Marine Environmental Quality Committee

ICES CM 1997/E:5

REPORT OF THE

ICES/HELCOM WORKSHOP ON QUALITY ASSURANCE OF PELAGIC BIOLOGICAL MEASUREMENTS IN THE BALTIC SEA

15–19 October 1996
Warnemünde, Germany

International Council for the Exploration of the Sea

Conseil International pour l'Exploration de la Mer

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1 OPENING OF THE WORKSHOP

The ICES/HELCOM Workshop on Quality Assurance of Pelagic Biological Measurements in the Baltic Sea was opened by Professor Bodo von Bodungen on behalf of the host, the Institut für Ostseeforschung in Warnemünde, Germany. Mr Lars Hernroth, Chairman of the Steering Group on Quality Assurance of Biological Measurements in the Baltic Sea (SGQAB), expressed his gratitude for being invited to hold the Workshop at the Institute, a site that during previous Workshops had proven ideal for these types of activities. The Chairman recognized the great help he had received from the local organizers, Mr Lutz Postel and Mr Norbert Wasmund. The participants were welcomed and asked to introduce themselves. The floor was then given to the hosts for information regarding practical matters.

2 ADOPTION OF THE AGENDA

A provisional timetable (Annex 1) for the Workshop activities was presented and adopted with some minor changes. A list of Workshop participants is attached as Annex 2.

3 APPOINTMENT OF RAPPORTEURS

The meeting decided that the Chairman of SGQAB should be responsible for the Report. Summaries from each day's sessions should be given to him from the two session Chairmen, Mr Lutz Postel (zooplankton) and Mr Franciscus Colijn (primary production).

4 TERMS OF REFERENCE

The terms of reference for the Workshop were (ICES C.Res. 1995/3:3) to:

a) *conduct an advanced study course on primary production measurements with the main emphasis on monitoring the state of the Baltic Sea;*

b) *review, under the Chairmanship of Ms G. Behrends, the present Guidelines for the Baltic Monitoring Programme (BMP) in the light of the new Zooplankton Manual produced by the ICES Working Group on Zooplankton Ecology and propose any changes needed to the BMP Guidelines.*

5 BACKGROUND AND AIMS OF THE WORKSHOP

The Chairman of SGQAB presented an introduction in which he pointed out the main aims of the Workshop. He stated that the overall objectives were to demonstrate equipment, sampling, and techniques for analyses for the two parameters mesozooplankton and primary productivity, viewed from the quality assurance aspect. Coupled to the demonstrations, there should be theoretical sessions where specific topics could be highlighted through lectures and discussions. The general outline of the Workshop was for the Primary Production Group and the Zooplankton Group to work in parallel sessions with a few plenary sessions during which items of general interest were presented and discussed. The outcome of these activities should be well-defined recommendations to HELCOM for the revision of the Baltic Monitoring Programme and its Guidelines.

A second task for the Workshop was introduced by HELCOM in late afternoon of the first day. It was an urgent request from the ongoing HELCOM Environment Committee meeting (EC 7) asking the Workshop to comment on a document (EC 7/96, 5/3) on the revision of the Baltic Monitoring Programme. Since this document contained a critical analysis of the present programme where the main core of objectives was discussed, the EC 7 Meeting felt that it must be given high priority during our Workshop. Many points in the document were actually topics that had been covered by the SGQAB during previous meetings and the Workshop welcomed the opportunity to provide HELCOM with its comments. The comments are compiled in Annex 3.

A third objective was to try to find time to discuss the conclusions reached in the HELCOM Third Periodic Assessment of the Baltic Marine Environment. The Workshop was attended by several persons participating in that Assessment.

6 OUTCOME OF THE SESSIONS

6.1 Zooplankton

During the first afternoon, detailed plans for the next day's field activities were made. It was agreed to follow the provisional outline for demonstrating and comparing sampling with WP-2 nets of three different mesh sizes, the Juday net and the Danish plankton pump. The comparison should include both qualitative and quantitative aspects. In addition, there should be a demonstration of German equipment and procedures for flow meters, wire angle measurements, fractionating samples, and preparations for wet mass/dry mass measurements.

A second topic for the first day was to study the videos of sampling that some of the participants had prepared. The purpose of the videos was to illustrate the general handling of nets, winches, closing devices, weights, etc. The video technique has successfully been used for QA matters by colleagues engaged in macrozoobenthos sampling. The video presentations triggered a lively discussion on details in sampling, highly relevant from the QA aspect.

The second day was devoted to field activities onboard R/V 'Alexander von Humboldt'. At a regular monitoring station, the zooplankton group sampled using three different WP-2 nets (55, 100 and 200 µm), the Juday net (160 µm), and the Danish plankton pump (100 µm). Four replicates were taken with each net and the pump, three for splitting and counting, and the fourth for ash-free dry mass determination. It was pointed out that this was not a true intercomparison, merely a practical demonstration of the gears and a possibility to give a rough illustration of the qualitative and quantitative performance of the gears. The results from this demonstration are found in Annex 4.

Following the practical activities, there was a comprehensive discussion on the different parts of the sampling procedure which led to a number of recommendations for clarifications, additions and changes to the present BMP Guidelines. The following items were discussed:

- It was concluded that the weight to be attached to the nets was in many cases too light to keep a vertical wire. The reason for this was obviously the lack of a sufficiently high frame to bring the net and the weight to the level of the deck. A low frame forces the crew to lift the net and the weight by hand, a hard work if one uses the recommended weight of 25 kg (40 kg when the angle tends to exceed 25° (UNESCO, 1968)).

- There were also inconsistencies in the reporting and corrections for wire angles. A simple device for measuring the angle by a clinometer was demonstrated and it was concluded that this step should always be included. A correction table is given in Annex 5.

- It was concluded that the optimum way of using the release mechanism on fractionated hauls is to send the messenger on an upward-moving wire to avoid a firm stop and potential loss of organisms from the net. However, this calls for experience from those handling the messenger to determine the time needed for the messenger to reach the net at the correct depth.

- The question of principles for fractionating the hauls was discussed intensely. It was obvious that the present guidelines leave room for considerable individual interpretations concerning at what depth the net should be closed. This is unacceptable from the QA point of view and the new Guidelines have to be more precise. The difficulties in using the data, experienced in the Third Periodic Assessment, have illustrated this. The general opinion was that the long records of fixed depth intervals must be given new consideration at the expense of the present Guidelines.

- The use of flow meters to measure the volume of water actually filtered was stressed. Presently, many institutes participating in the BMP still do not use flow meters and there are also some inconsistencies as to the correction of volumes in the final calculations of abundance and in the final data reporting. Technical problems of some types of flow meters were also reported, particularly performance during low temperature conditions.

- The towing speed of the winch was very similar among the countries and thus did not call for a change in the Guidelines.

- During the sampling on board, jellyfish appeared in the samples. The way to deal with such situations was discussed and a recommendation was agreed upon.

- Rinsing the net by use of a water hose was not found to be standard procedure on board all ships. It was concluded that such rinsing must be performed on all ships where zooplankton sampling occurs.

- Washing the plankton nets in warm fresh water with a detergent and finally rinsing them in pure fresh water after each cruise was another matter where differences between the countries were observed. It was agreed that this must be a standard procedure in order to keep the nets at optimum filtration capacity.

- Although the results from the sampling of the different gears and mesh sizes will not be available for consideration until later, there was a general opinion that in order to reduce the sources of error, there should be only one type of gear and one mesh size recommended. Not all countries use the recommended WP-2 net, some still use the Juday net, and the Danes use a pump. From a QA standpoint, this is another source of variability and potential error.

- Colleagues working in the southwestern Baltic informed the Workshop of the particular hydrographic conditions in this area. Since high salinity water is confined to layers very close to the bottom, the planktonic fauna in this water is

difficult to sample with the conventional nets. An alternative method would be to use a plankton pump or water bottles for these particular cases, but it was felt premature to propose any recommendations before the aim and structure of the new BMP were known.

- The need for regular training courses (about every five years) for staff engaged in sampling and analyses of zooplankton under the BMP was stressed.

The first part of the third day was devoted to a demonstration of the German method of analyzing and reporting the zooplankton samples. Following this, there was a session where all participants described their way of analyzing and reporting data. These descriptions revealed several inconsistencies and differences among the various laboratories. It was agreed to implement a common analysis and data reporting computer program that should also include a quality control of the data to be delivered to the HELCOM data bank. HELCOM should be approached to finance the development of such a program. Mr Günter Breuel and Mr Harri Kuosa were given the task to try to find an acceptable program based on already existing ones. Their proposal is found in Annex 11.

The performance of the present data bank was discussed. From the colleagues engaged in the Third Periodic Assessment as well as the information in the document from EC 7, it was obvious that the long-term data on zooplankton were very difficult to review and interpret, and there was thus an urgent call for improvements if all the efforts spent collecting the data should not be in vain.

The differences in microscopic analyses of the samples were also covered. It was concluded that there is a potential QA risk associated with the large variety of microscopes that are used within the HELCOM area. Even if the consequences might not be as great as for the analysis of phytoplankton, it was concluded that it should be a goal to standardize these procedures as well.

The last part of the third day was devoted to questions concerning subsampling and the numbers of organisms needed to be counted to obtain a statistically acceptable result. A lecture by Mr. Alexander Korshenko covered published studies on these matters and there was also a practical demonstration of different splitters. This lecture is found in Annex 12. The participants were asked to consider the studies presented in relation to the Guidelines and the document from EC 7 in order to be prepared for the next day's plenary session on recommendations for the revision of the BMP and its Guidelines.

During the last day, a demonstration of the Standard Size Class Method was conducted by Mr Zbigniew Witek. Following this, there was practical training using this method. An abstract of the method is found in Annex 6.

All activities carried out during the zooplankton sessions resulted in a summary which finally led to agreement on a number of recommendations for the revision of the BMP Guidelines.

The last day's sessions compiled all proposals. They were divided into the following sub-divisions:

- QA questions on sampling,
- sample treatment for a) abundance and b) biomass,
- data recording and reporting procedures.

The recommendations for revision of the BMP Guidelines are given in Annex 7 .

6.2 RUBIN Code

During the Workshop, a paper discussing the use of the RUBIN Codes was distributed for comment. The Zooplankton Group concluded that the RUBIN Codes are useful, but only as long as there is a continuous updating of the Codes. The fate of this system is presently unclear and it was stressed that there is a need for a rapid decision on the future handling of the Codes. A further consideration is the system to be used in the new OSPAR monitoring. Several participants favoured a joint system. This matter could be brought up during the next joint Steering Group meeting at ICES in February 1997.

