</td>
<td>Fill in same information as in Cols. 1-8 on the BioMaster Record.</td>
<td></td>
</tr>
<tr>
<td>9-11</td>
<td>Year</td>
<td>Insert last three digits of the year.</td>
</tr>
<tr>
<td>12-15</td>
<td>Parameter</td>
<td>Insert 05 (code for Zoobenthos).</td>
</tr>
<tr>
<td>14</td>
<td>Method</td>
<td>Insert the means of obtaining the sample according to the following code:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 - quantitative grab sampling</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 - diving (scuba)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 - dredge sampling</td>
</tr>
</tbody>
</table>
### Column 15: Specification
If samples have been obtained using more than one type of sampler or if more than one size of mesh or type of preservative has been used on the various samples, assign a number for each variation beginning with 1. If no different conditions have been used, insert a blank.

### Column 16-23: Unassigned
Fill with blanks.

### Column 24-38: Detailed coordinates
Insert detailed coordinates of sampling position, following the instructions given for the identical column numbers in the Phytoplankton Primary Production Type Master Record (Sec. 3.2.1).

### Column 39-40: Sampling time
Insert the time of sampling in GMT to the nearest hour.

### Column 41-42: Sampler
Insert code number for sampler used, as follows:
- 01 - 0.1m² van Veen grab.

### Column 43-46: Sampler area
Right justified. Insert in cm².

### Column 47-50: Mesh size
Right justified. Insert mesh size of sorting sieve in μm.

### Column 51: Preservative
Insert according to the following code:
- 1 - 4% formaldehyde buffered with hexamine
- 2 - 4% formaldehyde buffered with borax
- 3 - 1.4% formaldehyde buffered with borax.

### Column 52-68: Unassigned
Reserved for future definition, as needed. Fill with blanks.

### Column 69-76: Bottom type *
A code system will be developed to describe in detail the characteristics of bottom sediments in the area where the sample was taken.

### Column 77: Unassigned
Reserved for possible future use for coding purposes. Insert blank.

### Column 78-79: Type Master Record Code
Insert the figure 32.

### Data Cycle Record
One Data Cycle Record should be filled out for each species found in each sub-sample counted. The record should be completed as follows:

#### Column 16-17: Unassigned
Insert blanks.

#### Column 18-23: Observation depth
Right justified. Insert sampling depth in meters to one decimal place if possible. If not possible, insert a blank in Col. 23.

#### Column 24: Species identification code
Insert identification number for species code used, as follows:
- 1 - USNODC Taxonomic Code.
- 2 - Plain language abbreviation of Latin name (6 columns for genus + 6 columns for species).
- 3 - RUBIN Code List D Baltic Invertebrates.

#### Column 25-36: Species
Left justified. Insert code number of species or plain language abbreviation. Blanks should be inserted in columns not used. Fill with zeros if sample is totally devoid of fauna.

#### Column 37-38: Identifiers
Insert code for appropriate identifiers, e.g., developmental stage, sex, size, etc. (Code to be developed later).

#### Column 39-40: No. of parallel samples
Follow the instructions given for the identical column numbers in the Phytoplankton Primary Production Data Cycle Record (Sec. 3.2.2).

#### Column 41-42: Sample No.

#### Column 43: No. of sub-samples

#### Column 44: Sub-sample No.

#### Column 45-47: Portion counted
Insert the denominator of the fraction into which the sub-sample has been divided, e.g., 1/8 is recorded as 008.

#### Column 48-51: No. of individuals counted
Right justified. Insert the total number of individual organisms of the species which were counted in the sub-sample.

#### Column 52-56: Formalin wet weight
Insert the formalin wet weight of the individuals counted in mg. Scientific notation should be used and the number recorded as follows:
- Col. 52 integer
- Cols. 53-54 decimal places
- Cols. 55-56 exponent

See examples in the explanation of Cols. 52-56 of the Zooplankton Data Cycle Record (Sec. 3.5.2).

#### Column 57-61: Formalin dry weight
Insert the formalin dry weight of the individuals counted in mg. Scientific notation should be used and the number recorded as follows:
- Col. 57 integer
- Cols. 58-59 decimal places
- Cols. 60-61 exponent

See examples mentioned above.
### 62-66 Ash weight

Insert the ash weight of the individuals counted in mg. Scientific notation should be used and the number recorded as follows:

- Col. 62 integer
- Cols. 63-64 decimal places
- Cols. 65-66 exponent

See examples mentioned under formalin wet weight above.