6.3 Primary Production

All participants introduced themselves and mentioned their experience with the present ^{14}C method. After that, the meeting of the group started with an introduction by the chairman (F. Colijn, Germany) on the programme of the meeting. This included, according to the agenda, the following points:

- discussion of the field measurements;

the demonstration of the ^{14}C method using the standard 'ICES incubator' developed by the chairman together with several colleagues in the ICES Working Group on Phytoplankton Ecology, in which also colleagues from HELCOM countries were participating.

It was agreed to use the sea-going trip for measurements of irradiance profiles (for results, see Annex 8), which are needed to calculate the vertical attenuation coefficients, in conjunction with P-I measurements. Also, water samples were to be taken to use for subsequent incubations in the laboratory. It was decided to take the opportunity to run a test with the incubator to show its possibilities. The measurement of global irradiance was discussed later in more detail.

After setting these practical arrangements, the different aspects of the ^{14}C incubation technique and the additional data needed were explained by the chairman. The P-I incubator technique, including the measurement of global irradiance and incorporation of vertical attenuation coefficients, was discussed.

The incubators were shown during a short visit through the facilities in the IOW.

The next day was used for the field trip during which a document from the EC 7 meeting in Riga was discussed with all participants. This document was not available before the meeting, but was transmitted to Lars Hernroth during the meeting; nor was it part of the terms of reference of the meeting. The participants however agreed that as an expert group they were willing to discuss and comment on this paper. The outcome of this discussion is presented in Annex 3, including recommendations for improvements and/or changes to the BMP.

On Thursday, a demonstration of the incubator technique including all the different steps was given by one of the originators. The results of the P-I incubation using a water sample from the chlorophyll maximum at station 46 is given in Annex 9. The two-hour incubation showed that this technique gives reliable estimates of the P-I relationship which subsequently can be used to calculate potential production, daily production, and further can be used in estimates of annual production when enough incubations are performed over the year.

In between and after the practical exercise, several other aspects were dealt with, as discussed below.

Susanna Hietanen presented an overview of the results of the BMP questionnaire on primary production measurements. These results showed that only very few institutes follow the present guidelines for the measurement of primary production, and that very different procedures are used by the institutes involved in measuring primary production. Therefore, to increase the comparability of the measurements, a decision needs to be taken on this matter. This can only be guaranteed if participants are willing to adopt one single method for monitoring. A concept for this method will be worked out later.

In a second presentation, Harri Kuosa replacing Juha-Markku Leppänen introduced the Finnish Algaline project which includes high frequency observations on a wide spatial scale with automatic recording of several parameters on board ships of opportunity such as the Finnjet ferry between Helsinki and Travemünde.

The technical set-up as well as results of the measurements were presented and discussed.

The meaning of the very high fluorescence and chlorophyll values should be further evaluated.

It was recommended that the coordinators of the Algaline project be asked to explain the meaning of the extreme values in fluorescence in terms of spatial patchiness in physiological differences of phytoplankton populations.

A third contribution was given by Odd Lindahl on the different definitions of primary production and the possible meaning of new and regenerated production. The importance of new production in some areas was illustrated using examples of studies in the Gullmarfjord in Sweden, where primary production and sedimentation (exported production) have been measured simultaneously. Effects of eutrophication were presented in terms of higher sedimentation and possible consequences for oxygen deficiency.

Finally, the agenda for the next day was discussed and agreements were made on the preparation of a list of possible problems by Lars Edler. The following topics were discussed: the report section prepared by the chairman for the EC 7 meeting, the merits and pitfalls of sedimentation traps, the presentation of the results of the incubator experiment, and a final consideration of the present guidelines and the possible improvements needed. The points left open so far are sampling (depths), incubator techniques, particulate versus total production, and the whole complex of data handling, including a procedure for the calculation of production parameters.

The results of the discussion on a protocol for primary production measurements are compiled in Annex 10, which was agreed by the participants. All the different steps in the procedure were discussed and only a few questions could not be answered.

7 ADOPTION OF THE DRAFT REPORT

At the last plenary session, the Chairman presented the draft report to the Workshop and it was adopted with minor changes to be included.

8 CLOSING OF THE WORKSHOP

The Chairman thanked the hosts at the Institut für Ostseeforschung for providing excellent meeting facilities and hospitality. We particularly thank Prof. von Bodungen and the staff at the Institute for the generous service provided. Mr Wasmund was asked to deliver a special message thanking the crew of 'Alexander von Humboldt' for their kind assistance. The meeting also recognized generous support from the German Ministry of the Environment. The Chairman then thanked all participants for their contributions and brought the Workshop to a close.

ANNEX 1

AGENDA

Quality Assurance Workshop 1996

PRIMARY PRODUCTION

Chairman: Prof. Franciscus Colijn

TUESDAY 15 OCTOBER

13:00 h	*PLENARY*: opening of the Workshop, general introduction. Synchronizing next day's shiptime activities with the Zooplankton Group
14:00 h	Preparing specific Agenda for the activities, preparation of two different incubators in the laboratory
15:00 h	Coffee break
15:30 h	Loading equipment on board RV 'Alexander von Humboldt'; preparation of incubators continues

WEDNESDAY 16 OCTOBER

08:30 h	Departure to the vessel
09:00 h	Departure from the harbour, then water sampling and training in light measurements according to specific Agenda
16:00 h	Return to the harbour, transport to the Institute
16:30 h	Unloading, then presentation of and discussion on different types of incubators
20:00 h	Dinner

THURSDAY 17 OCTOBER

09:00 h	Practicing the incubator technique
10:30 h	Coffee break
11:00 h	Lecture on the state-of-the-art of Primary Production measurements within the BMP and results from the Finnish Primary Production Questionnaire
12:00 h	Lunch
13:30 h	Lecture on High Frequency Measurements
14:15 h	Presentation of the Finnish 'Baltic Sea Algaline Project'
15:00 h	Coffee break
15:30 h	Calculations of and discussion on the incubation results including drawing of light profiles, training on making P-I curves, calculation of daily production, interpretation of Pmax

FRIDAY 18 OCTOBER

09:00 h	Continued practicing of incubator technique and if needed, more incubations.
10:30 h	Coffee break
11:00 h	Lecture on New and Regenerated Production
12:00 h	Lunch
13:30 h	Lecture on the use of Sediment Traps
14:15 h	Discussion on revisions to the present BMP Guidelines and further activities regarding primary production measurements and Quality Assurance
15:00 h	Coffee break
15:30 h	Outlining of a Preliminary Report

SATURDAY 19 OCTOBER

09:00 h	*PLENARY*: Presentation of results from the two subgroups, suggestions for improving the BMP Guidelines, questions raised from the floor
10:30 h	Coffee break
11:00 h	Presentation of the two Draft Reports
12:00 h	Closing of the Workshop

AGENDA

Quality Assurance Workshop 1996

MESOZOOPLANKTON

Chairman: Dr Lutz Postel

TUESDAY 15 OCTOBER

13:00 h	*PLENARY*: opening of the Workshop, general introduction
	Synchronizing next day's shiptime activities with the Primary Production Group
14:00 h	Preparing specific Agenda for the zooplankton activities including priorities
15:00 h	Coffee break
15:30 h	Loading equipment on board RV 'Alexander von Humboldt'

WEDNESDAY 16 OCTOBER

08:30 h	Departure to the vessel
09:00 h	Departure from the harbour, then field sampling and demonstrations according to specific Agenda
16:00 h	Return to the harbour, transport to the Institute
16:30 h	Unloading, storing of samples
20:00 h	Dinner

THURSDAY 17 OCTOBER

09:00 h	Demonstration of three types of splitters
09:30 h	Lecture on the efficiency of different mesozooplankton splitters
10:30 h	Coffee break
11:00 h	Training on the use of different splitters
12:00 h	Lunch
13:30 h	Continued training on splitting samples
	Qualitative and quantitative analysis of the samples from the pump and the WP-2 net
15:00 h	Coffee break
15:30 h	Participants' Video presentations
16:00 h	Discussion on splitting and sampling techniques in the light of Quality Assurance

FRIDAY 18 OCTOBER

09:00 h	Presentation of the 'Standard Size Class Method' (SSCM) for biomass determination
09:30 h	Training on the 'SSCM'
10:30 h	Coffee break
11:00 h	Discussion on biomass determination in the light of Quality Assurance
12:00 h	Lunch
13:30 h	Discussion on the revision of the BMP Guidelines, further activities on QA for monitoring and research on zooplankton
15:00 h	Coffee break
15:30 h	Outlining of a Preliminary Report

SATURDAY 19 OCTOBER

09:00 h	*PLENARY*: Presentation of results from the two subgroups, suggestions for improving the BMP Guidelines, questions raised from the floor
10:30 h	Coffee break
11:00 h	Presentation of the two Draft Reports
12:00 h	Closing of the Workshop

ANNEX 2

LIST OF PARTICIPANTS

ICES/HELCOM Workshop on Quality Assurance of Pelagic Biological Measurements in the Baltic Sea 15–19 October 1996, Warnemünde (IOW), Germany

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ANNEX 3

COMMENTS FROM THE QA GROUP ON THE EC 7/96, 5/3 DOCUMENT

The Group was of the opinion that the problems described in the document from the seventh meeting of the HELCOM Environment Committee (EC) concerning missing information, missing or inadequately covered parameters, and inadequate BMP strategy are serious and therefore need considerable attention. Accordingly, the Group prepared the comments contained in this Annex.

The objectives and aims of the HELCOM BMP are still the same:

- to enable the assessment of the environmental state of the Baltic Sea including the identification of risks, and
- to identify changes, i.e., spatial and temporal variations, developments, tendencies and statistically significant trends

The problems of missing information and data discarded because of incorrect data reporting are those which can be dealt with immediately, irrespective of the new BMP. Our Group is of the opinion that the data reporting procedures for our parameters should be fully standardized. The Third Periodic Assessment has shown that many data had to be discarded because of incorrect reporting formats. This is a very serious waste of both money and effort. A uniform, quality controlled procedure would also facilitate the use of the data bank to a much higher extent than is the case today. Scientists seldom use the data of others because of these difficulties, indicating that the data bank is not used to the extent that it should be.

Missing information in the form of national data not being available or difficult to convert to a format comparable to that of others is also an organizational matter. It is associated with both data reporting formats and standardized methodology in sampling and analysis, both of which are closely connected to Quality Assurance procedures within the laboratories and between the laboratories. As coastal monitoring is to be integrated into the BMP, the synchronization of methodology and data reporting should be given high priority.

The primary question driving most of the monitoring work within the BMP has been, and probably will continue to be, the question of whether eutrophication is affecting the environmental quality of the Baltic Sea, locally or as a whole. Although nutrient inputs tend to decrease (perhaps not yet everywhere), the question coming up soon is how fast the system will recover to a more natural state. To follow this process, there is a need to concentrate on those areas which are most affected at present. These regions need to be defined on the basis of the present assessment. To follow the subsequent changes, one needs to continue to use the BMP methods, which have been shown during previous assessments to give reliable results. It does not make sense to change these methods drastically because, if these methods would be changed, the present reference data can no longer be used. If large changes or alterations are introduced, old and new methods should be intercalibrated and run parallel for 1–2 years. This test should be performed by a few laboratories only, to keep the additional costs limited.

Finally, as coastal monitoring is about to be included in the next phase of the BMP, it is of vital importance that the methodologies applied are the same as those for the open sea programme to as large an extent as possible.

RECOMMENDATION 1: continue the ongoing measurements, with improvements where needed (see below).

In the document from EC BETA transmitted from the EC 7 meeting, several problems with and critical remarks on the present BMP are listed. It is our feeling that most of these problems can be solved if the frequency of the time trend measurements can be increased to such a degree that the outcome of the measurements can be properly interpreted. This statement holds for the present parameters for phytoplankton. Chlorophyll is used as an indicator of phytoplankton biomass, primary production as the indicator for the conversion of nutrients into biomass and as the only rate measurement, species composition as an index of phytoplankton diversity and changes therein, phytoplankton biomass and as a means to detect new, introduced and possibly toxic species. Moreover, several improvements have already been suggested by the Phytoplankton Working Group.

The problem raised with the primary production measurements can be solved when a standard procedure is adopted by all HELCOM partners. The basis for such a standard technique is available through the work of the ICES Working Group on Phytoplankton Ecology; a manuscript on this method (Colijn *et al.*) was presented during the 1996 ICES Annual Science Conference and has now been distributed to all participants. During the workshop, all participants will

have the opportunity to work with this method, which originally was set up as an inexpensive and simple method for monitoring primary production.

The second point of critique can also be solved, i.e., that the frequency of the measurements is too low. Of course, primary production measurements are very useful if the frequency is kept at or increased to at least fifteen measurements per year at a few stations (offshore) and more than twenty per year in coastal areas to be able to observe different states of eutrophication. This kind of frequency is needed to enable calculation of annual primary production and trend analysis. In several reported cases (Arkona Sea, Eastern Gotland Sea), the frequency of measurements is already large enough to fulfill the requirements of the BMP. In other cases, the frequency is also high enough but the temporal coverage over the productive seasons is insufficient. Therefore, HELCOM is asked to organize the time schedule of the national cruises in such a way that this problem can be solved.

RECOMMENDATION 2: apply measurements of highly dynamic parameters with a high frequency.

Cooperative cruises sharing manpower and ship time can solve the problem of too high a work load for one group or nation/institute.

RECOMMENDATION 3: to incorporate the Finnish procedure to use ships of opportunity to obtain on-line information on phytoplankton dynamics. This information partly answers questions on possible changes in the primary part of the food web structure in the Baltic.

To solve the problem of large spatial heterogeneity and to obtain enough spatial coverage over the Baltic Sea, the introduction of the Finnish method of obtaining phytoplankton information by ships of opportunity should be further considered as an extension to the present programme. It also enables other scientists, because of the on-line information obtained, to study events which otherwise can hardly be covered in 'normal' operational monitoring programmes. The Finnish experience should be incorporated into the new monitoring programme. It would be useful to have another two or three transects (east-west) to increase the probability that the most important events are observed. The meaning of the present high exceptional peaks and events needs further investigation. The system also offers the opportunity to identify toxic blooms in surface layers in an early warning operational manner.

RECOMMENDATION 4: to include the measurement of silicate inputs and silicate concentrations in coastal regions as a mandatory parameter.

The strong linkage of the programme to eutrophication issues makes it necessary to include also silicate as a mandatory parameter. The arguments given in the EC BETA document speak for themselves. We support the idea that silicate plays an important role in eutrophication questions and effects on food web structure. Therefore, silicate should be taken into the programme as soon as possible.

RECOMMENDATION 5: to continue monitoring phytoplankton species composition and biomass.

Several arguments were raised in favour of continuation of the phytoplankton species monitoring, including measurement of phytoplankton biomass: they provide information on possible changes in the food web, or are a causal factor for food web changes. They give information on new and toxic species. However, the strategy should be changed and only surface waters should be sampled, plus an additional sample during stratification in deeper water. This should be registered using a CTD profile. The suggestion to revise and correct the present lists of species contained in the HELCOM data bank by a group of taxonomists was strongly supported.

RECOMMENDATION 6: to perform a revision of the list of species contained in the HELCOM data bank by a group of taxonomic experts in order to assure comparability of the whole data set.

An important argument to do this as soon as possible is that the originators can still be tracked and questioned on problematic identifications.

RECOMMENDATION 7: to investigate the possible power of sedimentary chlorophyll measurements to answer the questions related to the fluxes and transport of organic material to the sea bottom, including the fate of primary production and food availability for benthic organisms.

As a possible innovative method, the measurement of sedimentary chlorophyll was discussed. This would be a relatively easy, inexpensive method, but because of the uncertainty about the origin of the chlorophyll, a group of experts should

discuss this before a decision to incorporate this method can be taken. Moreover, this method does not represent a rate measurement, therefore setting limits on its use.

The issue of using sedimentation traps to measure pelagic-benthic coupling and to obtain a time-integrated value for new/export primary production was also discussed. The value of using sedimentation traps was accepted, but to keep the programme within financial limits this method can only be used at a few selected sites. This programme in offshore areas may be particularly helpful for understanding the trend of oxygen concentrations in deeper waters.

As an extension to the present programme, the measurement of bacterial production was discussed. These measurements would only make sense if primary production data were also available. However, the group has too little experience or expertise to answer this question. (As such, the method to measure bacterial production by thymidine incorporation is well established, so that from a technical point of view it would be possible.) A drawback is the high variability in bacterial occurrence. Therefore, more information is needed concerning which specific questions could be answered by introducing this method.

Arguments can be made for incorporating *in vivo* fluorescence as a further step in resolving the spatial distribution of phytoplankton. In those areas where steep gradients occur (e.g., eutrophied waters or upwelling areas), this method, which can easily be automated, may give very useful additional information.

As a final point, joint cruises were discussed. For monitoring purposes, these cruises would not be very helpful because of their low frequency and inter-annual variability, but for scientific reasons and quality assurance such cruises are of great importance.

A general statement from the Primary Production Subgroup on the revision of the BMP pelagic monitoring strategy

Following the argumentation in the document of EC BETA, we would stress that the questions to be answered are:

1. **How will the phytoplankton community respond to the expected decrease in nutrient concentrations? How is biodiversity of phytoplankton affected?**

The optimum parameters to be measured to answer these questions are:

- primary production;
- quantitative species composition;
- biomass (measured as chlorophyll-*a*);
- sedimentation rate of organic matter (export production).

The optimum strategy for each of these parameters is:

- a high frequency programme, use of the same sampling depths for phytoplankton parameters.

2. **How will decreased phytoplankton (production, biomass, species composition) affect the food availability for herbivore mesozooplankton?**

The optimum parameters to be measured to answer these questions are:

- primary production;
- quantitative species composition;
- biomass (measured as chlorophyll-*a*);
- sedimentation rate of organic matter (export production).

The optimum strategy for each of these parameters is:

- a high frequency programme, use of the same sampling depths for phytoplankton parameters.

3. Have new phytoplankton species been introduced to the Baltic Sea and what are their effects?

The optimum parameters to be measured to answer these questions are:

- quantitative species composition.

The optimum strategy for this parameter is:

- a high frequency programme, use of the same sampling depths for phytoplankton parameters.

4. Has the occurrence of toxic and potentially toxic species increased?

The optimum parameters to be measured to answer these questions are:

- quantitative species composition.

The optimum strategy for this parameter is:

- a high frequency programme, use of the same sampling depths for phytoplankton parameters.

5. What quantitative contribution does phytoplankton make in the sedimentation of organic matter and to what extent is sedimentation of phytoplankton involved in oxygen deficiency in deeper basins?

The optimum parameters to be measured to answer these questions are:

- primary production;
- biomass (measured as chlorophyll);
- sedimentation rate of organic matter.

The optimum methods for each of these parameters are:

- for primary production: the standard ^{14}C method using the protocol and incubator as developed by the ICES Working Group on Phytoplankton Ecology (ref. Colijn *et al.*);
- for chlorophyll: the standard Jeffrey and Humphrey method from the BMP Guidelines;
- for species composition: light microscopy with the inverse microscope, counting according to a standard protocol.;
- for sedimentation rate: sediment traps.

ANNEX 4

RESULTS FROM FIELD TEST OF FOUR PLANKTON NETS AND ONE PLANKTON PUMP

Assembled by Lutz Postel
Institute of Baltic Sea Research, Warnemünde, Germany

Zooplankton was sampled off Warnemünde (54°28.013 N, 12°12.840 E). The echosounder indicated a depth of about 26 m. The wind was weak, the weather slightly rainy.

We used five different gears:

- WP-2 nets, equipped with 100 µm mesh, as recommended in the HELCOM Guidelines of 1984 and 1988, and another with 55 µm mesh and finally one with 200 µm mesh (the latter is the original WP-2 net, tested and recommended by UNESCO (1968) for open ocean conditions).

- A submersible pump (Møhlenberg, F., 1987) equipped with a small net of 100 µm nylon gauze. This is used by the Danish scientists involved in the HELCOM monitoring programme.

- A Juday net, equipped with a 160 µm gauze. This has been used by laboratories in the eastern Baltic Sea for many years.

Hauls were done from 4 m above the bottom (22 m) up to the sea surface. The pump collected plankton closer to the bottom compared to the nets. Towing velocities were about 0.6 m/s, quite near the HELCOM (1988) recommendations. All gears were equipped with calibrated flow meters, the WP-2 nets with TSK flow meters, and the Juday net with Hydrobios type (including reversal break). Wire angles were determined with a clinometer and never exceeded 10°. Therefore, no wire length correction was needed to reach the depth of 22 m. The wire angle depends on the drifting of the ship and on the weight used. We used 30 kg, which is in the range of the recommendations of UNESCO (1968).