### 67-71 Carbon content

Insert the carbon content of the individuals counted in mg. Scientific notation should be used and the number recorded as follows:

- Col. 67 integer
- Cols. 68-69 decimal places
- Cols. 70-71 exponent

### 72-77 Unassigned

Reserved for definition later, as needed. Fill with blanks.

### 78 Unassigned

Reserved for possible future use for coding purposes. Insert blank.

### 79-80 Plain Language Record

Insert the figure 33.

#### Code Description

**Column 1-8**

Insert the information contained inCols. 1-8 of the associated BioMaster Record.

**Column 9-11**

Insert the last three digits of the year.

**Column 12-13**

Insert the code number of the parameter to which the record is relevant. If the record is associated with the BioMaster level, insert 00.

**Column 14**

Insert the appropriate figures from the relevant Type Master Records.

**Column 15**

Insert the figure 99.

### Code Description

- **Column 16-77**

Fill in with comments in the English language. If it is necessary to identify the record with a particular sample or sub-sample, the numbers of these should be inserted in Cols. 42-44 and 44, respectively. If further identification to a particular species is necessary, the species code should be inserted in Cols. 32-36.

- **Column 78**

Reserved for possible future use for coding purposes.

- **Column 79-80**

Plain Language Record Insert the figure 99.

### General Remarks for All Records

Except where stated otherwise in the above sections, whenever an element is missing this should be indicated by filling the whole field for that element with blanks.

All columns must be filled for every observation according to the scheme that columns for missing digits before a decimal point should be filled with zeros, while columns for missing digits after a decimal point should be filled with blanks. For example:

- A depth to bottom of 85m is recorded as 00085 blank
- A zooplankton wet weight biomass of \(3.9 \times 10^2\) g is recorded as 39 blank 02.

Many of the code listings for methods, samplers and preservatives are incomplete. Suggestions for additional items for these codes should be mailed to the Environment Officer, International Council for the Exploration of the Sea, Palægade 2-4, 1261 Copenhagen K, Denmark (after 1 January 1980).
<table>
<thead>
<tr>
<th>Country</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Belgium</td>
<td>11</td>
</tr>
<tr>
<td>Canada</td>
<td>18</td>
</tr>
<tr>
<td>Denmark</td>
<td>26</td>
</tr>
<tr>
<td>Finland</td>
<td>34</td>
</tr>
<tr>
<td>France</td>
<td>35</td>
</tr>
<tr>
<td>German Democratic Republic</td>
<td>96</td>
</tr>
<tr>
<td>Germany, Federal Republic of</td>
<td>06</td>
</tr>
<tr>
<td>Iceland</td>
<td>46</td>
</tr>
<tr>
<td>Ireland</td>
<td>45</td>
</tr>
<tr>
<td>Netherlands</td>
<td>64</td>
</tr>
<tr>
<td>Norway</td>
<td>59</td>
</tr>
<tr>
<td>Poland</td>
<td>67</td>
</tr>
<tr>
<td>Portugal</td>
<td>68</td>
</tr>
<tr>
<td>Spain</td>
<td>29</td>
</tr>
<tr>
<td>Sweden</td>
<td>77</td>
</tr>
<tr>
<td>Union of Soviet Socialist Republics</td>
<td>90</td>
</tr>
<tr>
<td>United Kingdom of Great Britain and</td>
<td>74</td>
</tr>
<tr>
<td>Northern Ireland</td>
<td></td>
</tr>
<tr>
<td>United States of America</td>
<td>32</td>
</tr>
<tr>
<td>Parameter</td>
<td>Method</td>
</tr>
<tr>
<td>-----------</td>
<td>--------</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Sample Information**
- **Sample No.:** 1997-10
- **Date of measurement:** 1997-10-01
- **Station No.:** 1997-10-01
- **Ship:**
- **Country:**

**Record Code:**
- **Record code:**
- **Part counted (%):**
- **Daily production (mg C/day):**
- **Carbon dioxide (mM):**
- **Relative nitrification:**
- **Depth to bottom (m):**
- **Incubation time (h):**

**Incubation Conditions**
- **Incubation temperature (°C):**
- **Incubation time (h):**
- **Incubation unit:**
- **Irradiance unit:**
- **Counting method:**
- **Attenuation determination method:**

**Other Information**
- **No. of parallel samples:**
- **Potential primary productivity (mg C/m^2 day):**
- **Daily production per m^3 (mg C/m^3 day):**
- **Relative primary productivity:**
- **Observation depth (m):**