Samples for the analysis of species composition, abundance and the calculation of wet mass were collected three times to diminish the influence of patchiness. The three samples of each gear were pooled and analyzed by one person. One additional sample was taken with each gear for biomass determination by weighing successively the fresh sample, the oven-dried sample (about 16 hours at 60 °C), and the remaining part of the sample, ashed at 500 °C .

The sampling was mainly done as a practical demonstration and evaluation of the different steps. The results, which are included in following tables, are only single observations but may be helpful for a preliminary evaluation.

Table A4.1. Amount of filtered sea water, total abundance, biomass in terms of calculated wet mass, directly measured wet mass, the comparison between both, finally the dry mass, the ash-free dry mass, and the ash content.

Sampler type	Average amount of filtered water (m^3)	Filtered water (m^3) according to wire length of 22 m and net opening area	Abundance (ind/m^3)	Calculated wet mass (g/m^3) using individual wet mass (according to PC soft-ware of KAHMA KY, Helsinki, SF)	Measured wet mass (g/m^3)	Measured dry mass (mg/m^3)	Measured ash-free dry mass (mg/m^3)	Calculated wet mass vs. measured wet mass	Ash %	Remarks
WP-2 (55 µm)	3.89	5.5	24 998	0.33	1.78	197	173	0.2	12	many *Ceratium* spp.
WP-2 (100 µm)	4.08	5.5	29 146	0.46	0.82	82	72	0.6	12	many *Ceratium* spp.
WP-2 (200 µm)	5,16	5.5	4 815	0.24	0.22	33	30	1.1	9	
pump (100 µm)	0.76		15 018	0.20	0.42	50	25	0.7	50	sand particles, fewer *Ceratium* spp. than in WP-2 (100 µm)
Juday (160 µm)	2.79	2.2	6 717	0.18	0.11	11	9	1.7	18	

The following results seem obvious:

- The original WP-2 net (mesh size 200 µm) and the Juday net (160 µm) had the best filtration performance, shown by the relationship between the volume of filtered sea water, calculated by wire length and multiplied by net opening area on the one hand, and those measured by flow meter on the other.

- The pump filtered a significantly smaller amount of water compared to the nets. This could increase the risk of not catching the rare species. According to Table 2, this seems to have been the case. The pump sampled five species less than the WP-2 net equipped with the same mesh size.

- The larger particles were less abundant than smaller ones.

- The WP-2 (100 µm) retained twice the number of organisms compared to the pump (100 µm). This might be caused by (1) **loss (escapement) of organisms through the meshes**, e.g., young stages of *Oithona similis,* and/or (2) **damage** of the fragile organisms, such as *Oikopleura dioica* and gastropod larvae, in both cases as a result of the comparatively larger filtration pressure of the pump. A third possibility could be (3) **avoidance** from the small pump entrance (e.g., *Acartia tonsa* (female, male, older copepodites) and *Centropages hamatus* females (cf. Table 2).

- Calculated wet mass includes only zooplankton. The comparison between calculated and the directly measured wet mass of (total) sample is satisfying for the nets of larger mesh size (160 µm and 200 µm); in the 55 µm and 100 µm mesh nets, the directly measured wet mass is remarkably larger than the calculated mass, probably caused by a significant amount of phytoplankton (*Ceratium* spp.) and/or sand particles in the case of pump (50% ash content; cf. Table 1).

The most abundant **nauplii** (*Oithona similis* and *Pseudocalanus* + *Paracalanus*) are best represented in the catch of the **55 µm WP-2- net**, followed by the 100 µm WP-2-net. The WP-2-net (55 µm) also showed the highest abundance of *Oikopleura dioica*. *Oithona similis* nauplii showed the above-mentioned effect of loss through the meshes during pump sampling (Figure A4.1).

The most abundant **copepodites** (*Oithona similis* and *Pseudocalanus* + *Paracalanus*) are best represented in the catch of the **100 µm WP-2- net**, followed by the 55 µm WP-2-net and the pump (100 µm).

The **adult copepods** are retained the best by larger-sized gauze **(200 µm)**, as expected.

The relatively high abundance of *Centropages hamatus* males in the 55 µm mesh and of females of the same species and of *Acartia tonsa* in the 100 µm mesh might be an overestimation. Only 4 to 6 organisms per sample were counted, divided by a lower amount of filtered water, compared to the case of the 200 µm mesh sample, where 17 and 39 individuals were behind the calculations.

Table A4.2. Differences in species composition in the samples from the different gears. Abundance of 36 taxonomic groups, which were caught in at least one of the gears used, sorted descending per gear.

	WP-2 (55 µm)		WP-2 (100 µm)		Pump (100 µm)		Juday (160 µm)		WP-2 (200 µm)	
1	Oithona_N	5652	Oithona_J	5877	Oithona_J	3236	Centham_J1	1283	Acarton_F	806
2	PseuPa_N	5223	PseuPa_J1	5224	PseuPa_J1	2945	Oithona_J	780	Centham_J2	496
3	PseuPa_J1	2826	Oithona_N	3591	PseuPa_N	2400	PseuPa_J1	604	Centham_M	352
4	Oithona_J	2740	PseuPa_N	3102	Centham_J1	1236	Acarton_F	579	PseuPa_J2	310
5	Oikopleura	1627	Centham_J1	1388	Oithona_N	945	Biv_L	428	Oikopleura	289
6	Acar_N	1199	Biv_L	1306	Biv_L	655	Centham_N	352	Oithona_F	269
7	Centham_J1	1113	Centham_N	1061	Centham_J2	618	Centham_J2	302	Centham_J1	207
8	Centham_N	856	Centham_J2	980	Centham_N	509	Oithona_F	277	Acarton_M	207
9	Centham_M	428	Oikopleura	816	Oithona_F	364	Acarton_M	252	Centham_F	185
10	Biv_L	428	PseuPa_J2	735	PseuPa_J2	364	Acar_J1	252	Acarbi_M	165
11	Centham_F	343	Oithona_F	490	Acar_N	255	Acar_J2	252	Podon	165
12	Oithona_F	257	Centham_F	490	Centham_M	218	Centham_M	176	Paracal_F	165
13	Acarbi_M	171	Acarton_F	490	Acar_J1	145	Centham_F	151	Acar_J1	145
14	Acarton_F	171	Podon	327	Oithona_M	145	Oikopleura	126	Acar_J2	145
15	Acarton_M	171	Gastrop_L	327	Centham_F	109	Cyphon_L	126	Cyphon_L	124
16	Acar_J1	171	Centham_M	327	Temo_N	109	Acarbi_F	126	Oithona_J	103
17	Centham_J2	171	Acarton_M	327	Paracal_F	109	Bala_N	126	Biv_L	103
18	PseuPa_J2	171	Temo_J2	245	Acarton_F	73	Acarbi_M	75	Bala_N	103
19	Temo_N	171	Cyphon_L	245	Acarton_M	73	PseuPa_J2	75	PseuPa_J1	83
20	Oithona_M	171	Acar_N	245	Podon	73	Temo_N	75	Oithona_M	62
21	Podon	171	Acar_J2	245	Temo_J1	73	Temo_J1	75	Temo_J1	62
22	Gastrop_L	171	Polychaet_L	163	Polychaet_L	73	PseuPa_N	50	Paracal_M	41
23	Cyphon_L	171	Paracal_F	163	Pseudo_F	73	Paracal_F	50	Acarbi_F	41
24	Acarlon_F	85	Oithona_M	163	Temo_J2	73	Pseudo_F	50	Pseudo_F	41
25	Paracal_F	85	Bala_N	163	Oikopleura	36	Oithona_M	25	Temo_J2	41
26	Paracal_M	85	Temo_J1	82	Cyphon_L	36	Acarlon_F	25	Gastrop_L	21
27	Temo_J1	85	Temo_F	82	Paracal_M	36	Temo_J2	25	Polychaet_L	21
28	Polychaet_L	85	Paracal_M	82	Bala_N	36	Oithona_N	0	Pseudo_M	21
29	Acarbi_F	0	Evadne	82	Acarbi_M	0	Acar_N	0	Temo_M	21
30	Acar_J2	0	Eurytem_J1	82	Gastrop_L	0	Podon	0	Evadne	21
31	Pseudo_F	0	Acarbi_M	82	Acarlon_F	0	Gastrop_L	0	Oithona_N	0
32	Pseudo_M	0	Acarbi_F	82	Acarbi_F	0	Paracal_M	0	PseuPa_N	0
33	Temo_F	0	Acar_J1	82	Acar_J2	0	Polychaet_L	0	Acar_N	0
34	Temo_M	0	Temo_N	0	Pseudo_M	0	Pseudo_M	0	Centham_N	0
35	Temo_J2	0	Temo_M	0	Temo_F	0	Temo_F	0	Temo_N	0
36	Eurytem_J1	0	Pseudo_M	0	Temo_M	0	Temo_M	0	Acarlon_F	0
37	Evadne	0	Pseudo_F	0	Eurytem_J1	0	Eurytem_J1	0	Temo_F	0
38	Bala_N	0	Acarlon_F	0	Evadne	0	Evadne	0	Eurytem_J1	0

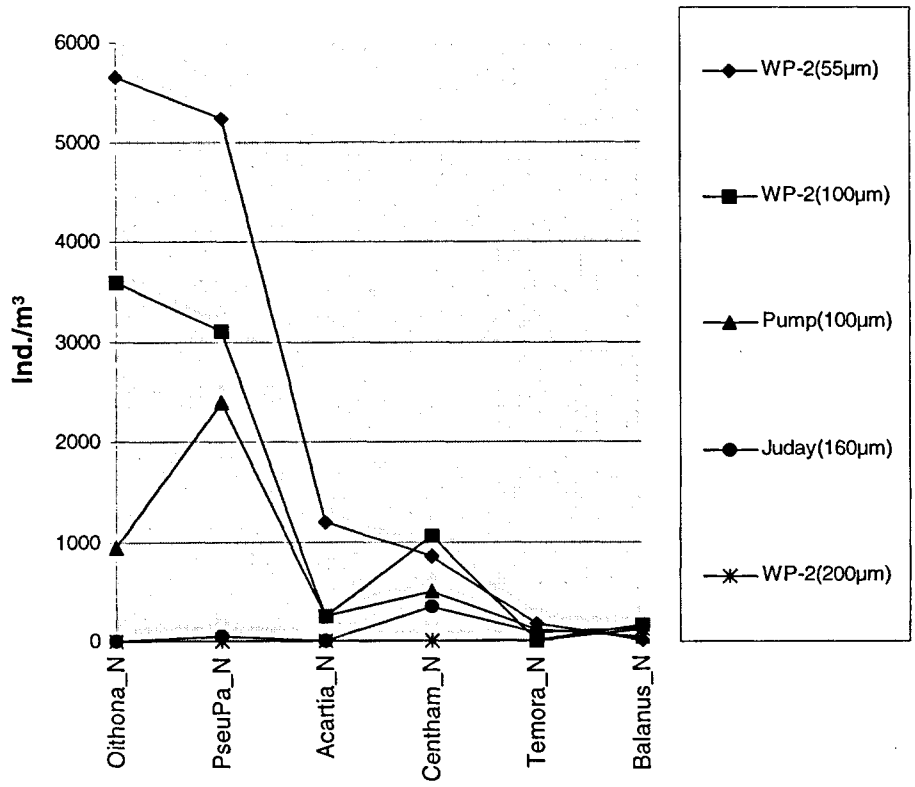

Figure A4.1. Abundance of nauplii in the samples caught by the different gears (cf. legend).

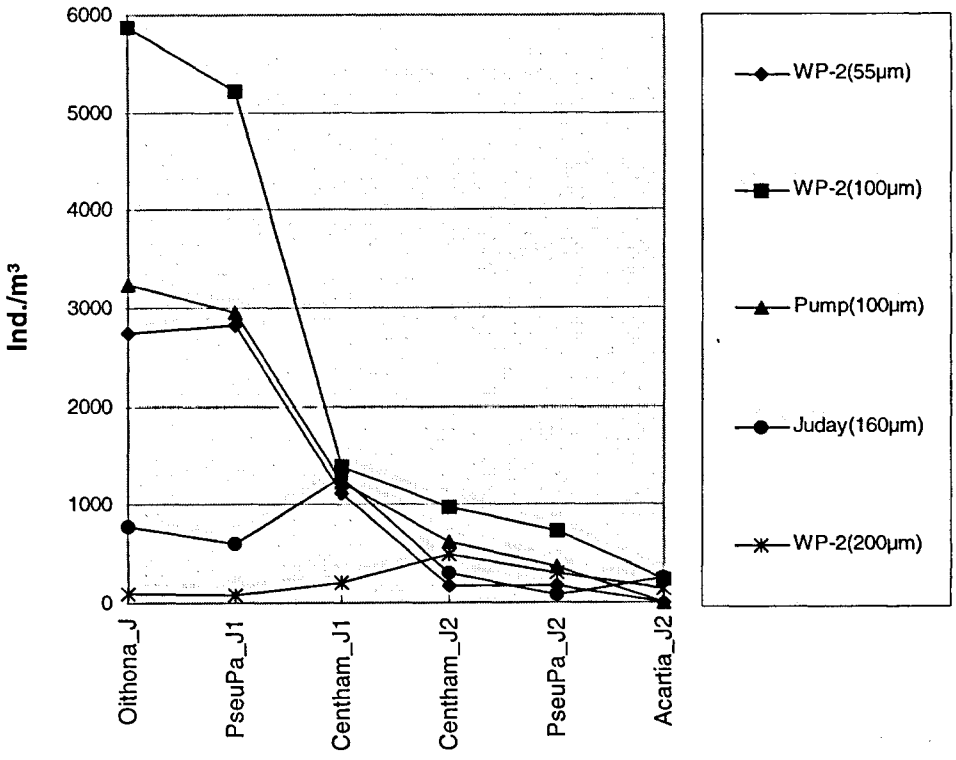

Figure A4.2. Abundance of copepodites in the samples caught by the different gears (cf. legend).

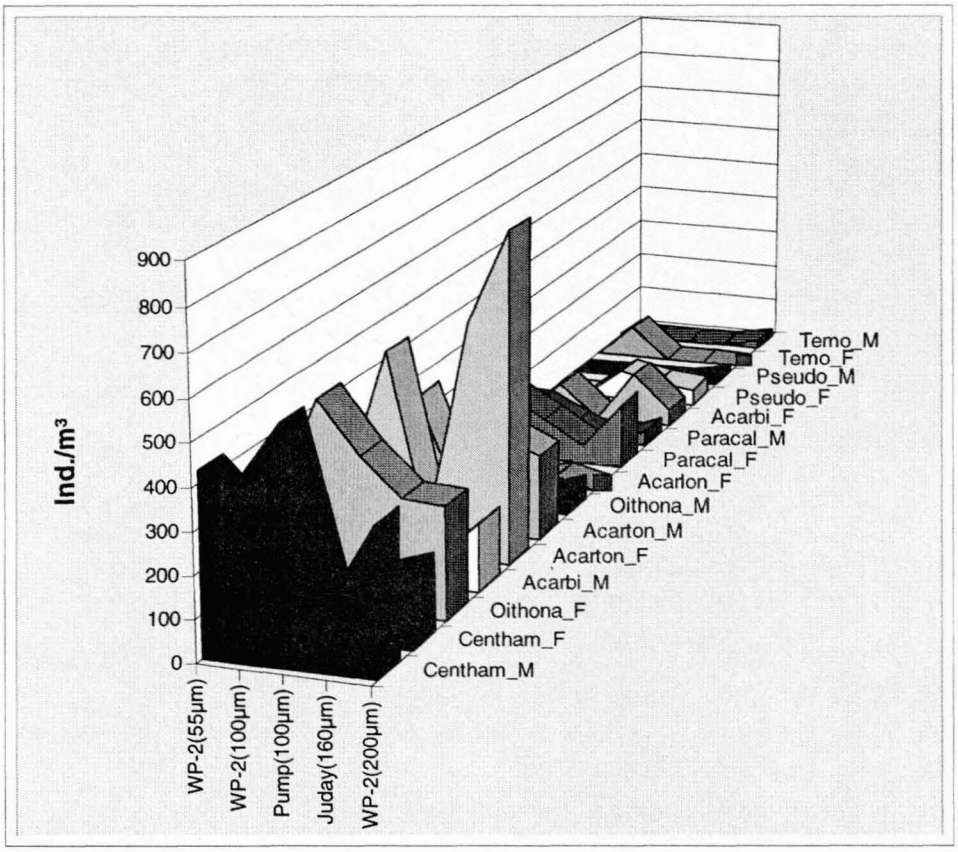

Figure A4.3. Abundance of adult copepods in the samples caught by the different gears.

Conclusions

The WP-2-nets equipped with a gauze of 55 µm and 100 µm mesh, respectively, do not have the optimal filtration performance. This promotes the avoidance of larger mesozooplankton, especially if phytoplankton clog the meshes. The geometry of the WP-2 net (100 µm), which is currently used in accordance with the HELCOM Guidelines (1984, 1988), should be optimized. Its geometry was originally designed for gauze of 200 µm mesh size and for the conditions of the open ocean (UNESCO, 1968).

The under-sampling of the nauplia (and in other seasons probably also the rotifers, which are in the same size range) by escapement from the 100 µm net is well known. It can only be avoided by the use of a smaller mesh size.

A combination of size fractionated samples from bottles for organisms ≤200 µm and from WP-2-net (200 µm mesh size) for organisms >200 µm, as recommended by UNESCO (1968), p. 174, would be the alternative for a proper collection of mesozooplankton and their developmental stages.

Although several discrete bottle samples could be pooled for the strata in which the net collects an integrated sample, the effort required would increase in both sampling and analysis.

A properly designed net of 55 µm mesh size, with an optimal filtration efficiency, catching nauplia and rotifera quantitatively, could be the choice instead of water bottles. It would exclude the problems of water samplers (time-consuming procedure, discrete sampling; limited amount of filtered water).

The pooling of size-fractionated samples, which are caught with the appropriate gear, would be the best solution, at least for studies of food web structure.

The current use of the submersible pump (100 µm) increases the above-mentioned problems: small organisms, which are retained by a 100 µm net, will escape owing to the higher water pressure. Fragile organisms will be damaged for the same reason. Additionally, mobile organisms avoid the small entrance of the pump, as partly described by Møhlenberg (1987). The lesser amount of filtered water seems to increase the loss of rare species. The advantage of near-bottom sampling includes the risk of touching the bottom by wave-dependent movements of the ship and thus it increases the amount of inorganic material in the sample. Some of the problems could be solved by proper handling.

The direct measurements of zooplankton biomass (drying, weighing) are affected by phytoplankton contamination, at least in nets with finer meshes (55 µm and 100 µm). The reduction by the determined phytoplankton mass via chlorophyll and phaeopigment determination and the successive use of conversion factors between the pigment content and carbon (Lorenzen, 1968) have already been used in eutrophic waters (e.g., Postel, 1990) and could be helpful.

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ANNEX 5

TABLE FOR CORRECTION OF DEPTH FROM WIRE ANGLE

Depth z, including trigonometric wire angle (α) correction, according to: $z = z_1 / \cos \alpha$						
depth z_1	wire angle α					
(m)	5°	10°	15°	20°	25°	30°
5	5	5	5	5	6	6
10	10	10	10	11	11	12
15	15	15	16	16	17	17
20	20	20	21	21	22	23
25	25	25	26	27	28	29
30	30	30	31	32	33	35
35	35	36	36	37	39	40
40	40	41	41	43	44	46
45	45	46	47	48	50	52
50	50	51	52	53	55	58
55	55	56	57	59	61	64
60	60	61	62	64	66	69
65	65	66	67	69	72	75
70	70	71	72	74	77	81
75	75	76	78	80	83	87
80	80	81	83	85	88	92
85	85	86	88	90	94	98
90	90	91	93	96	99	104
95	95	96	98	101	105	110
100	100	102	104	106	110	115
110	110	112	114	117	121	127
120	120	122	124	128	132	139
130	130	132	135	138	143	150
140	141	142	145	149	154	162
150	151	152	155	160	166	173
160	161	162	166	170	177	185
170	171	173	176	181	188	196
180	181	183	186	192	199	208
190	191	193	197	202	210	219
200	202	203	207	213	221	231

ANNEX 6

STANDARD SIZE CLASSES (SSC) METHOD

Organisms are classified into standard (fixed) size classes of width 0.3 $\log_{10}(V)$ (where V is a body volume), on the basis of their length and length:width (or length:diameter) proportions, with the help of a specially designed worksheet (Table 1). In this worksheet, lengths of bodies of different shapes but of the same fixed volume, corresponding to the boundaries between classes, are given. For use with a particular microscope, such a worksheet may be recalculated in a way that the dimensions are expressed in divisions of the eye-piece measuring plate, instead of in µm (mm) (Table 2). Volume (wet weight) which is assigned to the individual size class is a geometric mean of the lower and upper boundaries of the size class. For example, the mean volume (wet weight) in the size class from

$1*10^6$ µm^3 to $2*10^6$ µm^3 (1– 2 µg) is

$(1*10^6 * 2*10^6)^{1/2} = 1.414*10^6$ µm^3 (1.414 µg).