---

*Type the record, code 25.*

*Propagated Primary Production (parameter No. 01)*

---

*Type the code record, code 25.*

*Propagated Primary Production (parameter No. 01)*

---

*Type the record, code 25.*

*Propagated Primary Production (parameter No. 01)*
<table>
<thead>
<tr>
<th>Sample volume (dm$^3$)</th>
<th>Chlorophyll a (mg m$^{-3}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filter type</td>
<td>No. of sub-samples</td>
</tr>
<tr>
<td>Filter diameter</td>
<td>Parameter</td>
</tr>
<tr>
<td>Extraction volume (cm$^3$)</td>
<td>Method</td>
</tr>
<tr>
<td>Cuvette size (mm)</td>
<td>Solution</td>
</tr>
<tr>
<td>Calculation method</td>
<td>Method Specification</td>
</tr>
<tr>
<td></td>
<td>No. of depths</td>
</tr>
<tr>
<td></td>
<td>Depth to bottom</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Country</th>
<th>Ship</th>
<th>Station No.</th>
<th>Year</th>
<th>Parameter</th>
<th>Method</th>
<th>Specification</th>
<th>No. of sub-samples</th>
<th>Chlorophyll a (mg m$^{-3}$)</th>
<th>Phaeopigment (mg m$^{-3}$)</th>
<th>Observation depth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>

| Unassigned | Sampling time |
|            |               |

<table>
<thead>
<tr>
<th>Unassigned</th>
<th>Detailed coordinates</th>
<th>Unassigned</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Unassigned</th>
<th>Record Code</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Unassigned</th>
<th>Record Code</th>
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<th>Record Code</th>
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<tr>
<th>Unassigned</th>
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<table>
<thead>
<tr>
<th>Unassigned</th>
<th>Record Code</th>
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</thead>
<tbody>
<tr>
<td>Country</td>
<td>Ship</td>
</tr>
<tr>
<td>--------</td>
<td>-----</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: The table represents a data collection form for sampling and analysis, capturing various parameters such as country, ship, station number, year, parameter, method, specification, number of samples, number of individuals, wet weight, dry weight, ash weight, carbon content, filtration efficiency, wire angle, and record code.
<table>
<thead>
<tr>
<th>Country</th>
<th>Ship</th>
<th>Station No.</th>
<th>Year</th>
<th>Parameter</th>
<th>Method</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>No. of sub-samples</th>
<th>Sub-sample No.</th>
<th>Year</th>
<th>Parameter</th>
<th>Method</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Observation depth (m)</th>
<th>Species identification code</th>
<th>Species</th>
<th>Identifiers</th>
<th>No. of parallel samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Formalin wet weight (mg)</th>
<th>Formalin dry weight (mg)</th>
<th>Ash weight (mg)</th>
<th>Carbon content (mg)</th>
</tr>
</thead>
<tbody>
<tr>
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</table>

<table>
<thead>
<tr>
<th>Bottom type</th>
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<th>Unassigned</th>
<th>Unassigned</th>
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<tbody>
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<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Detailed coordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<table>
<thead>
<tr>
<th>Record code</th>
<th>(Unassigned)</th>
<th>(Unassigned)</th>
<th>(Unassigned)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Sampler</th>
<th>Sampler area (cm²)</th>
<th>Mesh size (µm)</th>
<th>Preservative</th>
<th>Bottom type</th>
<th>Unassigned</th>
<th>Record code</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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</tbody>
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<thead>
<tr>
<th>Observation depth (m)</th>
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<th>Species</th>
<th>Identifiers</th>
<th>No. of parallel samples</th>
</tr>
</thead>
<tbody>
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<tr>
<th>Formalin wet weight (mg)</th>
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</thead>
<tbody>
<tr>
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<tr>
<th>(Unassigned)</th>
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<table>
<thead>
<tr>
<th>Bottom type</th>
<th>Unassigned</th>
<th>Unassigned</th>
<th>Unassigned</th>
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<tbody>
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</table>

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<tr>
<th>Sampling time</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Detailed coordinates</th>
</tr>
</thead>
<tbody>
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<table>
<thead>
<tr>
<th>Record code</th>
<th>(Unassigned)</th>
<th>(Unassigned)</th>
<th>(Unassigned)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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</tbody>
</table>
SAMPLE DESCRIPTION FORM