The width of size classes (0.3 $\log_{10}(V)$) is almost equal to 1 $\log_2(V)$, which means that the volume (wet weight) at the upper class boundary is two times greater than at the lower boundary, as well as that the mean volume (wet weight) in class n+1 is two times greater than in class n.

Such a worksheet can be extended to every size range and can serve as a uniform basis for the whole community size structure studies.

TABLE 1

micrometres	Size Class No.:	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
	LOG [V,µm^3]:	4.8	5.1	5.4	5.7	6	6.3	6.6	6.9	7.2	7.5	7.8	8.1	8.4	8.7	9
	Volume,[10^6 µm^3]:	0.063	0.126	0.251	0.501	1	2	4	8	16	32	63	126	251	501	1000
	Weight:	63ng	126	251	501	1µg	2	4	8	16	32	63	126	251	501	1mg
	Mean weight:	89ng	178	355	708	1.41µg	2.82	5.62	11.2	22.4	44.7	89	178	355	708	
	L:D															
sphere	1 diameter:	49.4 µm	62.2	78.3	98.6	124	156	197	248	312	392	494	622	784	987	1.24
ellipsoid	1.25 length:	57.3 µm	72.2	90.8	114	144	181	228	287	362	455	573	722	908	1.14 mm	1.44
	1.5	64.7 µm	81.5	103	129	163	205	258	324	408	514	647	815	1.03 mm	1.29	1.63
	1.75 Bosm	71.7 µm	90.3	114	143	180	227	286	359	453	570	717	903	1.14	1.43	1.80
	2 npl	78.4 µm	98.7	124	156	197	248	312	393	495	623	784	987	1.24	1.56	1.97
	2.25 Temo	84.8 µm	107	134	169	213	268	338	425	535	674	848	1.07 mm	1.34	1.69	2.13
	2.5	91.0 µm	115	144	182	229	288	362	456	574	723	910	1.15	1.44	1.82	2.29
	2.75 Cent, Pseu	97.0 µm	122	154	193	244	307	386	486	612	770	970	1.22	1.54	1.93	2.44
	3	103 µm	129	163	205	258	325	409	515	648	816	1.03 mm	1.29	1.63	2.05	2.58
	3.25 Acar	108 µm	136	172	216	272	343	431	543	684	861	1.08	1.36	1.72	2.16	2.72
	3.5	114 µm	143	180	227	286	360	453	571	718	904	1.14	1.43	1.80	2.27	2.86
	4	124 µm	157	197	248	313	394	495	624	785	989	1.24	1.57	1.97	2.48	3.13
	5	144 µm	182	229	288	363	457	575	724	911	1.15 mm	1.44	1.82	2.29	2.88	3.63
	7	181 µm	228	286	361	454	572	720	906	1.14 mm	1.44	1.81	2.28	2.86	3.61	4.54
	10	229 µm	289	363	457	576	725	913	1.15 mm	1.45	1.82	2.29	2.89	3.63	4.57	5.76
	14	287 µm	361	455	572	721	907	1.14 mm	1.44	1.81	2.28	2.87	3.61	4.55	5.72	7.21
	20	364 µm	458	577	726	914	1.15 mm	1.45	1.82	2.30	2.89	3.64	4.58	5.77	7.26	9.14
	28	455 µm	573	722	909	1.14 mm	1.44	1.81	2.28	2.87	3.62	4.55	5.73	7.22	9.09	11.44

A. bifilosa	W=0.0224*L^2.75 where W - formalin wet weight (mg) and L - total length (mm)	L:	323 µm	415	534	686	882	1134	1457	1873	
A. longiremis	W=0.0183*L^2.43		302	402	534	709	943	1252	1664	2211	
C. hamatus	W=0.0246*L^2.73		309	398	513	661	851	1096	1412	1819	
P. minutus elor	W=0.0248*L^2.63		295	384	499	649	843	1097	1426	1855	
T. longicornis	W=00230*L^2.59		298	389	508	663	866	1131	1476	1928	

TABLE 2

1 div. = 20 µm	Size Class No.:	17	18	19	20	21	22	23	24	25	26	27	28	29	30
	Weight:	63ng	126	251	501	1µg	2	4	8	16	32	63	126	251	501	1mg
	Mean weight:	89ng	178	355	708	1.41 µg	2.82	5.62	11.2	22.4	44.7	89	178	355	708	
	L : D															
sphere	1 diameter:	2.5	3.1	3.9	4.9	6.2	7.8	9.8	12.4	15.6	19.6	24.7	31.1	39.1	49.3	62.0
ellipsoid	1.25 length:	2.9	3.6	4.5	5.7	7.2	9.1	11.4	14.4	18.1	22.8	28.7	36.1	45.4	57.2	72.0
	1.5	3.2	4.1	5.1	6.5	8.1	10.2	12.9	16.2	20.4	25.7	32.4	40.7	51.3	64.6	81.3
	1.75 Bosm	3.6	4.5	5.7	7.2	9.0	11.3	14.3	18.0	22.6	28.5	35.9	45.2	56.8	71.6	90.1
	2 npl	3.9	4.9	6.2	7.8	9.8	12.4	15.6	19.6	24.7	31.1	39.2	49.4	62.1	78.2	98.5
	2.25 Temo	4.2	5.3	6.7	8.5	10.7	13.4	16.9	21.3	26.8	33.7	42.4	53.4	67.2	84.6	
	2.5	4.5	5.7	7.2	9.1	11.4	14.4	18.1	22.8	28.7	36.1	45.5	57.3	72.1	90.8	
	2.75 Cent Pseu	4.8	6.1	7.7	9.7	12.2	15.3	19.3	24.3	30.6	38.5	48.5	61.0	76.8	96.7	
	3	5.1	6.5	8.1	10.2	12.9	16.2	20.5	25.7	32.4	40.8	51.4	64.7	81.4		
	3.25 Acar	5.4	6.8	8.6	10.8	13.6	17.1	21.6	27.2	34.2	43.0	54.2	68.2	85.9		
	3.5	5.7	7.2	9.0	11.4	14.3	18.0	22.7	28.5	35.9	45.2	56.9	71.7	90.2		
	4	6.2	7.8	9.9	12.4	15.6	19.7	24.8	31.2	39.3	49.4	62.2	78.3	98.6		
	5	7.2	9.1	11.4	14.4	18.1	22.8	28.7	36.2	45.6	57.4	72.2	90.9			

TAXON:

ANNEX 7

RECOMMENDATIONS FOR THE REVISION OF THE BMP GUIDELINES FOR MESOZOOPLANKTON

Sampling

As for all other parameters within the BMP, the aims of the programme should govern the strategy to be used. For the parameter mesozooplankton, this is crucial since the sampling gear used at present is a compromise with obvious drawbacks (e.g., loss of smaller organisms and avoidance by large organisms, susceptibility to clogging). For the next phase of the programme, this must be taken into account in order to optimize accuracy and precision. If microzooplankton will be included in the BMP, some of the present limitations could be solved.

For the present, only the WP-2 net of 100 µm shall be used (as is recommended in the present guidelines). The original WP-2 net was fitted and tested by UNESCO with a 200 µm mesh. The filtration efficiency of the 100 µm version used in the Baltic can be reduced during periods of high phytoplankton abundance. If this type of net will remain the standard gear, one may consider altering the construction in order to improve the efficiency.

The weight to keep the wire vertical should be 25 kg (40 kg when the wire angle tends to exceed 25°, UNESCO, 1968).

The wire angle should always be reported. A correction table is given in Annex 5. If the wire angle exceeds 40°, the sample should be discarded.

For fractionated hauls, the intervals should be (illustrated in Figure A7.1):

bottom to halocline (included)

top of halocline to thermocline (included)

top of thermocline to surface

If there is no thermocline, a standard haul of 25–0 m should be made.

If there is no halocline, there should be a standard haul of 75 m

to the thermocline (included) or to 25 m if there is no thermocline either.

No hauls shorter than 5 meters should be made.

Flow meters should always be used. They should be mounted at 1/4 of the diameter of the ring (UNESCO, 1968).

The net shall always be rinsed by use of a gentle flow from a hose. When fractionated, only the part below the strap should be rinsed. After emptying, the whole net shall be rinsed with the cod-end open.

After each cruise, the net shall be washed in warm fresh water with a detergent to secure optimum filtration capacity.

When jellyfish appear in the sample, it is recommended that the sample be discarded and a new sample taken. When it is impossible to avoid jellyfish, they should be rinsed from other zooplankton and then discarded. When applicable, these procedures should be recorded.

Sub-sampling and counting procedure

It was decided to recommend a calibrated Stempel-pipette or a Kott Splitter. The Kott Splitter is somewhat better in precision, but is time-consuming to handle.

A few drops of a detergent should be added to allow the cladocerans to mix in the sample.

Microscope

The microscopes used should have magnifications to at least 125 X.

Analysis

1 Abundance

a) One hundred individuals of the three most dominant⌖ groups (other than nauplii, rotifers and tintinnids⌖⌖) should be counted. This amounts to a precision of 20% (see Tables A12.1 and A12.2, Annex 12). If a total of 100 individuals is not reached in one sub-sample, additional sub-samples must be counted. The taxonomic group(s) that reached 100 individuals in the previous sub-sample, need not be counted in the next sub-sample(s).

b) An overview of the remaining part of the sample should be made in order to look for rare species that did not appear in the sub-samples. These qualitative findings should be reported as a comment. The same is valid for macrozoo- and meroplankton found.

⌖ The term "taxonomic groups" includes species, genera, families as well as different developmental stages of copepods.

⌖⌖ Although nauplii, rotifers and tintinnids fall outside the size range of mesozooplankton and are not all retained by a 100 µm mesh, there is a considerable amount of historic data on these groups. They should thus be reported, but from a QA point of view there is an urgent need to evaluate these results.

2 Biomass

2.1 Obligatory

It is obligatory to use biomass factors for the different taxonomic groups and developmental stages. To facilitate the calculations, these factors should be included in the computer software used for analysis and data reporting (see Annex 11). The present factors need to be improved taking into account the seasonal and geographical differences in individual volume*.

2.2 Voluntary

It is a voluntary option to use direct measurements of ash-free dry mass (AFDW) of one half the sample. Samples, which have been deep frozen (−18 °C) on pre-weighed glass fibre filters (GF/C, d = 47 mm), should be dried at 60 °C in an oven (Lovegrove, 1962, 1966) and ashed at 500 °C. The computer software should also be used for reporting these voluntary data **.

* Mrs M. Wolska -Pys and Mr Z. Witek of Poland have kindly offered to provide biomass factors for meroplankton of the southern Baltic Sea.

** Direct measurements of biomass have the advantage of being comparatively quick to perform and the method is less biased than that using factors for individual volumes, provided that the sample contains few phytoplankton. Not only the factors bias the method, van Guelpen *et al.* (1982) showed that the splitting procedure can introduce an additional error of about 13 %.

Recording and reporting procedures

All BMP laboratories analyzing zooplankton and reporting data should use the same type of computer software. This software should also contain a part that carries out a quality control of the data before they are forwarded to the data bank.

References

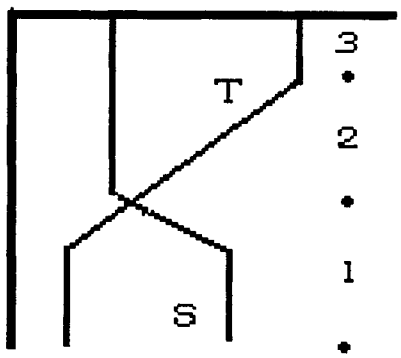
van Guelpen, L., Markle, D.F., and Duggan, D.J. 1982. An evaluation of accuracy, precision, and speed of several zooplankton subsamples techniques. J. Cons. int. Explor. Mer, 40: 226–236.

Lovegrove, T. 1962. The effect of various factors on dry weight values. Rapp. P.-v. Reun. Cons. int. Explor. Mer, 153: 86–91.

Lovegrove, T. 1966. The determination of dry weight of plankton and the effect of various factors on the values obtained. *In* Some contemporary studies in marine science, pp. 462–467. Ed. by H. Barnes. Allen and Unwin Ltd., London.

UNESCO, 1968. Monographs on oceanographic methodology 2. Zooplankton sampling. Paris. 174 pp.

Figure A7.1. Sampling depths during fractionated hauls.



ANNEX 8

MEASUREMENTS OF VERTICAL IRRADIANCE ATTENUATION

At station 46, underwater attenuation profiles were measured in duplicate, within 5 minutes. Measurements were made at every 0.5 m up to 5 m and in 1-m intervals below 5 metres. A LICOR underwater sensor was used with a spherical bulb. Both results were identical with a k value (m–1) of 0.43, with a r-square of 0.875 and 0.759, respectively. At a coastal station, the measurements were repeated at low global irradiance. Again both k-values were almost identical 0.61 and 0.58, but with a much higher r-square (0.994 and 0.997).

The measured hourly primary production, measured either by the incubator or by the *in situ* method, can be converted into daily production by the light factor method according to the recommendations given by the BMB (1976). It was pointed out that the type of instrument which is used to measure the daily flux of irradiance is important for the calculation of the light factor. In principle, there are two different spectra of irradiance measured depending on the instruments used: global irradiance (400–2000 nm) and PAR (photosynthetic available radiation, 400–700 nm). According to preliminary results where light-factors have been calculated from the two different measures of irradiance, it seems that there will be a difference of approximately 10–20%, where the light-factor calculated from the global irradiance always is larger. In turn, this will lead to a difference in the calculated daily primary production which will be in the same range, e.g., 10–20%. This difference is probably caused by a difference in the distribution of the light energy during the day as measured by the different instruments. Most likely there is also an annual trend between the light-factors depending on the two measures of the irradiance.

It was strongly recommended that only measurements of PAR should be used for the calculation of light-factors and that the laboratories using global irradiance should change their measurement equipment after an assessment has been performed of how the change should be done in order not to introduce a discontinuity in existing and ongoing time-series of primary production.

ANNEX 9

THE P-I INCUBATOR TECHNIQUE

Results

Twelve bottles were incubated for 2 hours after adding 200 µl of radioactive ^{14}C solution to each bottle. The mean irradiance in the incubator was 268.5 µE m^{-2} sec^{-1} (ranging from 199 to 308, and measured at six positions in the incubator). The transmission of the bottles ranged from dark to 100%, with the following intermittent values: 2.5, 5, 15, 25, 30, 35, 40, 50, 65, and 80%. After incubation, the bottles were filtered over cellulose-nitrate membrane filters, which were rinsed with 10 ml cold medium. After that they were dried at 40 °C in an oven. Then they were transferred into picovials and 5 ml filter count was added.

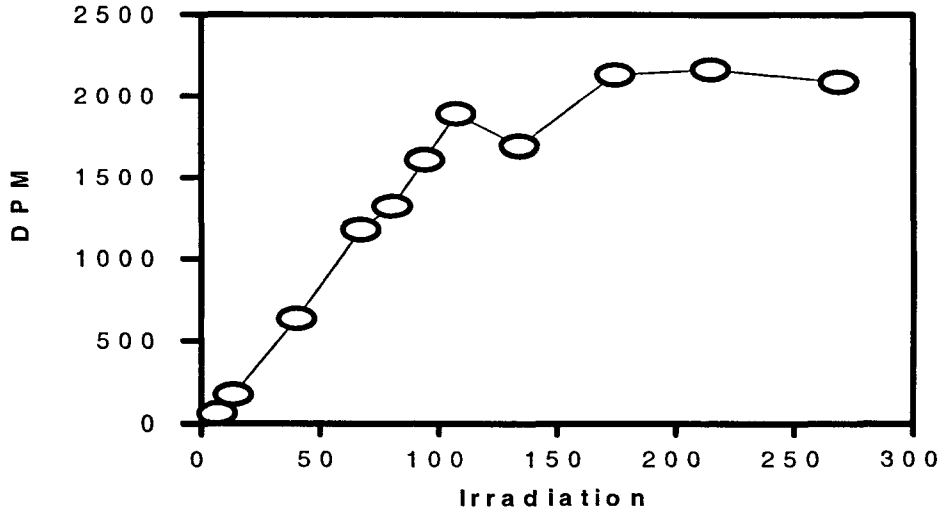
Total CO_2 (or DIC) was measured in duplicate according the standard procedure of Grasshoff *et. al.* (1.72 and 1.75 mMol C/l).

Chlorophyll-*a* was measured in triplicate after filtration of 500 ml of sample (results: 4.04, 4.33 and 4.23 mg m^{-3}, respectively).

In Figure A9.1 the results of the single incubation are presented:

Photosynthetic activity is given in dpm after subtraction of the dark value. Irradiances were calculated using the transmission percentages of the bottles. No further calculations were made.

Figure A9.1. Results of the P-I incubation test.



ANNEX 10

MANUAL FOR PRIMARY PRODUCTION MEASUREMENTS WITHIN THE FRAMEWORK OF THE HELCOM BMP

Agreed on 17 October 1996 in Warnemünde

INTRODUCTION

Primary production is the only rate measurement in the Baltic Monitoring Programme and measurements can be used to calculate the amount of newly formed organic material from light, carbon dioxide, and nutrients. Therefore, primary production has important links to eutrophication and sedimentation and, consequently, to deep-water oxygen concentrations.

1 METHOD

Primary production should be measured using the 'P/I method', in an incubator. With this method, the uptake rate of carbon is measured at a range of irradiance levels in order to obtain a relationship between photosynthesis and light. Pmax (maximum photosynthetic rate) and alpha (initial slope of the P/I relationship) can be calculated using this method.

The advantage of this method is that ecophysiological information on the phytoplankton assemblage can be derived from the P/I curve. It is also possible to calculate the daily production from these measurements and, with some assumptions, the annual primary production.

2 INCUBATOR

A standardized incubator* should be used for all measurements of primary production made in the Baltic Monitoring Programme (BMP). This includes the specially coated bottles** used for the measurement of photosynthesis at different irradiances. HELCOM should be approached to cover the expenses for identical bottles for all BMP laboratories.

3 INCUBATION TECHNIQUE

a) ^{14}C is added separately to each bottle.

b) The concentration of ^{14}C in the experimental bottles should allow for statistically sufficient counts of the radioactivity; at the same time, it should be kept as low and as precise as possible.

c) The incubation time is 2 hours and the bottles should revolve at an approximate speed of 10 rpm.

d) The incubation temperature should be kept at the *in situ* temperature. (For samples in stratified waters, two separate incubators may have to be used.)

e) The light level in the incubator should be high enough to ensure that light saturation of the transparent bottle is achieved. (preferably up to 400 $\mu E\ m^{-2}\ s^{-1}$).

f) The incubator should be placed in such a way that 'outside' light is not interfering.

4 SAMPLING TIME

Sampling should be performed during daylight.

* The incubator consists of a perspex rectangular box, illuminated from one side by TL tubes. (For a full description, the reader is referred to Colijn *et al.*, annex to the Report of ICES WG on Phytoplankton Ecology, ICES CM 1996/L:3).

** The bottles were specially prepared by ZEMOKO, (J. de Keyzer), Koudekerke, the Netherlands.

5 SAMPLING STRATEGY

At all stations, one sample should be obtained from the mixed euphotic layer (mandatory). In areas with a stratified euphotic zone, additional samples are recommended.

It is recommended to take integrated samples, using a silicon hose (Lindahl, O., 1986. A dividable hose for phytoplankton sampling. ICES CM 1986/L:26, Annex 3).

6 ^{14}C SOLUTION

It is recommended that the ^{14}C solutions used in this programme are standardized as well as the added concentration to each sample.

7 TERMINATION

At the end of the incubation, the water samples are filtered (mandatory). Whole water production is tentative. GF/F filters should be used. Some details of the procedure will be checked (rinsing, fuming HCl, drying of filters).

8 RADIOACTIVITY MEASUREMENT

Only the liquid scintillation counting technique should be used. Counting should be done to give a result of 3% accuracy. Quench curves should be established and the efficiency of the counter should be checked using an internal standard. Counting efficiency should be determined by occasional calibration using a ^{14}C standard (e.g., hexadecane).

9 TOTAL CO_2

DIC should be measured according to the standard procedure (Strickland and Parsons, 1972) or calculated from formulas (Buch, 1945). It is recommended that the formulas be checked .