<table>
<thead>
<tr>
<th>CHAIN SIZE</th>
<th>GOAL</th>
<th>COLoured</th>
<th>SUBSTITUTE</th>
<th>APPROX.</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

STONES
- GRAVEL (l) coarse
- SAND (sandy)
- CLAY (clayey)
- MUD (mud) (muddy)
- BIANCO (clean)
- ARTHRO (clean)

SOIL: good
- medium
- mixed

NOTES: sample CR

<table>
<thead>
<tr>
<th>INSTITUTE:</th>
<th></th>
</tr>
</thead>
</table>

SUBCORE no:...... Sample depth ......cm Equipment core length core print

DATE & TIME: Observer

DESCRIPTION: colour (Hunsell/USA colour chart)
- smell, H.S
- surface brown oxidized .... cm black grey up colour difference
- fluid layer .... cm disturbed mounts sessile organisms
- ripples height ... cm length ... cm

SURFACES:
- brown oxidized .... cm
- black grey up
- colour difference
- fluid layer .... cm
- disturbed mounts
- sessile organisms
- ripples
- height .... cm
- length .... cm

SEDIMENT STRUCTURE:
- firm
- medium
- soft
- fluid layer
- sketch overlap
- others

FELLETS:
- size cm

TUBES:
- organism: .... cm length .... cm ox open

SHELLS:
- organism(s): .... cm length .... cm layers at cm
- shells broken
- closed

PLANT LIFE:
- type:

BIOTA:
- type:

CONCLUSION:

- notes:
11. Forms to be used in relation to the biological data.
### PHYTOPLANKTON PRIMARY PRODUCTION

**DATA CYCLE RECORD**

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<th>Depth</th>
<th>Observation</th>
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<th>Daily Production</th>
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### CHLOROPHYLL A

**DATA CYCLE RECORD**

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12. Microbiological data reporting format

The microbiological data reporting format will be prepared by ICES, as requested by the Helsinki Commission (HELCOM 9/16, Paragraph 6.9).
BALTIC SEA ENVIRONMENT PROCEEDINGS


No. 2 REPORT OF THE INTERIM COMMISSION (IC) TO THE BALTIC MARINE ENVIRONMENT PROTECTION COMMISSION (1981)

No. 3 ACTIVITIES OF THE COMMISSION 1980


No. 6 WORKSHOP ON THE ANALYSIS OF HYDROCARBONS IN SEAWATER Institut für Meereskunde an der Universität Kiel, Department of Marine Chemistry, March 23 - April 3, 1981 (1982)

No. 7 ACTIVITIES OF THE COMMISSION 1981

No. 8 ACTIVITIES OF THE COMMISSION 1982

No. 9 SECOND BIOLOGICAL INTERCALIBRATION WORKSHOP Marine Pollution Laboratory and Marine Division of the National Agency of Environmental Protection, Denmark, August 17-20, 1982, Renne, Denmark (1983)


No. 12 GUIDELINES FOR THE BALTIC MONITORING PROGRAMME FOR THE SECOND STAGE (1984)

No. 13 ACTIVITIES OF THE COMMISSION 1983

No. 14 SEMINAR ON REVIEW OF PROGRESS MADE IN WATER PROTECTION MEASURES 17-21 October 1983, Espoo, Finland (1985)

No. 15 ACTIVITIES OF THE COMMISSION 1984
- HELCOM Recommendations passed during 1984 and 1985 (1985)

No. 16 WATER BALANCE OF THE BALTIC SEA A Regional Cooperation Project of the Baltic Sea States; International Summary Report (1986)


ACTIVITIES OF THE COMMISSION 1985
- HELCOM Recommendations passed during 1986 (1986)*

BALTIC SEA MONITORING SYMPOSIUM

FIRST BALTIC SEA POLLUTION LOAD COMPILATION
(1987)*

SEMINAR ON REGULATIONS CONTAINED IN ANNEX II OF MARPOL 73/78 AND REGULATION 5 OF ANNEX IV OF THE HELSINKI CONVENTION

SEMINAR ON OIL POLLUTION QUESTIONS

ACTIVITIES OF THE COMMISSION 1986
- HELCOM Recommendations passed during 1987 (1987)*

PROGRESS REPORTS ON CADMIUM, MERCURY, COPPER AND ZINC (1987)

SEMINAR ON WASTEWATER TREATMENT IN URBAN AREAS
7-9 September 1986, Visby, Sweden (1987)

ACTIVITIES OF THE COMMISSION 1987

* out of print