10 CALCULATION

The total carbon uptake is calculated from the equation given in the guidelines. Dark values should be subtracted but also reported (see Section 12, below). Temperature correction is not needed if samples are incubated at *in situ* temperature.

11 LIGHT ATTENUATION

The light attenuation at the sampling site should be measured with an instrument that measures PAR. In case this is not possible, the measured Secchi depth can be transformed to an approximate attenuation value according to the formula:

attenuation coefficient = 1.7/Secchi depth (Raymont, 1967)*.

* This formula may not be valid in all areas of the Baltic Sea. For instance, a test with 36 parallel measurements of the attenuation coefficient and the Secchi depth in the southeast Kattegat gave a mean factor of 1.84 (standard deviation: 0.39). It is likely that this factor may increase further into the Baltic (L. Edler, pers. comm.).

12 DATA HANDLING

New Type Master and Data Cycle Records for reporting data to HELCOM must be developed. In these both raw data and calculated data must be given. Supporting data for other conversions must also be included. It is recommended that the HELCOM Phytoplankton Expert Group applies for a project to develop a computer programme for the productivity calculations.

13 NECESSARY SUPPORTING PARAMETERS

In order to make all productivity calculations, the following parameters need to be measured:

PAR, vertical light attenuation

Salinity, temperature, pH, alkalinity,

Chlorophyll

Depth of chlorophyll maximum from fluorescence profiles

14 SAMPLING FREQUENCY

In order to obtain useful values for the primary productivity of the Baltic Sea, it is necessary to sample at least 15 times per year in the open sea. At coastal stations, an even higher sampling frequency is necessary; 20–25 times per year.

15 COSTS

The cost for the investment of material and the costs for one year of monitoring should be estimated and be put in relation to the cost of other parameters and ship time.

ANNEX 11

PROPOSAL FOR AN IMPROVED COMPUTERIZED ANALYSIS AND DATA REPORTING PROGRAMME

It was the opinion of the Workshop that, for zooplankton analysis and data reporting, there is a need for a common computer software similar to that used for phytoplankton. There are already some in existence that can be used as a basis, but improvements are needed. It was concluded that the following items should be considered;

1) It should be possible to correct the file during the input of data.

2) There should be an internal quality control in the program.

3) The program should automatically calculate the abundance per m^3 independent of sub-sample size used (e.g., when abundant and rare species are counted in different sub-sample volumes).

4) Rare species should be listed in table form instead of on a comment line.

5) Since the biomass factors vary with geographical area and season, this must be reflected in the matrices.

6) Biomass should also be given as ash-free dry mass.

7) It would be helpful to have a table in the N-files (plain language files) where a record of, e.g., number of counted individuals, abundance, biomass and % of total, is shown for the major taxonomic groups.

8) It should be possible to export the files to other formats, e.g., Excel.

9) When new factors for calculation are introduced (e.g., for biomass), the programme should automatically save the old version as a separate file.

ANNEX 12

ZOOPLANKTON SUB-SAMPLING AND COUNTING PROCEDURE

lecture by Alexander Korshenko, Marine Pollution Monitoring Laboratory, State Oceanographical Institute, Moscow, Russian Federation

I. Stempel pipette

1 Sample volume

At the beginning, the total sample should be diluted or concentrated according the experience of the investigator in order to reach an appropriate concentration of organisms. The volume of the sample should be measured in a graduated cylinder.

2 Sub-sampling

The sample should be mixed intensively until all organisms are distributed randomly in the sample volume. Lumps (aggregations of organisms) should be taken out of the sample and the organisms counted.

3 Microscope

Microscopes should have a range of magnifications up to at least 125 X. The field of view at low magnification should cover both walls of the chamber.

4 Counting procedure 1

The first counting portion should be small, possibly one Stempel pipette of 1 ml volume. All organisms, including rotifers and nauplii, which cannot be caught quantitatively by the net with 100 µm mesh size must be counted.

5 Counting procedure 2

The counting procedure continues with additional sub-samples until the first three most abundant groups, with the exception of rotifers and nauplii, reach the level of 100 counted specimens.

***Note**: For a Baltic mesozooplankton community with many dominant groups, the total amount of specimens counted, according the recommended procedure, can often reach 500–800 (rotifers + nauplii + 3 groups x 100 individuals + 'tail' of less dominant species).*

The term 'group' covers taxonomic groups of different levels (species, genus, family, etc.) as well as development stages (copepodites, nauplii, etc.).

7 Calculation of abundance

Since the sub-sampled volume will probably not be the same for each group, their abundance must be calculated separately from the number of counted specimens and the volumes of the sub-sample, sample and water filtered:

$$N\ (ind/m^3) = K \times \frac{m}{Vf}$$

where m is the number of counted specimens (ind.), Vf is the volume of water filtered (m^3) and K is the counted part of the sample.

For the Stempel pipette method: K = Vs / Vsub, where Vs is the volume of sample and Vsub is the total volume of sub-samples.

Finally, the total abundance is the sum of the abundance of all groups.

8 Precision of the counting procedure

It was assumed that the precision of the calculated abundance depends only on the number of specimens counted (Cassie, 1971). The upper and lower 95% confidence limits of counted numbers have been calculated and presented in Tables A12.1 and A12.2. The first table includes the confidence limits when the number of counted specimens is less than 17, assuming an asymmetrical Poisson distribution; Table A12.2 includes confidence limits when the number of specimens is more than 16, assuming Normal distribution. The limits are expressed both in absolute numbers and percentages. The latter is valid not only for counts, but also for the subsequently calculated abundance.

For example, the precision of calculated abundance for organisms of the first three groups, that will be counted up to 100 specimens, amounts to 20% (Table A12.2). The estimation of abundance for other groups ('tail') will be less precise. The groups with the number of counted specimens less than 8 cannot be considered to be counted quantitatively.

9 Counting procedure 3

The remaining part of the sample should be checked for taxonomic groups which were not found in the sub-samples already counted. Their presence should be reported as well.

10 Reporting

The number of specimens counted, the volume of water filtered, and the portion of the sample counted (K) must be reported in the data sheet. The precision of the counting procedure can easily be estimated from the number of specimens counted.

II Kott splitter

1 Sample volume

The sample should be diluted/concentrated to the appropriate volume.

2 Sub-sampling

The sample should be sub-sampled until the compartments contain the number of organisms needed for counting.

3 Counting procedure

One or, when necessary, more chambers from the same level of sub-sampling should be counted with the aim of reaching a level of 100 specimens for the three most abundant groups, with the exception of nauplii and rotifers. These last two should be counted only in the first chamber.

4 Calculation of abundance

The abundance of each group should be calculated as:

$$N\ (ind/m^3) = K \times \frac{m}{Vf},$$

where Vf is the volume of water filtered (m^3), m is the number of counted specimens (ind.), and K is the counted part of the sample.

For the Kott splitter $K = L^{-1}/n$, where L is the level of splitting = 1/10, 1/100, etc., and n is the number of counted chambers.

Finally, the total abundance is the sum of all groups.

5 Next steps

Steps 8 to 10 for the Stempel pipette should be followed.

REFERENCES

Cassie R.M. 1971. Sampling and Statistics. *In* A Manual on Methods for the Assessment of Secondary Productivity in Fresh Waters. Ed. by W.T. Edmondson and G.G. Winberg. IBP Handbook N 17, Oxford, p. 181.

HELCOM. 1988. Guidelines for the Baltic Monitoring Programme for the Third Stage. Part D. Biological determinands. Baltic Sea Environment Proceedings, No. 27D. 161 pp.

Kozova, O.M., and Melnik, N.G. 1978. (Instruction for plankton samples treatment by counting method.) Eastern Siberia Pravda, Irkutsk, 52 pp. (in Russian)

Veldre, S.R. (Statistical verification of counting method used for quantitative analysis of plankton samples). *In* (Application of mathematical methods in biology), Collected papers, Leningrad State University, Vol.2, pp.124–131. (in Russian)

Table A12.1. Lower and upper 95% confidence limits (in units and as a percentage) for number of counted specimens less than 17 (Kozova and Melnik, 1978).

N ind counted	Lower limit	Upper limit	Lower limit (%)	Upper limit (%)
0	0	3.7	!!!	!!!
1	0.03	5.6	97.0	460.0
2	0.2	7.2	90.0	260.0
3	0.6	8.7	80.0	190.0
4	1.1	10.2	72.5	155.0
5	1.6	11.8	68.0	136.0
6	2.2	13	63.3	116.7
7	2.8	14.4	60.0	105.7
8	3.4	15.7	57.5	96.3
9	4.1	17	54.4	88.9
10	4.8	18.3	52.0	83.0
11	5.5	19.6	50.0	78.2
12	6.2	21	48.3	75.0
13	6.9	22.2	46.9	70.8
14	7.6	23	45.7	64.3
15	8.4	24.7	44.0	64.7
16	9.1	25.3	43.1	58.1

Table A12.2. Lower and upper 95% confidence limits (in units and as a percentage) for number of counted specimens more than 17 (see also Kozova and Melnik, 1978; HELCOM, 1988; Veldre).

N ind counted	Lower limit	Upper limit	Lower limit (%)	Upper limit (%)
17	8.9	25.1	47.5	47.5
18	9.7	26.3	46.2	46.2
19	10.5	27.5	45.0	45.0
20	11.2	28.8	43.8	43.8
25	15.2	34.8	39.2	39.2
30	19.3	40.7	35.8	35.8
35	23.4	46.6	33.1	33.1
40	27.6	52.4	31.0	31.0
45	31.9	58.1	29.2	29.2
50	36.1	63.9	27.7	27.7
60	44.8	75.2	25.3	25.3
70	53.6	86.4	23.4	23.4
80	62.5	97.5	21.9	21.9
90	71.4	108.6	20.7	20.7
100	80.4	119.6	19.6	19.6
110	89.4	130.6	18.7	18.7
120	98.5	141.5	17.9	17.9
130	107.7	152.3	17.2	17.2
140	116.8	163.2	16.6	16.6
150	126.0	174.0	16.0	16.0
275	242.5	307.5	11.8	11.8
300	266.1	333.9	11.3	11.3
350	313.3	386.7	10.5	10.5
400	360.8	439.2	9.8	9.8
450	408.4	491.6	9.2	9.2
500	456.2	543.8	8.8	8.8
600	552.0	648.0	8.0	8.0
700	648.1	751.9	7.4	7.4
800	744.6	855.4	6.9	6.9
900	841.2	958.8	6.5	6.5
1000	938.0	1062.0	6.2	6.2
1500	1424.1	1575.9	5.1	5.1